

INTERNATIONAL SYMPOSIUM ON “GENOMICS IN HEALTH AND DISEASE”

&

40TH ANNUAL CONFERENCE OF INDIAN SOCIETY OF HUMAN GENETICS

28TH TO 30TH JANUARY 2015, NEHRU CENTRE, MUMBAI

ORGANIZED BY NATIONAL INSTITUTE OF IMMUNOHAEMATOLOGY (ICMR), MUMBAI

The Scientific Programme

Day 1: 28th January 2015 (Main Auditorium)

8.00 am to 10.00 am	Registration
10.00 am to 10.10 am	Welcome Address: Dr K Ghosh, Chairman, Organizing Committee Chairpersons : Prof Partha Majumder, Dr K Ghosh
10.15 am to 11.00 am	Key Note Address : Prof Stylianos E Antonarakis, Switzerland Transcriptome dysregulation and single cell genomics in Down syndrome
11.00 am to 11.30 am	Break for Tea/Coffee
11.30 am to 12.15 pm	Dr L D Sanghvi Oration
12.15 pm to 1.00 pm	Plenary Session-I Chairpersons: Dr Dipika Mohanty, Dr S V Chiplunkar
12.15 pm to 12.35 pm	Cancer genetics including Circulating Tumor Cells - Dr Purvish Parikh, Mumbai
12.35 pm to 12.55 pm	Genetics of complex diseases : From genome to epi-genome, a new perspective for understanding complex diseases - Dr Sanjeev Galande, Pune
1.00 pm to 2.00 pm	Lunch and viewing the posters
2.00 pm to 4.00 pm	Young Scientist Award Presentation (Y1 – Y8), Chairpersons: Dr. K. Thangaraj, Dr. Q. Annie Hasan
4.00 pm to 4.30 pm	Break for Tea/Coffee and viewing the Posters
4.30 pm to 6.30 pm	Plenary Session-II Chairpersons : Prof S. E. Antonarakis, Prof N K Mehra
4.30 pm to 4.55 pm	Structure and Evolution of Genes of the innate immune system - Prof Partha Majumder, Kolkata
4.55 pm to 5.20 pm	Post Genomics era: MHC is where the action is? - Prof N K Mehra, New Delhi
5.20 pm to 5.45 pm	Exome sequencing reveals new genes involved in genitourinary development - Prof Ken McElreavey, France
5.45 pm to 6.10 pm	Hereditary Cancers in South Asians: Bench to Bedside - Dr Rajiv Sarin, Mumbai
6.15 pm to 7.00 pm	Poster Presentation (P1 to P60)

Inauguration

7.00 pm to 8.00 pm	Dr Vishwa Mohan Katoch, Secretary DHR & DG, ICMR
8.00 pm to 10.00 pm	Dinner

Day 2: 29th January 2015 (Hall A)

8.00 am to 9.30 am	Registration
9.30 am to 11.00 am	Session 1: Cancer Genetics Chairpersons : Dr P S Chauhan, Dr Farah Jijina
9.30 am to 9.50 am	Is Cytogenetics of hematological malignancies still relevant in the genomic era? - Prof Susan Mathew, USA
9.50 am to 10.10 am	Clinical Applications of Molecular Genetics in Bone and Soft Tissue Neoplasia - Prof Meera Hameed, USA
10.10 am to 10.30 am	Translating Cancer Genomics to Medicine - Dr Amit Dutt, Mumbai
10.30 am to 10.50 am	Cytogenetics and molecular profiling in Multiple Myeloma - Dr Pratibha Amare, Mumbai
11.00 am to 11.30 am	Break for Tea/Coffee and viewing the Posters
11.30 am to 1.00 pm	Session III : Inborn Errors of Metabolism Chairpersons : Prof A Jyothy, Dr Jayesh Sheth
11.30 am to 11.50 am	Clinical Practice in NGS Era - Prof I C Verma, New Delhi
11.50 am to 12.10 pm	Mitochondrial Disease Diagnosis and Management - Dr Virginia Kimonis, USA
12.10 pm to 12.30 pm	Enzyme replacement therapy for lysosomal storage disorders - Dr Mamta Muranjan, Mumbai
12.30 pm to 12.50 pm	Screening & Diagnosis of IEM by Mass-Spectrometry - 15 Years of MILS Experience - Dr Usha Dave, Mumbai
1.00 pm to 2.00 pm	Lunch and viewing the Posters

2.00 pm to 3.30 pm
Oral Presentations (10 minutes each)

Oral Presentations (O1 - O9)
Chairpersons : Dr Rama Mittal, Dr Annie Hasan
1. Validation of Wilson's Disease DNA Microarray- Dr Manjula Mathur, Mumbai
2. Antioxidant gene variants and Type-2 Diabetes: A north Indian study - Dr Monisha Banerjee, Lucknow
3. Association study of inflammatory genes with Rheumatic Heart disease in North Indian population: A multi-analytical approach- Ms Usha Gupta, Lucknow
4. Parental CYP21A2 genotyping plays an important role in reduction of neonatal deaths in CAH families- Dr Eunice Marumudi, New Delhi
5. Association of D18S880 polymorphism of the CNDP1 gene with diabetic nephropathy in South Indian population- Dr Bodhini D, Chennai
6. Expression of CD133 and BM11 and its prognostic role in subjects with glioblastoma multiforms from Indian population- Dr Chetan GK, Bangalore
7. Insights on the functional impact of microRNA's in Autism- Associated copy number variants- Dr Varada Rajan Vaishnavi, Chennai
8. A clustering approach for mapping rare variants based on mutual association- Dr Saurabh Ghosh, Kolkata
9. Genetic markers in PCOS risk and phenotype progression - Dr Srabani Mukherjee, Mumbai
Break for Tea/Coffee and viewing the Posters

3.30 pm to 4.00 pm
4.00 pm to 6.00 pm

Session V: Clinical Genetics

Chairpersons : Dr Ruchi Nanavati, Dr Aparna Parikh
Genetic Disorders and Consanguinity: Indian Scenario – Dr Shubha Phadke, Lucknow
Noninvasive prenatal diagnosis - Dr Nilesh Dharajiya, USA
Disorders of imprinting - Dr Koumudi Godbole, Pune
Fetal dysmorphology in fetal autopsy - Dr Prakash Gambhir, Pune
Molecular Diagnosis of Genodermatoses in India - Dr Parag Tamhankar, Mumbai
Poster Presentation (P61 to P120)
General Body Meeting
Dinner

Day 2: 29th January 2015 (Hall B)

9.30 am to 11.00 am

Session II: Hemoglobinopathies

Chairpersons: Dr S L Kate, Dr Roshan Colah
Past, Present and future of Hemoglobinopathies in India- Dr Dipika Mohanty, Bhubaneswar
Spectrum of molecular and clinical heterogeneity in HbH disease in north Indian patients – Dr Reena Das, Chandigarh
Role of borderline Hb A2 in carrier detection of beta thalassemia - Dr Anita Nadkarni, Mumbai
Next generation technologies for rare mutation detection - Dr Prashant Khadke, Mumbai
Break for Tea/Coffee and viewing the Posters
Session IV: Genetics of Complex Diseases
Chairpersons: Dr AJS Bhanwer, Dr K Thangaraj
ITGAM and lupus susceptibility: From genetic association to causal variant identification - Dr Swapan Nath, USA
Genetic and epigenetic causes of congenital heart defects - Dr Uppala Radhakrishna, USA
Confused Confucius: The circle of complex disorders - Dr Dwaipayan Bharadwaj, New Delhi
Elucidation of genetic and cellular defect in Neurodevelopmental and Neuropsychiatric disorders – Dr Dhanjit Das, Mumbai
Lunch and viewing the posters
Oral Presentations (O10 to O18)

1.00 pm to 2.00 pm
2.00 pm to 3.30 pm
Oral Presentations (10 minutes each)

Chairpersons: Dr Hema Prasad, Dr. V. Babu Rao

1. Whole-exome sequencing helps characterize a rare highly penetrant familial disorder, turning epidemic in a village of Jammu and Kashmir, India- Dr Swarkar Sharma, Jammu
2. FISH for Prenatal, Post-natal, and Preimplantation Genetic Diagnosis:- Dr Lim Jee Hian, Malaysia
3. Masking of the commonest Indian beta-thalassaemia lesion IVS 1 nt 5 [G>C] by different α -globin gene defects- Ms Stacy Colaco, Mumbai
4. Molecular karyotyping for prenatal diagnosis- Dr Manjeet Mehta, Mumbai,
5. Effect of hydroxyurea on microRNA expression and its role in fetal hemoglobin induction in sickle cell anemia patients- Ms Madhavi Sawant, Mumbai
6. Validation of nsSNPs for pharmacogenetic analysis amongst hypertensive Punjabi population- Dr Praveen P Balgir, Patiala
7. Identification of novel disease genes/mutations for single gene disorders through homozygosity mMapping and whole exome sequencing- Mr Aneek Das Bhowmik, Hyderabad
8. Chromosomal aberrations and importance of genetic counseling: A study of 288 couples with bad obstetric history - Dr Shailesh Pande, Mumbai
9. Genomic studies of high altitude pulmonary edema, a critical condition in susceptible sojourners- Dr Soma Sarkar, New Delhi
Break for Tea/Coffee and viewing the Posters

3.30 pm to 4.00 pm

4.00 pm to 6.00 pm	Session VI: Pharmacogenomics
4.00 pm to 4.25 pm	Chairpersons: Dr Nilima Kshirsagar , Dr. B. R. Das,
4.25 pm to 4.45 pm	Pharmacoeugenomics of Malaria and its Relevance - Dr Satyamoorthy, Manipal
	Understanding therapeutic practice in Schizophrenia using pharmacogenomic approach; an Indian perspective –
4.45 pm to 5.10 pm	Dr. Moinak Banerjee, Thiruvananthapuram
5.10 pm to 5.35 pm	Optimal dosing of warfarin in Indian Population: Contribution of Genetic polymorphisms - Dr Shrimati Shetty, Mumbai
5.45 pm to 6.30 pm	Pharmacogenomics and Drug Discovery and Development - Dr Sanish Davis, Mumbai
6.30 pm to 7.30 pm	Poster Presentation (P61 to P120)
8.00 pm to 10.00 pm	General Body Meeting (Hall A)
	Dinner

Day 3: 30th January 2015 (Hall A)

9.30 am to 11.00 am	Session VII: Chromosomal Diseases
9.30 am to 9.50 am	Chairpersons: Dr Smita Mahale, Dr Pratibha Amare
9.50 am to 10.10 am	Understanding of chromosomal rearrangements: A challenge to Geneticist - Dr Frenny Sheth , Ahmedabad
10.10 am to 10.30 am	Assessment of DNA Microarray for evaluation of Microdeletion syndromes - Dr Ashutosh Halder, New Delhi
10.30 am to 10.50 am	Indigenous or Outsourcing? Make in India Molecular Diagnosis of Fragile X Syndrome - Dr Sarita Agarwal, Lucknow
11.00 am to 11.30 am	Intellectual developmental disorders: A molecular cytogenetic update - Dr Rajasekhar Moka, Manipal
11.30 am to 1.00 pm	Break for Tea/Coffee and viewing the Posters
Oral Presentations (10 minutes each)	Oral Presentation (O19 to O27)
	Chairpersons: Dr. Y. Z. Italia, Dr Ajit Gorakshakar
	1. Mitochondrial DNA diversity and its implications in the genetic history of three different populations of Amini Islander's of Lakshadweep- Dr MS Mustak, Mangalore
	2. Genetic association study and gene expression analysis revealed osteoprotegerin as candidate gene for otosclerosis- Dr Sourabh Priyadarshi, Bhubaneswar
	3. Clinical, Haematological, Molecular characterization and response to hydroxyurea treatment of symptomatic 17 HbSE cases in Eastern India: The largest series in world- Dr Siris Patel, Burla
	4. Mitochondrial somatic mutations in Human gastric Cancer- Ms Bhagya Bhavana, Hyderabad
	5. Clinical Exome sequencing to aid diagnosis- Case studies and lessons learnt- Dr Nandita M, Bangalore
	6. The etiopathogenesis of human genetic disease- Translating basic research to clinic- Dr Radha Saraswathy
	7. Association of Monocyte chemoattractant protein-1 (MCP-1) gene polymorphisms [-2518 A>G (rs1024611) and I/D (rs3917887)] with T2D and ESRD patients from population of Punjab - AJS Bhanwer, Amritsar
	8. Studies on obese breast cancer patients with leptin gene polymorphism- Dr Kaiser Jamil, Hyderabad
	9. Development of multiplex PCR based method for diagnosis of Duchenne Muscular Dystrophy (DMD) in the patients attending PGIMS hospital- Dr Daya Shankar Lal Srivastava, Rohtak
1.00 pm to 2.00 pm	Lunch
2.00 pm to 2.30 pm	Poster presentation (P121 to P185)

Day 3: 30th January 2015 (Hall B)

9.30 am to 11.00 am	Session VIII: Genetics Epidemiology
9.30 am to 9.50 am	Chairpersons: Prof Virginia Kimonis, Dr Kunal Ray
9.50 am to 10.10 am	Integrative genomics approach for identification of functionally informative regions in the human genome: Bridging the gap from genotype to phenotype –Dr Mitali Mukerji, New Delhi
	A global perspective on the functional genomics of congenital glaucoma using next generation sequencing –
10.10 am to 10.30 am	Dr Subhabrata Chakrabarti, Hyderabad
10.30 am to 10.50 am	Nutrient-Mediated Teratogenesis and Risk of Diabesity in Indians - Dr Giriraj Chandak, Hyderabad
11.00 am to 11.30 am	Palaeolithic Antiquity and Continuity of Indian Populations: Genomic and Non-Genomic Evidences – Prof V R Rao, New Delhi
11.30 am to 1.00 pm	Break for Tea/Coffee and viewing the Posters
Presentations (10 minutes each)	Oral Presentation (O28 to O36)
	Chairpersons: Dr Devila Sahu, Dr Manisha Madkaikar
	1. Surface Layer Protein-A (SlpA): A protein domain of the Lactobacillus sp. with anti-cancer activity on GIT cancer cell lines- Dr Ranjith Kumavath, Kerala
	2. Role of ADAM33-S2 (G/C) gene polymorphism in the etiology of COPD in south Indian population- Ms Vijaya Laxmi K, Hyderabad
	3. Association of PGC-1 α Gene with Type 2 Diabetes in Three Unrelated Ethnic Groups of North-West India – Dr Rubina Sharma, Amritsar

	4. Association of cytokine gene polymorphism with periodontal disease- Dr Manikandan G R, Trivandrum
	5. TP53 polymorphisms and chromosomal instability in Breast Cancer patients: A follow up study- Dr Sarika Sharma, Amritsar
	6. A Common Functional Genetic variant of Pancreastatin profoundly increases the risk for cardiometabolic diseases in Indian population- Dr Prasanna KR Allu, Chennai
	7. Genotypes of CYP1A1, SULT1A1 and SULT1A2 and risk of squamous cell carcinoma of the esophagus in Kashmir, India; outcome of a case-control study- Dr Idrees Ayoub Shah, J&K
	8. Role of Shh-Gli1 signaling Novel Transcription Factor BM1 in Medulloblastoma Development- Dr Mohammed Afzal, Aligarh
	9. Genetics of Fetal Hemoglobin- Dr Aparna Bhanushali, Mumbai
1.00 pm to 2.00 pm	Lunch
2.00 pm to 2.30 pm	Poster presentation (P121 to P185)
2.30 pm to 3.30 pm	Plenary Session-III (Main Auditorium) Chairpersons: Prof Ken McElreavey, Dr Balraj Mittal Darwin to DNA – Story of Evolution Retold - Dr K Ghosh, Mumbai Ethical issues in Human Genetics Research - Dr Roli Mathur, New Delhi
2.30 pm to 2.55 pm	Plenary Session-IV Chairpersons: Dr Malay Mukherjee, Dr Prochi Madon Next Generation Human Genetics: Opportunities and Challenges - Dr Arindam Maitra, Kolkata
2.55 pm to 3.20 pm	Innovations in chromosome analysis using automated digital imaging technologies and multi-color Spectral Karyotyping (SKY®) - Dr Michael Koehler, Germany
3.30 pm to 4.30 pm	Advances in Preimplantation Genetic Screening - Dr Alan Thornhill, UK
3.30 pm to 3.50 pm	Valedictory function followed by Tea
3.50 pm to 4.10 pm	
4.10 pm to 4.30 pm	
4.30 pm to 5.00 pm	

Invited Lectures

No	Title	Name
I1	Transcriptome dysregulation and single cell analysis in Trisomy 21	Stylianios E. Antonarakis
I2	Cancer Genetics including Circulating Tumor Cell	Parikh PM
I3	From Genome To Epigenome: A New Perspective For Understanding Complex Diseases	Sanjeev Galande
I4	Structure And Evolution Of Genes Of The Innate Immune System	Partha P. Majumder
I5	No Abstract	N. K. Mehra
I6	Exome sequencing approaches to understand the genetic basis of human urogenital anomalies	Ken McElreavey
I7	No Abstract	Rajiv Sarin
I8	Cytogenetics of Acute Leukemia and Non-Hodgkin Lymphoma	Susan Mathew
I9	Clinical Applications Of Molecular Genetics In Bone And Soft Tissue Neoplasia	Meera Hameed
I10	Cytogenetics And Molecular Profiling In Multiple Myeloma	Pratibha S. Kadam Amare
I11	Translating Cancer Genomics to Medicine	Amit Dutt
I12	No Abstract	I.C. Verma
I13	Mitochondrial Disease Diagnosis And Management	Virginia E. Kimonis
I14	Enzyme Replacement Therapy For Lysosomal Storage Disorders	Mamta Muranjan
I15	Screening & Diagnosis Of Inborn Errors Of Metabolism (Iems) By Mass Spectrometry - 15 Years Of MILS Experience	Usha P. Dave
I16	Genetic Disorders and Consanguinity in India	Shubha Phadke
I17	Noninvasive Prenatal Diagnosis	Nilesh Dharajiya
I18	Disorders of Imprinting	Koumudi Godbole
I19	Fetal Dysmorphology In Fetal Autopsy	Prakash Gambhir
I20	Molecular Diagnosis of Genodermatoses	Parag M Tamhankar
I21	The Past, Present and Future of Hemoglobinopathies in India	Dipika Mohanty
I22	Wide Spectrum Of Molecular And Clinical Heterogeneity In HBH Disease In North Indian Patients	Reena Das
I23	Role Of Borderline Hb A2 In Carrier Detection Of Beta Thalassemia.	Anita H Nadkarni
I24	Next Generation Technologies For Rare Mutation Detection	Prashant Khadke
I25	ITGAM And Lupus Susceptibility: From Genetic Association To Causal Variant Identification	Swapna K. Nath
I26	Genetic And Epigenetic Causes Of Congenital Heart Defects	Uppala R
I27	Confused Confucius!! The Circle of Complex Disorders	Dwaipayan Bharadwaj
I28	Elucidation of genetic and cellular defect in Neurodevelopmental and Neuropsychiatric disorders	Dhanjit Kumar Das
I29	Immunological Basis Of Schizophrenia: A Diagnostic And Therapeutic Viewpoint	Moinak Banerjee
I30	Pharmacoeugenomics of Malaria and its Relevance	H. Gupta
I31	Optimal Dosing Of Warfarin In Indian Population: Contribution Of Genetic Polymorphisms	Shrimati Shetty

I32	Pharmacogenomics and Drug Discovery and Development	Sanish
I33	Understanding Of Chromosomal Rearrangements: A Challenge To Geneticist	Frenny Sheth
I34	Assessment Of DNA Microarray For Evaluation Of Microdeletion Syndromes	Ashutosh Halder
I35	Indigenous Or Outsourcing? Make In India Molecular Diagnosis Of Fragile X Syndrome.	Sarita Agarwal
I36	Intellectual Developmental Disorders: A Molecular Cytogenetic Update	Rajasekhar M
I37	Bridging The Gap From Genotype To Phenotype Using Integrative Genomics Approach	Mitali Mukerji
I38	No Abstract	Subrata Chakrabarty
I39	Nutrient-Mediated Teratogenesis and Risk of Diabetes in Indians	Giriraj Ratan Chandak
I40	Palaeolithic Antiquity And Continuity Of Indian Populations: Genomic And Non-Genomic Evidences	V. R. Rao
I41	Darwin To DNA – Story Of Evolution Retold	K. Ghosh
I42	Ethical Issues in Human Genetics Research	Roli Mathur
I43	Next Generation Human Genetics: Opportunities and Applications	Arindam Maitra
I44	Innovations in chromosome analysis using automated digital imaging technologies and multi-color Spectral Karyotyping (SKY®)	Michael R. Koehler
I45	Advances In Preimplantation Genetic Screening	Alan Tornhill

Young Scientist Award

No.	Title	Name
YSA1	Role of apoptosis in benign and malignant tumors of breast.	Syeda Zubeda
YSA2	Investigation of insulin like factor-3 (<i>INSL3</i>) gene polymorphisms with PCOS susceptibility.	Nuzhat Shaikh
YSA3	Human frontal cortex is enriched for G: C>T: A somatic transversions mediated by physiological oxidative stress.	Anchal Sharma
YSA4	Deregulation of immunogenetic factors in Type 2 Diabetes Mellitus patients with impaired wound healing.	Kanhaiya Singh
YSA5	<i>CETN1</i> variations cause idiopathic male infertility.	D V Sudhakar
YSA6	Genetic epidemiology of pharmacogenetic variants associated with Warfarin and Clopidogrel in North Indians.	Anil K Giri
YSA7	Abnormal complement activation leads to pathological neovascularisation in subjects with Retinopathy of Prematurity.	Sonika Rathi

Oral Presentations

No.	Title	Name
O1	Validation of Wilson's Disease DNA Microarray	Manjula Mathur
O2	Antioxidant Gene Variants and Type 2 Diabetes: A North Indian Study	Pushpank Vats
O3	Association Study of Inflammatory Genes with Rheumatic Heart Disease in North Indian Population: A Multi-Analytical Approach	Usha Gupta
O4	Parental <i>CYP21A2</i> Genotyping Plays an Important Role in the Reduction of Neonatal Deaths in CAH Families	Eunice Marumudi
O5	Association of D18S880 polymorphism of the <i>CNDP1</i> gene with diabetic nephropathy in south Indian population	Dhanasekaran Bodhini
O6	Expression of CD133 and BMI1 and its prognostic role in subjects with glioblastoma multiforme from Indian Population	Chetan G K
O7	Insights on the Functional Impact of MicroRNAs Present in Autism-Associated Copy Number Variants	Varadarajan Vaishnavi
O8	A Clustering Approach for Mapping Rare Variants Based on Mutual Association	Saurabh Ghosh
O9	Genetic Markers in PCOS Risk and Phenotype Progression	Srabani Mukherjee
O10	Whole Exome Sequencing helps characterize a rare highly penetrant familial disorder, turning epidemic in a village of Jammu and Kashmir, India"	Swarkar Sharma
O11	FISH for Prenatal, Postnatal, and Preimplantation Genetic Diagnosis.	Lim Jee Hian
O12	Masking of the Commonest Indian β thalassaemia Lesion IVS I nt 5 [G>C] by Different δ Globin Gene Defects.	Stacy Colaco
O13	Molecular Karyotyping for Prenatal Diagnosis	Manjeet Mehta

O14	Effect of Hydroxyurea on MicroRNA Expression and its Role in Fetal Hemoglobin Induction in Sickle cell Anemia Patients	Madhavi V Sawant
O15	Validation of nsSNPs For Pharmacogenetic Analysis Amongst Hypertensive Punjabi Population	Praveen P. Balgir
O16	Identification of Novel Disease Genes/Mutations for Single Gene Disorders through Homozygosity Mapping and Whole Exome Sequencing	Aneek Das Bhowmik
O17	Chromosomal Aberrations and Importance of Genetic Counseling : A Study of 288 Couples with Bad Obstetric History	Shailesh Pande
O18	Genomic Studies of High Altitude Pulmonary Edema, A critical Condition in Susceptible Sojourners	Soma Sarkar
O19	Mitochondrial DNA Diversity and its Implications in the Genetic History of Three Different Populations of Aminil Islander's of Lakshadweep.	M Naveen
O20	Genetic Association study and Gene Expression Analysis revealed Osteoprotegerin as Candidate Gene for Otosclerosis	Saurabh Priyadarshi
O21	Clinical, Hematological, Molecular Characterization and Response to Hydroxyurea Treatment of Symptomatic 17 HbSE cases in Eastern India: The Largest Series in world	Siris Patel
O22	Mitochondrial Somatic Mutations in Human Gastric Cancer	Bhagya Bhavana V
O23	Clinical Exome Sequencing to Aid Diagnosis – Case Studies and Lessons Learned	Saroja M. K
O24	The Etiopathogenesis of Human Genetic Diseases – Translating Basic Research to the Clinic	Radha Saraswathy
O25	Association of Monocyte chemoattractant protein-1 (<i>MCP-1</i>) gene polymorphisms [-2518 A>G (rs1024611) and I/D (rs3917887)] with T2D and ESRD patients from population of Punjab.	AJS Bhanwer
O26	Studies on Obese Breast Cancer Patients with Leptin Gene Polymorphisms.	Kaiser Jamil
O27	Development of a Multiplex PCR Based Method for Diagnosis of Duchenne Muscular Dystrophy (DMD) in the Patients Attending PGIMS Hospital	Daya Shankar Lal Srivastava
O28	Surface Layer Protein-A (SlpA): A protein Domain of the <i>Lactobacillus</i> sp. with Anti-Cancer Activity on GIT Cancer Cell Lines	Ranjith Kumavath
O29	Role of Adam33-S2 (G/C) Gene Polymorphism in The Etiology of Copd in South Indian Population	Vijaya Laxmi K
O30	Association of <i>PGC-1α</i> Gene with Type 2 Diabetes in Three Unrelated Ethnic Groups of North-West India	Rubina Sharma
O31	Association of Cytokine Gene Polymorphosis with Periodontal Disease	Manikandan G R
O32	<i>TP53</i> Polymorphisms and Chromosomal Instability in Breast Cancer Patients: A Follow up Study	Sarika Sharma
O33	A Common Functional Genetic Variant of Pancreastatin Profoundly Increases the Risk for Cardiometabolic Diseases in Indian Populations	Prasanna K R Allu
O34	Genotypes of <i>CYP1A1</i> , <i>SULT1A1</i> and <i>SULT1A2</i> and risk of squamous cell carcinoma of the esophagus in Kashmir, India; outcome of a case-control study	Idrees Ayoub Shah
O35	Role of Shh-Gli1 Signaling Novel Transcription Factor BMI1 in Medulloblastoma Development	Mehdi H. Shahi
O36	Genetics of Fetal Hemoglobin: Relevance for Prognostication	Aparna A Bhanushali

Poster presentations

No.	Title	Name
P1	Dermatoglyphic Pattern Study in Morton's Toe/Royal Toe.	Vijaylaxmi Gupta
P2	<i>In Silico</i> Study for Interaction of Ligand Molecules with Ebola Virus VP40 Matrix Protein	Neha Srivastava
P3	Molecular Characterization of Atypical Deletion Contributing to Clinical Manifestation of Williams-Beuren Syndrome	Rajitha P
P4	A Systematic Molecular Cytogenetic Characterization of A Supernumerary Marker Chromosome in A Girl With Seizures	Usha R Dutta
P5	Genomic Approach to Etiology of Pediatric Autism and Intellectual Disability	Ashish Bahal
P6	Deletion Boundaries and Variable Phenotypic Expression in WHS	Chirag Modi
P7	The Role of Microsatellites – BAT26, D2S123 Flanking The Human Mismatch Repair Gene in the Genesis and Progression of Oral Squamous Cell Carcinoma	K Navin Nair

P8	Role of Basic Fibroblast Growth Factor (b FGF) as an Angiomodulator in Oral Submucous Fibrosis	Revathy Bharathan
P9	Microsatellite Instability in Oral Squamous Cell Carcinoma With Reference to Tumor Suppressor Genes	Ayswarya T
P10	Role of PTEN Mutation in Oral Squamous Cell Carcinoma.	Binisree SS
P11	Molecular Basis of Afibrinogenemia: Application for Prenatal Diagnosis in The Affected Families	Alfiya Mukaddam
P12	Coexistence of Glucose-6-phosphate Dehydrogenase Deficiency and Gilbert's Syndrome with Hereditary Spherocytosis	Manu Jamwa
P13	Selection of reference genes for qRT-PCR based mRNA expression analysis of human reticulocytes from patients with hereditary spherocytosis	Anu Aggarwal
P14	Pharmacogenetic Implications Of Adducin rs4961 Gene Amongst Hypertensive Punjabi Population	Harsimran Kaur
P15	A Study of Association of GSTM1, GSTT1, GSTP1, MDR1 and TPMT Gene Polymorphism with Susceptibility to Acute Lymphocytic Leukemia (ALL)	Satbir Kaur
P16	I/D Polymorphism in Angiotensin Converting Enzyme Gene and its pharmacogenetic implications amongst Hypertensive Punjabi Population	Jaspreet Kaur Bhullar
P17	Demographic and Clinical Profile of Major Depressive Disorder (MDD) Patients Attending AIIMS Psychiatry Department	Sweta Saha
P18	Current therapeutic approaches for Duchenne Muscular Dystrophy	Hemant Pandey
P19	Host IL28B Genotype Plays an Important Role as a Predictor for Determining Response to IFN Therapy and Spontaneous Clearance in HCV Infected Thalassemia Patients	Rushna Firdaus
P20	BMPRII Signalling in HEK293 Cells	Chandra Bhan Singh
P21	Genetic Factors Related to Unconjugated Hyperbilirubinemia Amongst Adults.	Ashish S Chidharwar
P22	Mutational Screening of <i>PINK1</i> and <i>LRRK2</i> in Indian Parkinson Disease Patients	Tamali Haldar
P23	Mutational screening of <i>PKD1</i> in North Indian patients	Sonam Raj
P24	Association of gene polymorphisms with Chronic Obstructive Pulmonary Disease in North Indian Population	Akantha Sahu
P25	Cytokine Gene Polymorphisms and their Association with Cervical Cancer: A North Indian Study	Maneesh Kumar Gupta
P26	Molecular Study of FANCA Complementation Group in Indian Population	Avani P Solanki
P27	Analysis of Microsatellite Markers in FSHD Families – A Simple and Indirect Approach to Pre-Implantation Genetic Diagnosis.	Manikandan Suraj
P28	Correlation of Immuno-haematological Concepts from murine model to human CNV	Raikwar Himanshu P
P29	Polymorphisms in <i>TP53</i> , <i>MDM2</i> , <i>HIF1a</i> , <i>BRCA1</i> and Rare Chromosomal Anomalies in a Male Breast Cancer Patient and His Unaffected Son	Vasudha Sambyal
P30	Determination of RHD Zygosity Based on Most Probable Genotype and PCR-SSP	Disha Parchure
P31	Vascular Endothelial Growth Factor (<i>VEGF</i>) Gene Polymorphisms and Breast Cancer Risk in Punjabi Population from North West India	Kamlesh Guleria
P32	Cytogenetic Aberrations as a Measure of Genotoxicity in Rural Population Living near River Beas	Jagjeet Kaur
P33	ABO Blood Group and Risk of Coronary Artery Disease.	Prerna K Chawla
P34	Gene Variants of Pro-Oxidant and Antioxidant Genes in Indian Coronary Artery Disease Patients	Sripriya Natarajan
P35	Common Variants of <i>SLCO1B1</i> , <i>CYP3A4</i> , <i>ABCB1</i> , <i>CYP3A5</i> , <i>ABCG5</i> and <i>CYP7A1</i> Genes as Predictors of Lipid-Lowering Response to Atorvastatin Therapy	Prajakta A Kadam
P36	Evidence for expression of ARL15, a novel GWAS identified G protein in Rheumatoid Arthritis Synovial Fibroblasts	Sujit Kashyap
P37	Identification and Characterization of D Variants in Apparently Rhd Negative Individuals	G Vidya
P38	BRCA 1 and 2 Mutations Detection by NGS in Breast Cancers Patients – Pilot Study.	Jaya Vyas
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P73	Mutation analysis of ARX and MECP2 genes in mental retardation	Gayatri Kulkarni
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P75	Effect of Novel Intronic Mutation c. 694+8_694+18del in LDLR Gene in the Pathogenicity of Familial Hypercholesterolemia	Nagarajan Arul Jothi
P76	Test For Transmission Disequilibrium Including Non-Informative Parents	Hemant Kulkarni
P77	Understanding the role of Trans acting genetic factor <i>KLF1</i> in Hereditary Persistence of Fetal Hemoglobin Syndromes in Indian population.	Neetu Rani
P78	Study of Various Polymorphisms in Indian Warfarin Treated Patients	Tejasvita Gaikwad
P79	Association of MMP-1 (-181A>G) and MMP-3(-1612 5A>6A) Promoter Polymorphisms and Risk for HIV progression.	Shruti D Marathe
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P83	A case of Lenz Majewski syndrome: report of the sixth case in literature with <i>PTDSS1</i> gene mutation	Shweta Kondurkar
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P116	Nucleophosmin Mutation in De-Novo Acute Myeloid Leukemia	Kumudesh Mishra
P117	Vascular Endothelial Growth Factor (VEGF) Polymorphisms in	
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P126	Role of Intronic SNP and Its Association with Oral Squamous Cell Carcinoma in the Populations of Western Part of India.	Sejal M Desai
P127	Analysis of Obesity Related Gene with Special Reference to Solute Carrier Family 6 (Amino Acid Transporter), Member 14 (Slc6a14): A Computational Approach	Rajan K Singh
P128	Fat Mass Obesity Associated (Fto) Gene: Computatioal Analysis of Non-Synonymous Single Nucleotide Polymorphisms (Nssnps)	Permendra Kumar
P129	Effects of Gender on The Relationship Between IL6 Promoter Polymorphism and Lymphocyte Gene Expression in Schizophrenia	Deepthi Venugopa
P130	A Novel and Recurrent Lynch Syndrome Associated Indian Founder Mutation in Mismatch Repair (MMR) Gene <i>MLH1</i>	Nikhat Khan
P131	Genetic Counseling an Essential Component of Thalassemia Management for Mitigating Disease Burden	Gayatri R Iyer
P132	A Case Report of Breast and Ovarian Cancer Family	Bibi Umae Salma
P133	Genetic Variability of 15 Autosomal STR Loci in Central Indian Populations	Pankaj Shrivastava
P134	Cytogenetic survey of Primary Amenorrhea - A study of 818 cases from Department of Cytogenetics, Metropolis Healthcare Limited.	Divya Shivaram shetty
P135	Genetic Polymorphism of Glutathione S-Transferase Genes <i>GSTT1</i> , <i>GSTM1</i> , <i>GSTP1</i> and Susceptibility to Breast Cancer.	Gurmeet Kaur
P135	Association of Cholesteryl Ester Transfer Protein (-629C/A) Gene Polymorphism with Non Diabetic Atherosclerosis.	Rajni Dawar
P137	A Rare Trisomy 18p Syndrome Characterised by Microarray Analysis and Metaphase FISH	Anju Joshi Sharma
P138	Negative Epistatic Interaction of Sickle Cell Trait and Alpha-Thalassemia Against Severe <i>P. falciparum</i> Malaria.	Prasanta Purohit
P139	A Novel Symptomatic B0-Thalassaemia Mutation Cd-15(-T) in Compound Heterozygote First Time with Sickle Cell Gene from Eastern India & Response to Hydroxyurea	Satyabrata Meher
P140	Genotype-Phenotype Correlation in Patients with Hbs β -Thalassaemia in Eastern India.	Snehadhini Dehury
P141	De-Novo Compound Heterozygous Mutations in MYH7 Gene in Patient with Restrictive Cardiomyopathy	Mitali Kapoor
P142	Sickle Cell Disorder- A Family Study with Ttotal Ten Children (8 Homozygous and 2 Heterozygous) from Satpuda Hilly Ranges of Maharashtra	GT Kulkarni
P143	Analysis of <i>Alu</i> Insertion/Deletion Polymorphisms in Four Endogamous Groups from North West India	Gagandeep Singh
P144	Improved Multiplex Ligation Dependent Probe Amplification Technique for Studying Copy Number Variations	Bhavyasri Vennapusa
P145	Molecular Testing for Inherited Genetic Diseases: A Metropolis Laboratory Experience	Pratiksha R Chheda
P146	Association of 14bp Insertion/Deletion (indel) Polymorphism of HLA-G Gene in Women with Recurrent Miscarriages from Amritsar Region of Punjab, India.	Vishali Kalotra
P147	DNA repair gene defects and its association with myelodysplastic syndrome	Dolly Joshi
P148	Glucose-6-Phosphate Dehydrogenase Deficiency- Who, When, Why And How.	Jummanah Jarullah
P149	Genetic Association of ISL1 Gene Polymorphism with Chronic Otitis Media with Effusion	Ranju
P150	Role of GSK3 β and Cyclin D1 in The Etiology of Esophageal Cancer	Mohammed Khaliq Mohiuddin
P151	Screening of Cervical Smears: Promoter Methylation of Hmlh1 & Rar β 2 Genes and Prevalence of HR-HPV Sub-Types in Cervical Pathologies	Sravya Thumaju
P152	Exome sequencing of a selected gene panel in patients with Autism	Sireesha D
P153	MMP9 polymorphism, prevalence and effect on cardiac iron overload in patients with beta thalassemia major.	Mable Misha Singh
P154	Are MTHFR and MTRR polymorphisms considered to be as risk factor in mothers of Down syndrome cases?	Ambreen Asim
P155	TP- PCR: Advancement in Diagnosis of Fragile X Syndrome	Deepika Delsa Dean
P156	Association Analysis of Various Anthropometric and Clinical Factors in Diabetic Retinopathy (DR) in Population of Punjab	Gurvinder Singh
P157	Fanconi Chromosomal Breakage Analysis Report of 98 Patients	Indhumathi N
P158	Spectrum of Hemoglobinopathies in Santhal's Tribal Community of Jamui District, Bihar	Ashish Kumar Singh
P159	Study of Risk Factors in Oral Carcinoma and the relationship between Tumor Thickness and Regional Nodal Involvement	Sama Rizvi
P160	Genetic Diversity in Reasi and Udhampur Districts of Jammu and Kashmir, India.	Indu Sharma

P161	Detection and Evaluation of BCR/ABL Gene mutations in CML Patients Treated with Imatinib Mesylate	Zaker F
P162	Clinical and Molecular Study of NPC in Iran: Report of 5 Novel Mutations	Amirabbas Parizadeh
P163	Identification of Genetic Loci for Uric Acid in North Indians using Genome Wide Association Study	Aarthi Ravindran
P164	Role of Fumarate Hydratase Gene Mutation in the Etiology of Uterine Fibroids	Ch. Bharathi
P165	Genetic Correlation of XmnI, MTHFR (C677T), ACE (I/D) Polymorphism with The Clinical Severity of Beta Thalassemia in Delhi-NCR Region.	Swati Chawla
P166	Variation in Exon-Intron Architecture of Autosomal and X-linked Genes in Dipteran Species	Shagun Shukla
P167	Association of Apolipoprotein E, A5 And Endothelial Nitric Oxide Synthase Gene Polymorphisms in Myocardial Infarction: A Hospital Based Study in North East India	Sukanya Baruah
P168	Non-Familial Sporadic Heritable Retinoblastoma and it's Correlation with Paternal Sperm DNA Damage	Shilpa B
P169	Deregulation of Mirnas in Cervical Cancer: Control by DNA Methylation	Vinay K. Varghese
P170	Amyotrophic Lateral Sclerosis (ALS) – An Overview	Varun Sharma
P171	Genetic and Environmental factors predisposing to Depression and Suicidal behaviour in Idu Mishmi Tribe of Arunachal Pradesh.	Piyooash Kumar Singh
P172	Molecular characterization of tetherin/BST-2 gene promoter in Indian HIV infected long term non progressors	Dhanashree D. Jagtap
P173	Epidemiology of Inbreeding Depression on Physical, Reproductive and Cognitive Behavior	Mohd Fareed
P174	Demographic and Clinical Profile of Dilated Cardiomyopathy Patients from North India	Soumi Das
P175	Genetics and Epigenetics Variations Can Influence Malaria Susceptibility	Himanshu Gupta
P176	Aberrant DNA Methylation in Early Oral Carcinogenesis: A Review	Astha Agarwal
P177	Genomics of Chronic Obstructive Pulmonary Disease and Lung Function: A Review	Astha Ranjan
P178	Family History of Cancer and Risk of Squamous Cell Carcinoma of Esophagus - An Outcome of a Case Control Study	Nazir Ahmad Dar
P179	Sickle Cell Anaemia- Community Control Program for Tribal Population Groups of Satpuda Hilly Ranges from Nandurbar, Dist Maharashtra	S.L.Kate
P180	Genome Wide Association Study of Glycemic Related Traits in Indians	Priyanka Banerjee
P181	Study of Bacterial Diversity in Beta-Thalassemia Patients of North Maharashtra Region by tRFLP Analysis.	Ranjeet Kumar
P182	Effect of Parental stress, Coping Strategy and XmnI- G _γ (HBG2) Polymorphism in Beta Thalassemia Disease Severity: An Evaluation of Gene- Environment Interaction.	Rajnish Kumar Singh
P183	Oxidative DNA Damage Assessment in <i>in situ</i> COPD Cases	Gurpreet Kaur
P184	Genomic Affinities Among Different Population Groups of Jammu Region of J&K State	Rakesh Kumar Panjalya
P185	Prevalence of various cardiac disorders in Pune population and associated physiological complications	Syed Abrar Ahmad
P186	Role of various variants of phase-1 xenobiotic metabolising genes in modifying esophageal squamous cell carcinoma risk in Kashmir- a high risk area.	Gulzar Ahmad Bhat
P187	Association Of TP53 Expression And GST & CYP1A1 Polymorphism In Oral Squamous Cell Carcinoma	Chandreyee Chakraborty
P188	Mutation in PAX3 associated with Neural Tube Defect	Rashmi
P189	Study of Abacavir hypersensitivity in Indian Subpopulation and its association with HLA-B*5701 and HCP5 rs2395029.	Yash Y Panchal
P190	Structural and Functional analysis of Protein-Protein interaction network in neurodegenerative disorders.	Nitin Thukral
P191	A Preliminary Study on Human Spermiation Defect	Manish Jain
P192	Genetic variant analysis in three Indian ethnically diverse human genome.	Seema Malhotra
P193	Genetic Variants of APOBEC3G-90C/G and -571G/C Polymorphism and Risk of acquiring HIV infection.	Sonam Lata
P194	Clinical and molecular characterization of Fragile X Syndrome	Shyla R Menon
P195	CYP1A1 MspI gene polymorphism and breast cancer susceptibility in females of Jammu region.	Ravi Sharma

Invited Lectures

I1

Transcriptome dysregulation and single cell analysis in trisomy 21

Stylianos E. Antonarakis

Trisomy 21 is the most frequent genetic cause of cognitive impairment. To assess the perturbations of gene expression in trisomy 21, and to eliminate the noise of genomic variability, we studied the transcriptome of fetal fibroblasts from a pair of monozygotic twins discordant for trisomy 21. We have shown that the differential expression between the twins is organized in domains along all chromosomes that are either upregulated or downregulated. These gene expression dysregulation domains (GEDDs) can be defined by the expression level of their gene content, and are well conserved in induced pluripotent stem cells derived from the twins' fibroblasts. Comparison of the transcriptome of the Ts65Dn mouse model of Down's syndrome and normal littermate mouse fibroblasts also showed GEDDs along the mouse chromosomes that were syntenic in human. The GEDDs correlate with the lamina-associated (LADs) and replication domains of mammalian cells. The overall position of LADs was not altered in trisomic cells; however, the H3K4me3 profile of the trisomic fibroblasts was modified and accurately followed the GEDD pattern. These results indicate that the nuclear compartments of trisomic cells undergo modifications of the chromatin environment influencing the overall transcriptome, and that GEDDs may therefore contribute to some trisomy 21 phenotypes. The study of gene expression in mammalian single cells via genomic technologies now provides the possibility to investigate the patterns of allelic gene expression. We used single-cell RNA sequencing to detect the allele-specific mRNA level in 203 single human primary fibroblasts over 133,633 unique heterozygous single-nucleotide variants (hetSNVs). We observed that at the snapshot of analyses, each cell contained mostly transcripts from one allele from the majority of genes; indeed, 76.4% of the hetSNVs displayed stochastic monoallelic expression in single cells. Remarkably, adjacent hetSNVs exhibited a haplotype-consistent allelic ratio; in contrast, distant sites located in two different genes were independent of the haplotype structure. Moreover, the allele-specific expression in single cells correlated with the abundance of the cellular transcript. We observed that genes expressing both alleles in the majority of the single cells at a given time point were rare and enriched with highly expressed. Overall, these results have direct implications in cellular phenotypic variability. Single cell transcriptome analysis in trisomy 21 will be discussed.

I2

Cancer genetics including circulating tumor cells

Parikh PM

The first human genome project was completed in 2003 at the cost of over \$ 3 billion. The decoded information was printed into 100 books of more than 1000 pages each, using font size that was barely readable. Today the same task can be done in less than \$ 1,000/- and the information requires 800 MB of

storage space on Google cloud that will cost \$ 25 per year. This is possible due to the explosive speed at which technology is becoming available in cancer genetics. Similarly the 5 year survival in acute lymphoblastic leukemia has increased from less than 10% in 1960s to more than 95% in 2010. This was due to better understanding of the tumor biology as well as drug combinations and scheduling. The next step was unravelling the molecular mutation driving tumor growth and designing a drug in-silico to specifically dock in the 3D receptor structure – the famous success story of imatinib and chronic myeloid leukemia. Today there are several such examples of deep dive into tumor mutations, downstream signalling and drug'gable targets that have led to meaningful prolongation of the life of patients with solid tumors as well – lung cancer, renal cell carcinoma and breast cancer being a few of the examples. With the help of technology available today, we can benefit cancer patient outcome by using the following strategies: Tumor specific driver mutation analysis

- Tumor drug handling kinetics
- Somatic cell pharmacogenomics
- Liquid biopsy and circulating tumor cell/ DNA monitoring

An article on the role of circulating tumor cells (CTCs) was first published in 2004. Ten years later, more than 15,000 articles exist in pubmed, more than 740 being published in 2014 alone. So this technology is well established, deeply studied and even US FDA approved for three cancers (breast, prostate and colorectal). A total of 15 ml of peripheral blood is required to identify CTCs. The sample is processed and stained to detect nuclear DNA, cytoplasmic cytokeratin and surface epithelial cell adhesion molecules (Fig 1). Such cells can be enriched, stained and examined under light/ digital microscopy to ascertain its morphological characteristics (Fig 2). This test is currently available in India and helps in cancer screening, has prognostic and predictive significance, enables us to identify response/ progression 8 to 12 weeks before it becomes evident on conventional imaging (CT Scan) and can also be used for further molecular testing (including gene profiling, cellular phenotyping and in-vitro chemosensitivity). The cut off level prognostic value of CTCs is 5 cells per 7.5 ml of blood for breast and prostate cancer. It predicts almost a doubling of overall survival when comparing outcome of patients below (favourable) and above (unfavourable) cut off values of CTCs (Table 1). This test is also considered of value in other cancers like lung cancer, bladder cancer, biliary duct cancer, liver cancer and pancreatic cancer. Thus measurement of CTCs is now an integral part of cancer patient evaluation and management. It can optimize the treatment strategy in individual patients allowing successful implementation of precision oncology and personalized medicine.

Table 1: Prognostic importance of CTCs in predicting overall survival in metastatic cancers

Metastatic (stage IV) cancers	Favourable CTCs (months)	Unfavourable CTCs (months)
Breast cancer overall survival	21.9	10.9
Colorectal cancer overall survival	18.5	9.4
Prostate cancer overall survival	21.7	11.5

CTCs: Circulating tumor cells

I3

From genome to epigenome: A new perspective for understanding complex diseases

Sanjeev Galande

Identical DNA sequence in the genome of complex organisms adopts cell type specific chromatin architecture in response to developmental programs and environmental cues. Such cell type specific conversion of genome to epigenome is associated with specific expression pattern of genes that eventually determines the cell fate. It is therefore becoming increasingly apparent that epigenetic marks reflect the functional state of the genome. The past decade has witnessed the explosion of information in biomedical sciences due to the availability of high quality genome sequences and the development of high throughput techniques that assay DNA and histone modifications. It is now possible to analyze epigenetic mechanisms that implement genetic programs of cellular differentiation and analyze epigenetic changes that take place in response to environmental changes including disease conditions. I will specifically focus on recent developments in the understanding of epigenetic regulation of complex diseases such cancer and diabetes. I will summarize the technological breakthroughs and also discuss how they would enable us to study disease susceptibility and presumably take us towards personalized medicine.

I4

Structure and evolution of genes of the innate immune system

Partha P. Majumder

Evolutionarily, the adaptive or acquired immune system is recent. It was built atop the innate immune system, which is phylogenetically more ancient and developed before the separation of vertebrates and invertebrates. Invertebrates and jawlessfish depend solely on the innate immune system. The innate immune system controls and assists the adaptive immune system, without which the adaptive immune response offers weak protection. We have tested the opposing views concerning evolution of genes of the innate immune system that (i) being evolutionary ancient, the system may have been highly optimized by natural selection and therefore should be under purifying selection, and (ii) the system may be plastic and continuing to evolve under balancing selection. We shall present results of an extensive and comprehensive study of genetic diversity, assayed by DNA resequencing, of 12 genes (CAMP, DEFA4, DEFA5, DEFA6, DEFB1, MBL2, and TLRs 1, 2, 4, 5, 6, and 9) in healthy volunteers recruited from various parts of India. For some of these genes, we have also compared data of the Indian populations with populations from other global regions. In these genes, there is (i) generally an excess of rare variants, (ii) high, but variable, degrees of extended haplotype homozygosity, (iii) low tolerance to nonsynonymous changes, (iv) essentially one or a few high-frequency haplotypes, with star-like phylogenies of other infrequent haplotypes radiating from the modal haplotypes. Purifying selection is the most parsimonious explanation operating on these innate

immunity genes. This genetic surveillance system recognizes motifs in pathogens that are perhaps conserved across a broad range of pathogens. Hence, functional constraints are imposed on mutations that diminish the ability of these proteins to detect pathogens.

I5

N. K. Mehra

No abstract

I6

Exome sequencing approaches to understand the genetic basis of human urogenital anomalies

Ken McElreavey

Exome sequencing has emerged as a very powerful tool to identify the genetic basis of rare human Mendelian disorders. This approach is particularly attractive for mutation detection in cases of Disorder of Sex Development (DSD) since these conditions are very difficult to study using classical genetic approaches. DSD covers a wide spectrum of phenotypes and is defined as "DSD is defined as 'congenital conditions in which the development of chromosomal, gonadal, or anatomical sex is atypical'. DSD cases with errors in sex-determination include 46,XY gonadal dysgenesis that is either complete or partial, or 46,XX testicular or 46,XX ovotesticular DSD, where testis develops in an XX individual. Other forms of DSD include male pseudohermaphroditism/ undervirilisation of an XY male or female pseudohermaphroditism/overvirilisation of an XX female as well as other genitourinary disorders such as cloacal extrophy, vaginal atresia, or MURCS. Excluding cases where the biochemical profile indicates a specific error in steroidogenesis, it is estimated that a specific molecular diagnosis is obtained in 20% of DSD cases and that only 50% of 46,XY children with DSD will receive a definitive clinical diagnosis. We have performed exome sequencing in >60 cases of DSD. Exon enrichment was performed using Agilent SureSelect Human All Exon V4. Paired-end sequencing was performed on the Illumina HiSeq2000 platform with an average sequencing coverage of x50. In this talk I will summarise our main findings and highlight some of the advantages and surprises that have resulted from this approach.

I7

Hereditary cancers in South Asians: Bench to bedside

Rajiv Sarin

Frequency of Mendelian inheritance of cancers ranges from <2% of Tobacco related cancer, 5% for breast cancer, 10% for ovarian cancers and sarcomas and 25% of retinoblastomas and some other childhood cancers. It is estimated that 50,000 hereditary cancers occur annually in India. However, due to lack of awareness, facilities and the cost of genetic analysis, comprehensive genetic services have not evolved in south Asia. Few studies have reported germline mutations in small cohorts of hereditary cancers from India, Pakistan and Sri Lanka and certain specific mutations occur commonly across countries in

south Asia. We have focused on identifying population specific founder and recurrent germline mutations and characterization of variants of unknown significance to develop rapid and cost effective geo-ethnic specific genetic analysis algorithms. The ICMR centre for Advanced Research in Cancer Genetics in ACTREC has established the largest south Asian cohort of ~3000 families with various inherited cancer predisposition syndromes. Using various genetic analysis approaches ranging from CSGE screening to NGS and with final validation with Sanger sequencing, we have identified germline mutation in one or more of the 20 high penetrance cancer predisposing genes (BRCA1, BRCA2, TP53, MLH1, MSH2, APC, RB1, RET, STK11, Nucleotide excision repair genes etc). Select novel, recurrent and unclassified sequence variants (Missense and intronic) have been further characterized by co-segregation, bio-informatics, structural and functional studies. A South Asian high penetrance cancer gene (SAHPCG) germline mutation database has been created. In addition to identification of large number of unclassified variants, known polymorphisms and known deleterious mutations, a large number of novel deleterious mutations in several high penetrance genes have been identified so far. Most recurrent germline mutations in South Asians, with maximum cases coming from our cohort, show geo-ethnic and community wise clustering with clear founder effect and suitable for population specific cost-effective mutation analysis algorithms. Targeted resequencing of commonly implicated genes reduces cost and time for genetic analysis. The penetrance and clinical outcome for RET, BRCA1 and Mismatch repair genes has been established for the first time for South Asian population.

18

Cytogenetics of acute leukemia and non-hodgkin lymphoma

Susan Mathew

An overview of the current World Health Organization (WHO) classification of acute leukemia and non-Hodgkin lymphoma will be presented. The primary focus will be on cytogenetic and molecular cytogenetic abnormalities in acute lymphoblastic leukemia, acute myeloid leukemia, and non-Hodgkin lymphoma and their significance in diagnosis, treatment, and prognosis. The relevance of Cytogenetics in the genomics era will be discussed.

19

Clinical applications of molecular genetics in bone and soft tissue neoplasia

Meera Hameed

Bone and soft tissue tumors are heterogeneous neoplasms of mesenchymal origin with diverse histologies and clinical behavior. Due to rarity of these tumors, pathological diagnosis can be challenging. In recent years the discovery of specific genetic abnormalities in these tumors has allowed for better classification, more diagnostic accuracy and understanding of mechanisms behind the pathogenesis.

Recent technological advances such as next generation sequencing have found their place in clinical labs and are

beginning to provide new insights into disease biology and discoveries of novel mutations and translocations applicable to diagnosis, prognosis and therapeutics. Based on our understanding so far, the genetics of bone and soft tissue tumors can be divided into three categories: Translocation associated sarcomas (tumors with simple karyotypes), sarcomas with complex karyotypes and those with point mutations and amplifications. Some examples include Ewing Sarcoma, the prototypic round cell sarcoma with its characteristic reciprocal translocation involving EWSR1 at ch22q12 to one of the ETS family of transcription factors, most commonly FLI1 resulting in t(11;22)(q24;q12) in the over 90% of the cases. While this highlights the importance of fusion genes as diagnostic markers, caveats remain in that these genes (for example EWSR1) are promiscuous and can have multiple partners for the same tumors and also the same fusion observed in more than one tumor type. In recent years, oncogenic driver mutations are increasingly being discovered with tumor specificity, a classic example being gastrointestinal stromal tumor driven by KIT and PDGFRA receptor tyrosine kinases with remarkable responses to targeted therapy and prolonged survival in these patients. The identification of IDH1 and IDH2 mutations in cartilage neoplasms has paved the way to explore the “cancer metabolome” and further explore the role of global methylation in these neoplasms. Within the genomic complexity of tumors one could still define specific alterations such as gene amplifications of CDK4 and MDM2 which are hallmarks of well-differentiated and dedifferentiated liposarcomas and low grade osteosarcomas. Thus we are in an era of modern medicine, where advances in molecular diagnosis have been most prolific and are reflected by the rapid discovery of fusion genes, tumor specific oncogenic drivers even in these rare disease entities.

110

Cytogenetics and molecular profiling in multiple myeloma

Pratibha S. Kadam Amare

Multiple Myeloma (MM) , is a clonal B-cell malignancy, characterized by heterogeneity at both clinical and genomic level. Despite the role of specific cytogenetic aberrations in the pathogenesis of disease, the prognostic significance of cytogenetic changes have been identified in MM and that has become an integral part of disease management. The genetic heterogeneity enabled the categorization of disease in to several subclasses. The most common are hyperdiploidy and non-hyperdiploid MM. The non-hyperdiploid MM frequently shows structural aberrations such as unique IgH translocations , aberrations of chromosome 13 , p53 deletion and 1q amplification. In comparison with conventional karyotyping, fluorescence in situ hybridization (FISH) could efficiently detect various genetic changes in non-cycling plasma cells in 50-90% of MM cases. Several high resolution platforms like CGH (comparative genomic hybridization) array, SNP (single nucleotide polymorphism) array, gene expression profiling (GEP) have provided comprehensive data at gene level and at RNA level. Genome expression profiling (GEP) has also

been used to identify high risk patients with multiple myeloma, and to further classify risk in poor prognosis multiple myeloma patients such as those with t(4;14). The Durie–Salmon Staging System and the International Staging System (ISS) are important for prognosis, but are not useful for therapeutic risk stratification. Independent prognostic markers provide a better estimate of differences in underlying myeloma biology. Either FISH or conventional cytogenetics, or preferably both, should be done at diagnosis in all individuals. Kumar and colleagues (2011) reported on the first study examining the utility of two GEP-based risk stratification systems in a cohort of individuals undergoing initial therapy in the context of a phase III trial of Lenalidomide in the treatment of multiple myeloma. The small number of patients in this study, however, prevents a comparison of the GEP and FISH-based risk stratification, and an assessment of the incremental value of GEP over FISH-based risk stratification” (Kumar, 2011). Mikhael and colleagues (2013) proposed a risk-adapted approach to the management of individuals with newly diagnosed symptomatic multiple myeloma. The Mayo Stratification of Myeloma and Risk-Adapted Therapy (mSMART) is an evidenced-based algorithm using conventional cytogenetic methods (karyotyping) and FISH to prognostically stratify individuals with multiple myeloma according to multiple recurrent chromosomal changes. The guideline suggests these factors must be considered to individualize the choice of therapy for persons with multiple myeloma. Although GEP analysis is included in the algorithms for prognostic factors and risk stratification, the consensus guideline does not currently recommend performing GEP analysis in a non-research setting. The National Comprehensive Cancer Network® (NCCN®) Clinical Practice Guidelines in Oncology™ for multiple myeloma (2013), states the role and application of gene expression profiling is emerging as a tool to further understand the molecular subtypes of multiple myeloma. However, standardized testing for GEP is unavailable and data is inadequate to determine how this prognostic information can improve health outcomes by directing care management for individuals with multiple myeloma. Data of Tata Memorial Hospital on comprehensive profiling of various cytogenetic markers by interphase-FISH will be discussed.

I11

Translating cancer genomics to medicine

Amit Dutt

Recent developments in genomic research and targeted cancer therapy provide a unique opportunity to improve cancer care. The identification of inherited and somatic mutations in cancer has revealed key biological pathways that underlie the initiation and progression of cancer. In turn, these advances are beginning to transform diagnostics (allowing cancers to be classified based on molecular mechanism and allowing clinical trials to be undertaken on more homogeneous groups of patients) and therapeutics (sparking a new generation of drugs targeted at the molecular alterations that cause cancer). At the same time, these discoveries have underscored the complexity of cancer genetics; at present, we know only

a subset of the genes that play a causal role in cancer. A comprehensive catalog of all such genetic lesions, together with a description of the cancer types in which they occur and the combinations in which they co-occur, is necessary for the effective development of targeted cancer therapy. However, despite much advances, it has not been technically feasible to interrogate the complete set of genomic alterations in a tumor in a systematic, comprehensive manner. This limitation is now set to change. Newer generation SNP arrays and massively parallel Next Generation DNA sequencing technologies already make possible the readout of millions and billions of nucleotides in a single run and will permit complete genome characterization of cancer. These methodologies and tools have been used to advance cancer sciences, leading to discovery of novel molecular subclasses, new therapeutic targets and biomarkers for clinical development. Using these and conventional technologies, my work led to the identification of causal alterations in human cancer and discovered novel somatic activating alterations at FGFR2, EGFR, AKT, PDGFRA and FGFR1 mutations in endometrial cancer, lung squamous carcinoma and cervical carcinoma. More recently, my laboratory has discovered and characterized some novel potentially therapeutic target in Indian samples of lung carcinomas and head and neck cancer.

I12

I.C. Verma

No abstract

I13

Mitochondrial disease diagnosis and management

Virginia E. Kimonis

Mitochondrial diseases are one of the most common inborn errors of metabolism, with a conservative estimated prevalence of approximately 1:5,000. Primary mitochondrial diseases are defined as disorders impacting the structure or function of the mitochondria as a result of either nuclear DNA (nDNA) or mitochondrial DNA (mtDNA) mutations. More than 1,400 nuclear genes are either directly or indirectly involved in mitochondrial function and contribute to 90% of primary mitochondrial disorders. There is currently no consensus on diagnosis and treatment of mitochondrial disorders therefore a panel was appointed to utilize a comprehensive review of the literature, surveys, and the Delphi method to reach consensus-based recommendations. These standardized recommendations on the evaluation, diagnosis, and care of patients with suspected or demonstrated mitochondrial disease will be discussed.

I14

Enzyme replacement therapy for lysosomal storage disorders

Mamta Muranjan

Lysosomal storage disorders (LSDs) are a group of 50 disorders, each resulting from a mutation of a gene coding for a lysosomal protein. The most frequent outcome of this mutation is a deficiency of a lysosomal enzyme (acid

hydrolase) or activator protein or transport protein or enzyme required for correct processing of other lysosomal proteins. The end result is disruption of metabolic pathways for degradation of several complex macromolecules. Incompletely degraded substrates of these deficient proteins accumulate progressively and ultimately cause cellular damage. As the distribution of the lysosomal enzymes and their substrate is widespread among several organs, LSDs are multisystem disorders. The concept of treatment for LSDs by replacement of the deficient enzyme (enzyme replacement therapy, ERT) was proposed by Brady and his colleagues in the 1960s. Dr. Brady's group at the National Institute of Health (NIH) developed the first product for treatment of Gaucher disease. This product, Alglucerase, was sourced from the human placenta. Alglucerase was approved for use by the FDA in 1991. The next generation ERT was human recombinant enzymes produced commercially by expression of a human gene in a cell culture system. The native recombinant enzyme is modified to expose mannose-6-phosphate moieties. This step is critical for efficient intracellular or intra-lysosomal uptake of the exogenously administered enzyme. The various LSDs treated by ERT and the currently available products are described in the following Table.

Disease	Product	Source (cell culture)	US FDA approval	In India
Gaucher	Imiglucerase	CHO	1994	1999
	Velaglucerase	HF	2010	2013
	Taliglucerase	CC	2012	NA
Fabry	Agalsidase β	CHO	2003	2005
	Agalsidase α	HF	Not approved	2014
Pompe	Alglucosidase α	CHO	2006	2008
Mucopolysaccharidosis type I	Laronidase	CHO	2003	2006
Mucopolysaccharidosis type VI	Galsulfase	CHO	2005	NA
Mucopolysaccharidosis type II	Idursulfase	HF	2006	2014
Mucopolysaccharidosis type IVA	Elosulfase α	CHO	2014	NA

CHO: Chinese hamster ovary, HF: Human fibroblasts, CC: Carrot cell,

ERT modifies the natural course of the disease and has to be taken lifelong. Optimum response is achieved with early institution of therapy. However, ERT does not cross the blood brain barrier and therefore CNS manifestations are not reversed or prevented. The major deterrent for widespread use of ERT in India is the prohibitive cost. The annual cost for therapy of Gaucher disease is US \$ 40 000–320 000 while the annual cost of ERT for Fabry disease is approximately US \$160 000. Therefore access is limited currently to a few patients through charitable programs of the manufacturers or isolated instances of indirect government funding through the organizations such as the armed forces or state police. Children with LSDs have been treated at KEM Hospital, Mumbai since the year 2000. This includes 11 children with Gaucher disease, 3 infants with classical Pompe disease and one child with Hunter disease.

I15

Screening & diagnosis of inborn errors of metabolism (iems) by mass spectrometry - 15 years of mils experience

Usha P. Dave

Most Inborn Errors of Metabolism (IEM) cause severe pathological sequelae like mental retardation, sudden infantile death or other irreversible neurological conditions. Hence, in the developed countries Newborn Screening programs (NBS) are being implemented to reduce the burden imposed by genetic disorders. Mass spectrometry – a high throughput method for selective IEMs was used for NBS which was soon recommended by WHO as a significant, preventive, public health strategy to decrease morbidity and mortality in infants and children. In the absence of newborn screening programmes in India, for the first time, the screening of IEMs in high-risk children using Gas Chromatography/ Mass Spectrometry (GC/MS) was started in 1997 by MILS International India with technical support from MILS Japan. This was then mainly used to confirm the clinical diagnosis. Till date, total 3341 high-risk babies from NICU & PICU with clinical symptoms like seizures, vomiting, poor feeding, metabolic acidosis, lethargy, and developmental delay were referred. Of the total, 39% (1297 of 3341) were diagnosed to have metabolic abnormality; of these amino and organic acidopathies accounted for 43%, followed by mitochondrial, nucleic acid & sugar metabolism disorders. Since the objective of metabolic screening is early & accurate diagnosis for prevention, the critically ill neonates within 30 days of age were particularly studied & found to have 41% (353/ 864) abnormality. The higher incidence of certain organic and amino acidopathies, mainly MMA, MSUD, PA, UCD, OTC, GA-I and Tyrosinemia was evident as a major cause of mortality and morbidity. Interestingly, 34 cases of GA-I & 10 cases of Canavan disease were diagnosed and further mutational studies for prenatal diagnosis in few families were carried out. A few interesting IEM cases will be illustrated considering religious, racial and ethnic diversity in Indian population with cultural and traditional misconceptions. Genetic counseling to the parents and family members was possible because of the confirmed diagnosis by GC/MS which helped in explaining the role of heredity (viz. autosomal recessive), risk of recurrence and scope of prevention. With over 28 million annual births, our MILS data offered important information to health policy makers on the frequency of certain IEMs in India to include in NBS programs. The present MILS method using GC/MS technology is not only useful for simultaneous screening of over 140+ metabolic conditions in one test but also gives confirmed diagnosis. Hence, MILS method can be used for both mass screening and diagnosis, thereby making it effective in the long run considering prevention and rehabilitation cost of the disabled child.

I16

Genetic disorders and consanguinity in India

Shubha Phadke

Association of genetic disorders and consanguinity is concerns autosomal recessive disorders mainly. Consanguinity being

highly prevalent in India upto 30 to 40% in some populations, higher prevalence of autosomal recessive disorders is often stated without strong evidence of population based data. The available data shows that the prevalence of autosomal recessive disorders may be one to one and half times in consanguineous populations. Our unpublished data shows that the consanguinity is 2 to 3 times common in families with rare autosomal recessive disorders (26.6%) as compared to common genetic disorders like beta thalassemia (10.7%). Though the consanguinity may not greatly influence the magnitude of the prevalence of autosomal recessive disorders greatly, these families with consanguinity provide an important diagnostic and research approaches. We used SNP microarray to identify candidate genes in autosomal recessive disorders of heterogeneous nature. In Osteogenesis imperfecta (OI) cases from consanguineous families SNP microarray was successful in identifying the causative gene in 4 out of 7 cases and missed one autosomal recessive gene in one. Exome sequencing in two cases of OI identified mutation in heterozygous state in COL1A2 gene, reinforcing the possibility of autosomal dominant disorders in consanguineous families. Illustrative cases stressing utility of SNP microarray in diagnostic approach to microcephaly will be presented. These families with new syndromes from consanguineous families also are good substrate for gene mapping using exome sequencing and gold mine of rare syndromes from consanguineous families need to be rapidly explored for identification of novel genes.

I17

Noninvasive prenatal diagnosis

Nilesh Dharajiya

Non-invasive prenatal testing for fetal aneuploidies by massively parallel sequencing has emerged as powerful tool in management of high-risk pregnancies. The high sensitivity and specificity proven by multiple clinical studies bundled with non-invasive nature and convenience of a blood draw has resulted in widespread adoption of the test. Since the publication of a committee opinion from The American College of Obstetricians and Gynecologists and the Society for Maternal-Fetal Medicine, non-invasive testing has become a part of the standard of care for aneuploidy testing. Since the introduction of NIPT to detect trisomy 21 by Sequenom Laboratories in October 2011, the technology has rapidly advanced to analyze other autosomal and sex chromosome aneuploidies, and now includes the detection of subchromosomal deletion and duplication events. As the first laboratory to design and clinically validate the test, here we offer a unique perspective on NIPT from the position of an experienced lab, which has processed more than 300,000 samples. This talk will encompass the fundamentals of NIPT by massively parallel sequencing. We will then delve into clinical performance of the assay including full trisomies and subchromosomal deletion/duplication events. Finally, we will go over interesting clinical cases that we encountered during our workflow.

I18

Disorders of imprinting

Koumudi Godbole

Genomic imprinting is a mechanism which allows monoallelic expression of an imprinted gene based on the parent of origin. This results in an unequal parental complement of imprinted genes with respect to their expression, despite both parents contributing equally. The mechanism of imprinting is complex and incompletely understood. The parental-specific imprint marks on a gene must be erasable in the germline when transmitted through individuals of the opposite sex, but maintained during somatic cell division. The expression of imprinted genes may be tissue- and stage specific with one of the parental alleles being differentially expressed only at a certain developmental stage or in certain cells. Imprinted genes are targets for environmental factors to influence expression through epigenetics whereby the expression level is altered without changing the DNA nucleotide coding structure. Imprinted genes are involved in many aspects of development including fetal and placental growth, cell proliferation, and adult behavior. Role of imprinting genes in classical genetic syndromes, assisted reproductive techniques, fetal /placental growth as well as complex diseases that present later in life, will be discussed.

I19

Fetal dysmorphism in fetal autopsy

Prakash Gambhir

10 to 15% of all conceptions end in a pregnancy loss. Some of these are spontaneous losses, some intrauterine fetal deaths and some terminated because of ultrasonologic detection of major congenital anomalies. It is important to reach a diagnosis in these instances as the terminated fetus will be disposed off and will not be available later. Fetal autopsy can lead us to diagnosis which will help in proper counselling and later prevention. This diagnosis exercise will consist of examination of the fetus with special attention to the dysmorphism, internal examination, fetal XRay and imaging, histopathology and relevant chromosomal and molecular investigations. Syndromic diagnosis is possible even in a fetus terminated at early second trimester. Special attention should also be given to placenta and cord especially in IUDs and IUGRs.

I20

Molecular diagnosis of genodermatoses

Parag M Tamhankar

Genodermatoses are genetic disorders which affect the skin structure and its function. These disorders have significant mortality, morbidity and may have multisystem involvement. Aim: To study the clinical and molecular characteristics of patients with genodermatoses. Methods: Patients with following clinical entities are classified as genodermatoses: epidermolysis bullosa, keratotic disorders, disorders of skin color, ectodermal genodermatoses, genodermatoses associated with connective tissue, vascular genodermatoses and genodermatoses with skin manifestation and elevated

cancer risk. Mutation analysis could be performed by PCR and Sanger sequencing for XPA, TGM1, RAB27A, COL7A1 genes. Results: During the period 2011-2015, a total of 169 patients were included as genodermatoses. Epidermolysis bullosa and autosomal recessive ichthyosis were the most common groups (21 patients each) followed by Griscelli syndrome (n-20), Xeroderma pigmentosum (XP) (n-15), ectodermal dysplasia (n-11), progeria (n-8) and infantile hyalinosi (n-9). Mutations could be identified in XPA, TGM1, RAB27A and COL7A1 genes. The mutation hotspots identified include p.R184X of RAB27A gene (Griscelli syndrome), exon 3 of XPA (neurological XP) and exon 72, 73 and 74 of COL7A1 gene (EB dystrophica). Conclusion: Indian patients with genodermatoses exhibit considerable genetic and allelic heterogeneity complicating molecular diagnosis. A next generation sequencing panel based testing would be ideal to increase sensitivity of diagnosis.

I21

The past, present and future of hemoglobinopathies in India

Dipika Mohanty

The inherited disorders of hemoglobin are by far the most common inherited single gene disorder not only India but in the whole world. Recent surveys suggest that between 300,00 and 400,000 babies are born with a serious hemoglobin disorder each year and that upto 90% of these births occur in low or middle income countries. India falls in this category. Early comprehensive treatment and improved technology have changed thalassemia from a fatal pediatric disease to one with which patients survive throughout adulthood and can enjoy productive lives. Advances in treatment have resulted in improved quality of life and a potential for a cure. The same applies to sickle cell anemia also. The first patient of thalassemia was detected at Calcutta in the year 1938 by Mukharjee and the presence of SCD in the tribals was first reported by Cut bush and Lehman in Nilgiri hill tribals in 1952. From those days onward the country has experienced a great move towards thalassemic care and cure and also control of birth of thal major babies in the last two decades. Similarly in the area of sickle cell disease care now the quality of life of the homozygous patients have significantly improved with therapy with Hydroxy urea and also with greater awareness amongst the communities. However, it is detected that the public awareness and care of cases of hemoglobinopathies in whole of India is not uniform. Because of poor awareness many states do suffer. In India in the field of diagnostics, the level of automation has reached a point where molecular techniques like PCR, RT-PCR and microarrays are available. Prenatal diagnosis and mutation detection by CVS analysis utilizing DNA technology in the first trimester of pregnancy is a reality since last 10-15years. It is heartening to know that many investigators have taken several research project on hemoglobinopathies and have generated significant data in Indians. The Govt. of India and also certain state Govts have initiated some control programme for hemoglobinopathies. Bonemarrow Transplantation for cure of β -thalassemia is now available in many centres at Vellore,

Mumbai, Chennai, Delhi. The improved component therapy has tremendously helped the & Thalassemia major patients. The availability of oral chelating agents in less prices is also helpful in bringing down the complications of thalassemia major patients and increased their survival significantly. Some of them live upto 40 to 50 years now. However, much research work remains to be done as regards the pharmacogenomics, newer drug use, understanding complexity of the disease and also genotype-phenotype correlation, Micro mapping also is also essential.

I22

Wide spectrum of molecular and clinical heterogeneity in HbH disease in North Indian patients

Reena Das

Background: Deletional α^+ -thalassemia comprising of $-\alpha 3.7$ and $-\alpha 4.2$ deletions is commonly encountered in the Indian sub-continent but HbH disease, resulting from the co-inheritance of α^+ -thalassemia trait and α^0 -thalassemia trait, is uncommon with just a few cases reported. Few recent reports on the molecular characterization of patients with HbH disease from India has been published which includes both deletional α -thalassemia and non-deletional α -thalassemia. This report highlights the clinical and molecular heterogeneity of HbH disease from north India. **Materials and Methods:** We encountered 34 patients with HbH disease in 28 families from north India over 15 years and performed the molecular analysis to determine the frequency of both deletional and non-deletional alpha gene defects. Patients as well as parents and affected siblings were also studied for automated complete blood cell counts, reticulocyte counts and HbH inclusions using 1% brilliant cresyl blue. Cation exchange HPLC (Bio-Rad Laboratories, Hercules, CA, USA) and hemoglobin electrophoresis at pH 8.6 were performed. Genomic DNA from peripheral blood leucocytes was extracted by the phenol-chloroform method. A multiplex Gap-PCR was carried out as an initial screen which tested for deletions $\alpha 3.7$, $\alpha 4.2$, α SEA, α MED and α SA. Southern Blot for alpha and zeta probes with Bam H1 digest was performed on 20 subjects. PCR followed by sequencing for both $\alpha 1$ and $\alpha 2$ genes were done. The α -GlobinXS MLPA kit (MRC Holland) with 35 probes was used to screen for the extent of deletions. **Results:** Homozygous Hb Sallanches ($\alpha 2$ codon 104 G>A; Cys $\rightarrow$ Tyr) in 6 cases in 5 families was found. Homozygous polyadenylation signal mutation of $\alpha 2$ (-AA) AATAAA $\rightarrow$ AATA—was detected in 3 cases. One patient each showed double heterozygosity for $-\alpha 3.7/\alpha$ Hb Sallanches α , $\alpha 76 + T\alpha/\alpha$ Hb Sallanches α . PolyA α/α Hb Sun Prairie α , α PolyA $\alpha/\alpha 76 + T\alpha$ and $-\alpha 3.7/\alpha$ Hb Seal Rock α and α Seal Rock α —/. Three patients were double heterozygous for $-\alpha 3.7/--SA$. Fifteen patients from 12 families had $\alpha 0$ deletion of varying lengths in combination with $-\alpha 3.7$ based on the MLPA analysis. The extent of deletions was variable ranging from $\alpha 0$ starts upstream of ξ gene & extends downstream of $\alpha 1$, deletion of probes 1-18, deletion of probes 1-22 and deletion of probes 8-18. The genotype phenotype correlation illustrating the clinical heterogeneity of HbH disease is detailed in Table 1. **Discussion:** This is the largest series of cases of

Table 1: Genotype and phenotype correlation of cases with HbH

	Number of cases	Clinical severity
Homozygous Hb Sallanches	6 cases (5 families)	Severe anemia with early presentation
Homozygous $\alpha^{\text{PolyA}}\alpha/\alpha^{\text{PolyA}}\alpha$	3 cases	Moderate anemia with presentation in the second decade
Double heterozygous $-\alpha^{3.7}/\alpha^{\text{Hb Sallanches}}\alpha$	1 case	Moderate anemia
Double heterozygous $\alpha^{76+T}/\alpha^{\text{Hb Sallanches}}\alpha$	1 case	Moderate anemia with presentation in the third decade
Double heterozygous $\alpha^{\text{PolyA}}\alpha/\alpha^{\text{Hb Sun Prairie}}\alpha$	1 case	Moderate anemia with early presentation
Double heterozygous $\alpha^{\text{PolyA}}\alpha/\alpha^{76+T}\alpha$	1 case	Moderate anemia with early presentation
Homozygous $\alpha 2$ Codon 19 (-G)	1 case	Severe anemia with early presentation
Double heterozygous for $-\alpha^{3.7}/-\alpha^{\text{SA}}$	3 cases	Moderate anemia with presentation in the third decade
Double heterozygous for $\alpha^{\text{Seal Rock}}\alpha/-$	1 case	Severe anemia with early presentation
Double heterozygous for $-\alpha^{3.7}/\alpha^{\text{Seal Rock}}\alpha$	1 case	Mild presentation at family screening in forth decade with mild anemia
Double heterozygosity for $-\alpha^{3.7}/-$	15 cases (13 families)	Larger deletions showed early presentation with severe anemia and shorter deletions showed mild anemia

HbH disease encountered in Indians. Since HbH disease is uncommon in India, the clinical recognition of the diagnosis is limited to few centres and molecular characterization is incomplete. Our study shows considerable heterogeneity both at the molecular level as well as the clinical presentation. Alpha2 gene sequencing revealed a novel mutation with addition of +T at codon 76 leading to a frameshift mutation. MLPA will be useful in characterizing the approximate location of the breakpoints and designing simple GAP PCR assays to identify the deletion. Increased awareness amongst the clinicians as well as more diagnostic laboratories will help to identify more patients with HbH disease.

I23

Role of borderline HbA2 in carrier detection of beta thalassemia

Anita H Nadkarni

Background: The increase HbA2 level is the characteristic of β thalassemia carriers. However, in some cases the level of HbA2 is not typically elevated and some difficulties may arise in making the diagnosis. For these reasons the quantification of HbA2 is very important and the results must be interpreted together with hematological and biochemical evidence. **Aims and Objectives:** The aim of the study was to find out the role of borderline Hb A2 in carrier detection of beta thalassemia and its prevalence in our population. **Results:** We report a retrospective analysis carried out on 16,590 subjects referred for thalassemia screening program. Of these subjects, 370 had borderline HbA2 values [3.0-3.9%], thus giving a prevalence of 2.2%. The genotyping in these subjects revealed presence of β thalassemia mutations in 72.6% of cases. Interestingly 25.5% of these showed HbA2 levels <3.5%. These findings are of significance since most laboratories in India rely on a threshold HbA2 level of 3.5% for diagnosing β thalassaemia carriers. These 48 cases would have been misdiagnosed if molecular analysis of globin gene was not undertaken. Milder mutations like Cap site +1 [A>C] and the Poly A tail defects accounted for most of the β thalassaemia alleles when HbA2 levels were <3.5% and β + IVS I nt 5 [G>C] alleles when HbA2 levels were >3.5%. Associated δ thalassaemia is one of the factors that leads to reduction of HbA2 levels. We also observed that the co-inheritance of α (14%) and δ thalassemia (17.5%) alleles contributed in reduction of the HbA2 levels in β thalassaemia

carriers **Conclusions:** Borderline HbA2 is not a rare event in a population with a high prevalence of β -thalassemia carriers. These data support the necessity to investigate these cases at a molecular level, particularly if the partner is a carrier of β -thalassemia.

I24

Next Generation technologies for rare mutation detection

Prashant Khadke

Background: HPLC is considered as the gold standard technology to identify beta thalassemia carriers. The HPLC technique gives highly accurate and reproducible results in the quantitation of Hb A2 and Hb F. However the dilemma occurs when the Hb A2 is normal (<3.5%) or in the borderline range. These situations require the use of DNA analysis techniques that can help identify the mutations in the patients thus helping in an efficient diagnosis. **Method:** Over 200 mutations leading to beta thalassemia have been identified worldwide. Of which approximately 50 mutations have been reported in the Indian population. Today CRDB and ARMS techniques very well established for the 6 common mutations but for the remaining 44 mutations a step wise approach to identify may prove long and cumbersome. Technologies like Multiplex Real time PCR and droplet digital PCR have been designed to help improve the process of identification of mutations. These technologies are not restricted to mutation detection and offer a wide range of applications. Real-time PCR system is based on the detection and quantitation of a fluorescent reporter. The signal increases in direct proportion to the amount of PCR product in a reaction. By recording the amount of fluorescence emission at each cycle, it is possible to monitor the PCR reaction during exponential phase where the first significant increase in the amount of PCR product correlates to the initial amount of target template. The higher the starting copy number of the nucleic acid target, the sooner a significant increase in fluorescence is observed. Droplet Digital PCR system is the third generation of PCR technology. Droplet Digital PCR provides an absolute measure of target DNA molecules with unrivaled accuracy, precision, and sensitivity. This technology enables to do absolute quantitation in the sample without any reference standard and without any standard curve analysis. This innovative technology from Bio-Rad will bring in many new solutions for copy number

variation, rare sequence detection, mutation detection and gene expression analysis. The ddPCR system provides single-copy PCR resolution to accelerate discoveries and new strategies for the research of genetic disorders, cancer, HIV, Tuberculosis, Thalassemia, viral and infectious diseases. **Conclusion:** In conclusion, the technologies like HPLC, Real time PCR and droplet digital PCR provided highly accurate diagnosis. The advanced technique like droplet digital PCR also offers additional benefits, including direct quantitation without requiring a calibration curve, minimal labor, rapid turnaround time, and decreased risk of PCR carryover contamination.

I25

ITGAM and lupus susceptibility: From genetic association to causal variant identification

Swapan K. Nath

Background and Aims: Systemic lupus erythematosus (SLE) is a complex, autoimmune disease with a strong genetic basis. Although, over 40 genetic associations have been identified through genome-wide and candidate-gene association studies, there is a major challenge identifying the causal variants and molecular mechanisms through which associated variants contribute to disease susceptibility. Using trans-ethnic mapping, we identified an ITGAM missense variant (rs1143679) strongly associated [$P=3.60 \times 10^{-90}$, $OR=1.76$, $N=28,439$] with SLE. ITGAM is a component of the MAC-1, which mediates leukocyte adhesion, migration and phagocytosis as part of the immune system. However, precise molecular mechanisms of this variant are incompletely understood. **Methods:** We used bioinformatics, gene expression and a series of in vitro and in vivo experiments to assess the molecular functions of rs1143679. **Results:** We used ENCODE data and discovered that the putative region is the most active region of chromatin regulation and transcription factor binding in ITGAM. Using luciferase assay, we showed that the risk allele carrying sequences act as enhancer for ITGAM. We quantitated ITGAM RNA and surface protein levels in monocytes from patients with each genotype. Transcript levels significantly decreased for the risk allele ('A') relative to the non-risk allele ('G'), in a dose-dependent fashion. Using EMSA and allele-specific ChIP-assay, we identified differential bindings with several transcription factors like NFKB1, EBF1 and Ku70/80 in monocytes. Proximity ligation assay detected a long range interaction between Ku70/80 and RNA Pol-II. **Conclusion:** The rs1143679 is most likely to be a causal variant; the reduced expression is attributed to reduced interaction of these proteins with risk allele carrying DNA sequences. Therefore, inefficient interaction between Ku70/80 and RNA Pol-II may affect the expression of ITGAM. This two-pronged contribution (nucleic acid- and protein-level) of the rs1143679 risk allele to decreasing ITGAM activity provides insight into the molecular mechanisms of its potent association with SLE.

I26

Genetic and epigenetic causes of congenital heart defects

Uppala R

Congenital heart defect (CHD) is the most common type of birth defect, and affects close to 1% of all live births. Over 50%

of children born with CHD will have one or multiple invasive surgeries in their lifetime. Every year, twice as many children die in the USA from CHD than from all forms of childhood cancers combined. The mortality rate of children with CHD-associated anomalies is 30%, and it approaches to 100% when chromosomal and other developmental anomalies are present. Over 40-50% of infants with CHD have accompanying birth defects involving neurodevelopmental, limb, muscle, defects and frequently are also susceptible to respiratory infections. Majority of CHD cases have no identifiable genetic abnormality, around 30% of CHD children are associated with chromosomal abnormality. Families with autosomal dominant, recessive and X-linked CHD have been reported; additionally many genetic syndromes have an association with CHD. About 80% of CHD are presumed to be multifactorial and arises through various combinations of genetic and environmental contributors. Among several candidate epigenetic mechanisms, DNA methylation may play an important role in the etiology of CHD. There are many known environmental risk factors including maternal risk factors and nutritional influence on epigenetics. We conducted a genome-wide DNA methylation analysis using an Illumina Infinium 450k human methylation assay in a large cohort of newborns who had various form of CHD including hypoplastic left heart syndrome, ventricular septal defect, atrial septal defect, pulmonary stenosis, coarctation of the aorta and Tetralogy of Fallot. Statistical and Bioinformatic analysis was performed followed by gene ontology analysis and functional enrichment analysis was done. The study identified significantly altered CpG methylation sites in many genes in affected subjects as compared to normal controls (either hypermethylated or demethylated). Using epigenetic analysis we were able to identify genes significantly involved in the pathogenesis of CHD.

I27

Confused Confucius!! The circle of complex disorders

Dwaipayan Bharadwaj

Phenotypes are not always a direct reflection of genetic inheritance. Unlike the monogenic diseases, the so-called "diseases of civilization" or complex diseases are multigenic, and they do not follow the mendelian inheritance pattern. Finding the root cause of these diseases have always been a major challenge in the field of biological research. In our lab, we have performed genome-wide association studies on complex diseases like Type 2 diabetes and childhood obesity in Indian population that yielded us many novel signals to validate further. However, all the genetic variants for type 2 diabetes in human from various populations across the globe have been cumulatively shown to explain only a small fraction (~7-8%) of disease risk. This interested us further to examine the potential reasons for this missing heritability that would help us to understand the etiology of type 2 diabetes in a better way. Through this presentation, we aim to illuminate the dark road of inexplicabilities of complex disorders through five case studies that are inquisitively picked up and analyzed from our experimental results from various lab projects.

I28

Elucidation of genetic and cellular defect in neurodevelopmental and neuropsychiatric disorders

Dhanjit Kumar Das

Neurodevelopmental and neuropsychiatric disorders are complex traits that result from multiple genetic determinants interacting with environmental factors to give rise to clinically diverse phenotypes. Neurodevelopmental disorders are associated with widely varying degrees of difficulty which may have significant mental, emotional, physical, and economic consequences for individuals. Among neurodevelopmental disorders, Rett syndrome (RTT) is an X-linked neurodevelopmental disorder, primarily affecting females. The prevalence is about 1 in 10,000 female births. Rett Syndrome is caused by mutations within methyl CpG-binding protein 2 (MECP2) gene. A total of 47 different MECP2 mutations and polymorphisms were identified in 32 patients. A total of 70 patients remained without molecular genetic diagnosis that necessitated further search for mutations in other genes like CDKL5 and FOXP1 responsible for causation of Rett phenotype. We also screened for mutation in CDKL5 gene and identified 5 mutations in 4 different atypical cases. Further investigation of FOXP1 gene indicated a frameshift novel mutation in one atypical patient. We have also identified an interesting case having mutation in MECP2 gene and 15q11.2-13.1 deletion also presented. This is the first case report having MECP2 mutation and 15q11.2-13.1del together. Schizophrenia is a common neuropsychiatric disorder. It is a hereditary disorder (~80%) with monozygotic twin concordance rates estimated to be as high as 40–65%. There have been significant efforts to identify possible common and rare genetic variants that might explain susceptibility to this disorder. Recent genome-wide association studies have confirmed a substantial risk of 15q11.2del associated with SZ. Copy number variants at 15q11.2 are also associated with intellectual disability, autism in addition to schizophrenia (SZ). This region, spanning BP1-BP2 is part of the Angelman/Prader-Willi region and encodes CYFIP1, NIPA1, NIPA2 and TUBGCP5 genes. From animal models, all four genes are important for brain morphogenesis. As these genes have not been investigated in human neurodevelopment, we have analyzed gene expression and neuronal morphology in differentiating induced pluripotent stem cells (iPSCs) from individuals with the 15q11.2 deletion. We have generated iPSCs line from a proband and his mother, both bearing a deletion at 15q11.2 and were compared with an unrelated control without deletion. The proband was also diagnosed with SZ, but not his mother. The deletions were identified using qPCR and array-CGH. Fibroblasts derived from skin biopsies were reprogrammed conventionally with Sendai viral vector expressing transcription factors Sox2, c-Myc, Klf4 and Oct4 (Yamanaka factor). Following quality control, the iPSCs were differentiated into neural rosettes and neuronal progenitor cells (NPCs) and then to glutamatergic neurons. The iPSC derived neurons stained for markers of glutamatergic neurons (vGLUT1, NR1, Calbindin and CART) and expressed functional ligand-gated channels. Since,

the dendritic spine architecture were shown to be altered in Schizophrenic post-mortem brain, we analysed dendritic spine morphology in iPSCs derived neurons carrying the deletion. Reduced expression of four CNV-related genes were observed in iPSCs, neural progenitor cells and iPSC derived neurons derived from the mother and the offspring, compared with control cells. CNV bearing iPSC derived neurons also showed decreased levels of CYFIP1 protein, reduced dendritic spine number and density, and enhanced proportions of immature-to-mature spines. The BP1-BP2 15q11.2 deletion is associated with reduction of mRNA expression of four deleted genes during iPSC to neuronal development and altered dendritic spine architecture. Further investigations are required to see whether haploinsufficiency of CYFIP1 causes developmental abnormalities that lead to the dendritic spine alterations.

I29

Immunological basis of schizophrenia: A diagnostic and therapeutic viewpoint

Moinak Banerjee

Schizophrenia is a severe and debilitating mental illness, affecting about seven to eight individuals per 1,000 of the general population. It is characterized by three broad spectrum behavioural domains such as positive symptoms, negative symptoms and cognitive domain. These behavioural domains tend to vary in their presentation based on ethnicity, culture or environmental conditions which might impact treatment and its outcome. These facts makes Schizophrenia a complex disorder which involves multiple genes with mild to moderate effect and non-genetic risk factors like environmental and psychological assaults, that alter the brain's chemistry. Numerous theories have been proposed regarding the cause of schizophrenia, ranging from developmental or neurodegenerative processes or neurotransmitter abnormalities to infectious or autoimmune processes. Schizophrenia patients experience elevated morbidity from infectious and auto-immune diseases; in addition, the factors that exacerbate schizophrenic symptoms also tend to impair immune function. Even the antipsychotics used in treatment have also been identified to alter immune regulation. Immune dysfunction can be proposed to be the common denominator for connecting multiple hypotheses in Schizophrenia with mild to severe effects, depending on the environmental cues. Cytokines can act as signals between cells to regulate the immune response to injury and infection. Besides providing communication between immune cells, they can also play a role in signalling the brain to produce neurochemical, neuroendocrine, neuroimmune, and behavioral changes. They also strongly influence the dopaminergic, noradrenergic and serotonergic neurotransmission. Therefore, cytokines being the key regulator of immune/inflammatory reactions and brain development emerge as a common pathway for genetic and environmental components of schizophrenia in monitoring diagnostic and therapeutic response. The role of cytokine in disease association and treatment response with specific reference to schizophrenia will be discussed.

I30

Pharmacoeugenomics of malaria and its relevance

H. Gupta

The molecular basis for the pathogenesis of malaria remains to be completely understood. Cytokines play an important role in the progression of malarial pathogenesis. Entry of malaria parasite in patients induces the synthesis of inflammatory cytokines such as TNF- α , IL-1, IL-6 and IL-10. Altered gene expression of cytokines known to be regulated by epigenetic mechanisms and cytokines by themselves may induce epigenetic changes. These may include changes in DNA methylation as well as miRNA expression. P-glycoprotein encoded by ABCB1 gene is class of drug transporter which has been found associated with drug resistance for many diseases including malaria. Malaria parasite invasion is facilitated by activation of the GPCR signaling pathway and also by the β 2-adrenoreceptor protein encoded by ADRB2 gene. However, available knowledge on their regulation is incomplete. We hypothesized that malaria susceptibility or its severity may be influenced by the SNPs in ADRB2, ABCB1 and other GPCR genes; and epigenetic changes at the critical CpG sites. We tested for global DNA methylation levels of human hosts using HPLC method. Promoter DNA methylation analysis of ADRB2 and ABCB1 genes were also performed using Bisulfite DNA sequencing. Significant SNP and DNA methylation differences were noticed in our study among cases and controls. The study provides evidence for the proposed role of ABCB1 and ADRB2 mediated mechanisms in etiology of malaria susceptibility and drug response, for clinical and therapeutic application. This study is the first to report altered DNA methylation patterns in distinct malaria severity conditions.

I31

Optimal dosing of warfarin in Indian population: Contribution of genetic polymorphisms

Shrimati Shetty

The narrow therapeutic range of warfarin and the inter-individual variation are the two important factors which necessitate warfarin pharmacogenomic studies with an ultimate objective of individualized treatment regimens based on the genotype of the patient. The pharmacodynamic and pharmacokinetic parameters of warfarin are largely influenced by genetic polymorphisms in two genes namely VKORC1 and CYP2C9. As there is a wide ethnic variation in the distribution of these polymorphisms, the mean warfarin dose also varies across populations. The African Americans need the highest dose of warfarin, followed by Caucasians and Asians. Among Asians again there is a wide discrepancy in the dosage prescribed; Indians need a higher dose of warfarin as compared to Japanese and Chinese. Differences in the allele frequencies have also been observed between Northern and Southern regions of the country. In addition, genes in the warfarin-vitamin K interactive pathways, vitamin K-dependent clotting factors were also studied. These genes include CYP4F2, CYP2C18, CYP2C19, APOE, EPHX1, CALU, GGCX, PROC, factor II, factor V, factor VII, Factor IX and NR1H2. Unlike CYP2C9

and VKORC1 the association of these genes to warfarin dose variation are not strong and the effects are too small to have any significant clinical use. Several new anticoagulants have been put to clinical use, for instance oral thrombin inhibitors (dabigatran), factor Xa inhibitors (rivaroxaban); however, there are still certain issues like the absence of a simple test like PT-INR for dosage monitoring and the exorbitant cost of these products. Thus at least in the near future, warfarin will remain as the most prescribed oral anticoagulant in India as also other parts of the world.

I32

Pharmacogenomics and drug discovery and development

Sanish

Pharmacogenomics is the study of the role of genetic inheritance in individual variation in drug response. Pharmacogenomics is moving from a candidate gene strategy to large scale approaches. This is in line with the new paradigm of linking a trait to (a) pathway(s) rather than to single genes. In addition, breakthroughs in genomics offer a non-a priori assessment of implicated genes, expanding the possibilities in pharmacogenomics research. The key issues that led to pharmacogenomic studies are lack of efficacy and serious adverse reactions. The potential benefits expected are (i) drugs may be tailor-made for individuals (ii) drugs with greater efficacy and safety (iii) advanced screening for disease and better vaccines (iv) improvements in the drug discovery and approval process and (v) decrease in the overall cost of healthcare. Pharmacogenomics studies are in use by clinical trials researchers for variations in cytochrome P450 genes to screen and monitor patients; by pharmaceutical companies to understand the drug metabolism by variant forms of CYP enzymes; and by doctors to screen patients for the deficiency of thiopurine methyl transferase, and its activity is monitored to determine appropriate thiopurine dosage levels. Personalized medicine—the right drug for the right patient—is often talked about as the future, maybe the only future, of the drug industry. The application of pharmacogenomic approaches during drug development is an evolving process that begins with discovery and continues through confirmation of clinical efficacy and safety outcomes. PGx studies can contribute to a greater understanding of interindividual differences in the efficacy and safety of investigational drugs. PGx research depends on the collection and use of biological samples to generate data. Across the drug development continuum, genomic data may be used for several purposes, including (1) identifying the basis for PK outliers and intersubject variability in clinical response; (2) ruling out the role of polymorphic pathways as clinically significant contributors to variable PK, PD, efficacy, or safety; (3) estimating the magnitude of potential drug-drug interactions; (4) investigating the molecular or mechanistic basis for lack of efficacy or occurrence of adverse reactions; and (5) designing clinical trials to test for greater effects in specific subgroups. Recent research in PGx and drug development has also focused on trial design or statistical analysis considerations for randomized, controlled clinical trials (early and late phase) that are intended to draw definitive conclusions about treatment

effects in a genomic subgroup (e.g., enrichment designs, adaptive enrichment designs), and also co-development of a drug and in vitro diagnostic. But does the industry run the risk of over-targeting drug development? By narrowing the focus too much on drugs for defined populations, will companies be ruling out therapies for other diseases and populations because they didn't work in one particular patient set? The talk will explore these and other facets of Pharmacogenomics and personalized medicine and its use in drug discovery and development.

I33

Understanding of chromosomal rearrangements: A challenge to geneticist

Frenny Sheth

Patients with congenital dysmorphic features and intellectual disability warrant cytogenetic investigation to identify causative chromosomal alteration or segmental aneusomy. Conventional G-banding provides whole genome coverage with a resolution limit of 5-10 Mb, which excludes diagnostic analysis of patients with sub-microscopic genetic alterations. On the other hand, high resolution FISH techniques, allow investigating sub-microscopic regions [gain/loss; 3-5 Mb] but the analysis is restricted to a specific locus. Availability of array-Comparative Genomic Hybridization (a-CGH) technique which incorporates the ability to interrogate the entire genome with a resolution of 20-150 Kb allows comprehensive characterization of structurally altered chromosomes. Identification of chromosomal breakpoints could help in mapping causative genes or regulatory sequences whose function is disrupted due to the chromosomal alteration; thus aiding in genotype-phenotype analysis and calculation of the recurrent risk for future pregnancies especially in the cases having multiple congenital anomalies/dysmorphic features with or without mental retardation. The practice of these technologies in precise characterization of chromosomal alterations in several case scenarios will be presented and discussed.

I34

Assessment of DNA microarray for evaluation of microdeletion syndromes

Ashutosh Halder

Background: Microdeletion syndromes are genomic disorders characterized by small & variable chromosomal deletion (<5Mb) in which many genes are involved. They are frequently associated with multiple congenital anomalies, copy number variations and loss of heterozygosity. The phenotype is the result of haploinsufficiency of genes in the critical interval. Fluorescent In-Situ Hybridization (FISH), Multiplex Ligation-dependent Probe Amplification (MLPA), Quantitative Fluorescent Polymerase Chain Reaction (QFPCR) and Array (microarray) Comparative Genomic Hybridization (aCGH) techniques are commonly used for precise genetic diagnosis of microdeletion syndromes. **Objective:** This study was conducted to assess the role of DNA microarray in the evaluation of suspected microdeletion syndrome. **Material & Method:** This study was comprised of 319 cases of suspected microdeletion syndromes. There were 193 cases of suspected

22q11.2 microdeletion syndrome, 49 cases of suspected Prader Willi syndrome, 34 cases of suspected William syndrome, 5 cases of suspected Tricorhinophalangeal syndrome and 38 cases of other microdeletion syndromes (Miller Dieker, Wolf Hirschhorn, retinoblastoma, etc). All cases were evaluated initially with FISH with specific probes. aCGH was carried out in all 150 cases using 300K DNA bead array on Illumina platform. **Result:** FISH was confirmatory in 36 cases only (11.3%). These were 22 cases of 22q11.2 microdeletion, 8 case of Prader Willi, 5 case of William and 1 case of TRP syndrome. There were 13 cases (36%) of mosaicism (6 cases of 22q11.2 microdeletion, 4 case of Prader Willi, 3 case of William syndrome). Microarray was picked up all FISH positive cases as well as smaller specific deletions, duplications and many more pathogenic/likely pathogenic CNVs. However, aCGH was filed to pick up mosaic cases with <50% deleted cell line. Clinically suspected specific locus CNV was detectable in approximately 26.3% cases and unsuspected CNV in another 14.4% cases by aCGH. Variation in deletion size, break points, other CNV and loss of heterozygosity were evident. **Conclusion:** We conclude that FISH in this format should not be the method of choice for clinically suspected microdeletion syndromes as cost, labor & time versus benefit is unjust. More strict clinical criteria should be followed. If clinical diagnosis is uncertain or doubtful then microarray should be first screening test. However, microarray is likely going to miss mosaic cases, if deleted cell lines concentration is less than 50%. Furthermore, aCGH provides associated CNVs (often many) as well as loss of heterozygosity and their significance is unknown at present. We think whole genome screening by aCGH should be carried out to investigation genotype phenotype co-relation/assessment so that consensus guideline can be derived for interpretation and counseling patient in coming years. FISH may be used for detecting mosaicism, screening family members and prenatal diagnosis in proven family.

I35

Indigenous or outsourcing? make in india molecular diagnosis of fragile x syndrome

Sarita Agarwal

Background: Fragile X Syndrome (FXS) is the second most common type of inherited intellectual disability after Down's syndrome that cause serious adverse effect on the affected individual and his family members. It is mostly caused due to silencing of FMR1 (Fragile X Mental Retardation) gene due to hyper expansion of trinucleotide repeat (CGG) in its 5' UTR region located at Xq27.3 chromosome. FXS is associated with mild to severe intellectual disability, social anxiety, attention/deficit hyperactivity disorder, autism spectrum features, and various physical and medical characteristics as caused by mutated FMR1. Presence of a FM (full mutation) allele causes FXS, but the carriers of PM (premutation) alleles does not exhibit any of the characteristic phenotypic features associated with FXS. PM is more frequent in population as compared to FXS and occurs in 1 in 113–259 females and 1 in 260–810 males. PM alleles can expand to full mutation in following generations, which signifies the screening of PM in the pregnant women

to offer genetic counselling if desired. **Aims and Objectives:** The intention of the present study is to do molecular screening of FMRI gene by using TP PCR technique. **Materials and Methods:** Genomic DNA was extracted from 75 subjects, which included 30 clinical suspects of FXS (both male and female) and 45 pregnant females. After bisulphite treatment the samples were subjected for methylation studies and the TP- PCR amplicons were subjected to fragment analyses and results were documented. **Results:** Out of 30 clinical suspects, 9 were found to have full mutation and a single female was found to be having permutation allele. Extended family screening was done for this female subject and it was deduced that she got permutation allele due to maternal transmission. Also in one of the full mutation subject family screening was done and his mother was found to have permutation allele. This permutation allele has expanded to full mutation in the concerned subject. Out of 45 pregnant female screened for FMR1 mutation none were positive. **Conclusions:** Present study validates the use of TP-PCR as a robust, rapid, sensitive and cost effective diagnostic tool for characterising FMR1 gene (pre-mutation or full-mutation) against the kits available in market. The risk couples could be offered for prenatal diagnostic options and genetic counselling.

I36

Intellectual developmental disorders: A molecular cytogenetic update

Rajasekhar M

Background: Cytogenetics has been the gold standard and the first tire for the screening of chromosomal rearrangements related to intellectual developmental disorders (IDDs) since the 1970's. The percentage of anomalies that can be screened using karyotyping alone accounts to >47% of all cases coming to the clinics. However, modern molecular technology has added to this detection percentage to an extent to which the detection of Copy Number Variations that contribute to IDDs, its pathogenesis and the classification of the corresponding genes which have been thought to be unknown have become possible. **Methodology:** Here, we report the screening of 385 children with intellectual developmental disorders using conventional cytogenetics followed by molecular screening using Multiplex Ligation dependant Probe Amplification (MLPA) employing probes for multiple micro-deletion/duplication and subtelomeric regions. **Results:** We detected gross chromosomal rearrangements in 184 cases which included Down syndrome accounting for majority of the cases and other abnormalities including like Edward syndrome, insertional inversion involving chromosome 1 and 2 and heteromorphic variant 15p+ and inversion 9qh. Further the samples which did not show chromosomal aberrations were subjected to MLPA and we found heterozygous microdeletions in 22 IDD related genes which were validated using RT-qPCR and FISH. **Conclusion:** Hence our strategy was to supplement conventional cytogenetic analysis by MLPA currently and replace it in a diagnostic set-up in the near future. In our study, cytogenetic findings have showed normal karyotypes in ~52% of cases and with MLPA, and we detected an additional 10.9% of the patients with sub- microscopic abnormalities.

I37

Sickle cell anaemia- community control program for tribal population groups of satpuda hilly ranges from Nandurbar, Dist Maharashtra

Y.S.Prabhune, P.N.Dalvi, G.T.Kulkarni, S.L.Kate

During last 10 years of 20th Century we screened most of the tribal population groups from state of Maharashtra and found that Madia, Pardhan, Otkar and other tribal groups from Gadchiroli Dist. and Bhill and Pawara tribal population groups from Nandurbar District have higher prevalence of sickle cell disorder. (Heterozygous >20%). We identified three voluntary health organization i.e. SEARCH of Dr. Abhay Bang, LOKBIRADARI project of Dr. Prakash Amte and AMHI AMCHYA AROGYASATHI of Dr. Satish Gogulwar, trained their staff members and encourage them to undertake work on Sickle Cell Anaemia. There was no any NGO working on this problem from Nandurbar District, hence in 1998 Maharashtra Arogya Mandal established Community Control Program Centre in Satpuda hilly ranges (Nandurbar district) with help of local tribal youths. The centre is popularly known as Sickle Cell Dawakhana (Roshmal Budurk, Taluka Dhadgaon, Dist. Nandurbar.) We provide all the following facilities

- Accurate diagnosis
- Possible Treatment and follow up
- Population genetic surveys
- Health education
- Improvement in the quality of life (QOL)
- Genetic counselling
- Marriage counselling
- Prenatal diagnosis
- Research
- Training

We are working in this area for last sixteen years. We screened more than 1.5 lakhs tribal people and more than 2000 patients are under our medical supervision. Patients and parents are happy with our medical treatment and we have good response. Our ten point programme will be presented.

I38

A global perspective on the functional genomics of primary congenital glaucoma using next-generation sequencing

Subhabrata Chakrabarti

Background: Primary congenital glaucoma (PCG) is an autosomal recessive disorder of the eye largely attributed to mutations in the CYP1B1 gene ranging from 20-100% worldwide. In India, PCG is largely attributed to 43% mutations in the CYP1B1 and to a small proportion across other glaucoma-related genes (MYOC, LTBP2 and FOXC1). **Aims and objectives:** The present study aimed to identify novel genes and functionally characterize them in PCG cases that are devoid of mutations in the known genes. **Materials and methods:** From a large number of clinically well characterized PCG cases who were devoid of other mutations (n=321), ten trios consisting of an affected child along with parents born out of consanguineous marriages were selected for whole exome sequencing (WES).

The WES was performed on an Ion Proton platform (Life Tech) using the whole exome AmpliSeq chemistry. We specifically determined the potential homozygous candidate gene variant(s) segregating in the affected children, which are being replicated in the additional cohort (n=311). Further, the expression of these genes are being assayed by quantitative real time PCR and their interactions through bioinformatic analysis. **Results:** The overall sequence data revealed >50,000 variants for each trio. This was cleaned up following standard quality control methods, wherein, polymorphic variants reported in the dbSNP and 1000 genomes were excluded. Further filtering based on ClinVar database, variant effects and homozygosity significantly reduced the number of potential variants (n~150). Hence, only the homozygous loci in the PCG affected children that were heterozygous in their unaffected parents were retained. Functional assessment based on bioinformatic analysis of some of these variants led to the identification of novel genes that may be involved in PCG and were absent in the normal controls. **Conclusions:** The different candidates genes including transcription factors, some of which have been earlier implicated in anterior segment anomalies. Pathway analysis of these genes have provided some interesting insights in delineating their precise role in PCG.

I39

Nutrient-mediated teratogenesis and risk of diabetes in Indians

Giriraj Ratan Chandak

India is known as the Diabetic Capital of the World and also suffers from the rare distinction of being capital of Low Birth Weight (LBW) in the World. This double burden is likely to provide an interesting link that can be exploited to restrain the ever-exploding burden of type 2 diabetes (T2D), metabolic syndrome and its related complications. Recent spurt of genome-wide association studies, mainly in Europeans has led to identification of many variants that predict risk of T2D; several of them are also associated with birth weight and other anthropometric parameters. Our studies in Indian population has interesting mix of results; some loci replicating, several others replicating with higher effect size and few not showing similar effect as identified in Europeans. The latter category comprise those which influence intermediate traits for T2D, such as obesity and insulin resistance. These observations provide an interesting link to the established phenotypic differences in Indians and Europeans, better known as "Thin-Fat Phenotype" for Indians. It is also established that this Indian phenotype characterized by less muscle mass and high fat percentage is present at birth and predicts future risk of cardiometabolic disorders. Such risk has been shown to be mediated through two interlinked pathways; Nutrient-Mediated teratogenesis and Fuel-Mediated teratogenesis. Strong evidence also exists that maternal hyperhomocysteinemia is risk factor for LBW, higher adiposity and insulin resistance. Since, Vitamin B12 is central to S-adenosyl methionine production, the universal methyl-donor to almost all kinds of methylation reactions in the cell, hence we have initiated attempts to understand the molecular mechanism of B12 action and genes/pathways influenced by B12 intervention, as a model of understanding molecular basis of Nutrient-mediated teratogenesis. I will discuss the advantages and limitations of genetic studies of

type 2 diabetes, especially in Indians and present preliminary data to advance suggestions that epigenetic regulation of key genes may underlie the fetal programming of Diabetes and thus may be an interesting strategy to rein the epidemic of type 2 diabetes in Indians and other developing countries.

I40

Palaeolithic antiquity and continuity of Indian populations: Genomic and non-genomic evidences

V. R. Rao

Out of Africa expansion of anatomically modern humans and Palaeolithic continuity of the present day Indian populations is the most parsimonious explanation as of now, based on maternal and paternal haploid DNA lineages and high density autosomal DNA markers. Archaeological dating corroborate with DNA clock of this expansion to about 1, 60,000 ybp (years before present). The rout of this expansion, whether north via Levant or south via horn of east Africa to Indian coast to east- Asia to euro-Asia is an intense debate. However, large amount of empirical data from India generated on complete mt DNA sequences of more than 3000 samples from 37 tribal populations by Anthropological Survey of India, irrevocable in support of the southern rout. Now, it is increasingly believed that the southern-rout is the only expansion by which modern humans moved out of Africa and peopled all other non- African continents. Further support to this tantalizing proposition, even pointing out that Indian populations have ancestral foot prints to Chinese, has come from high density DNA mapping of large number of populations from Asia- Pacific region by international collaborative study. While situating people of India as representatives of earliest anatomically modern human expansion that resulted in the peopling of the world, the Indian genetic data is also forth-with in explaining the socio-cultural and historical paradigms like Tribe, Caste, language categories, large scale north -west Indo-Aryan invasion etc. The results are emphatic that ancient genetic substratum and continuity resulted in sharing and Tribe-Caste continuum; language is later super-imposition and shifting of languages is a common phenomenon; large scale invasion which could have resulted in non-Indian genetic lineages in the hierarchical structure of Indian populations does not exist. DNA dating for Palaeolithic continuity in Indian scenario starts from 60-65 kybp (thousand years before present) and glacial –inter glacial climatic fluctuations could have affected the ancient anatomically modern humans largely surviving on hunter-gatherer subsistence. We do have populations like Jarawas, Onge and Sentinelese of Andaman and Nicobar Islands as direct descendents of human expansion, while all other populations share this ancient substratum. The paper finally discusses, the non-genomic evidences in terms of paleo-art and linguistics, that supports ancient human bio-cultural heritage of Indian populations.

I41

Darwin to DNA – Story of evolution retold

K.Ghosh

Darwin visited Gath pagers Island in HMS Beagles and changed the way we discuss biology. He found different animals, intermediate

links between two animals, looked at the environment around and following deep thought and introspection wrote his famous "Origin of species". We know that this epoch-making thought would never have been published in any scientific journal of today and was not even publishable then. Hence he had to write his own book and hide it for fear of persecution from the church, However the essence of Darwinism is that there is a continuous in the thread of life which became more and more complex from simple life form. This complexity arose because of interaction of organism with its environment which was ever changing. He thought the species tries to adapt with its environment through the process of variation, completion, extinction and occupation of different ecological niche. Sexual reproduction was found to be powerful natural tool by which some of the variation took place. Darwin's initial funding was that of naturalist. Later on anatomists found striking continuity in skeletal changes from fish to amphibians to snakes and birds. Biochemistry added some muscle to the theory of evolution showing that macro molecules in the living system i.e. various proteins across animals and plants show striking analogy showing that many of these molecules had common origin. Today's molecular biology which reads the DNA of various living system and by using powerful computers and various statistical and analytical algorithm Darwin's theory of evolution is strongly in the Drivers seat echoing Theodosius Dobazanky's view "Nothing in biology makes sense unless it can be explained through evolution"

I42

Ethical Issues in Human Genetics Research

Roli Mathur

Research in human genetics is scaling new heights with the availability of state of the art technology, tools and tests. The availability of enormous genomic information has ethical implications in terms of its basic understanding, probabilities, translational value, sensitivity to societal values and actual applicability for improvement of human health. There are concerns about the ability to handle and decipher such information. Clinical research in human genetics is subject to general ethical considerations and additionally not only concern the individual alone but also the close family, even the extended family or community as a whole. Additionally the harm may not only be physical, but also psychosocial which may produce anxiety or damage familial relationship if the information gets into wrong hands or if the communication is improper. There is great importance of spoken words since genetic counselling is akin to therapy, genetic manipulations have consequences for the future, some unknown; issues such as pedigree analysis, confidentiality, informed consent, genetic screening, prenatal testing, postnatal screening, gene therapy, DNA banking, pre-implantation diagnosis, pre-morbid diagnosis, forensic genetics, intellectual property rights etc are all very relevant and critical areas that need careful ethical considerations. In addition to these often the distinction between research and practice is quite blurred. Human genetics and genomics research in India should follow the Ethical Guidelines for Biomedical research on Human Participants (ICMR, 2006). The guidelines have a detailed chapter; "Statement of Specific Principles for Human Genetics

and Genomics Research" which discuss the ethical issues related to genetic research. Institutional Ethics Committees (IEC) reviewing genetics research should be abreast with necessary expertise and knowledge. It is thus important to have the policies to address ethical, legal and social issues (ELSI) so that the research leads to newer discoveries without any discrimination or potential harm to the participating populations.

I43

Next Generation Human Genetics: Opportunities and Applications

Arindam Maitra

DNA sequencing represents a single format of information over which diverse and multiple biological processes can be projected for high throughput data generation. Over the last few years, disruptive advances in DNA sequencing technologies have enabled us to take on challenges in genomics, especially human genetics, at levels which are not only unprecedented but which were never thought of earlier. This has made us to radically rethink on our knowledge and understanding of human disease genomics, especially cancer genomics. It has democratized the field of DNA sequencing, enabling even labs outside large genome centres to undertake challenges in complex biological problems by generating reasonably high throughput sequencing data. These technologies and applications are rapidly evolving. I will provide an overview of some of these approaches along with some recent examples to show how this new wave of technology is being deployed to generate valuable information on human health and disease.

I44

Innovations in chromosome analysis using automated digital imaging technologies and multi-color Spectral Karyotyping (SKY®)

Michael R. Koehler

For more than 40 years, chromosome banding has been the laboratory standard to detect and identify numerical and structural chromosome aberrations in pre- and postnatal cytogenetics as well as to analyze tumor cell chromosomes for cancer diagnosis and research. With the introduction of Fluorescence in situ hybridization techniques (FISH) and digital imaging techniques the resolution limit of chromosome banding was impressively improved. More recently the extension of the FISH technique by multicolor Spectral Karyotyping (SKY®), based on the simultaneous hybridization of all 24 chromosome-specific painting probes labeled with different fluorochrome combinations and using an innovative interference-imaging-spectrometer allowed the simultaneous differentiation of all 24 human chromosomes in 24 different colors and thus identifies even hitherto unknown or unclarified chromosomal aberrations unequivocally. In the last couple of years this development was complemented by automated imaging technologies ranging from metaphase finding systems, and FISH spot counters up to robotic high throughput machines which have streamlined the laboratory work and further improved the efficiency in cytogenetic laboratories. Despite its limited resolution, cytogenetic analysis techniques provide an

overview of the entire genome on a single cell level, which is still one of the major challenges of high resolution molecular methods such as Next Generation Sequencing (NGS) or array based tests, and therefore cytogenetics still maintains its position as a fundamental standard in genetic diagnostics. An overview about the applications of SKY®, its technique, and new developments in digital imaging techniques and their automation in cytogenetic analysis and research will be presented.

I45

Advances In Preimplantation Genetic Screening

Alan thornhill

The largest cause of failure for In-Vitro fertilization cycles is aneuploidy in embryos. As prospective mothers get older, their proportion of aneuploidy oocytes increases, with over 40% of the oocytes being aneuploidy at age 35, rising to over 70% aneuploidy at age 45. Aneuploidy screening has been advocated as a tool to address this issue. Until a few years ago, the only available method was via FISH, but this method is highly subjective and has even been shown to be detrimental in randomized controlled trials. Over the last few years, several methods have been used to address this issue with array comparative genomic hybridisation, being the leading technology. The latest development is the use of massively parallel sequencing technology in aneuploidy screening.

Young Scientist Award

YSA1

Role of apoptosis in benign and malignant tumors of breast

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Aim: To study the role of apoptotic factors in benign and malignant tumors of the breast. **Introduction:** A swelling or lump in any part of the body due to abnormal growth of tissue is known as a tumor these may be non-cancerous (benign) or cancerous (malignant). Benign tumor cells grow only locally cannot spread by invasion or metastasis, where as Malignant cells invade neighbouring tissues, enter blood vessels and metastasize to different sites and are often fatal. Breasts may develop benign tumors commonly fibroadenomas or malignant ones known as carcinomas. Benign tumors are responsible for morbidity but the malignant tumors cause both high morbidity and mortality. Benign breast disorders have an incidence of 8.1 /1000 of adult female admissions where as in India, the annual breast cancer diagnosis rate has now reached to 23/100,000 women. Both benign and malignant tumors have the potential to proliferate. It is well established that cell proliferation and apoptosis occurs simultaneously in tumors [Bowen, 1998]. This balance between proliferation and apoptosis is very essential for the normal growth of tissue and an imbalance between these results may be pivotal in benign versus malignant tumors. Hence

in the present work we have looked at apoptotic factors in fibroadenoma and malignant tumors of the breast. Apoptosis or programmed cell death, constitutes a systematic means of cell suicide during normal morphogenesis. Inappropriate apoptosis may cause various human diseases, making the control of apoptosis an important potential target for therapeutic intervention (Rudin, 1997). Apoptotic cell morphologies include membrane blebbing, nuclear and cytoplasmic condensation and appearance of apoptotic bodies (Schimmer et al., 2001). Apoptosis is induced by both intrinsic and extrinsic pathway. In the former Bcl2 and Caspase -3 play an eminent role while apoptosis inducing factor plays significantly in Caspase independent cell death. The control of apoptosis is an important potential target for therapeutic intervention. In this study three important apoptotic related genes AIF, BCL2 and Caspase-3 have been assessed in fibroadenoma and malignant tumors of the breast. Apoptosis Inducing Factor- Mitochondrion associated 1(AIFM1) is a flavoprotein involved in initiating caspase-independent pathway of apoptosis. BCL-2, the first gene found to be associated with inhibition of apoptosis and Caspase-3, a frequently activated death protease, catalysing the specific cleavage of many key cellular protein, is a hallmark of apoptosis and is indispensable for apoptotic chromatin condensation and DNA fragmentation in all cell types. **Hypothesis:** Apoptotic factors are up regulated in Benign tumors and down regulated in Malignant tumors. **Objectives:** 1] To screen for nuclear gene mutations specifically involved in apoptotic pathways such as AIF in exon 15 (F566A). 2] To evaluate the methylation status of AIF, Bcl-2 and Caspase-3 gene in benign and malignant tumor tissue and its adjacent normal tissue by Methylation Specific Restriction Assay. 3] To examine the transcript levels of the apoptotic factors AIF, Bcl-2 and Caspase-3 by quantitative RT PCR in both types of tumor tissues. 4] To study the expression of specific mitochondrial factors implicated in apoptosis like AIF, Bcl-2 and caspase-3 by Immunohistochemistry in fibroadenoma and breast cancer tissues. 5] To correlate the clinicopathological features like age, reproductive status, Estrogen Receptor [ER], Progesterone Receptor [PR] and Human epidermal growth receptor 2 [HER-2/neu] with fibroadenoma and breast cancer tissues. **Subjects:** 300 FFPE tissue samples which comprised of 100 breast cancer, 100 respective adjacent normal and 100 fibroadenoma are obtained. Clinical details along with family history and histopathological reports of the cases were collected after institutional ethical committee approval. **Ethical Approval:** Approval [VMRCE-3-2011] for genetic studies in Breast cancer was taken from the Ethics Committee of Vasavi Medical and Research Centre, Vasavi Hospital, Hyderabad, India. **Methodology:** DNA was extracted from 300 FFPE blocks by the salting out method. Methylation specific restriction assay (MSRA) was carried out by the method reported from our group earlier (Mohan et al 2006). Methylation status of promoter region of AIF, P2 promoter region of BCL2 gene and intronic CpG island of Caspase-3 genes were determined for both Benign and malignant tumors along with their adjacent normal tissues. RNA is isolated from the FFPE tissues of different pathologies of breast. SYBR® Green has been used as double-strand DNA-specific dye for real time PCR. Formalin-fixed Paraffin-

embedded tissue sections of malignant, benign and adjacent normal samples were used for immunohistochemistry. The Bcl-2, Caspase-3 and AIF expression have been analysed by using the commercially available anti- antibody from ABCAM. **Statistical Analysis:** The sub grouping of different protein expression and comparison of different demographical data such as age, stage, type of tumor, hormone receptors were made by t test using MedCalc software [12.2.1 version]. P values of < 0.05 were considered to be statistically significant. SISA online statistical software was used to derive the correlation co-efficient values with the expression ratios of different protein markers examined. **Results:** The mean age of breast cancer patients was 51.15 ±12.98 yrs and that of fibroadenoma patients is 28.755 ± 9.94. Increased transcription and expression of AIF in the tumor tissue but less methylated status of the studied promoter region gives insight into the involvement of mosaicism in the product of the gene due to dosage compensation i.e involving one X chromosome inactivation since this gene is present on X chromosome. Enhanced transcription and expression of BCL2 in the tumor tissue correlated with the hypomethylated status of the p2 promoter region studied. Superior transcription and expression in the normal tissue correlated with the hypomethylated status of the intronic CpG cluster of Caspase-3 studied. **Significance of the work:** Analysis of three apoptotic genes i.e AIF, BCL2, CAS-3 has given more understanding of how and why apoptotic process that take place in normal, benign and malignant tissues of breast are different. **Conclusion:** Consequently, Bcl-2 immunopositivity may connote a more treatable form of breast cancer, thus accounting for its association with longer survival among women with breast cancer as well as in women with metastatic disease. **Acknowledgements:** I am thankful to UGC for providing the financial assistance to carry out the work. I thank MNJ, Indo American and Kamineni Hospital for kind help and cooperation. I thank Prof Y.R. Ahuja and Vasavi hospital, VMRC and its staff for their support.

YSA2

Investigation of insulin like factor-3 (INSL3) gene polymorphisms with PCOS susceptibility

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Background: Polycystic ovary syndrome (PCOS) is a common, complex genetic disorder which affects 5-7% of women of reproductive age. It is the most common cause of anovulatory infertility characterized by irregular menses, abnormal gonadotropin dynamics with increased LH: FSH ratio. Hyperandrogenemia is one of the key features involved in its pathophysiology. Polycystic ovaries represent many small preantral follicles whose growths are arrested due to abnormal folliculogenesis. Insulin-like factor 3 (INSL3), a member of relaxin–insulin family is synthesized by the ovarian theca cells and its levels thus reflect the number of growing

follicles. INSL3 is implicated to be an important intraovarian factor which regulates androgen production by the theca cells. Serum INSL3 levels are elevated in women with PCOS. Familial linkage based studies have identified INSL3 as a potent candidate gene which could have role in PCOS predisposition. **Aims and Objectives:** To investigate association of INSL3 polymorphisms with PCOS and its related phenotypes in a case-control based study setting in Indian population. **Materials and Methods:** A total of 496 women were recruited and screened for presence of INSL3 polymorphisms and further genotyped by direct sequencing. Complete phenotyping of study recruits in terms of clinical, biochemical and hormonal parameters were carried out. Genotype association with PCOS susceptibility and its related traits were carried out by chi square and regression analysis in respective case and control groups. **Results:** The rs6523 polymorphism in exon 1 of INSL3 showed significant association with PCOS susceptibility while the other polymorphisms failed to show such relationship. The rs6523 in exon 1 also showed significant association with hyperandrogenemia and hyperinsulinaemia related traits of PCOS. **Conclusions:** Variation in INSL3 gene does influence PCOS and its related phenotypes. **Keywords:** PCOS, genetic, polymorphism and INSL3.

YSA3

Human frontal cortex is enriched for G:C>T:A somatic transversions mediated by physiological oxidative stress

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Functional heterogeneity in different tissues is majorly contributed by dynamic nature of gene expression across different cell types, whereas, genome is usually considered to be static. However, somatic cells have a tendency to acquire genetic changes as they undergo multiple divisions during lifetime of an individual either due to exogenous mutagens or inherent infidelity of DNA polymerase. Such genetic changes are termed as somatic variations. This phenomenon is expected to introduce intra-individual variability at the DNA level and impact phenotypic outcomes – cancer being the most drastic one. In order to have better understanding of consequences of intra-individual genomic variability, it is a prerequisite to know the rate of accumulation of such genetic changes in different cell types of normal healthy individuals. Somatic variations have been shown to play very important role in many Mendelian diseases. Recently, there have been reports showing presence of somatic variations in different tissues of healthy individuals, but their extent and role is still underexplored. Somatic variations could be of great importance for an organ like mammalian brain which has high complex structural and functional organization, high plasticity, and limited regenerative capabilities. Apart from harboring large scale retro-transpositions, Copy Number Variations and aneuploidy, it has also been shown recently that DNA double strand breaks acquired by neurons due to physiological activity of brain contribute in learning and adaptability. But so far it has not been considered whether

somatic changes at the resolution of single nucleotides may contribute to normal physiology of the human brain. Here, using exome sequencing of paired tissue samples, we show that the human brain, especially the frontal cortex, harbors somatic single base variations. Exome sequencing of paired. Samples from nine unrelated adults aged between 23 and 45 years was performed. Four pairs represented neuronal and non-neuronal parts of the brain - frontal cortex (FC) and corpus callosum (CC), respectively. These were from post-mortem, road accident victims. The other five pairs were DNA from blood and saliva of healthy individuals representing circulatory cell types with high turnover and therefore high likelihood of spontaneous somatic variations. We analysed about 1.5 billion reads from whole exome sequencing of these 18 samples with an average of 60 Mb coverage per exome and identified 473,336 single nucleotide variations (SNVs), with an average of ~50,000 sites per sample. The sequence depth for the samples ranged between 91x – 120x (average 100x) for the FC-CC samples and 25x – 86x (average 51x) for the blood-saliva samples. For technical confidence of the genotype calls all the samples were genotyped on Illumina Infinium 660W-Quad microarrays and we observed 98%-99.9% genotype concordance between the NGS and the microarray data. Sequence data from the paired brain samples were found to harbor somatic variations measuring up to almost 5% of the total variations. About 66% of these somatic variations in the brain are expected to lead to non-synonymous changes, and as much as 86% of these represent G:C>T:A transversion events. Further, upto 98% of the transversion events in the brain were mostly found in the frontal cortex (harboring GT and CA heterozygotes), whereas the corpus callosum from the same individuals harbor the GG and CC homozygous genotype. It is known that most common type of lesion found on DNA as a result of oxidative stress is 8-OHdG, modified adduct of deoxy guanosine, which can mis-pair with adenine and in further rounds of replication it can lead to G>T and C>A transversions on DNA. Given this background we investigated the possibility that the higher rates of transversion events in cortex relates to higher oxidative stress. In support, we found significantly higher amount of 8-OHdG (oxidative stress marker) in the frontal cortex compared to the corpus callosum of the same subjects ($p < 0.01$). A pathway based analysis of the genes where the G:C>T:A transversions have occurred revealed axon guidance and neurogenesis as significantly enriched pathways with p-values (FDR corrected) ranging from $6.03E-04$ to $2.67E-20$. This could represent either a directed selection of genetic variations in these pathways or increased susceptibility of some loci to oxidative stress. Upon analysis at the amino acid level, it was observed that out of all the non synonymous changes that are possible due to G:C>T:A variations at each of the three codon positions, Asp>Tyr and Glu>stop changes were much more frequent than expected by chance, constituting up to 21% (11% and 10% respectively; p value = 0.0004 and 0.004 respectively) of all non-synonymous changes. We found a bias for Adenine to be present immediately 3' of the somatic G>T sites. Based on this observation and supporting literature we speculate that the observed amino acid bias of the somatic sites towards Asp

(GAC/GAT) > Tyr (TAC/TAT) and Glu (GAA/GAG) > Stop (TAA/TAG) changes might have been contributed by the nucleotide sequence context of the G:C>T:A sites. Thus, in this study we show that normal human brain, especially the frontal cortex, is enriched for somatic SNVs with a clear bias for G:C>T:A transversion events most likely brought about by oxidative stress and that these changes likely contribute to neuronal development and plasticity. Our results also indicate that a natural metabolic stress that otherwise would be damaging for the cell, in moderation can also increase the diversity of neuronal genomes and be beneficial for the organism.

YSA4

Deregulation of immunogenetic factors in Type 2 Diabetes Mellitus patients with impaired wound healing.

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Wound healing is a dynamic process in which cells, tissues and structure damaged by any injury are restored. A wound healing cascade requires a perfect orchestrated system for proper healing of wounds. The inflammatory phase of wound healing is a vital phase in which pathogenic microbes are encountered along with the repairing of tissue. But this inflammatory phase should be short, self resolving and perfectly controlled. Prolonged inflammatory stage may abrogate normal healing and finally transform it into non healing chronic ulcers. The involvement of immune mechanisms also plays an important part in the process of wound healing. Toll like receptor 9 (TLR9) is one such pattern recognizing receptor (PRR) which is expressed primarily by subsets of B cells and dendritic cells in humans and is involved in both systemic as well as local inflammatory responses. It recognizes damage associated molecular patterns (DAMPs), for instance, S100A8 produced during tissue necrosis and acts by releasing a variety of proinflammatory cytokines like interleukin 8 (IL8). Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of myeloid cells comprising of macrophages, granulocytes, dendritic cells and other myeloid cells at different stages of differentiation which function as immunosuppressive agent by nullifying the effect of proinflammatory cells. Type 2 diabetes mellitus (T2DM) has its role to play in each and every step of wound healing as persistent hyperglycemic condition generally increases the inflammation through activation of TLRs and results in the spillage of the inflammatory phase into the proliferation phase. This enhanced inflammatory phase in diabetic wound may be contributed by TLR9 mediated signaling. S100A8 also add up to the inflammation by mediating the release of IL8 and is associated with the translocation of the transcription factor NF- κ B. In the present work we hypothesized that low levels of immunosuppressive cells like MDSCs and increased expressional levels of TLR9 and its proinflammatory signaling molecules like S100A8, IL8 are few causes of persistent inflammatory phase in chronic wounds like diabetic foot ulcer (DFU) and may contribute to

impaired wound healing in T2DM patients. Levels of TLR9 and its signaling molecules were analyzed in 84 diabetic wound tissue samples and 8 non diabetic control wound tissue samples by semi quantitative RT-PCR, quantitative RT-PCR, immunohistochemistry (IHC) and western blot. Systemic levels of CD11b+ CD33+ dual positive MDSCs were analyzed by flow cytometry. The analysis of semiquantitative RT-PCR data indicated that the TLR9 mRNA expression was significantly upregulated in the lower extremity wounds of DFU patients compared to that of control wounds (p value=0.03, t = 2.21). RT-qPCR analysis further validated the upregulation of TLR9 in diabetic wounds compared to control wounds (p value= 0.04, mean log (fold change) = 0.98, Standard error of mean=0.11). The expression of TLR9 mRNA was found to increase with the grade of wounds on Wagner's scale (p value < 0.001, R^2 = 0.196). The infection status of wound was also a regulatory factor for the expression of TLR9, which was found to decrease significantly in the presence of infection (p value = 0.01, t = 2.58, R^2 = 0.07). IHC expression analysis among groups also suggested significant difference of TLR9 between the wound biopsies of DFU cases and controls. The findings of IHC results were validated by western blot experiments using antibody against TLR9. A similar upregulation of TLR9 proteins was also found through western blot analysis (p value <0.001, t = 4.27). We also found that TLR9 protein was truncated in some cases of DFU patients and was present in 3 fragments while the TLR9 was intact in all of the wound samples of non diabetic wounds. We have also checked the expression levels of upstream and downstream molecules of TLR9 signaling pathway. The expression level of S100A8 protein, a DAMP, that brings about its proinflammatory role through TLR9 mediated signaling, was also found to be elevated in diabetic wounds compared to control wounds (p value = 0.003 by semi quantitative RT-PCR and p value = 0.01 by quantitative RT-PCR) which was further validated at protein level by western blot analysis. The expression levels of S100A8 was also dependent upon the grade of wound severity on Wagner's scale and was dependent upon the infection status of diabetic wounds, expressing more in infected wounds compared to sterile wounds. The downstream molecule IL8 expression was also significantly higher in diabetic wounds compared to control wounds (p value = 0.04, t = 2.04 by semi quantitative RT PCR and p value = 0.03, t = 2.31 by quantitative RT PCR) but was not dependent on the wound grade or wound infection. Systemic mRNA expression level of S100A8 and IL8 were also higher in diabetic individuals with impaired wound healing compared to controls. The systemic levels of MDSCs were low in diabetic individuals compared to controls. Among T2DM patients the levels of MDSCs were higher in DFU subjects compared to non ulcer subjects. The levels of MDSCs were directly correlated to the infection status of the wound and DFU cases with infected wounds displayed an increase in the MDSCs levels compared to non infected wounds. Our study suggested that the expression level of TLR9 gene was higher in diabetic wounds compared to control wounds both at transcriptional and translational level. The increase in expression of TLR9 with the increase in wound grade suggested that the expression level of TLR9

directly modulates the severity of diabetic wounds. This finding may be supported by the neurotoxic effects of TLR9. Levels of proinflammatory proteins S100A8 and IL8 transcript were also found to be higher in diabetic wound compared to control wounds. Systemic MDSCs levels were drastically reduced in the T2DM subjects compared to control subjects. DFU subjects also had low levels of MDSCs but their levels were higher as compared to T2DM subjects because of the presence of infection in the wounds of DFU subjects. Hence, in this comprehensive study we have clearly shown that there is an extended inflammatory condition in diabetic wounds mediated by TLR9 and its signaling partners with the lack of MDSCs. In conclusion, the upregulation of immune system derived proinflammatory molecules in combination with lower levels of anti-inflammatory cells may bring about the impairment of wound healing in T2DM subjects.

Key words: T2DM, Wound healing impairment, Toll like receptor, TLR9, S100A8, IL8, MDSC.

YSA5

CETN1 variations cause idiopathic male infertility

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Centrins are calmodulin-like, EF-hand containing calcium-binding proteins that are found in all eukaryotic cells from yeast to mammals. Centrins are encoded by 3 homologous genes (CETN1, CETN2, CETN3) in humans. The expression of centrin-1 (CETN1) is testis-specific, spermatogenic cell-specific and developmental stage-related. Our previous studies on several Y-chromosome, autosomal and mitochondrial genes revealed 25% of genetic causes responsible for male infertility. Therefore, our aim was to identify additional genetic factors that are associated with male infertility. We collected testicular biopsy from 6 obstructive and 5 Non-obstructive Azoospermic (NOA) men, isolated mRNA and performed gene expression analysis using Affymetrix array (2.0). We found several genes were differentially expressed, of which centrin was one among the highly down-regulated genes in NOA individuals. Moreover, recent studies have shown that *Cetn1* (-/-) male mice were infertile. Hence, we selected this gene for further studies. We have sequenced the coding region of CETN1 in ethnically matched 875 infertile (Azoospermic, Oligospermic and Oligoaesthenoteratozoospermic individuals) and 552 fertile Indian men and found five nucleotide substitutions in the entire CETN1 gene; of which one was 5'UTR variant g580393C>T (rs 367716858), one each was synonymous (rs114739741), non-synonymous (rs61734344) and two 3'UTR variants; g.581123T>C (rs568365) and g.581199_581200 delTGTTinsAGAGA (novel). Non-synonymous nucleotide substitution (rs61734344) was found to be strongly associated with male infertility (PCorr<0.005), replacing Methionine with threonine (pMet72Thr) in highly conserved region of CETN1 protein. Functional characterization of the above non-synonymous mutation (p.Met72Thr) was carried out using wild type and mutant proteins (overexpressed). Biophysical Properties of the wild and mutant CETN1 proteins were

studied using various approaches like Circular Dichroism, ANS Fluorescence and Iso Thermal Titration Calorimetry (ITC). These studies revealed that the mutant protein (p. Met72Thr) was less structured and had considerable differences in the surface properties like hydrophobicity. ITC study showed that mutant protein's (p.Met72Thr) ion binding thermodynamics and calcium binding affinity were different compared to wild type protein. Since, the mutant protein's spectroscopic and thermodynamic properties were hampered, we predicted that the mutation could probably lead to the compromised physiological functions of centrin1, which is a calcium sensor. In addition to the above said Non-synonymous substitution, we found a 5'UTR mutation, g580393C>T (rs367716858) which is also associated (**PCorr**=0.011) with male infertility in Indian infertile men. This is the first study on CETN1 gene mutations and their association with human male infertility.

YSA6

Genetic epidemiology of pharmacogenetic variants associated with Warfarin and Clopidogrel in North Indians

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Aims: The genetic variations in the genes involved in metabolism of pharmacogenetic drugs influences the individual's response to drug. Population-scale genome-wide genotype datasets provides an opportunity towards building allele frequency maps which could be potentially useful to understand differences in drug response. **Methods:** We mined a cross-sectional population-scale genome-wide dataset of 2128 Indo-Europeans residing in North-India enrolled as member of INdian Diabetes COnsortium (INDICO) for presence of variants associated with pharmacogenetics of Warfarin and Clopidogrel. **Results:** We determined allelic frequency map of genetic variations involved metabolism of Warfarin (CYP2C9*3, CYP4F2*3, VKORC1 (-1639G>A) and Clopidogrel (CYP2C9*2, CYP2C9*3, P2RY1 ABCB1, CYP1A2, CYP2C19*2C, CYP3A5 and PON1) among various regions of North India. The geographical distribution pattern of genetic variants (CYP2C9*3, CYP4F2*3, VKORC1 (-1639G>A) affecting Warfarin metabolism showed an increased trend of extensive metabolizers with a concomitant decreased trend of poor metabolizers of Warfarin from Himalayan region to Gangetic region. Allelic distribution of VKORC1 (-1639G>A) showed a greater degree of variation across North-Indians. Our analysis relating to genetic variants affecting Clopidogrel responsiveness reveals significant differences in population-scale allele frequencies between Indians and global-population. Indians had a higher allele frequency for variants in CYP2C9*2, CYP2C9*3 and P2RY1 genes whereas lower frequency for ABCB1, CYP1A2, CYP2C19*2C, CYP3A5 and PON1 compared to global-population. Further, study proposed a model to explain the higher prevalence of Clopidogrel metabolizers in North-Indians. **Conclusions:** This is the largest population-scale genetic epidemiological study providing

population-level allele frequencies that could be potentially valuable to clinicians to rationally plan appropriate dosage for therapy in resource-poor condition.

YSA7

Abnormal complement activation leads to pathological neovascularisation in subjects with Retinopathy of Prematurity

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Purpose: Retinopathy of Prematurity (ROP) is a proliferative retinal vascular disorder and the leading cause of blindness in premature children worldwide. It occurs in prematurely born infants of low birth weight (<1500 g) and low gestational age (<32 weeks). More than 50% of premature infants weighing less than 1250g at birth show evidence of ROP, and about 10% of the infants develop severe ROP. ROP is a progressive as well as self limiting disease, primarily characterized by impaired retinal vascular development, which may progress from stage I to V i.e., from vaso-obliteration to abnormal retinal vascular proliferation causing retinal detachment and finally leading to severe visual impairment. In some premature infants ROP regresses spontaneously but in some infants in spite of providing same antenatal and prenatal condition, it progresses to severe stages. The reason behind the progression of this disease only in a subset of premature babies is still not clear. Therefore the present study aims to find the biomarkers which are responsible for the progression of this disease. Although there are evidences for the genetic susceptibility of ROP, the underlying molecular mechanisms are unclear. Herein, we undertook a comprehensive analysis of candidate genes involved in the development of early retinal vasculature, neurodevelopment, inflammation and regulation of angiogenesis, in ROP-affected babies to identify genetic variants conferring disease susceptibility. Additionally, functional analysis were undertaken to substantiate the genetic data. **Methods:** Initially, intragenic variants (n=384) spanning 27 candidate genes were screened in a cohort of 400 premature babies that included clinically well characterized cases of ROP (n=200) and unaffected controls without ROP (n=200). Screening was accomplished by customized genotyping followed by validation through resequencing. Allele and genotype frequencies, linkage disequilibrium and haplotype analysis were done to delineate the ROP-associated variants. Furthermore, the vitreous humor levels of 27 growth factors and genes involved in angiogenesis were assessed in patients with advanced stages of ROP (n=30) and congenital cataract (n=30) by multiplex bead array method to assess the concordance between the protein expression and genetic association data. Differentially expressed protein in vitreous were further validated by western blotting and zymography while cells in vitreous were detected by H&E staining and immunofluorescence. **Results:** There were no deviations from Hardy Weinberg equilibrium for these 384 single nucleotide polymorphisms (SNPs) among the normal controls (p>0.05). There was a significant difference

in the alleles and corresponding haplotype frequencies of few intragenic SNPs in TSPAN12, CFH, C2/BF, IHH, and MMP9, between cases and controls that withstood Bonferroni correction for multiple testing ($p=1.3 \times 10^{-4}$). Variations in the other genes (OPTC, EPAS1, PRELP, AGTR1 GP1BA, VEGFA etc.) did not exhibit any association to ROP. The associated SNPs in CFH and C2/ BF genes conferred a significant risk and protection to ROP, respectively, as observed in AMD. Of the 28 analytes evaluated, 21 were detectable in the vitreous humor samples. Patients with ROP exhibited significant elevations in MMP9 ($p=0.035$), CFH ($p=0.002$), C3 ($p=0.006$), C4 ($p=0.0009$), Prealbumin ($p=0.012$), SAP ($p=0.026$), APO A1 ($p=0.002$) and APOC3 ($p=0.006$) compared to the controls. No significant differences were observed in vitreous humor concentrations of 14 other proteins between patients and controls ($p>0.05$). These results were further validated by western blotting that further provided interesting insights on their role in ROP development. Zymography revealed the activation of matrix metalloproteinases (MMP2 or MMP9) in ROP patients but not in controls. Vitreous staining with CD68 revealed the presence of activated macrophages/microglia while H&E staining showed the presence of inflammatory cells only in ROP vitreous but not in controls. **Conclusions:** The present study highlights the potential involvement of novel genes in ROP based on their allelic (and haplotype) associations and expression levels in the vitreous humor that underscore their potential role in disease susceptibility. The elevated levels of complement factors and extracellular matrix metalloproteinase proteins in the vitreous humor of patients indicated the abnormal immune environments and ECM components in ROP patients. Presence of macrophage in ROP vitreous suggesting that the activation of complement component further activates macrophages that results in the secretion of proangiogenic cytokines and ECM molecules which in turn leads to angiogenesis in ROP. This results illustrate the new mechanism by which activation of complement pathway and ECM components leads to aberrant neovascularization in ROP. While the activated macrophages may serve as a link between complement activation and the balance of angiogenic and anti-angiogenic factors. Present study, thus concludes that the activation of the complement components in ROP patients may have a role in neovascularisation. Therefore, the complement pathway specifically C3 molecule not only act as biomarker but can be used as therapeutic target for preventing neovascularisation which might be helpful in controlling the progression of ROP.

Oral Presentations

O1

Validation of Wilson's Disease by DNA Microarray Manjula Mathur, A.V.S.S. Narayana Rao, Rita Mukhopadhyaya

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Background: Wilson disease (WD) is a monogenic autosomal recessive disorder clinically manifested upon accumulation of copper at the median age of 12 to 23 years due to a mutant

ATP7B gene leading to defective copper homeostasis. Human ATP7B has 1465 amino acid consisting of eight trans-membrane domains, six copper binding domains, ATP-binding, transduction, phosphorylation and actuator domains. Nearly 500 mutations in ATP7B gene have been associated with WD across the world. We have attempted to develop microarrays for detecting a mutations specific to Indian population. **Aims & Objectives:** a) To understand the spectrum of mutations in the Indian population and, b) To validate the in-house developed WD Microarrays. **Material and Methods:** WD mutations in Atp7B that are specific to Indian population were selected from the available WD mutation database. DNA microarrays for detection of these 62 mutations were developed. Six different types of mutations in ATP7B were generated by site directed mutagenesis (SDM). The mutation status of these samples was verified by sequencing. DIG labeling of these mutant samples followed by hybridization with WD microarrays were used for validation of the microarrays. **Results:** Exon-wise mutation occurrence frequency in India was generated from the WD mutation database and is compared to the mutation pattern obtained world over. Hybridization efficiency of each of SDM amplicon was tested. Quantitative comparison of the detection efficiency of the probes was carried out using Discrimination Score (DS). All the simulated mutations could be detected by our arrays. **Conclusion:** SDM generated samples are one of the useful means for validating indigenously developed microarrays. To the best of our our knowledge, in India, this is a first attempt for diagnosis of multiple WD mutations simultaneously.

O2

Antioxidant Gene Variants and Type 2 Diabetes: A North Indian Study

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Background: Type 2 diabetes mellitus (T2DM), also called metabolic syndrome is a serious global health problem. The 6th edition of IDF (International Diabetes Federation) Diabetes Atlas, 2014, estimates show 382 million people worldwide suffering from T2DM between 20-79 years. Oxidative stress results from impaired antioxidant enzyme activities leading to insulin resistance (IR), β - cell dysfunction, glucose intolerance (IGT) and ultimately T2DM. Furthermore, oxidative stress has been implicated as the underlying cause of both macrovascular and microvascular complications associated with T2DM. **Aims and Objectives:** Our aim was to carry out genotypic, haplotype and gene-gene interaction analyses of GSTM1 del, GSTT1 del, GSTP1 +313A/G (105Ile/Val; rs1695), CAT -21A/T (rs7943316), SOD2 +47C/T (rs4880) and GPx1 +599C/T (rs3811699) in type 2 diabetes (T2DM) cases and controls. **Material and Methods:** In a case-control study, T2DM cases were selected as per inclusion/exclusion criteria. Whole blood samples were collected in 0.5M EDTA vials from T2DM cases ($n=410$) and normal healthy controls ($n=558$). All anthropometric and biochemical measurements

were performed. DNA was isolated and genotyping was performed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques. Genotype, allele and carriage rate frequencies were calculated using SPSS (ver 21.0). Haplotype and gene-gene interaction analyses were performed by SHEsis software to study association with T2DM. **Results:** On analyzing the genotypes of 06 SNPs showed that individuals possessing 'AT' of CAT -21A/T and 'I/V' of GSTP1 105I/V have 2.1 and 2.5 times higher risk of developing T2DM. SHEsis analysis showed 03 risk combinations of alleles viz. T C C* (2.1 times), N N V* (13.5 times) and P P I T C C* (5083 times) with significant association with T2DM. **Conclusion:** Antioxidant gene polymorphism studies involved in oxidative stress may provide prognostic markers for disease risk identification and help the clinicians to develop personalized regimens for treatment.

O3

Association Study of Inflammatory Genes with Rheumatic Heart Disease in North Indian Population: A Multi-Analytical Approach

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Background: Rheumatic heart disease (RHD) is an inflammatory, autoimmune disease; occurring as a consequence of group A streptococcal infection complicated by rheumatic fever (RF). The higher incidence of RHD has been partly attributed to inflammatory factors. Therefore the present study was designed to explore the role of genetic variants in inflammatory genes in conferring risk of RHD. **Materials and Methods:** The present case-control study recruited total of 700 subjects, including 400 RHD patients and 300 healthy controls. We examined the associations of 9 selected polymorphisms in seven inflammatory genes: IL-6 [rs1800795 G/C], IL-10 [rs1800896 G/A], TNF- α [rs1800629 G/A], IL-1 β [rs2853550 C/T], IL-1VNTR [rs2234663], TGF- β [rs1800469 C/T]; [rs1982073 T/C], and CTLA-4 [rs5742909 C/T] with RHD risk. Genotyping for all the polymorphisms was done using PCR-ARMS/PCR/RFLP methods. Multifactor dimensionality reduction and classification and regression tree approaches were combined with logistic regression to discover high-order gene-gene interactions in inflammatory genes. **Results:** On comparing the genotype frequency distribution in RHD patients with that of healthy controls, we found significant association of variant-containing genotypes (CT/TT) of TGF β 869 T/C [rs1982073]; [$p=0.004$ & 0.001 , OR (95% CI)= 1.65(1.2-2.3) & 2.25 (1.4-3.6) respectively], variant genotype (CC) of IL 1 β -511C/T [rs2853550]; [$p=0.001$, OR (95% CI)=2.33 (1.4-3.8)] and IL 1 VNTR [rs2234663];

[$p=0.03$, OR (95% CI)=5.25 (1.2-23.4)] SNPs with RHD risk. On performing the MDR analysis, TGF β 869T>C yielded the highest testing accuracy of 0.562. These results were further supported by the CART analysis which revealed that individuals with the combined genotypes of TGF β T/C_ rs1982073 (CT/TT) and IL 1 β _ rs2853550 (CC) had significantly higher susceptibility for RHD [$p=0.0005$, OR (95% CI) = 5.91(2.9-12.5)]. **Conclusion:** Using multi-analytical approaches, our study suggested important role of TGF β 869 T/C [rs1982073] in RHD susceptibility.

O4

Parental CYP21A2 Genotyping Plays an Important Role in the Reduction of Neonatal Deaths in CAH Families

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Background: Congenital adrenal hyperplasia (CAH) is an autosomal recessive metabolic disorder caused by mutations in the *CYP21A2* gene. If not diagnosed/ treated it can be fatal. **Aims & Objectives:** The present study was planned to identify *CYP21A2* gene mutations in CAH patients and in their families with a history neonatal deaths due to adrenal crisis. **Material & Methods:** CAH patients registered in our clinic were the subjects for this study. This study is approved by the Institutional ethics committee. Detailed family history and blood samples were collected after taking the informed consent from the patients, parents and siblings. PCR amplification was done using specific primers to amplify the *CYP 21 A2* gene. **Results:** There were 15 CAH patients who had a family history of neonatal (sibling) deaths. These 15 had 52 siblings. Out of these 52, 18 died at infancy due to adrenal crisis & 19 had normal phenotype according to parents. These patients' parents & siblings were genotyped to find out *CYP21A2* gene mutations. In 4 patients common mutations were not found. Out of these 4 patients, 3 had a family history of consanguinity. In one patient genotype has to be confirmed. Remaining 10 patients had abnormal genotype in heterozygous (p.P30L-1; In2-2; Δ 8 bp -1; gene duplication-2), homozygous (p.Q318X-1; p.I172N -1) and in compound heterozygous (p.P30L+ In2+ p.Q318X-1; p.P30L+ In2+ p.Q318X; p.R356W-1) state respectively. **Conclusions:** It is important to consider CAH who present with a family history of neonatal deaths. Therefore parents of CAH patients who are in reproductive age group should be counselled for genotype analysis for planning future pregnancies and the risk assessments. It is also advisable to perform genotype analysis of the CAH patients' siblings who present with a history of consanguinity & sibling deaths. **Acknowledgement:** This study is being supported by Department of Biotechnology (Ref.No.BT/PR7548/Med/12/593/2013), Government of India.

O5

Association of D18S880 polymorphism of the *CNDP1* gene with diabetic nephropathy in south Indian population

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Background: The *CNDP1* (carnosinase dipeptidase) gene is a potential candidate for diabetic nephropathy (DN). Genetic variants in this gene have shown association with DN in Caucasian population and have not been studied in Indian population. **Aims and objectives:** To test whether the D18S880 trinucleotide repeat polymorphism in the *CNDP1* gene is associated with DN in south Indian population. **Material and Methods:** Type 2 diabetic subjects with nephropathy (n=723) and without nephropathy (DM; n=560) were recruited from Dr.Mohan's Diabetes Specialities Centre, Chennai. D18S880 was genotyped by fragment analysis on capillary sequencer. Results were analysed using Genemapper software. **Results:** The frequency of the XL (6/7 repeats of Leucine) allele was significantly lower in the DN group (0.41) as compared to the DM group (0.48, $p=0.0007$). Logistic regression under a recessive model showed that the odds ratio for DN was 0.69 (95%CI: 0.53-0.90; $p=0.008$) for 6L/XL (6L/6L and 6L/7L) genotype when compared with the 5L/5L genotype. XL genotype remained significantly associated with DN even after adjusting for potential confounders such as age, sex, BMI, HbA1c, duration of diabetes, systolic blood pressure and smoking (OR: 0.85; 95%CI: 0.74-0.98; $p=0.031$). **Conclusion:** This is the first report on the role of *CNDP1* D18S880 polymorphism in DN in south Indian population. The 6L/XL genotype of D18S880 polymorphism shows significant protection against DN as compared to the 5L/5L genotype in this population. The finding gains importance as D18S880 Leucine repeat is present in signal peptide region of *CNDP1* gene which might play a role in secretion of carnosinase.

O6

Expression of CD133 and BMI1 and its prognostic role in subjects with glioblastoma multiforme from Indian Population

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Background: Glioblastoma multiforme (GBM) is the most common primary malignant brain tumor, generated by bulk of malignant cancer stem cells (CSCs). CSCs express a wide variety of proteins which are involved in the self renewal, differentiation of stem cells and it leads to the treatment failure in cancer patients. CD133 and BMI1 are the most common among them. Although several studies explained the importance of CD133 in cancer,

but the role of this protein and correlations of mRNA expression with BMI1 is not addressed before. **Aim and Objectives:** This study aims to analyze the role of CD133 mRNA and BMI1 protein expression and correlation with TP53 mutations from newly diagnosed GBM patients and its role in prognosis. **Materials and Methods:** CD133 mRNA expression has performed by SYBR green realtime PCR, TP53 mutations has analysed using sequencing of exon 5-9 and Bmi1 protein expression has analysed using western blotting. Survival analysis was estimated by Kaplan Meier curve and Cox proportional hazard ratio analysis. **Results:** Over expression of CD133 and BMI1 was found in 47.6% and 76.2% respectively and TP53 mutations were 57.1% in our cohort. There was no correlation between TP53 mutations and gene expressions of CD133 and BMI1. We found that high level of BMI1 expression is favourable for the patients survival ($P = 0.0075$) and high CD133 mRNA expression was unfavourable for the patient survival ($P = 0.0226$). **Conclusion:** CD133 and BMI1 expression could independently predict the glioblastoma patient survival in multivariate analysis.

O7

Insights on the Functional Impact of MicroRNAs Present in Autism-Associated Copy Number Variants

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Background: Autism Spectrum Disorder (ASD) is a complex neurodevelopmental disorder that appears during the first three years of infancy and lasts throughout the person's life. Recently, a large category of genomic structural variants denoted copy number variants (CNVs), were established to be a major contributor of the pathophysiology of autism. **Aims and Objectives:** Till date most of the CNV studies in autism has focused only on the protein coding genes. Therefore we aimed at analysing the miRNAs present in autism associated CNV regions. **Materials and Methods:** A series of computational tools and databases including AutDB, UCSC genome browser, TargetScan, and DAVID were utilized to elucidate the biological and functional significance of CNV-miRNAs and their target genes. **Results:** Nearly 11% of the CNV loci harbor miRNAs and a few of these miRNAs were previously reported to be associated with autism. A systematic analysis of the CNV-miRNAs based on their interactions with the target genes enabled the identification of top 10 miRNAs namely hsa-miR-590-3p, hsa-miR-944, hsa-miR-570, hsa-miR-34a, hsa-miR-124, hsa-miR-548f, hsa-miR-429, hsa-miR-200b, hsa-miR-195 and hsa-miR-497 as hub molecules. Further, the CNV-miRNAs formed a regulatory loop with transcription factors and their downstream target genes, and annotation of these target genes indicated their functional involvement in neurodevelopment and synapse. **Conclusion:** This is the first report to highlight the significance of CNV-microRNAs and their target genes to contribute towards the genetic heterogeneity and phenotypic variability of autism.

O8

A Clustering Approach for Mapping Rare Variants Based on Mutual Association

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In spite of successful identification of a large number of common variants associated in various complex disorders, a substantial proportion of the total variation in a trait still remains unexplained. It is becoming increasingly evident that the “Common Disease Common Variant” paradigm needs to be modified and the “missing heritability” can possibly be explained by rare variants, which could not be identified using genome-wide association studies. However, the major impediment in identifying rare variants is that one would require huge sample sizes to detect differences in allele frequencies between cases and controls. Thus, most existing methods are based on collapsing multiple variant sites using different statistical algorithms. Motivated by the combined multivariate and collapsing (CMC) algorithm, we propose a clustering of rare variant sites, but based on their mutual extent of association rather than similarity in allele frequencies as proposed in CMC, thereby reducing the possibility of combining functional and non-functional variants. The Fisher's exact test is performed to identify blocks of variant sites such that the initial site in each block is associated with all other sites in the block. The test for association is performed within each block by comparing the proportions of affected and unaffected individuals carrying at least one copy of a rare variant using a variance stabilizing sine transformation. We carry out extensive simulations under different rare variant models and compare the false positive rate and the power of our proposed method with some of the popular competing methods: CMC, adaptive SUM, WSS, TestRare, RareCover and Kernel-based Adaptive Clustering. We find that the proposed test procedure yields more power than the existing approaches, especially with increasing sample size, while maintaining the correct size.

O9

Genetic Markers in PCOS Risk and Phenotype Progression

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Background: Polycystic ovary syndrome (PCOS) is a common endocrinopathy that causes anovulatory infertility in women in their childbearing years. This complex syndrome has both gynecological and metabolic consequences and is typically characterized by hyperandrogenemia, insulin resistance, and menstrual irregularities. The pathophysiology is still elusive, but has been considered to be multifactorial and multigenic consisting of both genetic and environmental components. Familial aggregation of cases provides strong evidence for

hereditary pattern in PCOS. **Aims and objectives:** Our study has undertaken a candidate gene based approach to identify putative risk alleles of some important genes viz. INSR, PPAR α , INSL3 and PON1 in association with PCOS and its related traits. **Materials and Methods:** Genotyping was carried out by direct sequencing and genotype association with PCOS and its related traits were carried out by chi square and regression analysis. **Results:** In our study, the polymorphic genotype (CT+TT) of a silent C/T polymorphism at His1058 in exon 17 of INSR was significantly associated with PCOS as well as hyperinsulinemia and hyperandrogenemia in lean PCOS subgroup only. For the PPAR α polymorphisms, the most common variant Pro12Ala in exon B showed significant association with decreased PCOS susceptibility His447His polymorphism in exon 6 as well as the Pro12Ala were significantly associated with insulin related traits in women with PCOS. An exonic polymorphism L55M of PON1 gene was found to be significantly associated with PCOS susceptibility while the Q192R polymorphism showed no association. Exploring the association for coding region polymorphisms of INSL3 with PCOS revealed that only rs6523 SNP in exon 1 has significant association with PCOS susceptibility. **Conclusion:** Our genetic data establishes that in Indian women, variations in genes related to insulin resistance influence the pathophysiology of PCOS. We have also highlighted a strong implication of differential insulin resistance pathogenesis in PCOS women based on BMI.

O10

Whole Exome Sequencing helps characterize a rare highly penetrant familial disorder, turning epidemic in a village of Jammu and Kashmir, India”

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Background: Rare disorders are poorly understood, most often remain uncharacterized or patients are misdiagnosed due to lack of specific clinical resources. A rare mysterious skeletal disorder that remained unidentified for decades and rendered many people physically challenged and disabled for life has been reported in an isolated remote village ‘Arai’ of Poonch district of Jammu and Kashmir. This Village is isolated in mountains, lacking medical facilities and the population residing in the region is highly consanguineous. Lack of information about the disorder has led huge increase in number of affected in the region. **Aims and Objectives:** To identify the disorder and characterize the cause of the disease **Material and Methods:** In our survey of the region, 70 affected people were reported, showing similar phenotype, in the village with a population of approximately 5000 individuals. We could only carry 3 affected

and two unaffected members for radiological examinations, due to transport issues, inability of most of the affected to walk and resources limitations. However, we were able to collect information of members, belonging to two multigenerational extended families, from the village and generate pedigrees up to 6 generations. We were able to characterize the disorder as a skeletal dysplasia, with Autosomal Recessive Inheritance. However, exact diagnosis could not be made due to limited radiological and specific clinical details. We also managed to collect blood samples from multiple affected and unaffected members from these extended families. We elected to do Whole Exome sequencing (WES) in selected members from one of the family to characterize the disorder. **Results:** With WES, we successfully identified a rare variation NM_003880.3:c.156C>A NP_003871.1:p.Cys52Ter, which results in introduction of premature stop codon in WISP3 gene. We found this variation perfectly segregating with the disease in the family. However, this variation was absent in other family. We Sanger sequenced all the exons, splice junctions and UTRs of the WISP3 gene in the second family and interestingly, a novel splice site mutation at position NM_003880.3:c.643+1G>A of WISP3 gene, perfectly segregating with the disease was observed. **Conclusion:** Thus, exploiting Whole Exome Sequencing and putting different evidences together (familial histories and genetic data, available clinical, radiological and biochemical tests findings), the disease has finally been diagnosed as a rare, recessive hereditary skeletal disease “Progressive Pseudorheumatoid Arthropathy of Childhood” (PPAC) also known as “Spondyloepiphyseal Dysplasia Tarda with Progressive Arthropathy” (SED-T-PA). This genetic characterization and identification of the disease causing mutations will aid in genetic counseling, critically required to curb this rare disorder and to prevent its appearance in future generations in the population, where this disorder is attaining epidemic proportions. It could be implicated that WES is an affective method in understanding uncharacterized Mendelian Disorders and is of great help, especially in developing countries where such familial disorders exist in abundance but remain undiagnosed/misdiagnosed due to lack of specialized testing.

O11

FISH for Prenatal, Postnatal, and Preimplantation Genetic Diagnosis.

Lim Jee Hian

Malaysia

No Abstract.

O12

Masking of the Commonest Indian β thalassaemia Lesion IVS I nt 5 [G>C] by Different δ Globin Gene Defects.

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Background: An elevated HbA2 level is one of the characteristic

diagnostic hallmarks of a β thalassaemia carrier. Hence HbA2 levels play a clinically significant role in β thalassaemia prevention programs aimed at detecting carriers, especially in a country like India with a high prevalence of the disease. While δ thalassaemia has no clinical symptoms, its co-existence in β thalassaemia carriers may negatively impact the expression of HbA2 [a2d2]. This could lead to misdiagnosis of carriers and cause grave problems in genetic counselling and prenatal diagnosis for β thalassaemia major. **Aims and Objectives:** The main objective of the study was to evaluate the impact of the association of different δ thalassaemia alleles on carrier detection of β thalassaemia. **Materials and Methods:** HbA2 and HbF were measured on the Variant Haemoglobin Testing System. Serum iron was measured using ferritin and soluble transferrin receptor ELISA assays. Presence of IVS I nt 5 [G>C] was detected by CRDB technique and the common α globin deletions were ruled out using Multiplex PCR. The δ globin gene defects were identified using direct DNA sequencing. **Results:** Here we report the hematological and molecular analysis of ten unrelated β thalassemia heterozygotes showing presence of the common Indian β thalassemia mutation IVS I nt 5 [G>C]. All ten subjects showed HbA2 levels ranging from 2.4 - 3.9%. Interestingly, three of these carriers showed HbA2 levels $\leq 3.5\%$ and seven of them were married to a spouse carrying a haemoglobinopathy. Iron deficiency anaemia was not detected in any of these subjects but molecular analysis of the δ globin gene revealed the presence of δ chain variation in all the subjects [δ promoter region mutation -68 [C>T] – 6, HbA2 Yialousa [CD 27 G>T] – 2, HbA2 Pelendri [CD 141 T>C] – 1 and a novel δ globin mutation CD 46 [G>T] – 1]. All ten subjects showed presence of four intact α globin genes [$\alpha\alpha/\alpha\alpha$]. **Conclusions:** These findings accentuate that the co-inheritance of δ thalassaemia in β thalassaemia carriers is not an uncommon event in the Indian population. Our studies also emphasize the importance of performing a complete molecular work up in subjects showing borderline or normal HbA2 levels, especially those married to a spouse carrying a haemoglobinopathy so as to prevent the probable birth of affected offspring.

O13

Molecular Karyotyping for Prenatal Diagnosis

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Molecular techniques have been developed for prenatal diagnosis of the most common chromosome disorders (trisomies 13, 18, 21 and sex chromosome aneuploidies) where results are available within a day or two. This involves fluorescence in situ hybridization (FISH) or quantitative fluorescence polymerase chain reaction (QF-PCR) on fetal DNA. Both methods are quite reliable. These tests are used as a preamble to full chromosome analysis by microscopy. Cytogenetics is an indispensable diagnostic tool for the identification of chromosome abnormalities. It has become increasingly important for the identification of aneuploidy and unbalanced structural rearrangements

in the fetus. However, conventional cytogenetic analysis of amniotic fluid or Chorionic Villus cannot reliably detect rearrangements of genomic segments smaller than 5–10 million base pairs (Mb). In addition, microscopic examination of the chromosomes may not reveal the chromosomal origin of small supernumerary marker chromosomes and may not identify subtle rearrangements of the subtelomeric regions. Furthermore, the turnaround time for karyotyping is increased by the need for cells to be cultured 10–14 days before analysis. We report here a novel karyotyping assay, utilizing bead-bound bacterial artificial chromosome probes, providing a fast and easy tool for detection of chromosomal abnormalities in a cost-efficient high-throughput manner. KaryoLite is a multiplex assay which detects aneuploidies for chromosomes 1–22, X and Y. The assay is fluorescence based and uses encoded multiplex beads from defined loci on all 24 chromosomes. This allows detection of aneuploidies on all chromosomes from a clinical sample in a single well of a 96-well microtiter plate. The test is performed using minute amounts of genomic DNA extracted directly from amniotic fluid, chorionic villi or fetal tissue. This is potentially very useful as a first line test for aneuploidy detection because of its lower cost, rapid detection, and ability to generate a molecular karyotype for samples that are critically important, without the need for culture. KaryoLite as a 'first-tier' test in combination with other approaches would clearly enhance prenatal testing.

O14

Effect of Hydroxyurea on MicroRNA Expression and its Role in Fetal Hemoglobin Induction in Sickle cell Anemia Patients

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Background: Hydroxyurea (HU) is efficacious for children and adults with Sickle Cell Anemia (SCA), primarily due to its ability to increase fetal hemoglobin (HbF). However, HbF induction by hydroxyurea is highly variable among patients, and its mechanism of HbF reactivation remains unclear. Recently, miRNAs have been implicated in cellular regulation and differentiation including hematopoiesis and hemoglobin switching. But very few studies have focused on the importance of miRNA expression for HbF induction. **Aims and Objectives:** To see the effect of hydroxyurea on expression patterns of miRNA and its role on fetal hemoglobin induction. **Materials and Methods:** HbF levels of all samples were measured by HPLC. Each sample was enriched for CD71+ erythroid cells using an autoMACS magnetic cell sorter. miRNAs were extracted from CD71+ erythroid cells of the healthy individuals [n=3] and SCA patients, at baseline [n=7] and after 3 months of HU treatment [n=3]. Differential miRNA expression was studied using TaqMan chemistry and real-time PCR. **Results:** After HU therapy for 3 months in the 3 SCA patients the mean HbF level increased from $18.433 \pm 2.396\%$ at baseline to $30.766 \pm 3.7\%$ ($p < 0.01$).

Expression of all 6 miRNAs [miR494, miR29a, miR130b, miR210, miR16-1, miR144] were found to be up-regulated among SCA patients as compared to controls. After HU therapy miR494, miR29a, miR130b, miR210, miR16-1, miR144 showed > 3 fold increase in expression ($p < 0.05$). **Conclusion:** This preliminary study showed a significant association of miRNA expression in HU mediated induction of HbF among Indian sickle cell disease patients.

O15

Validation of nsSNPs For Pharmacogenetic Analysis Amongst Hypertensive Punjabi Population

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Background: Despite the availability of several antihypertensive drugs which include thiazide diuretics, beta blockers, Angiotensin-Converting Enzyme (ACE) inhibitors, angiotensin receptor blockers (ARB) and calcium channel blockers, global estimates suggest that less than 35% of hypertensives are able to achieve their target systolic and diastolic blood pressure with these drugs. Blood pressure response to antihypertensive drugs has a genetic component. A nsSNP result in actual changes in primary amino acid sequences, the function of the protein products might be altered, which can result in drastic changes in drug target interaction and, therefore, in dysfunctioning of the drug. Some studies are available amongst world populations, concerning genes in above systems and pathways but Indian studies of very few genes are available. **Aim and objectives:** In silico analysis of various antihypertensive drug target genes of RAAS, Beta Adrenergic receptor, Sodium transport system and Ion regulation system pathway genes have been analysed for prediction of possible impact of an amino acid substitution on the surface and function of the human protein. **Material and Methods:** Relevant gene sequences were downloaded from NCBI. Bioinformatic tools like Ensemble, PolyPhen, Sift, SNPs3D, SNPeffect and LS-SNP available at F-SNP have been used to predict the dysfunctional effect of nsSNPs. **Results:** RAAS (ACE- rs1799752, rs4343 and rs148193919, AGTR1- rs5186, rs12721226, CYP11B2- rs1799998), Beta Adrenergic receptor (ADRB1- rs1801253, rs1801252 and rs372579473, ADRB2- rs1042713, rs1042714 and rs1800888), Sodium transport system (NEDD4L - rs4149601, rs3865418, ADD1- rs4961, GNB3-rs5443), and Ion regulation system pathway genes (KCNJ1-rs 610155, rs 2846679, rs2186832, PRWNK1- rs765250, rs2277869, PRWNK4 -rs1156666 and C1163527T) were variants identified in the relevant pathways. Results will be presented pertaining to the analysis. **Conclusion:** Since the efficacy of the antihypertensive medications are influenced by nsSNPs in enzymes and proteins involved in ADME of the drugs affecting the overall safety and efficacy in an individual patient. The SNPs have been identified and validated in silico analysis and are ready for wet lab experiments.

O16

Identification of Novel Disease Genes/Mutations for Single Gene Disorders through Homozygosity Mapping and Whole Exome Sequencing

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Background and Objective: The application of homozygosity mapping followed by whole exome sequencing has led to rapid identification of the causative alleles for numerous single gene disorders with autosomal recessive inheritance. In the present study we report on results of these high throughput techniques in two patients affected with presumed single gene disorder.

Materials and Methods: Patient A presented with Spastic Paraplegia whereas patient B had features of Spinal muscular atrophy (SMA). Both patients were born out of consanguineous union and had similarly affected siblings. Array comparative genomic hybridization (CGH) was performed using Affymetrix CytoScan 2.7M Array. Exome capture was performed on genomic DNA sample from patient A using TargetSeq Exome Capture kit and sequencing was done on SOLiD 5500xL platform.

Results: In array CGH, patient A did not reveal any significant genomic deletion/duplication. However there were many loss of heterozygosity (LOH) regions present. Homozygosity mapping analysis identified a single gene responsible for Spastic Paraplegia i.e. GJC2 in the overlapped homozygous region of 1q41-q42.2. Exome sequencing and variant annotation analysis identified 5 potentially significant indels in patient A. Among these, a novel single base deletion was identified in GJC2 gene which causes a frameshift at codon 23 creating a stop codon (TGA) at codon 38 and thus is likely to be pathogenic mutation in the patient. Array CGH analysis in patient B also identified several LOH regions. A single gene responsible for SMA i.e. PLEKHG5 was found in the overlapped LOH region of 1p36.31 by homozygosity mapping analysis. Sequencing analysis of patient B is currently on progress. **Conclusion:** These findings illustrate the utility of homozygosity mapping followed by exome sequencing to detect the cause of autosomal recessive single gene disorders. These highly automated technologies are proving to be effective for several this type of disorders and gradually replacing the laborious conventional methods.

O17

Chromosomal Aberrations and Importance of Genetic Counseling: A Study of 288 Couples with Bad Obstetric History

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Background: Bad obstetric history (BOH) is defined as three or more consecutive pregnancy losses. Though almost in 50% cases the etiology is unknown, some of the factors are infectious diseases, endocrine dysfunction, auto immune disorders, parental age, environmental toxins, uterine anomalies and genetic abnormalities. Of all pregnancies 15–20% end up as spontaneous abortions of which chromosomal aberrations

(CA) are seen in 50%. Parental CA is an important etiology of BOH. CA can lead to meiotic errors resulting in gametes with abnormal unbalanced chromosomes leading to pregnancy loss or genetically abnormal pregnancy outcomes. Different studies published have shown 3% to 8% of couples with BOH have CA.

Aims and Objectives: To investigate the contribution of chromosomal aberrations (CA) in couples with bad obstetric history (BOH) and the role of Genetic counseling.

Material and Methods: A retrospective cytogenetic study was done from Jan - Nov 2014 on blood samples of 288 couples with BOH. Peripheral blood cultures were set & analyzed by GTG – banding at 450-550 band level and reported as per ISCN. FISH was done wherever necessary. **Results:** Out of 288 couples, CA were detected in 33 cases (11.48 %). Among those 09 cases showed balanced reciprocal translocations (27.27 %), which also included a couple with both partners. Robertsonian translocation was found in 01 (3.03 %) cases involving chromosome 13 and 14. As such couples are at high risk for fetal CA, extensive genetic counseling for couple and family members is essential. 24 cases showed normal polymorphic variants (72.72 %), genetic counseling for mode of transmission and clinical correlation may be helpful as their association with BOH is reported. Inversion Y chromosome observed in 3 cases, although usually familial its probable associated consequences need counseling. Yqh+ in 02 (06.06%), 1qh+ in 02 (06.06%), inv(9) in 06 (18.18%), 9qh+ in 08 (24.24 %), and 15ps+, 21ps+, 22ps+ in 01 (03.03%) each. Three couples with CA in the form of same polymorphic variation.

Conclusions: Our study for CA in BOH are consistent with other studies. As most CA are familial, detection and genetic counseling should be recommended by all the medical practitioners to help the couple have an informed choice and help other family members in reproductive age group.

O18

Genomic Studies of High Altitude Pulmonary Edema, A critical Condition in Susceptible Sojourners

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Background: Military and strategic requirements of Indian Army entail deployment of troops in the high altitude regions of the Himalayan mountains where individuals encounter hardships of whole body hypoxia and life threatening conditions of high altitude pulmonary edema (HAPE) and high altitude cerebral edema (HACE). The precise pathoetiology and inciting mechanisms regulating HAPE remain unclear. **Aim and Objective:** Identify the genetic variations and molecular mechanisms in susceptible individuals who develop high altitude pulmonary edema (HAPE). **Material and Methods:** Genetic variations in HAPE susceptible were compared to those who did not succumb to the high altitude stress (acclimatized) by differential gene expression analysis, candidate gene polymorphism profiling and whole genome association studies. **Results:** Pathways regulating vasoconstriction through smooth muscle contraction, cellular actin cytoskeleton rearrangements

and endothelial permeability/dysfunction were found to be modulated in HAPE. Several regulators of systemic/pulmonary hypertension including ADRA1D, ECE1, and EDNRA were upregulated in HAPE. Genotype and allele frequencies of adrenergic beta receptors (ADRB) and angiotensin converting enzyme (ACE) were significantly different in the HAPE individuals compared to acclimatized sojourners. Two SNPs, rs 994960 (position 73682765) and rs 1355469 (position 73664989) on chromosome 3 had p value less than 1.5E-05 in HAPE. **Conclusion:** High Altitude Pulmonary Edema was characterized by concurrent modulation of multiple pathways that regulate vascular homeostasis and lung fluid dynamics suggestive of multi-genic nature of regulation of HAPE. Individuals with DD genotype and D allele of ACE were prone to developing high altitude pulmonary edema. The associated SNPs rs 994960 and rs 1355469 lie near PDZ domain containing ring finger 3 (PDZRN3). Genomic studies on physiology of hypoxia, which is at the basis of high-altitude medicine, should elucidate important pathways in lung and heart diseases.

O19

Mitochondrial DNA Diversity and its Implications in the Genetic History of Three Different Populations of Amini Islander's of Lakshadweep.

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Lakshadweep consists of group of coral islands shattered along south-western frontiers of India in the Arabian Sea. Amini Island is one of the first inhabitant islands of northern side of Lakshadweep. Entire indigenous population in Amini Island of Lakshadweep has been classified to three groups- Koya, Malmi and Melacheri based on their occupation and social status. These populations are highly endogamous in nature. The Migration of population, social structure and caste endogamy of Amini Island has been not traced yet genetically. In the present study, we focused on three populations (Koya, Malmi and Melacheri) from Amini Island for tracing their maternal ancestry using the approach of complete mtDNA sequencing. We have isolated the DNA from blood samples of unrelated males from above three populations and subjected to PCR amplification and whole mt-DNA sequencing. We report that Koyas, the land owner group which stands upper hand in caste stratification has high frequency of South Asians affinity (60%) with less haplogroup diversity(M33a), while in contrast Melacheris, the core working group which stands lower hand in caste stratification has high frequency of West Eurasian affinity (79.48%) with more haplogroup diversity(R30b2a). Malmis, the sailors stand middle in the stratification, has almost equal contribution of West Eurasians and South Asians ancestry (40% and 53% respectively). Genetic data from mtDNA infers that Melacheris, the first settlers with West Eurasian ancestry admixed with koyas, while Malmis served as intermediate people.

O20

Genetic Association study and Gene Expression Analysis revealed Osteoprotegerin as Candidate Gene for Otosclerosis

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Background: Otosclerosis (OTSC) is a late-onset hearing disorder characterized by increased bone turnover in the otic capsule. Disturbed Osteoprotegerin (OPG) expression has been found in the otosclerotic foci which may have an important role in the pathogenesis of otosclerosis. **Aims and Objectives:** The present study was aimed to identify the genetic risk factors in the OPG gene and to investigate its possible role in otosclerosis development. **Materials and Methods:** In this study, we recruited a total of 254 otosclerosis patients and 262 controls for sequencing the coding region of OPG gene. To assess the role of OPG gene in otosclerosis, we studied the gene expression of OPG and related genes (RANK and RANKL) in otosclerotic tissues (N=25) compared to normal stapes tissues (N=5) and incus bones (N=2) applied as negative controls for otosclerosis. **Results:** Sequence analysis of OPG gene revealed five known single nucleotide polymorphisms (SNPs) c.9C>G, c.30+15C>T, c.400+4C>T, c.768A>G and c.817+8A>C in both cases and controls. Significant associations were found for two SNPs c.9C>G and c.30+15C>T with OTSC. Testing of these SNPs for sex specific associations revealed significant association of c.9C>G in males while c.30+15C>T in females with OTSC. The underrepresentation of minor alleles for these SNPs in cases compared to controls indicates their protective effect towards otosclerosis development. Gene expression analysis detected significantly missing OPG expression in otosclerotic tissue ($\chi^2=4.693$, $P=0.0151$, one-tailed χ^2 -test). However, majority of the patient stapes were featured by considerable RANK (84%) and RANKL (80%) mRNA expression. The transcript level of OPG was significantly lower ($P=0.0002$) in cases compared to controls. **Conclusion:** The results obtained from this study suggest that genetic variations in OPG and its altered expression in disease tissues may be the plausible cause of otosclerosis susceptibility.

O21

Clinical, Hematological, Molecular Characterization and Response to Hydroxyurea Treatment of Symptomatic 17 HbSE cases in Eastern India: The Largest Series in world

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Background: Sickle cell Hemoglobinopathy is a major public health problem in our region. Literature shows HbSE cases are less symptomatic. A compound heterozygote state of 17 cases

of HbSE, the largest number hitherto reported in our centre, most of them were severely symptomatic and they responded well to Hydroxyurea treatment. **Aims and Objectives:** Clinical, hematological and molecular study of HbSE cases & their response to low and fixed dose of Hydroxyurea (10 mg/kg/day). **Material and Methods:** Detailed clinical data, Sickling test, alkaline electrophoresis, CBC, Biochemical examination, CE-HPLC, ARMS-PCR, haplotype analysis and α -thalassemia were done at Sick Cell Clinic & Molecular Biology Laboratory, VSSMCH, Odisha. DNA sequencing was done. Hydroxyurea was administered at a low and fixed dose (10 mg/kg/day) based on frequency of painful crisis (VOC) and hospitalization and their response was studied. **Results:** Thirteen out of 17 cases were symptomatic with repeated VOC and hospitalization. Presented at an early age (10 yr) they had moderate anemia. MCV, MCH and MCHC were low. 87.5 % showed Southeast Asian haplotype for Hb E (----+). One was asymptomatic having Atypical haplotype (+---+) with α -4.2 α / α . Other one having Atypical haplotype (+--+) with α -4.2 α / α was on HU from two year of age. 50% (6/12) cases had α -thalassemia (three each with α -3.7 & α -4.2 allele) in heterozygous state. XmnI polymorphism (presence of 'T' allele) was found in 90.9 %. **Conclusion:** This is the first report of 17 cases of HbSE, the largest cohort studied ever. Majority are symptomatic with 100% response to HU treatment. Presented at early age. Other associated genetic factors may be responsible for their severe symptoms which needs further research. Finding of different compound heterozygote SCD with symptoms may need consideration for Prenatal Diagnosis & pre-marital screening in our area.

O22

Mitochondrial Somatic Mutations in Human Gastric Cancer

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Background: Cancer strikes people of all age groups. However, some cancers are often more frequent in individual aged >50 years. Gastric Cancer is one of the important age associated cancer which remains a third leading cause of cancer death in both sexes worldwide (8.8% of the total cancer cases). **Aims and Objectives:** DNA damage has proven to be the most independent cause for both aging and cancer. Apart from the human genomic alterations, mitochondrial dysfunction also plays a significant role in aging and cancer independently. Hence, it is very essential to identify the mitochondrial dysfunction triggered by mitochondrial genetic mutations. Mitochondrion is an important extra cellular organelle which involves in large spectrum of cytological events such as metabolism, cell cycle, growth, differentiation, signaling and apoptosis. The human mitochondrial genome is 16,569 bp in length which harbors few spontaneous mutations and many

induced in various pathological conditions. Many inherited diseases are developed by pathogenic mtDNA mutations. Also there are adequate evidences associating mtDNA mutations with stomach, prostate, ovarian, breast, colon, liver and kidney cancer. **Material and Methods:** In our present study, we profiled mtDNA mutation status of 47 tumor tissues and paired normal samples from gastric cancer subjects. Average age of the gastric cancer patients was 55.27 $\pm$ 4. All samples were subjected to complete mtDNA sequencing and compared with the revised Cambridge Reference Sequence (rCRS). **Results:** Interestingly, we identified a total of 16 novel mutations among all samples upon the comparison of both the tumor and normal tissue samples. These mutations were located in coding regions of various genes including 16S rRNA, tRNA, COI, ATP6, ND2, ND5, ND6 and also in the Control region HVS-I. The newly identified mutations were compared with other samples from different diseases to make it clear that these are novel and unique. All the mutations were compiled together and simultaneously Haplogroup association was performed. Samples were assigned with different Haplogroups among which "M" Haplogroup was highest & remaining were "R" and "U" Haplogroups respectively. **Conclusion:** Our Preliminary data suggests that mtDNA of gastric cancer patients yield some novel mutations which functional consequence should be thoroughly dissected out on comparing with clinical manifestation of the tumor. Collectively, we conclude that our study subjects exhibited a mitochondrial heteroplasmy which is a very common phenomenon in gastric tumorigenicity.

O23

Clinical Exome Sequencing to Aid Diagnosis – Case Studies and Lessons Learned

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Background: Genomic approaches to aid in diagnosis of genetic disorders are gaining popularity in the current times. Focused exome sequencing kits have a high diagnostic yield as they represent a subset of the human exome containing only clinically relevant genes. We will present case studies (E.g.: Waardenburg-Shah, Metabolic disorders, DMD) utilizing NGS approaches in a diagnostic setting, and discuss findings, challenges and the scope and utility of these tests. **Aims and Objectives:** To determine causative genomic aberrations in patient samples. Typical scenarios include when conventional methods gave opposing results, when a genetic disorder was suspected but exact causative gene was not determined, or, when the case as presented was difficult to diagnose. **Material and Methods:** Illumina TruSight One Exome capture and sequencing kits, Illumina MiSeq and Next Seq500 Sequencer. DNA extracted from patient samples. **Results:** Exome sequencing aided in the identification of causative DNA variants in a variety of cases. The results help validate a diagnosis and, in some cases aid in correct diagnosis in the face of conflicting results from other tests. **Conclusion:** We present a few different case studies on the use of clinical exome sequencing for

diagnosis, and describe the challenges and efficient workflows to adopt genomics technologies in diagnostics. We conclude that exome sequencing based tests have significant utility in molecular diagnosis of genetic disorders, Their use and interpretation, given that they are rapidly evolving, needs to be examined carefully by qualified personnel.

O24

The Etiopathogenesis of Human Genetic Diseases – Translating Basic Research to the Clinic

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One of the practical approaches in the expansion of the knowledge of etiology and pathogenesis of the human diseases at this juncture is the Translation of basic research to the Clinics. Human health improvement requires the basic research studies to be application oriented for the welfare of the society. Various sophisticated techniques are being developed for early diagnosis and to identify the predisposed individuals or high risk groups for an early treatment or management. Using biochemical, molecular and cellular tools for developing disease markers and/ or biomarkers such as disease related polymorphisms, micronuclei assay, serum markers, DNA damage and integration with bioinformatics approach will be discussed. Various precancerous and fragile chromosome syndromes were analyzed with respect to their nature of predisposition to cancer using clinical, cytogenetic and molecular studies to understand the severity of DNA damage induced genomic instability according to the nature of the disease. The diabetes samples were clinically classified into their respective types and analyzed. It was observed that the cytomolecular studies could be used as biomarker for evaluating the human diseases for an early diagnosis along with clinical and biochemical parameters. Thus advancing the diagnosis for management of human genetic diseases and utilizing genetic counseling tools will be appropriate for translation to the clinics.

O25

Association of Monocyte chemoattractant protein-1 (MCP-1) gene polymorphisms [-2518 A>G (rs1024611) and I/D (rs3917887)] with T2D and ESRD patients from population of Punjab.

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Background: Type 2 diabetes (T2D) is a chronic and complex disorder leading to end stage renal disease (ESRD) which increases mortality and morbidity within the diabetic patients. Monocyte chemoattractant protein (MCP-1) gene is an important gene implicated in the pathophysiology of T2D and ESRD. **Aims and Objectives:** The present study examines the role of -2518 A>G (rs1024611) and Insertion/deletion (rs3917887) polymorphisms in MCP-1 gene in T2D and ESRD in the population

of Punjab. **Material and Methods:** A total of 776 patients (257 with T2D, 204 T2D with ESRD) and 315 healthy controls were genotyped using ARMS-PCR and Insertion/deletion PCR for -2518 A>G and Insertion/Deletion polymorphism of MCP-1 gene followed by agarose gel electrophoresis. **Results:** For rs1024611, AG and GG genotype tends to provide 1.5-2.6 fold risk towards development of ESRD when T2D with ESRD were compared with only T2D cases, however no association was found with T2D. For rs3917887, comparison of T2D with ESRD and only T2D cases with controls under dominant model analysis revealed 1.48 and 1.80 fold risk towards T2D and ESRD development, respectively. While codominant model observed that ID genotype provided nearly 2 fold risk towards ESRD development. **Conclusion:** The -2518 A>G and I/D polymorphism of MCP-1 gene seems to be associated with risk of development of ESRD among T2D cases in the population of Punjab.

O26

Studies on Obese Breast Cancer Patients with Leptin Gene Polymorphisms.

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Background: Obesity is linked to elevated circulating levels of adipocyte-derived hormone leptin a recently established risk factor for breast cancer incidence and mortality. However, the mechanisms involved are poorly understood, except that leptin is a 16 kDa polypeptide hormone which is secreted by adipose tissue that acts at the brain to regulate energy expenditure and food intake and has an important role in energy balance, insulin pathway and inflammation. In the present study FAC (5-Fluorouracil, Adriamycin and Cyclophosphamide) Combination chemotherapy was most frequently used than the other combinations. **Aims and objectives:** In view of the importance of this gene our investigation has been to determine the impact of Lep-2548G/A polymorphism in breast cancer therapy of patients and compare to polymorphism in control group. **Patients and Methods:** This study was approved by the Institutional Ethics Committee. Biopsies and blood samples were collected after informed consent. The breast cancer patients were receiving various combinations of chemotherapy for their treatment. We determined the polymorphism of leptin gene for variants by a screening this gene in obese subjects (n = 100 cases and n=100 controls). The Gln2548Arg polymorphism was determined by using PCR-RFLP method and the association between the leptin gene and breast cancer risk were evaluated. **Results:** We recorded demographic parameters of patients and control subjects, to correlate this data to determine the risk factors of the disease. We also determined the significance of all the parameters for statistical evaluations. The distributions of all three genotypes GG (18.8%), GA(44.8%) and AA (36.4%) in breast cancer cases were compared to that of the controls as 33.0%(GG), 29.6% (GA), and 37.4% (AA). We found that postmenopausal breast cancer cases showed

statistically significant association with GA genotype when compared with premenopausal women without the disease ($p=0.001$). This difference was between the cases and controls in the Gln2548Arg genotypes. **Conclusion:** This study has demonstrated a modestly increased risk of postmenopausal breast cancer in women harboring Gln2548Arg polymorphism of the leptin gene. Our findings suggest that the LEP Gln2548Arg polymorphism may be a useful biomarker associated with the risk of breast cancer women in obese Indian population.

O27

Development of a Multiplex PCR Based Method for Diagnosis of Duchenne Muscular Dystrophy (DMD) in the Patients Attending PGIMS Hospital

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Background: Duchenne muscular dystrophy (DMD) is an inherited X-linked recessive disorder. It has estimated worldwide incidence of 1:3500 in children. The disease is caused by mutations in the dystrophin gene, which results in muscle degeneration and eventually death. At PGIMS Rohtak, creatine kinase, serum calcium, electromyogram, and muscle biopsy have been used routinely for clinical diagnosis of DMD. **Aims and Objectives:** As of now, molecular diagnosis has not been in clinical practice at PGIMS Rohtak for specific diagnosis of DMD. Since, there is no treatment available for DMD, carrier detection and prenatal diagnosis remains important considerations for families with history of DMD. In the present study, by using appropriate set of primers, a multiplex PCR based diagnostic test has been established. **Material and Methods:** We have screened 7 suspected DMD patients who were referred to our laboratory by outpatient department of our hospital. Genomic DNA was extracted from human peripheral blood and 21 exons deletions in dystrophin gene were tested by multiplex PCR method. **Results:** Out of seven, 6 patients demonstrated at least one exon deletion in the dystrophin gene. However, in one suspected patient no deletion mutation was observed. **Conclusion:** The method carries significance because of its specificity, sensitivity and rapidity, as several exons can be detected in a single run.

O28

Surface Layer Protein-A (SlpA): A protein Domain of the *Lactobacillus* sp. with Anti-Cancer Activity on GIT Cancer Cell Lines

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The surface layer proteins are found in the adherence bacterial strains viz. *Lactobacillus* sp. and *Clostridium* and they are secreted for purpose of the evoking immune responses during the active pathogenic infection in the host organism. It had a wide variety of applications in probiotics, cytotoxic and apoptotic activities against yeast system. Indeed we had found with SlpA a domain protein binding

efficacy on the GPCR, RTK, TNFR, JNKR, and p38 receptors with protein-protein interaction on Clus pro 2.0 and showed distinctive near-native structures nested within a multitude of false positive structures on surface complementarities to provide atomic interactions, and electric charges. We have depicted the pathways leading to the apoptosis by the SlpA. The binding efficacy of the SlpA with the GPCR had made to conformation excitation of the G-linked proteins by the GTP and related kinases. GPCR leads to the phosphorylation of the G-Linked and also the β - γ couple proteins. However, these leads to the cascades of the phosphorylation and electron transfer to on PKC proteins. In contrast, to JNK and p38 signaling pathways could negotiate inflammatory responses by interaction over their expression and binding energies. **Conclusion:** The significant activities of SlpA with specific sites of pathogenic cellular responses like cell proliferation, stress induced responses on the apoptosis modulation on the gastrointestinal track (GIT) cancer cell lines.

O29

Role of ADAM33-S2 (G/C) gene polymorphism in the etiology of copd in south indian population

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Background: COPD is characterized by air flow limitation that is not fully reversible and is associated with an influx of neutrophils, macrophages and CD8 T lymphocytes in the airways. ADAM33 is a member of the ADAM family involved in cell adhesion, cell fusion, cell signalling, tissue remodelling and proteolysis. Recent researches are elucidating the role of ADAM33 SNPs in lung function loss and COPD. ADAM33-S2 polymorphism effect gene regulation influence susceptibility to the disease and leads to progressive destruction of alveolar tissue and enhance accelerated decline in lung function. **Aim & Objectives:** The present study is aimed to evaluate the role of ADAM33-S2 gene polymorphism in the etiology of COPD in South Indian population. The objective of the study is to identify the association of S2 polymorphism in the progression of disease. **Materials and Methods:** The present study included 120 patients diagnosed with COPD from Govt. Chest Hospital, Hyderabad and an equal number of age and sex matched healthy controls. Genomic DNA was extracted from all the subjects by phenol-chloroform method. Genotyping was carried out by PCR -RFLP followed by agarose gel electrophoresis. Results were analysed for statistical significance using chi-square and risk was predicted by odds ratio. **Results:** Genotype frequencies GG, GC and CC were found to be 20.8%, 47.5% and 31.6% in controls and 19.1%, 31.6% and 49.1% in COPD cases respectively. The statistical analysis of genotypic and allele frequencies revealed a statistically significant association of G allele in COPD patients in comparison with controls [GG Vs GC: X^2 7.514, $p=0.006$, OR= 2.329, 95% CI=1.306-4.153] and [(GG Vs CC: X^2 = 1.684, $p=0.194$, OR= 1.688, 95% CI= 0.842-3.391]. Allelic frequencies are found to be C=44.58%, G=55.41% in controls and C=35%,

G=65% in patients respectively [(G Vs C: χ^2 4.209, $p=0.040$, OR= 1.494, 95% CI= 1.035-2.158]. **Conclusion:** Our study is first to reveal the association of ADAM33-S2 'G' allele increased risk by 2-fold in the etiology of COPD. However, an increase in sample size and haplotype analysis with other SNPs in proximity further strengthens the study.

O30

Association of PGC-1 α Gene with Type 2 Diabetes in Three Unrelated Ethnic Groups of North-West India

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Background: PGC-1 α is a multifunctional transcriptional protein which plays a central role in the control of genetic pathways that result in glucose homeostasis in liver and muscle, fatty acid β -oxidation, mitochondrial biogenesis, insulin secretion and adaptive thermogenesis. **Aim:** To investigate the association of PGC-1 α gene with Type 2 Diabetes (T2D) among endogamous groups (Bania, Brahmin and Jat Sikh) of North-West India and to understand the interaction between these SNPs. **Methods:** The present study investigates the association of six polymorphisms: Thr612Met (rs3736265), Thr528Thr (rs3755863), Gly482Ser (rs8192678), Asp475Asp (rs17574213), Thr394Thr (rs2970847) and IVS2+C>A (rs2946385) in PGC-1 α gene with T2D. Total of 1125 samples including 554 diabetic patients and 571 controls (202 cases and 207 controls from Bania, 151 cases and 158 controls from Brahmin, 201 cases and 206 controls from Jat Sikh groups) were enrolled in this study. **Results:** Higher risk of AA genotype for rs3736265 and rs3755863 was observed for T2D susceptibility in Jat Sikh group. AA genotype of rs8192678 tends to provide 2-fold risk towards T2D predisposition in Jat Sikh and on contrary, individuals with GG genotype give 2.3-fold risk in Bania group. CT genotype of rs2970847 in Brahmin; TT genotype in Jat Sikh group confers risk for T2D. For rs2946385, CA+AA and CA genotypes in Brahmins provide 2 fold risk towards T2D aetiology. No association of rs17574213 was seen in any group. Haplotypes GGGGCA, GGGGTC in Brahmin; GAAGTC, GGGGTC in Jat Sikh group provide ~1.5-5 fold increased T2D risk. Global p-values of haplotypes in Bania, Brahmin and Jat Sikh are 0.01, 2.08×10^{-8} and 1.34×10^{-7} respectively implicate significant role of observed haplotypes in T2D etiology. MDR analysis revealed significant interaction (high synergy) between 3-locus model involving rs3755863, rs8192678 and rs2970847 polymorphisms in the progression of T2D. **Conclusion:** Differential pattern of association of various SNPs and haplotypes of PGC-1 α suggests the role of ethnicity in susceptibility to T2D.

O31

Association of Cytokine Gene Polymorphism with Periodontal Disease

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Periodontal disease comprises of a heterogeneous group of conditions that affect millions of people worldwide.

Cytokines play a key role in all stages of the immune response to periodontal disease. A cross sectional study design was employed in which the frequency of specific alleles and genotypes at specific locus of IL-1RN and IL-6 genes were compared between individuals with the disease and a comparison group of healthy people with clinical attachment loss of less than 3 mm. Restriction fragment length polymorphism was carried out. The pattern of distribution of alleles and genotypes showed wide variations when compared to data from other ethnic population in literature. As the number of genetic studies increases, allowing for assembly of polymorphism maps for a given population the chance of developing strategies towards prevention or determination of prognosis of the condition will increase proportionally.

O32

TP53 Polymorphisms and Chromosomal Instability in Breast Cancer Patients: A Follow up Study

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Background: Genomic Instability is a hallmark feature of solid tumours including breast cancer. Alteration of p53 is an important step in genomic instability and susceptibility to neoplastic state transformation. Polymorphisms in TP53 and chromosomal instability can be a biomarker for aggressiveness of cancer and response to therapy. **Aims and objectives:** To explore possible interaction between TP53, p.R72P (rs1042522), PIN3 Ins16bp (rs17878362) polymorphism and chromosomal instability as biomarker for prognosis of breast cancer. **Materials and Methods:** DNA samples of 200 patients and 200 controls were screened for TP53 polymorphisms. Cytogenetic analysis was done for 56 patients (pre and post-op) and 56 unrelated healthy controls. **Results:** Frequency of RR, RP and PP genotype of p.R72P was 23.5% vs 33.5%, 51.5% vs 45.5% and 25% vs 21% in patients and controls respectively. RP genotype showed 1.61 fold increased risk for breast cancer. Frequencies of A1A1, A1A2, A2A2 genotypes of PIN3 Ins16bp polymorphism were 67.5 vs 68.5%, 26% vs 27.5%, 7% vs 4% in patients and controls respectively. PR-A1A1 genotype combination showed significant risk of breast cancer ($p=0.05$). Significantly higher frequency of aberrant metaphases in the post-op patients as compared to the pre-op patients ($p<0.0001$) and controls ($p=0.0001$) was noted. Chromosomal instability was increased in patients with PR-A1A2 genotype as compared to the controls. The patients with RR-A1A1 genotype had increased chromosomal instability after administration of chemo-radiotherapy. **Conclusion:** RR-A1A1 genotype combination of p.R72P and PIN3 Ins16bp polymorphism of TP53 is associated with increased chromosomal damage in post-op patients.

O33

A Common Functional Genetic Variant of Pancreastatin Profoundly Increases the Risk for Cardiometabolic Diseases in Indian Populations

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Background: Pancreastatin (PST) is a potent physiological dysglycemic peptide derived from the pro-hormone chromogranin A (CHGA). **Aims and Objectives:** Re-sequencing of the PST region in the exon-7 region of CHGA. **Material and Methods:** 3676 Indian subjects (n=7352 chromosomes) were studied. **Results:** Occurrence of three naturally occurring variants: C9226T causing substitution of Arg253 by Trp253, G9328A causing substitution of Glu287 by Lys287 and G9358A causing substitution of Gly297 by Ser297 was noted. While the Glu287Lys (PST-287K) is a novel variant in our population, the Gly297Ser (PST-297S) and Arg253Trp (PST-253W) variants have previously been reported in other populations. Among these PST variants, PST-297S is the most common one occurring in ~15% of our study population. Gly/Ser subjects displayed significantly higher levels of plasma glucose (fasting/post-prandial/random), insulin resistance (HOMA-IR), HbA1c, total cholesterol, triglycerides, LDL-cholesterol, diastolic blood pressure and catecholamines (epinephrine and norepinephrine) as compared to wild-type Gly/Gly counterparts. Consistent with such effects on biochemical/physiological phenotypes, the PST 297Ser allele was detected at markedly higher frequencies (by 1.3- to 1.4-fold) in subjects with type-2-diabetes/hypertension/coronary artery disease than healthy controls. **Conclusion:** Thus, PST-297S variant of physiological dysglycemic peptide increases the risk for type-2-diabetes, hypertension and coronary artery diseases via enhanced interaction with PST receptors.

O34

Genotypes of CYP1A1, SULT1A1 and SULT1A2 and risk of squamous cell carcinoma of the esophagus in Kashmir, India; outcome of a case-control study

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Background: Polymorphisms of various genes encoding xenobiotic metabolising enzymes have been associated with different cancers, but the overall results are mixed and inconclusive. Esophageal Squamous Cell Carcinoma (ESCC) is a leading malignancy in Kashmir India, and the studies on genetic predisposition towards ESCC are limited. **Aims and objectives:** We conducted a case control study to evaluate the association of CYP1A1, SULT1A1 and SULT1A2 genotypes with ESCC in Kashmir India, and their role in modulating the risk associated with different risk factors. **Methods:** We recruited 404 pairs of histopathologically confirmed ESCC cases and controls individually matched for sex, age and district of residence. Information on various dietary, lifestyle and environmental factors was obtained in face to face interviews using a structured questionnaire. Genotypes were analysed by polymerase chain reaction and restriction fragment length polymorphism and sequencing in a randomly selected samples. Conditional logistic regression models were used to calculate odds ratios (ORs) and 95% confidence intervals (95% CIs). **Results:** A higher risk was observed in the subjects who harboured variant genotype of CYP1A1*4 (OR = 2.08; 95% CI: 1.27 – 3.41); and the risk was further enhanced in the subjects who were smokers (OR = 3.47; 95% CI: 1.62 – 7.42) and were adobe dwellers (OR = 6.71; 95% CI: 3.02 – 14.89). There was a non-significant difference in the polymorphic variants of SULT1A1*2 and SULT1A2*2 between cases and controls and both variants do not modulate risk in smokers or adobe dwellers. **Conclusion:** Unlike SULT1A1*2 and SULT1A2*2, the polymorphism of CYP1A1*4 is associated with ESCC risk. The findings need to be substantiated from other high risk populations with large sample size.

O35

Role of Shh-Gli1 Signaling Novel Transcription Factor BMI1 in Medulloblastoma Development

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Medulloblastoma is the most common malignant pediatric brain tumor. Brain tumor stem cells (BTSCs) are responsible for the poor survival outcome of the treated medulloblastoma patients. Medulloblastoma has been divided into four categories on the basis of expression pattern of genes and the major cell signaling pathways in the development. Medulloblastoma Group A (Wnt signaling mediated), Group B (Sonic hedgehog signaling, Shh) while non-Wnt/Shh include Group C and Group D. Interestingly, Sonic hedgehog (Shh) signaling pathway is one of the crucial signaling pathways to regulate stem cell self-renewability. Glis activates many downstream target gene/transcription factors including BMI1 to promote brain tumors (medulloblastoma and

glioblastoma). BMI1, a polycomb complex protein is also known as polycomb group RING finger protein 4 (PCGF4) or RING finger protein 51 (RNF 51). Interestingly, BMI1 showed high expression in both brain tumors, medulloblastoma and glioblastoma. Shh treatment induced BMI1 expression besides its downstream gene Gli1 and high Gli1 expression also promotes BMI1 expression. High expression of BMI1 in tumor cells indicates high capacity of self-renewing characteristics. Most of the BTSCs showed high expression of BMI1. Inhibition of Glis with specific inhibitor GANT61 and Glis siRNAs mediated knockdown inhibits the brain tumor cell proliferation and also decreased the expression of BMI1 in medulloblastoma and glioblastoma. Interestingly, recent studies indicated that medulloblastoma of group C and group D showed high expressions of BMI1 and forkhead box protein G1 (FoxG1), respectively. Interestingly, it is reported that BMI1 is also downstream target of FoxG1. Personalized medicine to treat different groups of medulloblastoma is not feasible in India, rather we need to focus on very specific and crucial transcription factor, which is in the downstream target of the most of the signaling pathways responsible for medulloblastoma development.

O36

Genetics of Fetal Hemoglobin: Relevance for Prognostication

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Background: Though SCD is a monogenic disorder, at the phenotypic level it is a multigenic disease, with different clinical outcomes. These variable outcomes could be attributed to genetic modifiers. Several studies have revealed fetal hemoglobin (HbF) as a major genetic modulator in SCD. Recently, Genome wide association studies have indicated three major quantitative trait loci; Xmn-HBG2, HBS1L-MYB intergenic region and BCL11A locus to account for 20-50% of the variation in HbF levels in SCD patients. **Aims and Objectives:** There is minimal information about genetic factors influencing the disease course in Indians. The current study is the one of the first report investigating genetic variants at these loci affecting HbF levels. **Materials and Methods:** The current study was conducted on 240 SCD and 60 sickle cell trait individuals. Genotyping was performed for the BCL11A rs11886868, rs1427407; HMIP rs9399137, rs6934903; HBG2 Xmn1 polymorphism rs7482144; -68 C>T HBD promoter polymorphism. Frequency and association of these variants with HbF levels was analyzed. **Results:** All the 3 loci were associated with HbF levels in Indian SCD patients. The BCL11A rs1427407 was significantly associated with HbF levels contributing to ~23% of the trait variance, the BCL11A rs11886868 for 3.65%, HMIP rs9399137 for 3.8% and XMN1 accounted for 11% of the trait variance. Interestingly no association of the HBS1L-MYB rs6934903 with the HbF levels was seen. This study also determines the presence of the promoter polymorphism -68C>T in the HBD gene which was exclusively seen in individuals with Arab-Indian haplotype and

has hitherto been unreported in Indians. **Conclusions:** The present study indicates the BCL11A, HMIP and β -Globin region to be associated with elevated HbF levels. Further interrogation of these variants with respect to pain crisis is warranted, as they are also known to associate with pain crisis and hospitalization rates. This may aid in prognostication.

Poster Presentations

P1

Dermatoglyphic Pattern Study in Morton's Toe/Royal Toe.

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Background: In 1980, Francis Galton suggested fingerprints as a useful tool in personal identification. The term Dermatoglyphics was coined by Harold Cummins in 1926 which refers to the study of epidermal ridges. **Aims and Objectives:** Evaluation of sexual dimorphism in dermatoglyphic traits. **Objective:** Study of fingertip pattern of individuals having Morton's toe. **Material and Methods:** 77 students (49 females and 28 males) aged 20-30 years from Banaras Hindu University those specially having Morton's toe were enrolled for the present study. Both fingerprints and footprints were taken on white paper using inkpad. However, presence of insufficient literature and an accepted classification of foot prints only fingerprint data was tabulated analyzed statistically and discussed. **Results:** While all the participants in the study group have Morton's toe different fingertip patterns were observed in males and females. Whorl was found in 42.04% female and 40.00% of male, radial in 28.57% female and 24.642% males, ulnar in 24.285% female and 24.571% male, and arch in 5.102% female and 6.785% male. **Conclusion:** It appears that whorl impression is more prominent in both the sexes. Followed by radial (28.57%) in female and ulnar in male (28.57%). Lowest frequency was found for arch pattern in both sexes i.e. 6.78% in male and 5.10% in female respectively. Study is underway for identifying unique foot print pattern in future.

P2

In Silico Study for Interaction of Ligand Molecules with Ebola Virus VP40 Matrix Protein

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Background: Ebola virus is a negative strand RNA filamentous virus of filoviridae family, which causes severe hemorrhagic fever in human and non human primates. This virus is naturally resistant to various drug and vaccines. The virus matrix protein VP40 is a structural protein that plays a central role in virus assembly and budding at the plasma membrane of infected cells. **Aim:** Identification of potential drug targets for Ebola virus matrix protein VP40. **Objective:** Qualitative assessment for various potential drug targets for Ebola virus. **Materials and Methods:** Sequence of VP40 protein of Ebola was taken from

the NCBI database. The structure of VP40 Ebola virus protein was taken from abstract of All India Cell Biology Conference 2014 held at CDRI, Lucknow with authors consent. Active site prediction for interaction with ligand was performed using Zinc and Pubchem databases and eventually drug designing was performed by iGEMDOCK Finally the merit of the potential drug target/s was evaluated by the "Lipinski rule of five". **Results:** Active site was found in the targeted protein which interacts best with 5-bromo-N'-(3-chlorophenyl) nicotinohydrazide (ligand) molecule. Docking analysis of reveals that VP40 protein with the best hits of the ligand molecules could possibly inhibit the target protein. **Conclusion:** VP40 matrix protein of Ebola virus plays an essential role in virus assembly and budding at the plasma membrane of infected cells. The ligand molecules are interacting with this protein to inhibit the process of assembling and budding.

P3

Molecular Characterization of Atypical Deletion Contributing to Clinical Manifestation of Williams-Beuren Syndrome

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Background: Williams-Beuren syndrome (WBS) is a microdeletion syndrome caused by haploinsufficiency of 26-28 genes at 7q11.23, with multisystemic manifestations and mainly characterized by vascular stenoses, distinctive craniofacial features, and mental retardation. **Aims and Objectives:** Here, we report the phenotypic and molecular characterization of a boy (2years) with MR, dysmorphism, joint laxity and delays in fine motor skills. We report on the utility of cytogenetic and molecular cytogenetic techniques (MLPA and ArrayCGH) used to characterize the breakpoint in this patient. **Materials and Methods :** Chromosome analysis was done by GTG-banding techniques in the patient as well as both parents. MLPA (Multiplex ligation-dependent probe amplification) was performed using Salsa MLPA kit P064-B3 MR-1 to detect aberrant copy numbers of several chromosomal regions known to cause mental retardation syndromes Cytogenetic microarray was performed using the HumanCyto SNP-12 Beadchip (Illumina, San Diego, Calif) which includes 300,000 markers targeting all regions of known cytogenetic importance. **Results:** Chromosomal analysis revealed normal karyotype for patient and the parents. MLPA showed the deletion of only CYLN2 and RFFC2 genes among 7 (CYLN2, FZD9, STX1A, ELN, LIMK1 and RFC2 gene) included in the detection of Williams Beuren syndrome The breakpoint was resolved in detail through cytogenetic microarray which detected a de novo 588 kb deletion in the WBS critical region. **Conclusion:** Deleted region included CYLN2 encoding cytoplasmic linker protein and general transcription factors (GTF2I and GTF2IRD1) are expressed in the brain and may contribute to the distinct neurological and cognitive deficits in WS patients. This case highlights the utility of high resolution molecular cytogenetic techniques like array CGH and MLPA. Confirmation of clinical suspicion is essential for clinical monitoring of the patient and genetic counseling of the family.

P4

A Systematic Molecular Cytogenetic Characterization of A Supernumerary Marker Chromosome in A Girl With Seizures

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Background: Small supernumerary marker chromosomes (sSMCs) are chromosomal fragments or markers whose origin often cannot be identified by conventional cytogenetic methods alone and require molecular approaches for definitive characterization. sSMCs are almost the size of chromosome 20 or smaller. sSMCs are found in 0.043% of live births and approximately 77% of sSMCs are de novo and 23 % are inherited. **Aims and Objectives:** A 11-year old girl was referred to our genetic clinic for evaluation. The aim of the present study was to identify and characterize the chromosomal abnormality. **Materials and Methods:** Here we report a 11 year old girl with seizures, low IQ, white strips on whole body and hands and with less hair growth. The conventional cytogenetic techniques like GTG banding, and molecular cytogenetic techniques like Fluorescence in situ hybridization (FISH) using WCP probes and bacterial artificial chromosomes (BAC) clones were performed. Additionally, array CGH was also performed. **Results:** The GTG banding revealed a karyotype of 47,XX+marker. Array CGH identified the marker to host a 33Mb of chromosome 3q region and a 38Mb of chromosome 9p region. This was confirmed by WCP FISH. Further characterization was done by 4 BAC clones from 3q region and 4 BAC clones from 9p region. **Conclusion:** The application of the combined cytogenetic methods like array CGH and FISH helped in the systematic characterization of the sSMC. The accurate characterization of the SMC helps in the identification of chromosomal origin, gene content or any other imbalances in the genome.

P5

Genomic Approach to Etiology of Pediatric Autism and Intellectual Disability

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Background: Intellectual disability (ID) and Autism are life-long disabilities with a major impact on the lives of the children and their families. The prevalence of ID is 2-3% in population, and the underlying cause remains unknown in 65-80% of patients. Genomic imbalances play a major role in causation of these disorders. Using routine cytogenetics techniques, chromosomal imbalances larger than 5–10 Mb in size can be detected. Microarray-based comparative genomic hybridization allows for a high-resolution evaluation of DNA copy number alterations associated with chromosome abnormalities. **Aims and objectives:** To recruit and evaluate patients with Intellectual Disability and Autism with molecular cytogenetic techniques. To characterize the regions of microdeletion / microduplication identified. **Material and Methods:** After recruitment, patient's

blood sample was collected and cultured to obtain metaphase spread using standard procedure. These metaphases were analysed using cytovision software. Patients without identifiable chromosomal anomalies were tested for Fragile X syndrome. Array CGH testing was done in fragile X negative patients using Illumina and Affymetrix platforms. The results were validated using MLPA / FISH / qPCR. **Results:** Patients were divided into following groups. Patients with intellectual disability (n=55) - a total of 96 copy number variations were observed, out of them 15.62% were pathogenic, 57.29% were of uncertain significance and 27.08% were benign in nature. Patients with autism (n=21) - a total of 55 copy number variations were observed, out of them 7.27% patients showed pathogenic copy number variations, 61.81% were of uncertain significance and 30.9% were benign in nature. **Conclusion:** Use of Array CGH has led to increased yield of cytogenetic anomalies in patients of Intellectual disability / Autism. Higher resolution feature leads to better delineation of genomic imbalances and characterization of previously unidentified cytogenetic anomalies is also made possible by use of this technique.

P6

Deletion Boundaries and Variable Phenotypic Expression in WHS

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Background: Deletion encompassing 4p16.3 causes Wolf-Hirschhorn syndrome (WHS). This is a contiguous gene syndrome caused by the deletion of more than one gene and is associated with wide spectrum of phenotype in patients. **Aims and Objective:** To compare and correlate the size of deletion with phenotypic presentation. **Materials and Methods:** Three cases having multiple congenital anomalies/dysmorphic features were initially investigated using conventional cytogenetic [CC] GTG-banding technique. Minimum 25 metaphases were scored in each case to rule out mosaicism. Parents were investigated to confirm the mode of inheritance. Fluorescence in situ hybridization [FISH] was carried out using overlapping BAC clones in one case clinically suspected having WHS and in cases of dysmorphic features, developmental delay and failure to thrive; oligonucleotide array comparative genomic hybridization [aCGH] was carried out. **Results:** Microscopically visible terminal deletion on 4p was detected in case-1 46,XX,der(4)del(4)(p16pter) whereas remaining two cases were apparently normal at 550-band resolution. Clinically suspected case was further investigated using various BAC FISH probes covering whole #4p arm including RP11-262P20 encompassing WHSC 1 and WHSC 2 genes and control BAC probe RP11-195L6 positioned at 4q26. This has facilitated to narrow down and confirm the deleted region of 193Kb covering both the genes WHSC1 and WHSC2 i.e. 46,XY. ish 4p16.3(RP11-262P20x1, RP-195L6x2). The third case was having severe facial dysmorphic features along with significant failure to

thrive and developmental delay hence, aCGH was carried out and 5.5 Mb deletion was detected at 4p16.3p16.2 i.e. arr 4p16.3p16.2(71,552-5,506,588)x1(hg19). All three cases showed de novo alterations as parental karyotype study was normal. **Conclusion:** The study demonstrates that an individual with a strong clinical suspicion of chromosomal abnormality and a normal CC study should be further investigated using molecular cytogenetic techniques such as FISH or aCGH.

P7

The Role of Microsatellites – BAT26, D2S123 Flanking The Human Mismatch Repair Gene in the Genesis and Progression of Oral Squamous Cell Carcinoma

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Background: Oral squamous cell carcinoma is the sixth most common human cancer and accounts for at least 90% of all oral malignancies.(1) The increased occurrence of oral squamous cell carcinoma in the Indian subcontinent is primarily due to the habit of betel quid chewing. The prognosis of oral cancer is dependent on the early detection of cancer. The choice of the treatment modalities and whether a radical or conservative approach should be considered may also depend to a certain extent on the stage of tumour cell invasion and histopathological grade of cancer. **Aims and Objectives:** Considering the various contradicting propositions regarding the role of microsatellites in oral squamous cell carcinoma (OSCC) our study tried to determine the role of Big Adenine Tract (BAT 26) and D2S123 flanking the Human Mismatch Repair (hMSH2) gene, in the genesis and progression of oral squamous cell carcinoma. **Materials and Methods:** Tumor tissue and 3ml blood were collected from each patient diagnosed with oral squamous cell carcinoma (n=37). DNA was isolated and amplified by monoplex Polymerase Chain Reaction using appropriate markers. Microsatellite instability was assessed by comparing the peak patterns of matched tumor and blood in the electropherogram. **Results:** The frequency of microsatellite alteration, microsatellite instability and loss of heterozygosity at D2S123 were calculated as 43.2%, 21.6% and 27% respectively and was in various forms. There was a significant association between microsatellite alterations at D2S123 with histopathological differentiation (p < 0.05). There was no microsatellite alteration at BAT 26 in any of our samples. Though BAT 26 is reported to be quasimonomorphic, interestingly one patient in this study has shown polymorphism which is worth reporting as this allele was previously not reported in Indian population. **Conclusion:** This study did not find a role of BAT 26 in oral squamous cell carcinoma on the other hand microsatellite instability at D2S123 might play a role in progression of oral squamous cell carcinoma. By the virtue of this study we could identify a never reported allele of BAT 26 in Indian population. which strengthens the concept of routine evaluation of both normal and tumour specimens must be done to avoid mistakes in classifying an allelic variation as microsatellite alteration

P8

Role of Basic Fibroblast Growth Factor (b FGF) as an Angiomodulator in Oral Submucous Fibrosis

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Background: Oral sub mucous fibrosis (OSF) may be considered a collagen metabolic disorder that involves oral mucosa. **Aims and objectives:** To elucidate the role of basic fibroblast growth factor in angiogenesis in oral submucous fibrosis To find the polymorphism in bFGF gene in oral submucous fibrosis by PCR and enzymatic digestion of products **Materials and Methods:** This study is focused on polymorphism in FGF2 gene in the promoter region (-921 C/ D) in patients with oral submucous fibrosis (OSF), belonging to south Indian ethnic population. The sample size selected is 31 of which 22 female patients and 9 male patients were selected. DNA samples from 31 subjects of the same ethnic group and comparable demographic features who have had practiced the habit of areca-chewing of almost 5 year duration, and 52 remained free of disease constituted the controls. DNA from isolated blood samples and PCR was performed by specific primer to amplify specific target. All DNA samples were collected progressively and purified from peripheral blood employing standard protocols and tested for SNPs . The extracted DNA samples along with the primers underwent PCR amplification and the genotypic and allelic frequencies were calculated. **Results:** allelic frequencies for this C to G polymorphism in patient were 58.06% and 41.94% G (9 CC , 18CG, 4GG) and in controls were 60.57% and 39.43% G (17 CC, 29CG, 6CG). **Conclusion:** our study showed no significant variation in expression of SNP, -921(C/G) of the bFGF gene In case and controls.

P9

Microsatellite Instability in Oral Squamous Cell Carcinoma With Reference to Tumor Suppressor Genes

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Background: Microsatellite instability(MSI), a key factor in several cancers like colorectal, endometrial, ovarian, and gastric carcinoma have been studied extensively. But studies relating the same in oral squamous cell carcinoma(OSCC) in Kerala population are limited. This study is aimed at detecting the microsatellite alterations in OSCC in tumor suppressor genes p53 and BRCA1 present on chromosome 17 using two highly polymorphic dinucleotide microsatellite markers D17S796 and D17S579. **Aims and objectives:** To assess the association of MSI in OSCC with respect to tumor suppressor genes. To assess the association of MSI in relation to tumor suppressor genes with clinicopathologic findings in OSCC. Identification of prognostic microsatellite markers for early detection and management of OSCC. **Materials and methods:** DNA samples of histopathologically proven OSCC patients were extracted

using standard protocols. Primers were designed based on functionality and relevance of PCR. Capillary electrophoresis was done to analyse the loss of heterozygosity(LOH) and MSI. **Results:** The MSI frequency of D17S796 denoting the locus close to p53(17p13.1) was 76.7%. The frequency of LOH in relation to D17S796 was 23.3% and the total MSI frequency was 80%. The MSI frequency of D17S579 denoting the locus close to BRCA1(17q12-21) was 60%. The frequency of LOH was 16.7%. The microsatellite alteration frequency including both MI and LOH was 66.7%. Significant correlation with tumor location(buccal mucosa) and MSI was found(p value <0.005). No correlation between clinicopathologic parameters and MSI was observed. **Conclusion:** Microsatellite alterations in relation to D17S796 and D17S579, located in proximity to tumor suppressor genes, have a strong association in oral carcinogenesis.

P10

Role of PTEN Mutation in Oral Squamous Cell Carcinoma.

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Background: Several studies have been carried out on oral squamous cell carcinoma(OSCC) and its relationship with various genes. Though many studies have been conducted with regards to mutation profile of OSCC, the studies relating the role of PTEN mutation in OSCC in Indian has not yet been done till date. **Aims and Objectives:** This particular study was done to relate the role of mutation of PTEN gene in OSCC cases in south Indian population. Most of the PTEN mutations are concentrated in exon 5 of the gene, this study is also concentrated to this same site. **Material and Methods:** DNA samples of histopathologically proven OSCC patients were extracted using standard protocols. Primers were designed based on functionality and relevance for PCR. Sequencing was done to analyze mutation status. **Results:** With the study a novel mutation was found in the exon where the T allele was replaced by the C allele. We found four such cases in control population while there was only a single case with heterozygous CT genotype in the study population . This variant has not been reported till date. We have also screened three other variants rs 34904041, rs 35560700, rs34826144 which are monomorphic in our population. Study result showed that polymorphic variant of PTEN gene between cases and controls are not statistically significant. **Conclusion:** Initial observation from our study even though led to a novel mutation, functionality of this mutation and association with this disease has to be further investigated with larger sample size which could help us to find the real impact of this variant in the population as well as its association with OSCC.

P11

Molecular Basis of Afibrinogenemia: Application for Prenatal Diagnosis in The Affected Families

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Congenital afibrinogenemia is a rare autosomal recessive bleeding disorder with a prevalence of 1 in 1 million population, characterized by highly reduced or absence of fibrinogen in plasma. The disease is caused by defects in 3 genes FGA, FGB and FGG which encode for 3 polypeptide chains A α , B β and γ chains. We report the spectrum of mutations in 16 patients with fibrinogen deficiency detected by direct DNA sequencing. Mutations were detected in all the 16 cases; seven in FGA, six in FGB and 3 in FGG. Eleven were novel mutations which included 2 deletion mutations (FGA: c.22_30del; FGG: c.449_464del), 1 splice site (FGA: c.G180A), 5 missense (FGA: c.T2387G, c. T1294C; FGB: c.C1132T, c.A820T; FGG: c.A947C), 2 Indel (FGB: c.989_999delCCAGGATGGGACTT TTTCTATCAAAAGinsCCAGG ; FGG: c.73(-8)indel) and 1 insertion (FGB: c.819_820insT). Three recurrent mutations were also detected in two patients each (FGA: c.22_30del ; c.G180A; FGB: c.A820T). Two families were subsequently offered antenatal diagnosis in the first trimester of pregnancy. In the first case, the fetus was heterozygous for c.C464T mutation similar to the parents, whereas in the second family, the index case was homozygous for the deletion mutation c.22_30 and the fetus was normal. In conclusion, molecular basis in a large series of patients with afibrinogenemia was detected and successfully applied for genetic diagnosis in two affected families

P12

Coexistence of Glucose-6-phosphate Dehydrogenase Deficiency and Gilbert's Syndrome with Hereditary Spherocytosis

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Background: Hereditary Spherocytosis (HS) is a common inherited hemolytic anemia with heterogeneous clinical presentation. This is attributable predominantly to the underlying genetic defect. Some of the variability may also be due to other co-inherited factors like enzymopathies, thalassemia and Gilbert's syndrome (GS). An association of HS with glucose-6-phosphate dehydrogenase (G6PD) deficiency and GS in isolation has been reported previously. We describe for the first time three cases of HS with concomitant G6PD deficiency and GS. **Aims and Objective:** To describe the phenotype and genotypic findings in cases of HS with coinheritance of both G6PD deficiency and GS. **Materials and Methods:** HS was diagnosed by incubated osmotic fragility test (OFT) and EMA dye binding test. Methylene blue reduction test was used for screening G6PD deficiency and was confirmed by RFLP. Gene specific PCR and sequencing for the promoter region of UGT1A1 was performed to confirm GS. Gap PCR was used to detect alpha triplication and deletion. **Results:** **Case 1:** 40 year old male presented with fever and severe hemolysis. He was diagnosed with G6PD deficiency. Findings suggestive of ongoing hemolysis in the form of splenomegaly and gallstones

were noted during the follow-up period. Further testing revealed coexisting HS. Sanger sequencing of UGT enzyme showed homozygosity for [TA]7 repeats. **Case 2:** 25 year female with features of hemolytic anemia was initially diagnosed with HS. Patient was later found to be heterozygous for the G6PD Mediterranean and homozygous for [TA]7 repeats. **Case 3:** 15 year male patient presented with anemia and was diagnosed as HS. Patient was hemizygous for the G6PD Mediterranean and homozygous for [TA]7 repeats. Gap PCR for alpha gene deletion also revealed 4.2 kb deletion. **Conclusion:** Both G6PD deficiency and GS can coinhere with HS. In our three reported cases, the coinheritance did not lead to any significant alteration in the severity of anemia or increase in comorbidities. However, stressors which precipitate hemolysis in individuals of G6PD deficiency can exacerbate the anemia in these individuals. Therefore, more number of such cases needs to be studied and genotyped before concluding definitively regarding this association.

P13

Selection of reference genes for qRT-PCR based mRNA expression analysis of human reticulocytes from patients with hereditary spherocytosis

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Background: Reference genes (internal control or housekeeping genes) are essential elements used to normalise mRNA levels across different samples in real-time (qRT) PCR experiments. Selection of these genes is critical as expression levels vary among tissues and may depend on conditions like the amount of starting material as well as efficiencies of RNA extraction or reverse transcription. Reticulocyte mRNA is an attractive target for gene expression profiling of erythroid cells. There is a paucity of literature on suitable reference genes for these specialized cells, especially with regard to patients with membranopathies.

Aims and Objectives: To compare 8 potential reference genes for reticulocyte mRNA analysis by qRT-PCR in patients with hereditary spherocytosis (HS). **Materials and Methods:** Peripheral blood from 10 cases of moderately-severe HS and 10 normal individuals were studied using qRT-PCR. Eight candidate reference genes, namely, hemoglobin G2 (HBG2), membrane-palmitoylated protein 1 (MPP1), phosphoglycerate kinase 1 (PKG1), hypoxanthine phosphoribosyl transferase (HPRT1), β -actin (ACTB), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 18S, succinate dehydrogenase (SDHA) were studied. All except HBG2 have been used as internal controls in prior studies. Δ CT values were compared using Genorm, Normfinder, delta-CT and Bestkeeper software. **Results:** The most stable reference genes were HBG2, SDHA and MPP1 by BestKeeper, HPRT, HBG2 and SDHA by Genorm, and SDHA, HBG2 and HPRT by both delta-CT and Normfinder. When ranked comprehensively, HBG2 was identified as the most suitable reference gene (1.682) followed by SDHA (1.732). PKG1 (7.737) was the least stable

reference gene under our experimental conditions. **Conclusion:** We propose HBG2 as a novel stable reference gene for reticulocyte mRNA-based gene expression studies in HS. It is superior to both MPP1 and HPRT that have been used in earlier publications in this disorder. PKG1 was the least stable housekeeping gene in our experience and should be avoided.

P14

Pharmacogenetic Implications Of Adducin rs4961 Gene Amongst Hypertensive Punjabi Population

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Background: An inappropriate increase in peripheral vascular resistance relative to the cardiac output is a cause of essential hypertension. Numerous genetic markers have been identified in the regulation of blood pressure and essential hypertension. Alpha adducin (ADD1) is one such marker. ADD1 protein, found in the renal tubule, is involved in cellular signal transduction and interaction with other membrane skeleton proteins that affect ion transport across the cell membrane. Mutated ADD1 may affect the regulation of some factors in the Na transport system in the luminal part of the cell and hence, affects blood pressure. Diuretic agents are drugs that increase renal excretion of water and solutes (mainly sodium salt). The rs4961 (Gly460Trp) variant of the alpha-adducin gene influences the constitutive capacity of the kidney to reabsorb sodium, thus implying a modulation of the Blood pressure responsiveness to a diuretic, which inhibits such renal mechanism. Presence of Gly at the site results in more responsive therapeutic effect. **Aims and objective:** This study is carried out to survey Gly460Trp SNP prevalent among hypertensive patients from Punjab to predict their response to antihypertensive drugs such as diuretics. **Material and methods:** Blood samples from patients and normotensive individuals were collected with their informed consent under ethical clearance No ICEC/4/2011. Genomic DNA isolation was carried out by Salting out method. Genotyping of SNPs predicted by Insilco analysis of this gene has been carried out by PCR- RFLP analysis. **Results:** The study included 100 clinically diagnosed hypertensive patients with/without associated metabolic disorders and 100 normal healthy subjects. Results will be presented in presentation. **Conclusion:** The study was a way to “measure” the overall clinical impact of the ADD1 Trp allele distribution in Punjabi Population. The population variability then allows to estimate the size of the population that may be affected by the variable response to diuretics and help to discern the genetic mechanism.

P15

A Study of Association of GSTM1, GSTT1, GSTP1, MDR1 and TPMT Gene Polymorphism with Susceptibility to Acute Lymphocytic Leukemia (ALL)

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Background: Detoxification enzymes play a significant role in biotransformation of many xenobiotics including environmental carcinogens, pollutants and drugs. Both the genetic polymorphisms and expression pattern of these genes may have a major impact on cancer susceptibility, inter-individual variability in the prognosis, drug response and toxicity. We hypothesized that these polymorphisms might affect the risk of acute lymphocytic leukemia (ALL). **Aims and Objective:** To investigate the influence of GSTM1, GSTT1, GSTP1, MDR1 and TPMT gene polymorphisms in susceptibility to ALL. **Material and methods:** The frequencies of GSTM1 and GSTT1 homozygous deletions, GSTP1 (Ile105Val), MDR1 (3435 C>T) and TPMT*2 allele were examined in 27 ALL patients and 47 control subjects by allele specific PCR and PCR-RFLP method. **Results:** The frequency of individuals carrying GSTM1 null genotype was significantly higher among ALL patients (40%) as compared to controls (19%) (p=0.04). Although frequency of GSTP1 Val/Val genotype was higher among the patients (18%) in comparison to controls (6%), the difference was not statistically significant (p> 0.05). Individuals with both GSTM1 and GSTT1 null genotypes had a 3.12 higher risk for ALL. In case of MDR1 (3435 C>T), both homozygous and heterozygous mutant genotypic frequencies were more in controls than in ALL patients. **Conclusion:** The data suggests that individuals with GSTM1 null genotype appear to be more susceptible to ALL.

P16

I/D Polymorphism in Angiotensin Converting Enzyme Gene and its pharmacogenetic implications amongst Hypertensive Punjabi Population

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Background: Renin angiotensin system (RAS) has been a drug target as the most important regulator underlying hypertension which is mediated by the key component; angiotensin converting enzyme (ACE). ACE a zinc metallo-peptidase converts the inactive decapeptide, angiotensin I to the active octapeptide, a potent vasoconstrictor angiotensin II. Hence ACE inhibitors are a main line of drugs developed to treat hypertension. It has been demonstrated that the ACE expression related to genetic variations. Presence or absence of 287bp element in ACE leads to Insertion/deletion polymorphisms. The D allele of ACE gene has been correlated with high plasma level which has been observed to predispose individuals to disorders like Alzheimer disease, Diabetes mellitus, polycystic kidney disease, hypertension, coronary artery disease and pregnancy loss. The D/I and I/I genotypes results in intermediate or low plasma level of ACE. **Aim and objectives:** Present study sought to determine distribution of genotypic variability amongst hypertensive and normotensive Punjabi Population. It further explores variability in response to ACE inhibitors (Envas, Ramipril, Meoxipril, Lisinopril) amongst various genotypes of I/D among Punjabi hypertensive subjects. **Material and Methods:** The study included 200 clinically diagnosed hypertensive patients with/without associated metabolic disorders and

100 normal healthy subjects with their informed consent. Study has been approved by Institutional clinical ethical committee (ICEC/4/2011). Genotyping performed using PCR amplification analyzed on 10 % PAGE. **Results:** We found a 29 II, 106 ID and 65 DD in the hypertensive subjects and 25 II, 57 ID and 18 DD allele in normal subjects. The variability in response to ACE inhibitors was also observed amongst patients of various genotypes. **Conclusion:** The results suggested that ACE (D/D) genotypes are more prone for the development of hypertension. With these preliminary data at hand, the population-based screening risk assessment results that could possibly affect metabolism and efficacy of drugs that are commonly used by physicians; will be discussed in the presentation.

P17

Demographic and Clinical Profile of Major Depressive Disorder (MDD) Patients Attending AIIMS Psychiatry Department

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Background: Major Depressive Disorder has been considered as one of the leading causes of morbidity in India and worldwide. It has often been conceptualized as a response to overwhelming stress and associated psychiatric disorders. However, being a complex phenomenon, the etiology of Major Depression is still not very clear and Suicide as a major cause of mortality among MDD is one of the research questions being investigated. **Aims and Objectives:** The present study aims to understand the phenotypic characteristics of Depressive patients with and without suicide ideation. **Material and Methods:** 100 clinically established Major depressive disorder patients aged 18-65 years attending Psychiatric OPD at All India Institute of Medical Sciences were recruited and assessed for Psychiatric, Clinical and Sociodemographic variables. DSM-IV based MINI Screen, Mini International Neuropsychiatric Interview (MINI), Hamilton Depression rating Scale (HAM-D 17 item), Columbia-Suicide Severity rating Scale (C-SSRS) and Presumptive Stressful Life Event Scale (PSLES) were administered by clinical psychologist to assess Depression severity, suicidality and stressful life events among the patients. Clinical data included age at onset, total duration of illness, other comorbid disorders, Precipitating factors and family history while socio-demographic variables included Age, sex, ethnicity, marital status, literacy, occupation, monthly income and religion. **Results:** Among the total sample, 56% were having a current suicidal risk. Significant differences were also found among MDD with and without suicide risk behavior with respect to various demographic variables (Sex, marital status, occupation, education and Monthly family income) and psychiatric variables (Hopelessness, childhood adversity, past history, family history, Stress score). **Conclusion:** The findings of the present study suggest a possible sub-phenotype

mediating the susceptibility for risk of suicide among MDD patients. It would possibly lead to better understanding of the disease etiology which could be used for improved effective medication of the patients.

P18

Current therapeutic approaches for Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) is a devastating recessive X-linked muscle degenerative disease that affects 1 in 3600–6000 live male births. DMD caused by frame-shift deletions, duplications, or point mutations in the dystrophin gene (locus Xp21.2) which lead to an absence of or defect in the protein dystrophin. Dystrophin forms the dystrophin-associated glycoprotein complex (DGC) at the sarcolemma linking the muscle cytoskeleton to the extracellular matrix. When dystrophin is absent, muscle fibers become vulnerable to mechanical stretch and results in loss of independent ambulation with the progression of age of the patient. The molecular basis of DMD has been known for over 20 years. Many promising therapeutic strategies have since been developed in animal models. Human trials of these strategies have started, leading to the hope of definitive treatments for this currently incurable disease. Currently, a drug 'glucocorticoid' is the prominent medication and rehabilitative interventions have led to improvements in function, quality of life, health, and longevity, with children who are diagnosed today having the possibility of a life expectancy into their fourth decade. Several therapeutic approaches like gene replacement with virus vector, induction of protein expression by exon skipping, compensation with dystrophin surrogates and delivery of muscle stem cells or pluripotent stem cells have been investigated so far. The phase -3 clinical trial for Drisapersen, an antisense oligonucleotide for exon skipping could not meet the endpoint of statistically significant improvement, although it could still be most promising therapeutic approach. Recently, the modulation in the molecular pathways like VEGF/VEGFR pathway and constitutive activation of Notch pathway in animal models suggested the plausible cure of DMD. Here we present the updated information on the current therapeutics approaches for DMD.

P19

Host IL28B Genotype Plays an Important Role as a Predictor for Determining Response to IFN Therapy and Spontaneous Clearance in HCV Infected Thalassemia Patients

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Background: β -thalassemia major have a higher risk of hepatitis C virus infection due to repeated blood transfusions. Single Nucleotide Polymorphisms at rs 12979860 C/T and rs 8099917 T/G are strongly associated with Sustained Virological Response in hepatitis C patients. **Aims and Objectives:** To determine the potential effects of SNPs at rs1297960 and rs8099917 variation on outcome to HCV infection in a natural as well as IFN treated settings in β -thalassaemic patients. **Material and Methods:** 250 HCV sero-reactive thalassaemic patients were assessed for host and viral genotypic analysis. 33 patients were administered Peg IFN- ribavirin therapy. Viral genotype was determined by nested RT-PCR of viral genome followed by direct sequencing methods. Host IL28B genotyping was performed using Real Time PCR based SNP analysis. **Results:** 61% individuals were male, 39% were female. Out of 250 seropositive individuals, 59.2% were found to be RNA positive. Out of the 250 individuals, 90 (36%) individuals achieved sero-clearance without any IFN treatment, out of them 41 (45.5%) reported favourable CC allele at rs12979860, 22 (24.4%) individuals reported partially favourable CT genotype, 27 (30%) reported unfavourable TT genotype. C allele frequency was 0.58 whereas T allele frequency was 0.42. The distribution of allelic frequencies was in accordance to the Hardy–Weinberg equilibrium. In cases of IFN treated patients also, CC allele was predominant with 21 (63.6%) individuals carrying the favourable allele, 10 individuals reported CT (30.3%) whereas 2 (6.1%) individuals reported unfavourable TT genotype. **Conclusion:** IL28B CC genotype has a marked impact in the natural clearance of HCV in thalassemia patients, especially in low age group.

P20

BMPR1B Signalling in HEK293 Cells

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Limb deficiency has found in 3-8 per 1000 births, whereas congenital upper limb anomalies are second abnormalities in frequency and account for 10% of all malformations at birth. Limb development has been studied for decades, but very little is known about the molecular networks which regulate limb development. Since, none of the model till date is able to unravel the precise and accurate molecular mechanisms of limb development. Therefore, there is need to establish the signaling pathways that interplay during limb bud formation and digit patterning. Limb development are well-coordinated process regulated by fibroblast growth factors (FGFs) (Delgado et al., 2008), bone morphogenetic proteins (BMPs) (Ganan et al., 1996) transforming growth factor-beta (TGF- β) (Ganan et al., 1996), Msx-2 (Marazzi et al., 1997), Wnt (Grotewold and Ruther, 2002), Shh (Buscher et al., 1997), retinoic acid (Khan and Hales, 2006), and other signaling pathways.

BMPs are multifunctional growth factors belonging to the TGF- β multigene family. BMPs act as a central role during limb formation. It has been reported that BMPs may play different and even antagonistic roles at different stages of limb development. The BMP receptor family of transmembrane serine/threonine kinases are involved in endochondral bone formation and programmed cell death (PCD) during

embryogenesis. However the identities of the intracellular mediators of these signals are unknown. The aim of this study is to find out the particular signals that transduce apoptosis and chondrogenesis. To fulfil the objective BMPR1B gene has been knocked-down in HEK293 cells using 21 nucleotide long siRNA and confirmed the silencing by quantitative real-time PCR (SYBR Green) and western blot. The genes regulated by BMPR1B will be identified by whole-genome expression microarray followed by Real-Time PCR and Western blotting experiments. The results will be discussed in the presentation.

P21

Genetic Factors Related to Unconjugated Hyperbilirubinemia Amongst Adults.

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Background: Gilbert's syndrome (GS) is a benign unconjugated hyperbilirubinaemia occurring in 2-10% of the general population, and often detected in adulthood during routine blood tests, which unmasks the hyperbilirubinaemia. Earlier studies suggested that (TA)_n repeat polymorphism in the UGT1A1 gene promoter is associated with GS. However it remains unclear whether polymorphisms in other genes involved in bilirubin metabolism pathway are associated with hyperbilirubinemia. **Aims:** The present study was undertaken to investigate the genotype and allele frequencies in four bilirubin metabolism genes (UGT1A1, OATP2, HMOX1 and BLVRA) and their association with hyperbilirubinemia. **Material and Methods:** Genotyping of 16 SNPs was performed in 150 adults with hyperbilirubinemia and 115 controls by PCR-RFLP, Gene Scan analysis and direct DNA sequencing. **Results:** Genetic polymorphisms of the UGT1A1 promoter, specifically the T-3279G phenobarbital responsive enhancer module and (TA)₇ dinucleotide repeat as well as the intron and coding region variants of the OATP2, HMOX1 and BLVRA genes were significantly higher among the cases than the controls. Further, nearly 58% of the cases showed the presence of more than 4 variants as compared to 21% of the controls and the mean total serum bilirubin levels also increased according to the number of variants co-expressed. Exon-wise sequencing of the UGT1A1 gene revealed a variant at nt 6846 A $\rightarrow$ G in exon 2, with a predicted amino acid change of Ile $\rightarrow$ Val at codon 322 in heterozygous condition among the hyperbilirubinemia cases. **Conclusions:** This study demonstrates that polymorphisms in the bilirubin metabolism genes had a significant effect on bilirubin levels and could be genetic risk factors for hyperbilirubinemia.

P22

Mutational screening of PINK1 and LRRK2 in Indian Parkinson disease patients

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Background: Parkinson's disease (PD; OMIM #168600) is the most common neurodegenerative movement disorder affecting 1 to 2% of the population above age of 65 years with higher incidence in male than female. The vast majority of PD cases are sporadic in nature. Molecular genetic analyses have identified more than 500 distinct DNA variants in five disease genes associated with familial Parkinson disease; α -synuclein (SNCA), parkin (PARK2), PTEN-induced putative kinase 1 (PINK1), DJ-1 (PARK7), and Leucine-rich repeat kinase 2 (LRRK2). **Aims and Objectives:** Identification and characterization of causal genetic mutation underlying Parkinson's disease in North-Indian patients. **Objectives:** Mutational screening of candidate genes viz. autosomal dominant LRRK2 and autosomal recessive PINK1 in north Indian patient cohort. **Material and Methods:** A total of 100 clinically diagnosed PD patients apparently sporadic in nature including 28 early onset (<40 year) were enrolled. Peripheral blood was collected from patients with their informed consent. Genomic DNA was isolated for performing PCR followed by DNA sequencing. Exons encoding Roc (Ras of complex proteins), COR (COOH terminal of Roc) and Kinase domains of LRRK2 were screened in 60 patients irrespective of the age of disease onset and all exons of PINK1 were screened in 28 early and 20 late onset patients. **Results:** A novel homozygous nonsense variant Gln267Stop in kinase domain of PINK1 resulting a truncated protein was detected in a single PD patient, while it was absent from the rest 99 PD patients and 50 age matched healthy controls. Another reported heterozygous mutation resulting Arg276Gln was observed in two different patients in addition to 6 SNPs in the same PINK1. Screening of LRRK2 results in identification of two novel synonymous changes viz. Tyr1527 and Val1615 and a novel intronic deletion IVS36+67 del A. In addition to these four mutations reported earlier including two nonsynonymous (Pro1542Ser and Ser1647Thr) and two synonymous (Gly1624 and Lys1637) along with two intronic SNPs (rs1896252 and rs7137665) were observed. **Conclusion:** Mutation/s of PINK1 and LRRK2 are prevalent in northern Indian PD patients. Functional study of the novel mutations those identified in the present study will provide the opportunity to understand the molecular mechanism of Parkinson's disease pathogenesis. In vitro study for functional validation of the novel Gln267Stop is underway.

P23

Mutational screening of PKD1 in North Indian patients Sonam Raj¹, R G Singh² and Parimal Das¹

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Background: Polycystic kidney disease (PKD) is a systemic disorder and prime cause of end stage renal disease (ESRD) in renal patients. Linkage analysis revealed some closely linked loci two of which are identified as PKD1, PKD2 and an unidentified locus to Autosomal Dominant Polycystic Kidney Disease (ADPKD). ADPKD is genetically heterogeneous, Mendelian disorder, shows late onset and contributes 8-10% cases of End Stage Renal Disorders (ESRD). Protein product

of these identified candidate genes are central regulatory molecule of different signaling pathways controlling cell homeostasis and function. **Aim:** Establishing quick DNA based unique diagnostic test/s for PKD for specific Indian population. **Objectives:** (1) Mutational screening of PKD1 in clinically diagnosed PKD patients (2) Finding the spectrum and occurrence of genetic mutations in PKD1. **Materials and methods:** After informed consent, family history, clinical reports and blood sample was collected from 30 patients. DNA was extracted from collected peripheral blood for further molecular diagnosis. Mutational screening of PKD1 was performed using PCR followed by DNA sequencing. **Results:** In 3' single copy region of PKD1, four reported likely neutral (T3510M, A3512V, D3782D, IVS41+5_+6insGGG), one definitely pathogenic (P3582fs22X) and one novel likely neutral (A3772A) sequence variations were detected. Remaining 5' duplicated region have shown eleven reported likely neutral (A341A, L373L, IVS12-15C>T, A1516T, A1555A, A1724A, V2026V, R2200C, L2389L, L2481L, IVS30+54A>G), two novel pathogenic (S383X, c.9899delA) and two novel nonpathogenic (S1081L, V1800V) sequence variants. **Conclusions:** Occurrence of mutations was more (2X) in duplicated region of PKD1 compared to single copy region. Presence of more than one likely neutral variant in single individual suggests allelic heterogeneity which may contribute in variable expressivity at individual level while other pathogenic mutations leading to frame shift and truncated protein (polycystin1) production ultimately affect the cellular function and hence causes to disease manifestation.

P24

Association of gene polymorphisms with Chronic Obstructive Pulmonary Disease in North Indian Population

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Background: Chronic Obstructive Pulmonary Disease (COPD) is characterized by progressive decline in lung function and abnormal inflammatory response of lungs to noxious particles and gases. Two pro-inflammatory cytokines, IL-1RN and IL-6 and ADAM33, expressed in airway smooth muscle cells and fibroblasts of lungs play important roles in pathogenesis of COPD. **Aims and objectives:** The aim of present study was to assess the association of IL-1RN, IL-6 and ADAM33 gene polymorphisms with COPD. **Material and Methods:** COPD patients and healthy sex/age matched controls (n=100 each) were subjected to genotyping of IL-1RN VNTR (intron 2), IL-6 -597G/A (rs1800797), ADAM33 S2 (rs528557) and F+1 (rs511898) by polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism (PCR-RFLP). Genotype and allele frequencies were calculated and statistically analyzed by chi-square using SPSS (ver. 21.0). Haplotype analysis and gene-gene interaction, pairwise linkage disequilibrium (LD) based on 'D' statistics and correlation coefficients (r²) of frequencies were analyzed using SHEsis

(ver. Online). **Results:** Genotype frequencies of IL-6-597 G/A and ADAM33 (S2 C/G, F+1 C/T) as well as allele frequencies of IL-6-597 G/A and ADAM33 S2 C/G were significantly associated with COPD ($P < 0.001$). Haplotype analysis in individuals with ADAM33 G T* combination showed 2.919 times increased risk of disease. In gene-gene interaction, G II* combination of IL-6 -597G/A, IL-1RN showed 12.949 times while A II G C* and G I G T* of IL-6-597 G/A, IL-1RN, S2 C/G and F+1 C/T increased the risk upto 5.37 and 530.79 times respectively. **Conclusion:** Haplotype and gene-gene interaction analyses give a better understanding of disease risk in case of polygenic diseases. There was more than 500 times increased susceptibility to COPD in individuals with G I G T* in our study population. Prior knowledge of risk alleles will enable individuals to take precautionary measures to prevent or delay the onset of disease.

P25

Cytokine Gene Polymorphisms and their Association with Cervical Cancer: A North Indian Study

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Background: Inflammation, angiogenesis and thrombosis are involved in cancer development leading to the production of cytokines, growth factors and adhesion molecules. They promote tumor progression by signaling and providing optimal conditions for cervical cancer. **Aims and Objectives:** The present study was undertaken to evaluate association of cytokine gene polymorphisms with cervical cancer. **Material and Methods:** Genotyping of four SNPs viz. IL-1RN VNTR (in intron 2), IL-1 β -511C/T (rs16944), IL-6-597G/A (rs1800797) and TNF- α -308G/A (rs1800629) was carried out in 100 each of cervical cancer patients and healthy age matched control subjects by polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism (PCR-RFLP). Genotype and allele frequencies were calculated and statistically analyzed by chi-square (χ^2) using SPSS (ver. 21.0). Gene-gene interaction, pairwise linkage disequilibrium (LD) based on 'D' statistics and correlation coefficient (r^2) of frequencies was analyzed using SHEsis (ver. Online). **Results:** Epidemiological studies have shown that women of age >40 years have higher risk of cervical cancer due to greater susceptibility of cervical epithelium to Human Papilloma Virus (HPV) the reason during adolescence, improper hygiene and poor socio-economic conditions. Genetic studies showed that genotypic frequencies of IL-6-597G/A and TNF- α -308G/A as well as allelic frequency of IL-6-597G/A showed significant association with cervical cancer in North Indian population ($P < 0.001$). Gene-gene interaction analysis showed that G T G I* and G T A I* combinations of all four SNPs taken together increase the risk upto 11 and 5 times respectively in the study population. **Conclusions:** Genetic polymorphisms in associated genes can be used as markers to predict cervical cancer susceptibility. The knowledge of risk alleles will enable

individuals to take precautionary measures before hand and prevent or delay the onset of disease. This will provide a lead for other cancers as well.

P26

Molecular Study of FANCA Complementation Group in Indian Population

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Background: Fanconi anemia (FA) is a clinically heterogeneous disorder with incidence of 1 in 350,000 births and is characterized by bone marrow failure (aplastic anemia), developmental delay, physical abnormalities, increased incidence of solid tumors and leukemia, and cellular sensitivity to DNA interstrand cross-linking agents such as diepoxybutane (DEB) or mitomycin C (MMC). Sixteen genes (FANCA-Q) have been identified that can be mutated in FA. Molecular analysis is required for characterization of FA patients, demonstrating pathogenic mutations in the FA genes, and genotype-phenotype correlation. **Aim and Objectives:** Detection of FANCA gene mutations and genotype-phenotype correlation in Fanconi anemia. **Material and Methods:** The study was carried out on 449 patients with FA. Chromosomal breakage study was done using Phytohemagglutinin (PHA) stimulated lymphocyte cultures induced with MMC (40ng/ μ L). Molecular analysis of FANCA gene was carried out using direct sequencing and Multiplex Ligation-dependent Probe Amplification (MLPA). **Results:** Among 449 patients studied, high frequency chromosomal breaks were detected in 67 cases; of which 30 patients (44%) were found to belong to FANCA complementation group. The major clinical features in our study group were; skin pigmentation abnormality (57%), thumb anomalies (53%), and short stature (70%). Molecular study revealed 13 patients (43%) with deletions harbored in FANCA gene including 5 large deletions and 8 small deletions, and 17 patients (57%) with point mutations including 13 patients with missense mutations and 4 patients with non-sense mutations resulting in early-stop gain in FANCA protein. The study could detect 6 novel FANCA mutations including c.3679 G>C, c.3678 C>G, c.3993 G>A, c. 1274 C>G, c.2630 C>G and exon11 deletion. **Conclusions:** Chromosomal breakage investigation is a gold standard for diagnosis of FA. However, molecular analysis is still required for accurate diagnosis and management of the disorder. The FANCA is the major complementation group in Indian population. A combination of MLPA and direct sequencing methods is highly useful for detection of gene mutations in FA. This can expand our knowledge towards genotype-phenotype relationship in FA.

P27

Analysis of Microsatellite Markers in FSHD Families – A Simple and Indirect Approach to Pre-Implantation Genetic Diagnosis.

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Background: Facioscapulohumeral Dystrophy (FSHD) is an autosomal dominant disease associated with the heterozygous contraction of tandem repeats called D4Z4 arrays at 4q35.2. The disease is associated with contraction of the repeats to 10 or less compared to the normal individuals who have about 11 – 150. The present diagnosis of the disease depends on identifying the contraction of the repeats at D4Z4, however several complications such as discrimination of pathogenic 4q alleles from highly homologous 10q arrays by Southern blotting, cis-segregation of D4Z4 contraction with a 4qA allele and D4Z4 silencing at 4qB and 10q pose a problem in the molecular diagnosis of FSHD. Pre-implantation Genetic Diagnosis of FSHD involves analysis of highly informative microsatellite markers proximal to the D4Z4 including D4S2390, D4S16592, D4S2930 and D4S1523. These markers show tight linkage to the D4Z4 locus and tracking their movement through generations would help predict the movement of the contracted D4Z4 repeats. These markers give good amplification even in multiplex conditions and have good electrophoretic profiles. However a case of recombination poses a risk of producing accurate results in PGD. **Aim:** The work aims for analysing highly informative markers distal to the D4Z4 region and to verify their significance in an approach to Pre-Implantation Genetic Diagnosis. **Objectives:** Collection of blood samples from FSHD families and isolation of genomic DNA. PCR amplification of highly informative markers proximal to D4Z4 under multiplex conditions. Differentiating the banding patterns formed after electrophoresis. Sizing the alleles to determine their transfer from parents to progeny and tracking the events of recombination if any. **Material and Methods:** Isolation of DNA from peripheral blood lymphocytes, Agarose Gel Electrophoresis of DNA samples, Spectrophotometric analysis of DNA Samples, Primer Standardization and Multiplex PCR reactions set up, AGE of Multiplex PCR samples, PAGE and Silver Staining procedure, **Results:** The base pair sizes of each allele were determined using the software “LabImage” and the approximate sizes of bands were used for constructing a pedigree chart showing the transfer of alleles from the parents to the progeny. **Conclusion:** The microsatellite markers considered for the analysis were easy to amplify even under multiplex conditions. The allelic variations observed among the families were used to track the movement of the markers. Such information can provide us with the movement of the contracted D4Z4 repeats through generations. No recombination events were observed as far the movement of the alleles through a single generation was concerned.

P28

Correlation of Immuno-haematological Concepts from murine model to human CNV

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Background: Choroidal Neovascularisation (CNV) or wet

AMD mostly is a late sequel of dry AMD which is result of drusen deposition and characterized by loss of central vision if unchecked. The pathology develops owing to development of new blood vasculature between the bruchs' membrane and retina which is leaky and leads to retinal detachment and hence total vision loss. Our preliminary work in mouse model and other published reports in AMD patients have substantiated this finding reiterating the fact that certain myeloid cells are increased in human AMD. Hence there is imperative need to explore this myeloid cell population. **Aims & Objectives:** This is a prospective study targeting to find the systemic hematological changes in AMD/CNV. Study murine CNV with to identify the any changes systemically in blood/lymphnodes. Compare changes in blood of aged patients suffering from dry AMD, CNV with healthy age matched controls. **Material and Methods:** BD FACS Cantoll will be used to conduct all the flowcytometry using standard antibodies from commercial vendors. Commercial ELISA kit would be used to asses levels of VEGF and other growth factors or cytokines. **Results:** The mouse model clearly showed increase in the CD11b population of cells further more these cells had activation markers CD69. Studies from other groups have also shown increase in CD11b cells which were CD200 positive. This is an ongoing prospective study and several myeloid and lymphoid cells will be visited in great molecular detail. **Conclusion:** Preliminary studies suggest good correlation between murine and human CNV. While the data are from the western world they need to be correlated in Indian population. Also a detailed dissection of the changes in the CNV patient's blood is warranted and is being pursued. This study might reveal certain key targets for better treatment modalities of the disease.

P29

Polymorphisms in TP53, MDM2, HIF1a, BRCA1 and Rare Chromosomal Anomalies in a Male Breast Cancer Patient and His Unaffected Son

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Background: A first degree relative of a cancer patient may have 2-3 fold higher risk of developing cancer at same site as family members tend to share genetic background along with environment. **Aims and objectives:** In the present study genetic risk for cancer was estimated in the healthy male offspring of a Male breast cancer (MBC) patient using classical cytogenetic analysis, SNP-A and study of 13 polymorphisms in four genes i.e., four in BRCA1 (c.2612 C>T, c.190 T>C, 1307del T, g.5331G>A), five in TP53 (p.R72P, PIN3 16 bp Ins, p.P47S, p. R213R, r.13494g>a), three in HIF1a (g.C1772T, g.G1790A, g.C111A) and one in MDM2

(309T>G). **Material and Methods:** A 35 year old healthy male and his 60 year old father with infiltrating ductal carcinoma of breast and a family history positive for cancer were taken as study subjects. From each subject, G-banded karyotypes were made after standard 72 hour peripheral blood culture. Genomic DNA was extracted by standard phenol-chloroform method. The DNA samples were analyzed using Illumina Human Cyto SNP array and data was analyzed using Karyostudio v1.2. Genetic polymorphisms were assessed by PCR-RFLP. **Results:** Karyotypic analysis by G-banding showed increased aberrations in MBC patient (79.9%) than his healthy son (73.9%). The SNP-A of MBC patient showed a loss of Yq11.22.2 and gain in Yq11.22.1 in the Azoospermia (AZF) region. The son had rare copy neutral LOH in adrenal hyperplasia associated region on 6p22.3-6p21.2, duplication in Yp11.2 and Yp11.3 and deletion in 7q11-21 pericentromeric region. Among the SNPs analyzed, the MBC patient and his son were homozygous for MDM2 (309T>G) and either homozygous for wild type allele or heterozygous for rest of the studied polymorphisms. **Conclusions:** SNP-A revealed rare chromosomal anomalies in the MBC patient and his healthy son which had not been previously reported in breast cancer patient. Coupled with increased cytogenetic aberrations it indicates a higher genetic risk for the son.

P30

Determination of RHD Zygosity Based on Most Probable Genotype and PCR-SSP

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Background: In RhD negative women with anti-D alloimmunisation, determination of paternal RHD zygosity is useful in clinical practice for prenatal diagnosis and evaluation of risk of hemolytic disease of the newborn. Currently in India, paternal RHD zygosity assignment is inferred from serologic phenotypes by testing with antisera against C, c, D, E and e antigens of Rh blood group system. From the antigen phenotype expressed and the frequency of these antigens in Indian population the Most Probable Genotype (MPG) is deduced and thus the D zygosity. In all Rh phenotypes if the first MPG is homozygous for the D antigen, then the second MPG is heterozygous and vice versa. Hence the discrimination between RHD+/RHD+ and RHD+/RHD- is indirect and therefore not very accurate and reliable. D zygosity determination in populations studied so far has shown varying results between serological and molecular genotyping. **Aims:** To determine RHD antigen zygosity by serology (MPG) and Polymerase Chain reaction using Sequence Specific Primers (PCR-SSP) in blood donors. **Methods:** Three hundred and fifty four donors from Mumbai blood bank were tested with anti-C, anti-c, anti-D, anti-E and anti-e and MPG of the individuals deduced. Extracted DNA was tested by the PCR-SSP method which allows the detection of the hybrid Rhesus box and thus the determination

of the D zygosity. **Results:** Out of three hundred and fifty four donor samples tested, 45.5% were homozygous and 35% were heterozygous for D antigen by serology and PCR-SSP. 19.5% of cases gave discrepant results by the serological and molecular genotyping. **Conclusion:** This is the first study comparing the RHD zygosity by Rhesus box PCR-SSP with MPG in Indian population. In the discrepant cases it can be presumed that the second MPG by serology is the actual genotype. The results in this study show that PCR-SSP should replace MPG in prediction of the D zygosity. The availability of molecular tests for assessing RHD zygosity can be used for genetic counseling for better management of sensitized pregnant RhD negative women.

P31

Vascular Endothelial Growth Factor (VEGF) Gene Polymorphisms and Breast Cancer Risk in Punjabi Population from North West India

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Background: Vascular endothelial growth factor (VEGF), an endothelial cell specific mitogen, has been implicated as a critical factor influencing tumor related angiogenesis. **Aims and objectives:** To evaluate the association of seven VEGF promoter polymorphisms with breast cancer risk in Punjabi population from North West India. **Materials and Methods:** We screened DNA samples of 102 sporadic breast cancer patients and 102 unrelated healthy, gender and age matched individuals for seven VEGF promoter polymorphisms [-417C/T, -172C/A, -165C/T, -160C/T, -152G/A, -141A/C and -116G/A] by direct sequencing. **Results:** The frequency of GG, GA and AA genotype of -152G/A polymorphism was 26.47 vs 38.34%, 46.08 vs 51.96% and 27.45 vs 9.80% respectively in patients and controls respectively. VEGF -152 AA genotype was significantly associated with increased risk for breast cancer (OR=4.04, 95%CI, 1.69-9.68, p=0.001; recessive model OR=3.48, 95%CI, 1.59-7.63, p=0.001). For VEGF -116G/A polymorphism, G and A allele frequencies were 65.2 vs 76.47% and 34.8 vs 23.53% in patients and controls respectively. Individuals having -116AA genotype (OR=3.40; 95%CI, 1.24-9.37; p=0.014) and A allele (OR=1.73; 95%CI, 1.12-2.67; p=0.012) were associated with increased risk for breast cancer. There was significantly decreased frequency of CT genotype (4.90 vs 18.63%; p=0.002) and T allele (2.45 vs 9.31%; p=0.003) of -165C/T polymorphism among breast cancer patients as compared to controls. Significant reduced risk for breast cancer was observed with AC genotype (OR=0.34, 95%CI, 0.14-0.86; p=0.019) and C allele (OR=0.37; 95%CI, 0.15-0.89; p=0.023) of -141A/C polymorphism. We did not observe association of VEGF -417T/C, -172C/A, -160C/T polymorphisms with breast cancer risk in the studied subjects

($p > 0.05$). **Conclusions:** The VEGF -152G/A and -116G/A polymorphisms were found to be significantly associated with increased risk for breast cancer while -165C/T and -141A/C polymorphisms were found to be associated with decreased risk for breast cancer in Punjabi population from North West India.

P32

Cytogenetic Aberrations as a Measure of Genotoxicity in Rural Population Living near River Beas

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Background: Waters of the rivers Satluj and Beas in Punjab are reportedly polluted. Environmental impacts and occupational exposures are frequently assessed through cytogenetic markers. Chromosomal abnormalities are the commonly used biomarkers for human biomonitoring studies as chromosomal aberrations may act as the intermediate processes in the pathway of the progression to any genetic disorder. **Aims and objectives:** The present study was an attempt to determine the chromosomal instability with special reference to long term genotoxic effects of exposure to polluted water, if any, in rural subjects living near river Beas in Punjab. **Material and Methods:** In this study, 48 rural subjects (24 males and 24 females) living on bank of river Beas of Punjab, were screened for the cytogenetic aberrations in their cultured peripheral blood lymphocytes. Standard in-vitro cell culturing technique of 72 hours in RPMI-1640 medium in the presence of phytohemagglutinin (PHA-M) was used. In each case, 100 metaphases were examined for numerical as well as structural aberrations and karyotypes were made according to ISCN 2009. **Results:** A large number of cytogenetic aberrations including aneuploidy, polyploidy, translocations, marker chromosomes, ring chromosomes, dicentric chromosomes, premature centromeric division, interstitial deletions, acrocentric associations and terminal deletions were seen in subjects residing nearby river Beas. Chromosome gaps and breaks were found repeatedly in chromosome 1, 2, 3, 4, 5, 6, 7, 8, 10, 12 and 17 both in males as well as females. However, frequency of breaks and gaps were more in case of females as compared to males ($p < 0.05$). **Conclusion:** Increased chromosomal instability in the subjects residing near river Beas indicated that they might be at a higher genetic risk.

P33

ABO Blood Group and Risk of Coronary Artery Disease.

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Background: Human blood group antigens are glycoproteins

and glycolipids expressed on the surface of red blood cells and a variety of human tissues including epithelium, platelets and vascular endothelium. Plasma levels of various metabolites like IL-6, adhesion molecules, selectins, von-Willebrand factor, hs-CRP etc are influenced by the type of blood group. **Aims and Objectives:** The objective of the study was to test the genetic variants that determine the blood type A1 (normal sequence), A2 (deletion of C), B (G803C) and O1 (deletion of G) and associate them with coronary artery disease (CAD). Additionally, hs-CRP levels were recorded to assess the risk of CAD. **Material and Methods:** 160 patients visiting the P.D. Hinduja Hospital and Medical Research Centre's catheterization laboratory were recruited for the study. These included 80 angiographically verified CAD patients age and gender matched with 80 angiographically verified controls. Genotyping was performed by Allele specific PCR & hs-CRP levels were recorded using Beckman Coulter DXC Analyser. **Results:** Frequency of the A1 allele (Cases – 18% ; Controls – 19.3%) and O1 allele (Cases – 34.3% ; Controls – 53.1%) was observed to be higher in controls over the cases. Frequency of A2 allele (Cases – 6.8% ; Controls – 4.3%) and B allele (Cases – 40.6% ; Controls – 22.5%) was seen higher in cases over the controls. Patients of the B blood group showed about two times higher hs-CRP levels in cases over the controls while the AB blood group patients had hs-CRP levels higher in controls over cases. **Conclusion:** This is an ongoing study and the preliminary results suggest the B blood group to be a risk marker for CAD while the O1 blood group to be atheroprotective.

P34

Gene Variants of Pro-Oxidant and Antioxidant Genes in Indian Coronary Artery Disease Patients

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Background: Oxidized low density lipoproteins (ox-LDL) induce atherosclerosis by triggering an inflammatory cascade within the vascular wall. The role of genetics with oxidative stress in multifactorial coronary artery disease (CAD) has not been substantially explored in the Indian population. **Aims and Objectives:** The aim of the current study was to analyze the correlation of the pro-oxidant gene variants of the pro-oxidant genes, myeloperoxidase (MPO) and cytochrome b-245, alpha polypeptide (CYBA) of the NADPH oxidase complex and antioxidant genes, manganese superoxide dismutase (SOD2) and catalase (CAT) with the development of coronary artery disease. **Material and Methods:** A total of 150 angiographically proven cases and 150 age and gender matched angiographically negative, proven controls who underwent coronary angiography at the catheterization lab in P. D. Hinduja National Hospital & MRC were included into the study. Their clinical data along with traditional risk factors for CAD were noted down. Genotyping of MPO-463G>A variation was done using PCR-RFLP, while SOD2 47T>C, CAT -262C>T and CYBA G640A were genotyped by Tetra-ARMS PCR. **Results:** Family history of CAD was

seen in 40% cases as compared to 23% in controls and was found to be statistically significant ($p < 0.05$). No difference in the frequency of the variant allele of MPO-463G>A and SOD2 47T>C was seen in controls and cases (13.75% and 49.67% respectively). The frequency of the variant allele of CAT -262C>T was however higher in controls over cases (26.67% vs 23.82%) but was not statistically significant whereas the variant allele frequency of CYBA G640A was significantly higher in cases as compared to controls (56.67% vs 48.67%, $p = 0.04$). **Conclusion:** This is an ongoing study and the preliminary data suggests that CYBA G640A variation is associated with coronary artery disease and the variant "A" allele increases the risk of developing CAD in Indian subjects.

P35

Common Variants of *SLCO1B1*, *CYP3A4*, *ABCB1*, *CYP3A5*, *ABCG5* and *CYP7A1* Genes as Predictors of Lipid-Lowering Response to Atorvastatin Therapy

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Background: Statins are widely prescribed and are established as first-line therapy for the primary and secondary prevention of coronary artery disease (CAD). However, there is large inter-individual variability in clinical response to statin treatment. Several studies have reported genetic variation contributes to variable reduction in low-density lipoprotein-cholesterol (LDL-C) levels in response to atorvastatin therapy. Genetic variations in genes involved in statin and lipid metabolism are proposed as important determinants of statin response. **Aims and Objectives:** To study the association of known variations in *SLCO1B1*, *CYP3A4*, *ABCB1*, *CYP3A5*, *ABCG5* and *CYP7A1* genes and lipid levels in response to atorvastatin therapy. **Material and Methods:** Genotypes were determined by using multiplex allele specific-polymerase chain reaction (AS-PCR) in 100 hypercholesterolemic patients, treated with 10 mg of atorvastatin for 8 weeks. Baseline & after 8 weeks low-density lipoprotein cholesterol (LDL-C) levels were determined for subjects. **Results:** The genotype distribution for all polymorphisms investigated was in Hardy–Weinberg equilibrium. Our results show that, the variant allele (C) for *CYP7A1* (rs3808607) polymorphism is associated with $26 \pm 1.8\%$ LDL-C reduction, while wild type allele (A) showing $32 \pm 4\%$ reduction in LDL-C. We also see that, for *CYP3A4* promoter variant (rs2740574), individuals with AA genotype exhibited a greater reduction in LDL-C as compared to AG+GG genotype ($-30 \pm 1\%$ Vs. $-27 \pm 1.3\%$). However we did not observe any difference in LDL-C reduction for *CYP3A4* missense variant (rs4986910). We found for both the polymorphisms of *SLCO1B1* (rs2306283, rs11045819) variant allele showing higher reduction in LDL-C as compared to wild type. No differences were observed for *CYP3A5*, *ABCG5* and *ABCB1* variants. **Conclusion:** These results suggest that polymorphisms in lipid and statin pathway genes are associated with variable reduction in LDL-C. Inclusion of pharmacogenetic data along with clinical parameters would

assist in atorvastatin dosage in Indian patients.

P36

Evidence for expression of *ARL15*, a novel GWAS identified G protein in Rheumatoid Arthritis Synovial Fibroblasts

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Rheumatoid arthritis (RA) is an inflammatory autoimmune joint disorder that affects more than 1% population globally. A genetic basis for this disease is undisputed with HLA being the major risk factor. Results from several recent Genome-wide association studies (GWAS) of RA in Caucasian populations and their meta-analysis have identified many more risk variants but their contribution to our understanding of the disease biology and for developing effective treatment remains limited. Recently, in the first ever GWAS on RA from north India, we identified a novel gene *ARL15*, a small G protein to be associated with RA. *ARL15* variant has previously been shown to be correlated with levels of adiponectin, a cytokine involved with RA. Therefore, In this study, we investigated its possible expression in Rheumatoid arthritis synovial fibroblast (RASf), which is a major aggressive cell type implicated in cartilage and bone degradation in RA. RASf was isolated from the synovial fluid collected from three RA patients undergoing total knee replacements at AIIMS, New Delhi with informed consent and institutional ethical committee clearance. Primary cultures established using these cells was first confirmed to be true RASf by Fluorescence-Activated Cell Sorting. They were then tested for the expression of *ARL15* using multiple approaches. Western blot analysis and RT PCR confirmed the expression of *ARL15* in RASf. Considering the pharmacological potential of G proteins, our novel preliminary finding of *ARL15* expression in RASf seems to hold promise for lead molecule development.

P37

Identification and Characterization of D Variants in Apparently RhD Negative Individuals

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Background: RhD variants are of clinical importance and should be identified as they may produce anti-D antibodies upon exposure to normal D positive red cells either through transfusion or pregnancy. RhD variant individuals are reported as RhD negative or positive depending on the commercial monoclonal anti- D reagents and techniques used in different laboratories. Weak D and Partial D are the most commonly encountered RhD variants. Weak D are quantitative variants with less number of D antigenic sites while partial Ds are qualitative variants which lack one or more epitopes of D antigen. A panel of monoclonal anti-D antibodies can be used for serological identification and characterization of D variants.

Aims and objectives: The aim of this study is to identify D variants amongst apparently RhD negative individuals reported as RhD negative in their respective hospitals. **Material and Methods:** A total of 1400 apparently RhD negative individuals, were tested by ALBAclone Advanced Partial RhD typing kit (12 epitope specific monoclonal anti-D reagents) and Rh phenotyping was done using anti-C, c, D, E, and e antisera. Depending on the reactivity pattern with the anti-D reagents, weak D type 1 & 2 and 15 common partial D variants can be identified. **Results:** 3.57% of these apparently RhD individuals were identified as D variants. 93% of D variants could be characterized with this kit. Out of 1400 RhD negative individuals tested, 161 (11.5%) were "C" antigen positive. 31% of C positive apparently D negative individuals were identified as D variants. **Conclusion:** RhD variant individuals were reported as RhD negative by routine commercial monoclonal anti-D reagents. Screening for the presence of 'C' antigen in apparently RhD negative individuals and advanced partial D typing kit was very useful for identification of D variants and confirmation of RhD status.

P38

BRCA 1 and 2 Mutations Detection by NGS in Breast Cancers Patients – Pilot Study.

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Background: Breast cancer is the commonest cause of cancer death among females, accounting for 23% of the total cases and 14% of the total cancer deaths. While most breast tumors are sporadic, about 5 to 10% are caused by germ line mutations in BRCA1 and BRCA2. BRCA genes are responsible for approximately 20-40% of inherited breast cancer. BRCA genes are large and as almost 2000 mutations are reported in both BRCA 1&2 and there are no hotspots regions, the entire gene needs to be screened by sequencing for mutations. Various methods have been adapted to detect these mutations of which Sanger Sequencing method is the most comprehensive and gold standard but has its limitations. Next gene sequencing is the new technique quickly being adopted by the diagnostic community and has its advantages and limitations. **Aims and Objectives:** In the present study we have used next-generation sequencing (NGS) technique to determine the prevalence of germ line mutations in BRCA1 and BRCA2 gene in breast cancer patients. **Materials and methods:** We report our preliminary experience of 9 cases studied by Sanger's method (2) or Next gene sequencing techniques (7). **Results:** 2 of 7 cases for which we have received, the reports have shown likely pathogenic mutations. The First case had a likely pathogenic mutation C 5074+1g>A in BRCA 1 gene, confirmed by Sangers.c.9380G>A, nonsense mutation has been detected in the 2 case in BRCA2 gene which is a stop codon mutation resulting in pathogenic change. Other 5 cases have shown no reported pathogenic mutations. **Conclusion:** This is just a preliminary study and many more cases need to be studied. Both challenges and

benefits of testing BRCA genes by NGS will be discussed.

P39

Association of MTHFR C677T and A1298C Polymorphisms with Male Infertility in South Indian Population.

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Background: Infertility is a worldwide reproductive health problem that affects 10-15% of married couples, and approximately half of these cases are due to the male factor. Several etiologies have been identified including chromosomal abnormalities, Y-chromosome microdeletions, CFTR mutations, endocrinopathies, immunological factors, environmental exposures and other genetic abnormalities. Methylenetetrahydrofolate reductase (MTHFR) is one of the main regulatory enzymes of folate metabolism, DNA synthesis and remethylation reactions. MTHFR C677T variant decreases enzyme activity and increases the homocysteine level which is considered as a risk factor for male infertility. Similarly the MTHFR A1298C variant also reduces enzyme activity but to a lesser extent. **Aims and Objective:** It was proposed to examine the possible association of these two polymorphisms in MTHFR gene with male infertility. **Materials and Methods:** A total of 208 infertile men exhibiting a normal karyotype without any classical AZF deletions and 100 men with proven fertility were investigated. MTHFR C677T and A1298C polymorphisms were analysed by PCR-RFLP method using HinfI and MboII enzymes respectively. Two-sided Fisher's exact test and Pearson chi-square test were used to determine significance between groups. **Results:** The genotype frequencies of MTHFR C677T were CC = 0.79, CT = 0.20 and TT = 0.01 in infertile men and CC = 0.74; CT = 0.26 and TT = 0.0 in controls. No significant difference was observed in the distribution of mutant 677T allele between infertile cases and controls (0.11 vs. 0.13) (OR = 0.74; 95% CI = 0.43-1.29; P = 0.31). Similarly in case of A1298C polymorphism, the frequency distribution of AA and AC and CC genotypes and C allele between cases and controls were 0.29, 0.49, 0.22, 0.46, and 0.32, 0.45, 0.23, 0.45 respectively (OR = 1.07; 95% CI = 0.95-1.16; P = 0.79). **Conclusion:** Both MTHFR C677T and A1298C polymorphisms were not significantly associated with increased risk for male infertility.

P40

Association of rs363039 and rs3630350 SNPs in SNAP25 Gene with Intellectual Disability

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Background: Intellectual disability (ID) is characterized by significant sub-average intellectual functioning co-existing with deficits in adaptive behaviour. It occurs in 1-3% of general population and the etiology remains elusive in about 40-50% of the cases. Genetic polymorphisms associated with variation in normal intellectual ability have been suggested to be involved in some cases of ID. The SNAP25 gene lies in a linkage area implicated previously in human intelligence. This synaptosomal-associated protein is involved in regulating neurotransmitter release and has been associated with memory and learning. Alleles previously reported to be associated with ID were T for rs363039 and A for rs363050. **Aim and Objective:** It was proposed to compare the distribution of the SNPs rs363039 and rs363050 in SNAP25 gene in 146 ID cases and in 101 sex-matched healthy control subjects. **Material and Methods:** Genotyping using self-designed primers was performed for the SNP rs363039 by PCR amplification followed by sequencing and for rs363050 by PCR-RFLP using Sau3AI enzyme. Deviation from Hardy-Weinberg equilibrium for the SNPs was tested and a possible association of the minor alleles with ID was determined by both the recessive and dominant genetic models. **Results:** Genotypic analysis revealed that only the SNP rs363050 was in Hardy-Weinberg equilibrium in both cohorts. The dominant model indicated a significant “protective” association (OR = 0.45; P = 0.007) in which GG+AG individuals had lower odds of developing ID than AA individuals, while no significant association was observed for the recessive model (OR = 0.9; P = 0.73). For the other SNP rs363039, the dominant model indicated a significant “protective” association (OR = 0.55; P = 0.03). The recessive model (TT+CT) showed a significant association with ID (OR = 2.35; P = 0.03). **Conclusion:** Extended research incorporating a larger sample size is required to confirm the association of SNAP25 gene variants with ID.

P41

Molecular Screening in Two Cases of 46,XX Males

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Background: The 46,XX male syndrome (de la Chapelle syndrome or 46,XX testicular disorder of sex development) is a rare phenotype with a frequency of 1 in 20,000–25,000 males. XX males present as different clinical entities with ambiguous genitalia or partially to fully mature male genitalia, coupled with complete or incomplete masculinization. **Aim**

and Objectives: The aim of this study was to delineate phenotypic features in two male patients diagnosed with this syndrome. **Material and Methods:** The patients were referred with clinical findings as Ambiguous Genitalia at age 30 days (MI-06) and 4 years (MI-18). Cytogenetic analysis was carried out on peripheral blood lymphocytes as per the modified method of Hungerford (1965). Karyotypes were designated following the International System for Cytogenetic Nomenclature (2013). DNA isolated using PCI method will be amplified using specific primers to screen for the presence of the SRY gene. **Results:** Both the cases had poorly developed external genitalia. MI-06 exhibited labioscrotal fusion and absence of testis. MI-18 presented with hypospadias and small penile shaft. No scrotum or vaginal orifice was observed. Abdominal pelvic ultrasonography demonstrated presence of Mullerian structures in both. Magnetic resonance imaging of abdomen of MI-18 showed convoluted cerebriform appearance of bilateral adrenal gland while that of MI-06 revealed normal adrenal structures. Hormonal evaluation of MI-18 showed elevated levels of plasma ACTH (221pg/ml) and serum DHEA (60µg/dl), with normal levels of cortisol. 17-OH progesterone value of MI-06 was more than 100ng/dl. Renal function test revealed increased levels of urea and normal creatinine values in both probands. Chromosomal analysis showed 46,XX karyotype in both of them. **Conclusion:** The present study emphasizes the need for cytogenetic, hormonal and molecular evaluation in cases of ambiguous genitalia for subsequent clinical management such as assignment of gender, appropriate reconstructive genitoplasty and surveillance of the tumor potential of the gonad. Financial support by UGC-UPE and UGC-BSR as PF to BG is acknowledged.

P42

Preliminary Screening for Yq Microdeletions, Polymorphisms and Chromosomal Abnormalities in Indian Infertile Men

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Background: About 20% of the couples fail to achieve pregnancy after one year of unprotected intercourse. A male sole or co-factor is found in approximately 50% of these cases. An abnormal karyotype, a gene mutation/polymorphism, medical conditions, life-style factors, mental stress and environmental causes are usually responsible. It is imperative to rule out the common risk factors before commencement of any form of assisted reproductive technology. **Aim and Objective:** It was proposed to carry out a preliminary cytogenetic and molecular genetic investigation on Indian infertile men attending a fertility centre in the city. **Materials and Methods:** Chromosomal analysis was performed on GTG-banded metaphases from PHA-stimulated leukocytes. Multiplex PCR was carried out using two STS primer sets for each of the classical AZFa, AZFb

and AZFc microdeletions. Polymorphisms in Glutathione S-transferase genes (GSTM1, GSTT1, GSTP1) and C677T and A1298C polymorphisms in methylenetetrahydrofolate reductase gene were also examined. **Results:** All 35 men revealed 46,XY karyotype except one who showed a pericentric inversion of Y chromosome. AZFc microdeletions were detected in three men and one of them also showed a partial AZFb microdeletion. While six men showed a MTHFR CT genotype, the numbers of men exhibiting AC and CC genotypes were 15 and 8 respectively. Four men were double heterozygotes. Two individuals showed null genotypes for both GSTM1 and GSTT1. GSTP1 wild-type genotype in combination with GSTM1 null or GSTT1 null genotype or both was observed in three, two and one individual respectively. A variant Val/Val genotype was recorded in only one case. These results will be discussed in the light of existing literature. **Conclusion:** While AZF microdeletions constitute a known genetic cause of spermatogenic failure, association studies of GST and MTHFR polymorphisms with male infertility have been controversial. The study will be extended to include larger sample size for a significant phenotype-genotype correlation.

P43

Cytogenetic Abnormalities in Patients with Multiple Myeloma Identified by Interphase FISH

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Background: Multiple myeloma (MM) is a clonal B-cell neoplasia characterized by the accumulation of malignant plasma cells in bone marrow, monoclonal protein in the blood or urine and associated organ dysfunction. Monoclonal Gammopathy of Unknown Significance (MGUS) and Smoldering myeloma (SMM) are premalignant states that progress to MM. Several genetic abnormalities have been described to play important roles in the pathogenesis of MM and are contributory to tumor survival, proliferation, metabolism and drug resistance. **Aim and Objective:** It was proposed to screen for specific abnormalities of prognostic significance using interphase FISH in newly diagnosed multiple myeloma patients. **Material and Methods:** A total of 100 multiple myeloma patients diagnosed on the basis of pathological, radiological and clinical features were studied for the presence of non-random chromosome abnormalities using interphase fluorescence in situ hybridization (iFISH). Commercially available probes IGH@, LSI IGH/FGFR3, LSI IGH/CCND1 XT, LSI IGH/MAF, LSI TP53/CEP17, LSI D13S319/13q34 in combination with CEP9 (Vysis) were used. Slides were prepared from cell pellets obtained from direct harvesting of bone marrow aspirates. **Results:** The frequencies of chromosomal abnormalities among the

patients tested were as follows: IGH rearrangements (26%), t(4;14)(p16;q32) (14%), t(14;16)(q32;q23) (6%), t(11;14)(q13;q32) (3%), deletion 17p13.1 (7%), del(13q14.3)/monosomy 13 (25%) and hyperdiploidy as detected using CEP 9 probe (56%). It was observed that 49 of the 69 patients exhibited two or more abnormalities at the time of diagnosis. Evaluation of the clinical implications of these chromosomal abnormalities in terms of time to progression and overall survival is under progress. **Conclusion:** The frequencies of the different cytogenetic abnormalities screened in our subset of the Indian population are similar to those reported in other countries. A larger number of patients have to be examined using more probes to generate data on the types of genetic abnormalities and their prognostic impact in our population.

P44

A Rare Interaction of Homozygous β -thalassemia with HbH disease.

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Background: HbH disease as the severest form of α -thalassemia compatible with life. It usually presents as a chronic non transfusion dependent moderate anaemia, with far less clinical complications than β -thalassemia major. β -thalassemia major with associated HbH disease is very rare. **Aims and Objective:** To evaluate the haematological and clinical presentation due to co inheritance of homozygous β -thalassemia and HbH disease. **Material and Methods:** Investigations included CBC, HPLC and cellulose acetate electrophoresis for Hb analysis and heat stability test. The β -genotype was determined by direct sequencing, the α -genotype by multiplex PCR and the Xmn I [G γ -158 (C $\rightarrow$ T)] polymorphism was determined using PCR-RFLP. **Results:** A 25 years old female was referred to us with low haematological indices (Hb: 7.4 g/dl; MCV: 45.8 fL; MCH: 12.2 pg; MCHC: 26.7 %, high RDW: 27.8%). There was no enlargement of the liver and spleen and no growth retardation. She had no history of any blood transfusion but had complaints of weakness. On HPLC, the HbA2 level was 15.0% and HbF 0.9%. The peripheral smear showed anisocytosis and poikilocytosis with microcytic and hypochromic RBCs. Reticulocytes count was found to be 1.5%. Heat stability test for unstable haemoglobins was negative and no fast moving or abnormal band was observed on alkaline haemoglobin electrophoresis. DNA sequencing of the β -globin gene showed a homozygous β^+ mutation at 5' UTR +20 (-C). The Xmn I [G γ -158 (C $\rightarrow$ T)] polymorphism was absent (-/-). Her α genotype showed the deletion of three α genes (-a3.7/--SEA). **Conclusion:** The co-inheritance of homozygosity for a β -thalassemia mutation along with HbH disease may have led to more balanced α /non α globin ratios leading to a milder form of the disease.

P45

Vitamin D Receptor Gene Polymorphisms in Gallbladder Cancer Patients of North Central India

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Background: Gallbladder cancer is an aggressive malignancy and the most common biliary tract tumor in the World. It is much more common in the women of North and Central India. The etiology of the gall bladder cancer remains ambiguous. The VDR is a crucial mediator for the cellular effects of Vitamin D and additionally interacts with other cell signaling pathway and is involved in cell proliferation. **Aim and Objective:** The aim of the present study is to elucidate possible association of Apal, FokI and TaqI polymorphism of VDR in gallbladder cancer patients of North-Central India. **Material and Methods:** A total of 75 controls and 60 cases (GBC) were genotyped for the three different VDR polymorphisms, viz Apal, TaqI, & FokI and comparison in different groups were made between cases & control. For Apal Cases (n=55) Controls (n=72), For TaqI Cases (n= 55) Controls (n= 66), For FokI Cases (n= 60) Controls (n= 76). χ^2 test was performed to test the statistical significance. **Results:** Different proportion of the three genotypes i.e. AA, Aa, and aa of VDR Apa I polymorphism, TT, Tt, and tt of VDR TaqI polymorphism, and FF, Ff, and ff of VDR FokI polymorphism were observed. In our case-control study, we found no significant association was found between the genotypes and allele frequencies in gallbladder cancer cases and control for VDR gene Apal ($p=0.7893$ for "aa" and $p=0.7748$ for "a"), TaqI ($p=0.4398$ for "tt" and $p=0.3650$ for "t") and FokI ($p=0.8570$ for "ff" and $p=0.8676$ for "f"). **Conclusion:** From the above study we infer that VDR might not be involved significantly in GBC. It is also not reflected in the combined genotype and allele frequencies of Apal and TaqI polymorphisms, which are known to be in linkage disequilibrium.

P46

Expression Analysis of S100 Calcium Binding Protein A9 and ERBB2 in Gallbladder Cancer.

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Background: Gallbladder cancer is an uncommon malignancy and a rare neoplasm with high incidence in North India. Cholecystectomy is the only way of treatment left. Lack of diagnostic biomarker(s) and being chemotherapy resistant in nature, make this disease fatal. Therefore for improving the survival rate of GBC patients, identification of biomarker is a necessity. S100A9 is known to modulate signal pathways to directly promote invasion, migration and metastasis, probably

via activation of NF- κ B, Akt or MAP kinases and ERBB2 has a role as tyrosine kinase. **Aims and Objectives:** The aim of this study is to analyze the expression of S100A9 and ERBB2 in order to understand their likely role in gallbladder carcinogenesis. **Material and Methods:** Approx 70 samples of cancers (GBC) and 34 of non tumor samples were recruited for IHC and 28 samples for methylation study. Immunohistochemistry was carried out on Tissue Microarray (TMA) in gallbladder cancer tissues and compared with that of non tumor tissues. Bisulfite modification, followed by MS PCR, was performed to identify the promoter methylation. χ^2 analysis was performed to test the statistical significance. **Results:** IHC revealed underexpression (33/71 of cases; $p=0.6$) and hypermethylation (14/25 of cases; $p=0.4$) of S100A9 in GBC in contrast ERBB2 (49/74 of cases; $p=0.1$) was found overexpressed. **Conclusion:** Downregulation and upregulation of S100A9 and ERBB2 respectively were shown by immunohistochemistry and MS PCR which seem to be involved in the progression of GBC directly or indirectly. We propose that there two genes may be used as diagnostic and prognostic biomarkers for GBC in future therapeutics. However, these results require further validation by Real Time PCR in large number of samples size.

P47

Study on the Whole Genome Methylation Profiling in Gall Bladder Cancer and Gall stone Disease.

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Background: Gallbladder cancer is a serious fatal disease, ranking sixth amongst gastrointestinal cancers with gall stone as major risk factor. Therefore, we intended to study the relation of gallbladder cancer with gallstone in terms of differential methylation pattern and changes in the expression pattern of the corresponding gene. **Aim and Objective:** To investigate the role of differential methylation regions (DMRs) in the whole genome of gallbladder cancer and gall stone disease. **Material & Methods:** Whole genome methylation profiling was performed on 24 samples using Illumina Infinium HD450K assay, Data normalization and analysis was done through Genome studio software version 1.8. **Results:** Analysis of the whole genome methylation data was performed on three data sets, i.e. Chol (Cholelithiasis) vs ANT (Adjacent non tumor), Tumor vs ANT and Tumor vs Chol to reach any significant relation. To reduce any biasness, the most possible sources of variation, viz. sex chromosomes, SNP probes and DMRs with $p>0.05$ were excluded. After filtering we obtained a total of 33,343 CpG sites (DMRs). A subsequent change in the proportion of the sites was observed for the three data sets, as 1818/33443 (5%), 6968/33443 (21%) and 24657/33443 (74%) for CHOL vs ANT, TUMOR vs ANT and TUMOR vs CHOL respectively. Also, we found that most of the DMRs were present on CpG

islands (50%). and at the 1st exon and proximal promoters. The functional annotation and pathway ontology revealed that most of the genes fall under the signaling pathways with DNA binding and transcription factor activity. **Conclusion:** The analyzed data, revealed a differential methylation pattern in our sample groups. We infer that methylation is likely to play a role in the pathogenesis of gallbladder cancer and that cholelithiasis is a potential risk factor in the molecular pathogenesis of GBC.

P48

ADH1 and ALDH2 gene polymorphisms among the Koraga population of South Karnataka, India.

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Koragas are aboriginal tribe of Dakshina Kannada, Udupi districts of south Karnataka. Recent census studies show that the size of this population is decreasing annually due to many factors and among which high addiction to alcohol might be the leading contribution factor. The metabolism of alcohol is significantly influenced by the human behaviour of alcohol consumption and their adaptation. Alcoholism leads to alcohol-induced organ damage. To understand pattern of resistance/susceptibility of these population to alcoholism, we have undertaken gene targeted SNP study. Four functional polymorphisms included in this study are at genes class I alcohol dehydrogenase (ADH 1) and aldehyde dehydrogenase2 (ALDH2) that plays a role in metabolism of ethanol to acetaldehyde and acetaldehyde to acetate respectively. Two SNPs are present in ADH1B gene (Exon3-Arg47His and Exon9-Arg369Cys), one SNP at ADH1C gene (Exon8-Val349Ile) and one SNP in ALDH2 gene (Exon 12-Glu487Lys). Our result showed that these SNPs are associated with alcoholism with significant P-value in Koraga population of Dakshina Kannada and Udupi district.

P49

Identification of Hepatocyte nuclear factor-1beta (HNF1B) gene deletions in Indian diabetic patients with renal abnormalities

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Background: Heterozygous mutations in the transcription factor HNF1B have been reported to be responsible for maturity-onset diabetes of the young (HNF1B-MODY or MODY5) associated with various renal abnormalities. Whole HNF1B gene deletions accounts for 50% of HNF1B-MODY. **Aim:** To investigate the prevalence of whole or partial HNF1B gene deletion mutations in young Indian diabetic patients with various renal abnormalities, selected from Dr.Mohan's Diabetes Specialities Centre, Chennai. **Material and Methods:** Fifty unrelated young diabetic patients with renal abnormalities were screened for

whole or partial gene deletion using Multiplex ligation probe amplification assay (MLPA) MODY P241-B1 kit (MRC-Holland, Amsterdam, The Netherlands). Fragment analysis was carried out using standard methods on the ABI 3500 analyser. Results were analysed using Genemarker software. **Results:** The fragment analysis revealed heterozygous whole gene deletion of exons 1-9 (Met1_Trp557del) in two unrelated patients. One of the patients had mildly contracted kidneys with multiple cysts and congenital short pancreas. The other patient showed evidence of absence of right kidney with mildly enlarged left kidney. Both the patients had no history of renal abnormalities or diabetes among any of the family members. The deletion mutation was found to be de novo in origin in one of the patients whose family was tested for the inheritance. Therefore, patients with diabetes and renal cysts in the absence of any family history should also be considered for genetic testing for HNF1B-MODY. **Conclusions:** This is the first major study of HNF1B-MODY from India and shows that about 4% of young diabetic subjects with renal abnormalities seen at a tertiary diabetes centre harbour whole gene deletion mutation.

P50

Do δ -Globin Gene Variants Contribute in Substantial Reduction in HbA2 Levels?

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Background: Mutations in the δ -globin gene are not pathogenically relevant, but co-inheritance of δ -globin variants along with β -globin gene defects can complicate the diagnosis of β -thalassemia trait. Hence identification of δ -globin variants is critical for identifying couples at risk of β thalassemia particularly when one partner is a classical carrier. **Aim and Objective:** To determine the effect of δ globin gene variations on outcome of HbA2 levels. **Materials and methods:** Haematological analysis was carried out on a Sysmex K-500 analyser. The HbA2 and HbF levels were measured by HPLC. β and δ globin gene analysis was carried out by CRDB and by direct DNA sequencing respectively. **Results:** Here we report a family who had a child clinically presenting like β -thalassemia major. On investigation, the father had a classical β thalassemia trait picture having HbA2 level of 4.4% and was a carrier of IVS 1-5 G $\rightarrow$ C mutation. Interestingly the mother showed substantially reduced HbA2 level (1%) with an additional elution peak after the normal HbA2 peak on HPLC. Molecular analysis of the β globin gene showed that she was a carrier of the β thalassemia mutation IVS 1-5 G $\rightarrow$ C. The δ -globin gene analysis showed the presence of HbA2 St. George (CD 81 C $\rightarrow$ T) mutation. She showed absence of α thalassemia. Another case referred for haemoglobin electrophoresis showed near absence of HbA2 (0.2%). Molecular analysis showed one gene deletion in α globin gene (-a3.7/a α) and β globin gene was found to be normal, but the delta globin gene analysis showed presence of HbA2 Saurashtra (CD 100 C $\rightarrow$ T). **Conclusion:** These results show that few δ chain variants can remarkably reduce the HbA2 levels creating a problem in carrier detection of β -Thalassemia.

P51

Altered DNA Methylation in Young Coronary Artery Disease Patients Identified Using Whole Genome Methylation Profiling

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Background: Coronary artery disease (CAD), is a complex metabolic disorder characterized by development of plaques in the coronary arteries in the heart According to WHO, mortality due CAD in India was 2.03 million in 2010 and is expected to be 2.6 million by 2020. We had earlier reported that genomic DNA methylation is significantly higher in CAD patients as compared to healthy individuals. In this study we attempted to identify the regions that were differentially methylated in young CAD patients. **Aims and Objectives:** To identify differentially methylated regions in Coronary Artery Disease patients. **Materials & Methods:** A total of 17 angiographically proven CAD and 16 controls were selected for this study. Genomic DNA from these individuals was enriched for methylated DNA using 5-methylcytidine antibody. The methylation enriched DNA was sequenced for 50 cycles on an Illumina HiSeq (2500) analyzer to sequence methylated regions. Sequenced raw reads were then mapped back to the human genome reference sequence (hg19) using Bowtie2 aligner, and then diffReps software was used for finding potential differentially methylated regions (DMRs) between cases and controls. **Results:** A total of 2220 DMRs were identified in these CAD patients. Of these, 75 were in the promoter region, 312 in the gene body, 749 in pericentromeric region, 39 in subtelomeric region and 1044 in other regions including repeats. A total of 148 were differentially methylated in CAD patients of which 91 were up regulated and 57 down regulated. Genes involved in one carbon metabolism pathway, MAP kinase pathway etc. were found to harbour the DMRs. **Conclusion:** This is the first whole genome methylation map for CAD cases. Our data has lead to the identification of a few novel regions that could be associated with CAD in Indian population. Validation of the data is currently underway.

P52

DNA Copy Number Changes in Myelodysplastic Syndromes

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Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal haematopoietic stem cell disorders. The International Prognostic Scoring System (IPSS) introduces cytogenetic abnormalities together with cytopenia and percentage of marrow blasts as independent prognostic factors for both survival and progression to AML. Chromosomal

abnormalities have been reported at a frequency of 20-50% in MDS cases at presentation with common abnormalities such as (-5/del(5q), -7/del(7q), +8, +9, +11, del(11q), del(12p), del(17p), -18, +19, del(20q), +21). We designed a dedicated multiplex PCR assay (quantitative multiplex PCR of short fluorescent fragments or QMPFSF) to measure the copy number of genes located at several loci susceptible to gains and losses in MDS. **Aims and Objectives:** Detection of DNA copy number changes in MDS subgroups using QMPFSF assay. **Material and Methods:** A total of 60 primary MDS patients were included in our study. We designed two distinct QMPFSF assays which target 20 genes at several loci implicated in pathogenesis of MDS. QMPFSF is based on simultaneous amplification of multiple target sequences using dye labeled primers under semi-quantitative conditions that allow rapid and reliable comparison of the fluorescence of each amplicon in test samples and in controls. Allele dosage was calculated using: visual sample-to-control comparison and numerical sample-to-control comparison. **Results:** A total of 20 (33.3%) patients showed deletions majorly involving 7q loci gene EPO (15%) and 7p loci gene SEC61 (10%) while 5 (8.3%) patients showed duplication in EPO, SEC61, EGR1, CD69 and CDKN1B genes by QMPFSF previously undetected by CC or FISH. **Conclusions:** These results demonstrate that QMPFSF assay targeting 20 genomic regions located on the six chromosomes most frequently involved in MDS can be helpful for the detection of chromosomal abnormalities in MDS patients for whom cytogenetic data are not available. This method cannot replace CC. However, a prospective evaluation should confirm the clinical impact of this non-invasive and inexpensive approach.

P53

Association of Alu-ACE and CYP1A1 genes with colon cancer

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Introduction: Globally more than 1 million people get colon cancer every year resulting in about 715,000 deaths as of 2010 from 490,000 in 1990. As of 2012 it is the second most common cause of cancer in women and the third most common in men with it being the fourth most common cause of cancer death after lung, stomach, and liver cancers. It is more common in developed than developing countries. **Objective:** The objectives of the present study are to analyze the Alu-ACE and CYP1A1 markers for their allelic variation and to estimate the association between colon cancer and these markers. **Materials and Methods:** In the present study, 30 cases (15 males and 15 females) presenting colon cancer and 30 cases of age and sex matched healthy controls were included. After isolation of genomic DNA, genotyping of ACE and CYP1A1 genes was done by PCR followed by restriction fragment length polymorphism [PCR-RFLP], using specific primers. The statistical significance of differences between patients and controls were tested. Analysis of the data was carried out using Epi Info 5 software. Relative risk

was calculated by the random-effects method. For odds ratio, confidence interval was calculated. The significance level was 5%. **Results:** The results revealed that the chi-square test for homogeneity for ACE and CYP1A1 systems was found to be non-significant in patients with respect to controls. The inter group heterogeneity for ACE system was found to be a significant (ACE: $\chi^2 = 5.5786$; d.f. = 2; $0.05 > p > 0.10$), indicating a significant association between colon cancer and ACE marker. Whereas, the inter group heterogeneity for CYP1A1 system was found to be non-significant. Risk estimates show a significant association of ACE – ID phenotype (RR = 1.90) with an overall odds ratio of 3.45 and an increased risk of 50% and more, indicating that individuals with ACE-ID phenotype were more likely to get the disease when compared with the other phenotypes. Likewise, Risk estimates show significant association of homozygote (m2m2) and heterozygote (m1m2) phenotypes with colon cancer individuals (RR = 1.75 and 1.27), with an increased risk of 50% and more, indicating that individuals with these phenotypes were more likely to get the disease when compared with the other phenotype of the CYP1A1 polymorphism. **Conclusion:** Thus, our study concludes that there is no association found between the Alu-ACE and CYP1A1 markers with colon cancer. However, in Alu-ACE and CYP1A1 markers, the odds ratio and relative risk estimates of DD and m2m2 phenotypes showed an increased risk to have the disease when compared with the other phenotypes.

P54

PON1 gene polymorphisms and its activity in Indian women with PCOS

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Background: Polycystic ovary syndrome (PCOS) is a complex multigenic endocrinopathy with long term health implications like Type 2 diabetes mellitus and cardiovascular disease. It is the most common cause of anovulatory infertility and its cardinal features include hyperandrogenemia, menstrual dysfunction and insulin resistance. This multifaceted syndrome is influenced by genetic and environmental factors. Paraoxonase is a calcium dependent antioxidant enzyme which exerts a major cardioprotective effect via its ability to inhibit LDL oxidation and promote macrophage cholesterol efflux. The serum PON1 activity is partially determined by genetic variations in coding and non-coding region of PON1. **Aims and Objectives:** The present study aims to delineate the association of PON1 genomic variants with enzyme activity and cardiovascular disease risk factors in women with PCOS. **Materials and Methods:** Two coding region polymorphisms, L55M and Q192R, have been evaluated by PCR amplification of genomic DNA followed by RFLP in both PCOS and age matched healthy control women. Clinical, anthropometric and biochemical parameters related to metabolic and hyperandrogenemic traits have been also estimated. We have measured the arylesterase

and paraoxonase activity of PON1 towards phenylacetate and paraoxon respectively. **Results:** L55M polymorphism showed significant association with PCOS risk; however there were no difference between distribution of genotype and allelic frequency between controls and PCOS for the Q192R polymorphism. PON1 arylesterase activity is reduced in PCOS women compared to controls while paraoxonase activity is comparable between the two groups. **Conclusion:** Our study supports the role of PON1 as an important predisposing factor in PCOS development and will be helpful to unravel PCOS pathophysiology.

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In silico DNA Protein interaction study on Wild and Mutant sequence of PAX9

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Background: PAX9 is a transcriptional regulator of PAX family which has highly conserved DNA-binding region, the paired domain. PAX9 plays an important role during mammalian tooth development and its mutation leads to tooth agenesis. Attempt has been made to study the DNA protein interaction of a missense mutation ser43lys (S43K) and Phe151le (F151I) in paired domain of PAX9, recently reported in a study conducted in Chinese family and European descent segregating autosomal dominant nonsyndromic tooth agenesis. **Aim:** In Silico Prediction and Identification for cause of destabilized interaction/s between target 3D-DNA and mutant PAX9 protein. **Objectives:** (1) In Silico homology modeling of 3D structure of wild and mutant PAX9 protein with target DNA (2) Assessment of physiochemical properties in wild type and mutant protein while interacting with target. **Material and Methods:** 3D structure of wild and mutant PAX9 was modeled using Mod web and energy minimization was done by YASARA server. Physiochemical properties were calculated through Protparam tool. 3D DNA structure was modeled with the EMSA (Electrophoretic Mobility Shift Assay) e5 probe "CACC GCACGATTAGCACC GTTCC GCTCAGGCTGTCCCC". Modeling of DNA was performed by 3D-DART. The optimized model for protein and DNA were docked to illustrate the interaction between wild and mutant protein with DNA using HADDOCK Easy interface. **Results:** Study of Physiochemical properties on wild and mutant PAX9 protein are encouraging and revealed that the molecular weight, isoelectric point, positive residue, negative residue, instability index and, GRAVY value get diverse in wild and mutant PAX9. The DNA protein interaction study showed that the mutant PAX9 (S43K and F151I) protein cause decrease in affinity for DNA than wild type protein however mutant PAX9 (S43K) has more DNA binding affinity than mutant PAX9 (F151I). **Conclusions:** In silico studies are proven significant to delineate the molecular mechanism underlying mammalian tooth agenesis and being experimentally validated. **Keywords:** PAX9, Physiochemical properties, DNA-Protein interaction, HADDOCK Easy interface.

P56

Cytogenetics in Bad Obstetric History and Genetic counseling.

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Background: About 15% to 20% of pregnancies end in spontaneous abortion, mostly in the first trimester. At least 50% of clinical abortions result from chromosomal abnormalities. Chromosomal translocation in either parent is the most common structural rearrangement involved in spontaneous abortions. **Aims and Objectives:** The aim was to identify chromosome abnormality as an etiological factor in couples with bad obstetric history (BOH). **Material and Methods:** Our study included couples with BOH referred to the Departmental Clinic for Karyotyping and genetic counseling. A detailed proforma was filled for each case with detailed pedigree, family history etc. Peripheral blood lymphocyte cultures and G banding was done as per the routine protocol. **Result:** Case I: The karyotype of the husband was normal (46,XY) and the wife had a reciprocal translocation involving chromosome 2 and 10, with karyotype 46,XX,t(2;10)(q31;q25). Case II: The karyotype of the wife was normal (46,XX) and the husband showed a reciprocal translocation with karyotype 46,XY,t(5;9)(q13;q32). **Conclusion:** The cases highlight the importance of cytogenetic analysis in all couples with BOH. The identification of a balanced chromosomal rearrangement in a parent is useful because it provides not only an explanation for the miscarriages, but also information about the future pregnancies. Genetic counseling should be provided to such couples, which includes an explanation of the findings, risk for miscarriages and live birth with congenital anomalies and a discussion of reproductive options, including prenatal diagnosis. Hence, in cases of BOH, cytogenetic examination of both partners should be routinely done which will facilitate genetic counseling and management accordingly.

P57

Inherited and Acquired Thrombophilia in Women with Recurrent Pregnancy Loss

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Background: 15% of reproducing couples suffer from pregnancy loss (PL) and recurs in 2-3%. The most frequently hypothesized cause of unexplained PL refers to a defective maternal haemostatic response leading to uteroplacental thrombosis. **Aims and objectives:** The present study aims to investigate the prevalence of different genetic and acquired thrombophilia markers in a large series of Indian women with RPL. **Material and Methods:** The study comprised of 578 women with no apparent etiological causes of RPL and 115 healthy women with at least one live child and no history of PL or thrombotic episode. Genetic thrombophilia- PC, PS,

antithrombin (ATIII), Factor V Leiden mutation (FVL) were done in 578 patients and EPCR 23bp insertion, MTHFR polymorphism, PAI-1 4G/5G polymorphism in 488 patients. Acquired thrombophilia- LA and ACLA were done in 550 patients; A β 2GP1 and AAnnVA could be analyzed only in 532 patients. p values were calculated with two tailed Fisher's exact test, and statistical significance was assumed at $p < 0.01$, 99% confidence interval. **Results:** Among genetic thrombophilia, the risk of PL was highest with PS deficiency (16%, 99% CI, $p=0.006$) followed by PAI-1 4G/4G (19%) polymorphisms, PC deficiency (6%). Among antiphospholipid antibodies, the risk of PL was the highest in women with ACLA i.e. 24% and 2.6% in patients and controls, respectively. This was followed by AAnnVA - 18% against 1.7%, A β 2GP1- 11% against 1.7% and LA- 8% against 0.86% in patients and controls, respectively (99% CI, $p<0.01$). **Conclusion:** All Hematologists and Gynecologists should support thrombophilia screening as thrombophilia, both genetic and acquired, is an important contributing factor in RPL and women with unexplained PL should be screened in a cost effective method in the order of their prevalence.

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Biomonitoring and Genetic risk assessment in Petrol Pump attendants

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Background: Biomonitoring of exposure in workplaces has gained importance in evaluation of human health hazards. Petroleum fumes contains major organic products (Phenolic compounds, polycyclic aromatic hydrocarbons, benzene, toluene etc), which on chronic exposure can result in many disorders such as inflammation, lung function, asthma and oxidative stress, and exposure to gasoline vapors is classified by the International Agency for Research of Cancer as possibly carcinogenic to humans. Petrol station attendants are chronically exposed to petroleum derivatives through inhalation of petrol during vehicle refueling. **Aim and Objectives:** The aim and objective of present study is to determine the genotoxicity risk amongst Petrol pump attendants exposed to gasoline fumes and its in vitro amelioration. **Materials and Methods:** Urine sample analysis was done to evaluate the Phenol concentrations. Free radical toxicity parameters such as Superoxide Dismutase (SOD), Catalase (CAT), Glutathione (GSH) and Glutathione peroxidase (GPx) were measured to check the effect of gasoline exposure. While probable genetic damage was determined by Sister Chromatid Exchanges (SCEs) in peripheral blood lymphocytes of study subjects. Further in-vitro amelioration was also studied using antioxidants such as Vitamin C and Vitamin E. **Results:** Urinary phenol levels were increased significantly in sample populations as compared to controls; ROS parameters were also found to be significant amongst exposed individuals. Moreover, SCE frequencies were also found significantly increased due to exposure of gasoline fumes which showed amelioration by addition of antioxidants (Vitamin

C and E) to the exposed blood cultures. **Conclusion:** The data showed a direct co-relation between increased urinary phenol levels and ROS parameters due to occupational exposure. Moreover, it also displayed a potential to cause genetic changes in the exposed subjects. Vitamin C and E revealed protective effect against genotoxicity caused due to prolonged exposure. The data highlights the need to maintain safety measures and intervention to minimize exposure.

Keywords: Genotoxicity, Urinary Phenol, ROS, Vitamin C, Vitamin E.

P59

Mitigating effects of curcumin and vitamin C against cadmium induced toxicity

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Background: Cadmium (Cd) is a toxic, ubiquitous heavy metal of continuing occupational and environmental concern with a wide variety of adverse effects. It persists in the environment as highly toxic metal to humans. It has an extremely long biological half-life that essentially makes it a cumulative toxin. It has been listed as number 7 in its top 20 list of hazardous substances by Agency of Toxic Substances and Disease Registry and as a group 1 carcinogen by the International Agency for Research on Cancer. Cadmium exposure mainly occurs in industrial settings, cigarette smokes and cadmium contaminated water, air and food are other non-occupational source of human exposure to Cadmium. In contrast to the activity of Cadmium, Curcumin the yellow coloured polyphenolic super antioxidant derived from powdered rhizome of *Curcuma longa* is useful in various debilitating conditions. It is also recommended in cancer prevention and as a treatment adjunct in various diseases. While Vitamin C is a water-soluble dietary antioxidant that plays an important role in controlling the oxidative stress. It can also protect against damage induced by reactive oxygen species. **Aim and Objectives:** The aim of present study was to evaluate the genotoxic and cytotoxicity effects of Cadmium on the Peripheral Blood Lymphocyte Culture (PBLC) and to study the mitigating role of Curcumin and Vitamin C as potent antioxidants against cadmium induce toxicity. **Materials and Methods:** Genotoxicity parameters such as Sister Chromatid Exchanges, Cell Cycle Proliferative Index, Average Generation Time and Population Doubling Time is being studied by PBLC while, free radicals toxicity parameter such as Lipid Peroxidation, Glutathione as well as enzyme require to overcome oxidative stress i.e. Glutathione peroxidase, Glutathione reductase, Glutathione-S-transferase, Superoxide dismutase and Catalase were measured to check the effect of Cadmium induced oxidative stress influenced cell cycle and genetic aberrations. **Results and Conclusion:** The results reveals a significant toxicity in dose dependent manner in Cadmium added cultures while co-administration of Curcumin and Vitamin C along with Cadmium shows a significant protection against cytogenetic damage, indicating their combined protective role which was calculated

by percentage of amelioration. The significance of these results will be discussed.

Keywords: Cadmium, Genotoxicity, Oxidative Stress, Vitamin C, Curcumin.

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Role Of Some Antioxidants On Cyclosporine Induced Cytogenetic Alterations In Human Blood Cultures

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Background: Cyclosporine, an oligopeptide from the fungus *Tolypocladium inflatum*, consists of eleven amino acids. It is a potent immunosuppressant administered primarily during immunosuppressive therapy to prevent graft-versus-host reactions and to control diseases associated with immunological defence. Several studies indicate that cyclosporine target transporter family of proteins and exhibit affinity towards Ca²⁺ channel which results in blocking of T-cell activation. Immunosuppression is thought to be the result of impairment of T-cell function. Hence, this study was conducted to investigate Cyclosporine induced genotoxic effects on human lymphocytes. Further, in vitro amelioration was also assessed using mitigating antioxidants such as Curcumin and Melatonin as they are free radical scavengers possessing anti-oxidative and anti-inflammatory properties. **Aims and Objectives:** To demonstrate the ameliorative effects of curcumin and melatonin against cyclosporine induced genotoxicity in blood lymphocytes at various dose levels. **Materials and Methods:** Peripheral Blood Lymphocyte cultures (PBLC) were set up from blood samples of healthy donors (aged 18 to 25 years). The study was divided into ten groups consisting of untreated Control, Curcumin alone (3.8µM), Melatonin alone (0.2mM), Low dose (2.93 × 10⁻² µM), Mid dose (5.86 × 10⁻² µM) and High dose (0.12 µM) of cyclosporine in combination with and without Curcumin and Melatonin as well as positive control treated with Ethyl Methyl Sulphonate (EMS; 1.93mM). The genetic damage indicators studied were Cell Cycle Proliferative Index (CCPI), Sister Chromatid Exchanges (SCEs), SCEs/plate, SCEs/chromosome, Population Doubling Time (PDT) and Average Generation Time (AGT). Statistical analysis was done by student's t-test. **Results:** A dose dependent reduction was seen in CCPI of treated cultures in comparison to the controls. An increased frequency of SCEs was significant due to exposure to cyclosporine. Curcumin and Melatonin supplementation to treated cultures were found to ameliorate the genotoxic effects induced by cyclosporine. A comparison of all groups with untreated control and pro-oxidant group with respective antioxidant groups was also evaluated. **Conclusions:** Curcumin and Melatonin led to a significant decrease in the genotoxic indices exerted by Cyclosporine exposure to blood cell cultures. The outcome of this study is useful in addressing the adverse effects of cyclosporine in various medical conditions and promotes the supplementation

of antioxidants in cyclosporine-based therapies.

Keywords: Genotoxicity, Cyclosporine, Antioxidants, PBLC, CCPI, SCE.

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Some cytogenetic aspects of infertility in western India

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Background: The term infertility encompasses a wide variety of disorders. The identification of genetic factors causing infertility in humans remains noticeably deficient at present and hence the identification of genetic factors causing infertility has become important. **Aim and Objectives:** The aim of the present study was to analyse some etiological aspects of infertility in western Indian population. **Materials and methods:** The infertile males, infertile females and similar number of control individuals were recruited in the present study after taking their due consent. Controls were age matched fertile and chromosomally normal male and female individuals. The standard procedure of Hungerford (1965) was followed with slight modifications for metaphase chromosome preparations. G-banding was done by the method of Sun-Chu-Chang (1973) and karyotyping done. Hundred metaphases were analyzed in infertility patients and age matched control individuals for the various parameters. The statistical analysis was done by student's t-test. **Results:** Karyotype study revealed that one of the male individual had a balanced translocation involving chromosomes 5 and 9 and all the other cases were showed normal karyotype. The hypoploidy rate, the hyperploidy rate and total aneuploidy rate was significantly increased in infertile males as compared to control males. In infertile females the rate of hypoploidy and total aneuploidy was non-significant, while hyperploidy was significantly increased when compared to the control females. The single chromatid telomeric association frequency, double chromatid telomeric association frequency and the total telomeric association frequency in infertile males and females was non-significant as compared to the respective control individuals. **Conclusions:** The data of our study supports the hypothesis that aneuploidy is common in infertile patients and even significant etiologic factor leading to fertility problems. Therefore this should be confirmed in a larger group of infertile cases.

Keywords: Infertility, aneuploidy, telomeric association.

P62

Genetic variants of ABCA1 and coronary artery disease

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Background: Coronary heart disease is the leading cause of death worldwide, responsible for over 7 million deaths annually. Low HDL-C levels constitute an independent risk factor for Coronary artery disease. The ATP-binding cassette transporter A1 (ABCA1) is crucial in the initial step of HDL formation and in reverse cholesterol transport. **Aims and Objectives:** To test the SNP's of the ABCA1 gene and to associate the variations with CAD. **Materials & Methods:** Sample Collection: 150 angiographically verified CAD patients with 150 angiographically negative, age and sex matched CAD controls, visiting the P. D. Hinduja Hospital and Medical Research Center's catheterization laboratory were recruited for the study. Genotyping: DNA extraction was done by modified Miller et al method. 183 ABCA1 variants were genotyped with the Human Cardiometabo Beadchip using the Infinium II Assay. 8 variants not included in the microarray were genotyped by conventional allele specific PCR. **Results:** Of the variants genotyped by conventional PCR, frequency of the variants of I883M, V771M and V825I were observed to be higher in controls over cases, while T1427 variant was seen in more cases than controls. Among the variants genotyped by DNA microarray, 51 variants were seen more in cases over controls, while 36 variants were observed in controls over cases. The ABCA1 variant chr9: 10669854 significantly correlated with the cases ($p=0.027$). **Conclusion:** Preliminary results suggest that ABCA1 variant at position chr9:106698544 may be associated with CAD.

P63

Study of Chromosomal Aberrations and Molecular Mutations in Myelodysplastic Syndrome

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Background: Myelodysplastic syndrome (MDS) is a heterogeneous group of clonal hematopoietic stem cell disease. Chromosomal aberrations, frequency ranging from 20-50% have been reported in MDS. Various molecular mutations are associated with MDS. We have carried out a study to detect chromosomal abnormalities and molecular mutations in ASXL1, TET2 genes in MDS. **Aim and Objective:** To study the chromosomal aberration and molecular mutations in MDS patients and its correlation with prognosis. **Material and Method:** Cytogenetic study was carried out in 100 clinically diagnosed MDS patients. Cytogenetic study was done using GTG- banding and Fluorescence in situ hybridization (FISH). DNA was isolated and mutation analysis of TET2 and ASXL1 gene was carried out using specific primers and sequencing was done according to standard procedure. **Results:** The MDS subgroups in our study were RA (35%), RCMD (40%), RAEB-1 (20%), RAEB-2 (4%) and MDS-U (1%). The chromosomal aberrations were detected in 54% of MDS patients and the type of chromosomal aberrations were deletions 7q (17%), del 20q (18%) and trisomy 8 (16%). Molecular analysis of ASXL1 gene mutations including 3 novel mutations were detected

in 5 and 2 novel mutations were detected in TET2 gene. **Conclusions:** Confirmation of conventional cytogenetic is important in the diagnosis and prognosis of the disease. The molecular mutations such as TET2 and ASXL1 gene play an important role in understanding the progression of the disease.

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A Novel Phenotype in Complete Androgen Insensitivity Syndrome due to Δ Phe583 in Androgen Receptor Gene

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Accurate Androgen Receptor (AR) function is crucial for male sex development and maintenance of secondary male characteristics. Mutation in AR gene leads to androgen insensitivity syndrome. Individual bearing the complete form of Androgen Insensitivity (CAIS) present a female phenotype and lack of pubic and axillary hairs. Here, a case was presented with oligoamenorrhoea with complete androgen insensitivity syndrome (CAIS). Mutational analysis by Sanger sequencing of complete AR gene revealed an in frame 3 nucleotide deletion at codon 583 (or 584) in DNA bonding domain (Exon 2). This deletion removes one of the phenylalanines that invariably occupy adjacent positions in the N-terminal α -helical region of DBD of AR receptor. Mutation lies in P-box region responsible for the base-specific contacts with the DNA major groove. Patient had most of the features of CAIS like-high level of FSH and LH, both ovaries absent, hypoplastic uterus and female like physical appearance but milder form of amenorrhoea i.e Oligoamenorrhoea. This mutation may affect the binding of androgen receptor completely or partially to its downstream genes.

P65

Syndromic and Non-Syndromic Polydactyly

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The GLI3 protein is a zinc finger transcription factor, expressed early in development. GLI3 exhibit allelic heterogeneity as mutations in this gene are associated with several developmental syndromic and non-syndromic polydactyly. The present study reports a familial case of Greig Cephalopolysyndactyly Syndrome (GCPS), showing an autosomal dominant pattern of inheritance and a sporadic case with both postaxial polydactyly (PAP) type A and B. Resequencing of GLI3 gene reveals a previously reported nonsense truncation mutation g.42007251G>A (rs121917714, p.R792X) in GCPS family and a novel single nucleotide insertion g.42004239_42004240insA (p.E1478X) in the sporadic case of PAP. Both nonsense

truncation mutations p.R792X (in GCPS) and p.E1478X (in PAP) introduce a premature stop codon within its N-terminal and C-terminal portions respectively. The N-terminal truncation causes the loss of both downstream trans-activation (TA) domain and CBP-binding module, whereas C-terminal truncation leading to the loss of one TA domain (TA1) of GLI3. C-terminal truncation of GLI3 results in the inability of the protein to activate transcription of the target genes which might be leading to postaxial polydactyly type A and B (PAP) in the patient.

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Essentiality Verses Affordability Of Genetic Tests: Two Ends Of Spectrum In India

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Introduction: Prenatal screening tests are essential tools in prevention of fetal anomalies or inherited disease. With the evolution of newer tests in genetic field like microarray and mutation analysis, diagnosis for many unknown genetic disorders is not beyond the reach now. However because of poor socioeconomic status, affordability of these diagnostic tests has important negative impact on its practical applications. This is especially true for developing country like India. In this situations of moral crisis, the usual decision taken by most of parents is that of Neutrality. Here we have tried to present some of these symbolic cases. **Methods:** For all the cases referred, detailed history was assessed. Depending upon the requirement of individual case the prenatal screening test was offered along with non-directive counseling. Case 1: 25yr old lady [G3, P1, L2, A0], with 6yr old healthy daughter and 3yr old son affected with Down's syndrome. In presented with gestational age 19-20wk, USG showed hypoplastic nose and VSD in fetus. Advised amniocentesis and FISH during counseling but patient did not come for follow-up. Case 2: 19yr old primigravida came with the report of hydrops secondary to cystic hygroma. Advice of amniocentesis was given but patient directly came for follow-up during 28wk of gestation. At that time she was having USG reports from some centre out of Maharashtra, showing hyperechoic bowel, bones not corresponding to their parameters. Case 3: 28yr old lady [G5, P1, L2, A 2] came at 8th wk of gestation. She has history of first son 9yr old with mental retardation and dysmorphic features. With the history of 2 spontaneous abortions and previous child having MR, advice of karyotyping of affected son was given. As they were not affording the cost, they went for routine screening test of NT scan at 12wk but it gave the diagnosis of missed abortion. **Result:** Almost for all cases, where invasive procedure was required, financial status of the patient, played important role for decision making. **Conclusion:** Affordability issue limits the use of genetic tests. Though advanced and newer tests are now available, use of the test is beyond reach of needy patients. Funds should be raised and tests made available at concessional rate at private and government hospital.

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Cytogenetic Analysis For Chromosome Instability In Fanconi Anemia – SRL Experience.

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Background: Fanconi Anemia is an inherited, complex, heterogeneous disorder characterized by the presence of genomic instability. Chromosome breakage study which is based on exposure to low dose DNA-cross linking agents such as diepoxybutane (DEB) or Mitomycin C test is the gold standard method to diagnose Fanconi anemia cases. **Aim/Objective:** The aim of this study is to determine the frequency of chromosome breakage in cases suspected for Fanconi anemia which were referred to SRL. **Material and Methods:** A total of 646 cases with the history of aplastic anemia suspected for Fanconi anemia during the period 2010-2014 were referred from various hospitals from all over India. Chromosome breakage analysis was carried out as described previously using mitomycin C with varying concentrations. Age and sex matched normal whole blood was used as control for comparison. A total of 100 cells were scored from the test and control for the presence of chromosome breaks and radials. GTG banding was also performed simultaneously. **Results:** Among 646 cases studied, 466 were males while the remaining 180 cases were females. The male to female sex ratio was found to be 2.5:1. Out of 646 cases, 349 were below 15 yrs age group and 297 were above 15 years age group. Overall, chromosomal breakage analysis revealed that 52 out of 646 (8%) showed genomic instability and hence confirmed as fanconi anemia. Among the 349 cases which were below 15 yrs, 12.9% showed chromosomal breakage, in-contrast, of the 297 cases above 15 years of age, only 2.5% harbored chromosomal breakage. Interestingly, nine cases showed chromosome abnormalities like inv (12), rob translocation and polymorphic variants. **Conclusions:** This study revealed 8% cases with chromosomal breakage, based on which they were confirmed as Fanconi anemia. The current study highlights the importance of chromosomal breakage analysis for confirming the diagnosis of fanconi anemia.

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Association of Chromosomal Component in Primary Amenorrhea - SRL Experience

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Background: Primary amenorrhea is defined as the absence of menstruation and secondary sexual characteristics in phenotypic women aged 14 years or older, or aged 16 years or older if secondary sexual characteristics were present. Chromosome abnormality is one of the most important causes for Primary amenorrhea apart from hormonal disorders and the presence of abnormality affects subsequent management. **Aims And Objectives:** Present cytogenetic evaluation was undertaken to determine the prevalence of chromosomal abnormalities in patients of primary amenorrhea referred

to SRL. **Materials And Methods:** A total of 2351 women who were older than 14 yrs with absence of menarche were studied for chromosomal analysis referred during the period 2001-2013. Conventional cytogenetic analysis by Peripheral blood lymphocyte culture followed by GTG banding of the metaphase chromosomes were done as per standard protocol. Minimum of twenty metaphases were analysed in normal and 50 metaphases in mosaic cases. **Results:** Chromosome analysis revealed abnormal karyotype in 39.8%(935/2351) of the analyzed cases. Out of which 22.8% exhibited numerical aberrations (n=269) and 40.9% demonstrated structural aberrations (n=383). 30.3% of cases were with XY male karyotype (n=283), followed by 20.8% with complete monosomy 45, X (n=195). Structural abnormalities of X-chromosome included isochromosomes, deletions, rings, inversions, isodicentrics, duplications, and translocation between X chromosome and autosomes. Chromosomal abnormalities involving autosomes like insertion, Robertsonian translocation, derivative chromosomes were also observed indicating role of autosomes in sexual development. **Conclusions:** This is one of the largest chromosomal study in 2351 women with primary amenorrhoea from Indian subcontinent. The current study demonstrated chromosomal aberrations in 39.8% of the referred cases. Appropriate diagnosis, evaluation of clinical history and physical examination should be a routine practice for early detection of primary amenorrhea. Early cytogenetic investigation is prudent and Genetic counseling should be given to the patients for further clinical management.

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Partial Trisomy of Chromosome 9 with Congenital Anomalies

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Background: A case of trisomy for the short arm of chromosome 9 in patient is described, with particular emphasis on multiple congenital anomalies including microstomia, micro-opthalmia and an additional finger at pre-axial position, with chief complaint in breathing. The difficulty of providing comprehensive treatment in such case has been discussed. **Materials &Methods:** We investigated a large five-generation pedigree of Indian origin with ten affected family members. The patient was analyzed by classical karyotyping and applied; Chromosomal microarray and Fluorescence in situ hybridization (FISH). **Results:** The karyotyping results from both parents was karyotyped and the mother was found to be a carrier of balanced reciprocal translocation, 46, XX,t(9,10)(p13;p15). Therefore, the child was trisomic for the region 9p13--pter and monosomic for the telomere region of 10th chromosome. Following this, the diagnosis was changed to trisomy of the short arm of chromosome 9. **Conclusion:** The development of differential banding techniques for human chromosomes has resulted in the reporting of large numbers of partial duplication syndromes. We conclude that chromosome number 9 is particularly susceptible to breakage and rearrangement.

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Association of Toll Like Receptor and CD14 Genes Polymorphisms With Neonatal Sepsis

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Background: Despite significant advances in supportive care, neonatal sepsis is the commonest cause of mortality and morbidity. Recent studies suggest that sepsis is likely to be due to an interaction between genetic and environmental factors. Therefore identification of single nucleotide polymorphisms (SNPs) in the genes involved in sepsis may help to understand the pathophysiology of neonatal sepsis. Mutations of genes involved in the innate immune system have been reported to be associated with an increased sepsis rate. **Aims:** The present study was undertaken to investigate the genotypes and allele frequencies of the variants in the innate immune receptor genes (TLR2, TLR4, TLR5 and CD14) and their association with neonatal sepsis. **Material and methods:** Genotyping of eight SNPs (rs121917864, rs5743708, rs3804099 and rs38040100 in TLR2, rs4986790 and rs4986791 in TLR4, rs5744168 in TLR5 and rs2569190 in CD14 genes) was performed in 97 neonatal sepsis cases along with 50 controls by PCR-RFLP. **Results:** A complete absence of the mutant alleles for rs121917864 and rs5743708 was observed in both the cases and controls. Of the remaining six SNPs, a variation in the mutant allele, either in heterozygous or homozygous condition was observed only for the rs2569190 in CD14 gene in a higher proportion of the sepsis cases and was significantly associated with the risk of sepsis as compared to control group (88.0% vs 68.0%, $p < 0.004$). Haplotype analysis indicated that one haplotype (CTTACC) was significantly higher in the sepsis group than the controls (38.0% vs 12.0%, $p < 0.03$). **Conclusions:** The present study demonstrates that a variation in the CD14 gene and a haplotype could be associated with increased susceptibility with neonatal sepsis.

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A Complex Rearrangement of Chromosome 4p16 and 3p26.3 with Divergent Clinical Presentations: Report of an Extensive Indian Pedigree

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Background: The deletion of the chromosome 4p16.3 Wolf-Hirschhorn syndrome (WHS) critical region typically result in a characteristic facial appearance, severe mental retardation and growth delay, while gains of the same chromosomal region result in a more variable degree of intellectual deficit and dysmorphism. Similarly the phenotype of individuals with terminal deletions of the distal portion of the short arm

of chromosome 3 (3p deletion syndrome) varies from mild to severe intellectual deficit, micro- and trigonocephaly, and a distinct facial appearance.

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Renin Angiotensin System and Metabolic Syndrome: An Association Study on an Endogamous Tribal Population of Uttarakhand

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Background: Of the estimated 57 million global deaths in 2008, 36 million were due to NCDs (WHO 2011). Metabolic syndrome (MS), often acting as a predecessor for various NCDs like diabetes, CVDs, cancer etc. has become a major public health concern. Metabolic syndrome (MS) is characterised by a constellation of risk factors encompassing abdominal obesity, hypertension, hypertriglyceridemia, depressed plasma HDL cholesterol and elevated glucose. Studies have reported to confer twice the risk of developing cardiovascular disease and five times the risk for type 2 diabetes among individuals with MS. Renin Angiotensin System pathway (RAS), found to be associated with blood pressure as well as CVD, might be a potential pathway for the therapeutic treatment of metabolic syndrome. **Aims and Objectives:** The present study attempts to examine the association of RAS related gene polymorphisms (Insertion/Deletion of ACE, M235T, T174M and A6G of AGT and A1166C of AGTR1) with metabolic syndrome in an endogamous tribal population of Uttarakhand i.e. Rang Bhotia. **Material and Methods:** The study was conducted on unrelated subjects (age group 18-60) of an endogamous tribal population Rang Bhotias of Uttarakhand. Intravenous Blood was collected, from 254 individuals with informed written consent, for DNA extraction and other biochemical analysis. The polymorphisms were genotyped by PCR and PCR-RFLP methods. Statistical tools were used for data analysis using SPSS 16.0 ver. **Results and Conclusion:** Of all the five polymorphisms, none of them was found to be significantly associated with metabolic syndrome.

P73

Mutation analysis of ARX and MECP2 genes in mental retardation

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Background: Mental retardation (MR) is a common neurological disorder that affects 1-3 % of population. It can be divided into syndromic and non-syndromic form. Syndromic MR is characterized by distinctive and consistent clinical findings in addition to mental retardation. Whereas in non syndromic patients MR is the only primary symptom. On the observation of high ratio of affected males over females in MR it has been considered that X linked gene defects are the important cause of MR. ARX and MECP2 genes are most frequently mutated in MR patients. **Aims and Objectives:** Screening of ARX and

MECP2 genes in syndromic and non-syndromic MR patients and studying possible role of these genes in mental retardation. **Material and Methods:** Six families of non syndromic MR and five families of CdLS patients were selected for mutation analysis of ARX and MECP2 genes. Peripheral blood leukocyte DNA was extracted from patient, family members and control samples. Mutation screening for hotspot region of ARX and MECP2 genes were performed by PCR followed by conformation sensitive gel electrophoresis (CSGE). Positive results of CSGE were confirmed by sequencing. **Results:** G>A polymorphism is observed at genomic position 25031781 which leads to change of amino acid A111T. Polymorphism T>A is observed in patient at genomic position 25031330. This polymorphism changes amino acid K261M. This change does not affect protein functioning. Polymorphism T>C is observed in patient at genomic position 25031654. This polymorphism changes amino acid A153V. This change does not affect protein functioning. **Conclusion:** From this data we conclude that these mutations may affect the intellectual development of patients and cause syndromic or non-syndromic MR.

P74

Urine Metabolite Profiling for Detection of in Born Errors of Metabolism in Case of Intellectual Disability Disorders using UPLC-MS

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Background: Intellectual disability (ID) is characterized by impairment of skills manifested during the developmental period, which contribute to the overall level of intelligence. Inborn errors of metabolism (IEM) appear to be rare cause of ID. These are disorders in which there is a block at some point in the normal metabolic pathway caused by genetic defect of specific enzyme. Many authors emphasize the approach that has potentially significant treatable impact on patient outcomes. **Aims and objectives:** To explore the potential of non-invasive emerging technology of Ultra-High Performance Liquid Chromatography-Mass Spectroscopy (UPLC-MS) for global urine metabolic profiling in detection of IEM in ID. **Material and methods:** Informed consent forms were collected from the parents. Patients with ID were screened. The IQ reports were obtained for statistical interpretation. Urine samples were collected from normal individuals as control. These samples were used for optimization and as standard against patients. Similarly samples were collected from patients. The metabolite profiles of control and patients were compared using software for diagnosis of potentially treatable IEMs. **Results:** Urine samples were collected from control and patients with age groups matched. High throughput global metabolite profiling was carried out by the newly emerging UPLC-MS technique. Approximately 300 metabolites were screened for every individual. The sample and control chromatograms were matched using software and results were obtained for statistical analysis. **Conclusion:** The technique of UPLC-MS is a sensitive and reproducible approach to metabolic profiling because of improved chromatographic

performance providing superior metabolome coverage. It has an advantage of using urine as test sample, hence being non-invasive and easy to access.

P75

Effect of Novel Intronic Mutation c.694+8_694+18del in LDLR Gene in the Pathogenicity of Familial Hypercholesterolemia

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Background: FH is a polygenic genetic disease which is mainly caused by the autosomal dominant mutations in LDLR, APOB, PCSK9 and few other lipid metabolism genes. The heterozygous condition is common in 1/500 and homozygous 1 in a million worldwide. The exact frequency of FH in India is undetermined. **Aims and Objectives:** The study was aimed on screening LDLR, APOB and PCSK9 genes in South Indian population. The observed variations were scrutinized using various insilico tools to affirm their pathogenicity. **Material and Methods:** 350 patients enrolled at SRM Medical College Hospital and Research Centre were considered for the study. Based on UK-Simon Broome criteria, 10% of them were in the definite FH criteria and the remaining 90% were in probable / possible criteria. The genomic DNA was isolated from blood by organic extraction method and exon specific primers were used for amplifying respective exons. The exons were screened using High Resolution Melt analysis (Accumelt HRM mix, Qiagen). The exons with heterozygous melt pattern were sequenced. **Results:** The patient SInFH 146 with characteristic heterozygous FH features had an intronic novel mutation c.694+8_694+18del which was predicted to be pathogenic by insilico tool ESE finder and splice site prediction tool in BDGP. The tool predicted that the exon splice enhancers SRSF1, SRSF6 and SRSF1 which are essential for the splicing mechanism lost their site on the DNA due to the deletion of 11 bases, 8 bases downstream of the exon intron junction. This variation in LDLR gene has never been reported so far in any other population. **Conclusion:** However, mRNA analysis needs to be carried out to substantiate the pathogenicity of the mutation in causing FH. The APOB and PCSK9 genes were negative for any other mutation in this patient. This study emphasize the importance of critical analysis of intronic variation using insilico and invitro approaches.

P76

Test For Transmission Disequilibrium Including Non-Informative Parents

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Background: - The classical TDT (Spielman et al.1993) tests for genetic association in the presence of linkage and hence, protects against population stratification. The test has

been extended for quantitative traits using logistic regression (Waldman et. al. 1999). While these tests deal only with allelic transmission from parents who are heterozygous at a marker locus, the phenotypes status of an offspring, in practice, depends also on the allele transmitted by the other parent.

Aims and Objectives: - We modify the TDT procedure (binary as well as quantitative traits) including non-informative parents. We also compute the type-I errors and powers of our tests under a wide spectrum of genetic models and compare with the classical TDT. **Material and Methods:** - For binary traits, we construct 2 x 2 tables to model the probabilities of allelic transmissions by the heterozygous parent and the other parent at a marker locus. We compare the observed frequencies with those expected under no linkage or no association and use a goodness of fit test. For quantitative traits, we assume a multiple response logistic model and use a likelihood ratio framework to test whether the regression coefficients are zero. Extensive simulations are performed to evaluate type-I errors and powers of the proposed tests for different inheritance models and varying probability distributions. **Result:** - The power of the test increases with increasing similarity in allele frequencies at the marker and the trait locus. We observe that the power of the test is comparable across different probability distributions. However, there is no gain in power for the proposed test compared to the classical TDT procedure. **Conclusion:** - Our results suggest that non-informative parents carry much less information on genetic association compared to informative parents and hence, inclusion of both parents in a transmission-based model does not lead to increase in power to detect association.

P77

Understanding the role of Trans acting genetic factor KLF1 in Hereditary Persistence of Fetal Hemoglobin Syndromes in Indian population.

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Background: Hereditary Persistence of Fetal Hemoglobin (HPFH) is a rare hereditary condition characterized by persistent high level of fetal hemoglobin in adults. HPFH is caused by two classes of mutations, one involving large deletions at the β -globin cluster and the other involving point mutations in the promoters of the γ -globin genes. A variety of transcription factors such as GATA1, KLF1 (Kruppel-like factor1) have been identified which play role in globin gene regulations. Mutation which affects the activity of KLF1 gene may lead to high level of fetal hemoglobin which in turn can reduce the severity of β -hemoglobinopathies. Various types of mutations have been identified in KLF1 which leads to HPFH condition. **Aims and Objectives:** Analysis of spectrum of mutations of KLF1 gene in association with HPFH. **Material and Methods:** Blood sample of recruited subjects diagnosed with HPFH was collected in EDTA vials after getting their informed consent. Cases of HPFH-3 deletion and Asian Indian inversion deletion has been excluded. Total of 7 HPFH suspected subjects have been done. Genomic DNA

was isolated from collected blood samples using Phenol-Chloroform method. Whole KLF1 gene has been amplified by PCR and sequenced. **Results:** One pathogenic mutation has been identified in exon2 of KLF1 in one HPFH subject. Mutation is present in the 2nd zinc finger domain of KLF1 gene and first time reported in Indian patient. We also found one polymorphism in KLF1 gene in most of our cases. **Conclusion:** We found a pathogenic mutation in KLF1 gene in one HPFH subject which shows that there is some association between KLF1 gene mutation and HPFH condition. Similar study will be progressed with further collected samples.

P78

Study of Various Polymorphisms in Indian Warfarin Treated Patients

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Introduction: Warfarin (Coumadin®) is a widely used anticoagulant to prevent thromboembolic disorders. Management of anticoagulation therapy is difficult as there is a wide inter- and intra-individual variability that possibly leads to hemorrhagic or thromboembolic events despite careful dosage monitoring. Several polymorphisms in VKORC1, CYP2C9, CYP4F2, EPHX1, Calumenin, GGCX, factor IX, factor II and factor VII have been shown to be associated with warfarin dosage and other associated adverse events. **Method and Material:** 89 warfarin treated patients were genotyped for VKORC1 haplotype by direct sequencing method. 250 warfarin treated patients were genotyped for VKORC1 and CYP2C9 genotype using PCR-RFLP method. 200 warfarin treated patients were genotyped for EPHX1 T113C (rs1051740) and CYP4F2 rs2108622: G>A polymorphism using allele specific PCR method. 150 warfarin treated patients were genotyped for CALU c.*4A>G (rs1043550) polymorphism using allele specific PCR method. 100 patients were screened for GGCX 12970 C>G genotype using allele specific PCR method. 50 patients were screened for Factor VII c.-401 G>A and Factor VII c.-402 G>T genotype using direct sequencing method. **Results:** VKORC1 haplotype H1 and H2, VKORC1-1639 G>A, and CYP2C9*3 were found significantly associated with therapeutic warfarin dose requirement and risk of overanticoagulation. VKORC1 haplotype found strongly linked with VKORC1-1639 G>A polymorphism, hence addition of this haplotype does not add more information. CYP4F2 G>A (rs2108622), EPHX1 T113C (rs1051740), and CALU c.*4A>G(rs1043550) SNPs added minor contribution in warfarin pharmacogenomics. No contribution was noted for FVII promoter region SNPs. GGCX 12970 C>G variant was not found in Indian patients. **Conclusion:** VKORC1 -1639 G>A, CYP2C9 genotype, CYP4F2 G>A (rs2108622), EPHX1 T113C (rs1051740), and CALU c.*4A>G (rs1043550) polymorphisms will be beneficial to formulate warfarin pharmacogenetic algorithm in Indian patients. Skipping of four VKORC1 haplotype tag SNP's, Factor VII promoter region polymorphism and GGCX 12970 C>G genotype can make warfarin pharmacogenetics more cost effective in Indian population.

P79

Association of MMP-1 (-181A>G) and MMP-3(-1612 5A>6A) Promoter Polymorphisms and Risk for HIV progression

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Background: Matrix metalloproteinase (MMP-1) is known as interstitial collagenase and MMP-3 is known as secreted stromelysin 1. MMPs are involved to degrade the collagen, proteoglycon of extracellular matrix, cleave the blood brain barrier and rupture the neuronal synopsis thus plays an important role in neuro-inflammatory diseases such as HAND. The mRNA and protein levels of MMP-1 were found increased in the tissue of HAND patients. Also MMP-3 is found in the saliva of HIV-infected individuals. **Aims and Objectives:** We aimed to investigate the association of MMP-1-16072G/1G and MMP-3-1612 5A/6A gene polymorphism in HIV infected patients on ART. **Materials and Methods:** In the present cross sectional study, we enrolled a total of 130 HIV infected patients on ART and 150 unrelated healthy individuals. Polymorphism for MMP-1-16072G/1G and MMP-3-1612 6A>5A genes were genotyped by polymerase chain reaction and restriction fragment length polymorphism. **Results:** Frequency of MMP-1-1607 heterozygous 2G1G and variant 1G1G genotype differed significantly between HIV infected patients on ART and healthy controls ($P=0.05$; $OR_{2G/1G}=2.90$ and $P=0.02$; $OR_G=3.48$). Also, individual with 1G/1G genotype in recessive model conferred significantly higher risk of HIV infected patients on ART ($P=0.019$, $OR_{1G/1G}=4.00$). Individual with variant genotype 6A/6A of MMP-3-1612 gene polymorphism is associated with higher susceptibility to HIV infected patients on ART ($P=0.02$, $OR=2.52$). Individual with 1G/1G genotype in recessive model were at higher risk of HIV infected patients on ART ($P=0.09$, $OR_{1G/1G}=2.17$). None of the haplotype MMP-1-1607 2G1/G, MMP-3-1612 5A/6A gene was significantly associated with higher susceptibility to HIV infected patients on ART. **Conclusion:** MMP-1-1607 2G1/G gene polymorphism may have role in HIV progression. Similarly, MMP-3-1612 5A/6A gene polymorphism is associated with higher risk in HIV infected patients on ART.

P80

Association of DNA Repair Pathway Gene XRCC1 Variants with Human Male Infertility

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Background: Infertility is defined as a failure to conceive a

child after 1 year of regular intercourse without contraception. Endogenous and exogenous factors can give rise to germ cells DNA damage. An integrated DNA repair pathway exists in the human genome to repair a series of DNA damage in somatic and germ cells. Large number of studies reported that the single nucleotide polymorphisms (SNPs) in XRCC1 may be associated with the change of DNA repair capacity, which may affect genetic susceptibility to complex diseases and the sensitivity of individuals to treatment. In the present case-control study we have evaluated the association of XRCC1 gene polymorphisms with male infertility. **Material and Methods:** 100 azoospermic patients and 100 fertile men as controls were recruited for the study. Genomic DNA was isolated from peripheral blood. Genotyping of XRCC1 Arg280His G>A (Exon 9) and XRCC1 G>A Arg399Gln (Exon 10) was performed using PCR-RFLP. Insilico analysis was carried out using Polyphen2 software. **Results:** Statistical analysis of XRCC1 Exon10 G>A showed significant association ($p<0.05$) with male infertility whereas XRCC1 Exon9 G>A did not show a significant association. Insilico analysis by Polyphen2 software indicated that XRCC1 Arg280His G>A showed no risk with a score of 0.000 (sensitivity: 1.00; specificity: 0.00) while XRCC1 G>A Arg399Gln showed a probably damaging effect with a score of 0.979 (sensitivity: 0.76; specificity: 0.96). **Conclusion:** Results clearly indicates that G>A Arg399Gln variant of XRCC1 confer an increased risk in the development of infertile phenotype. The BRCT1 domain of XRCC1 protein is the most likely region of interaction with other key components of BER pathway. The Arg399Gln amino acid variant of XRCC1 is located within the region comprising the BRCT1 domains, and therefore may be associated with altered level of DNA repair in infertile men with impaired spermatogenesis.

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IL-17a Cytokine Polymorphisms and Risk of Early Miscarriage

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Background: IL-17a has been shown important inflammatory cytokine in pathophysiology of various autoimmune diseases, cancer and other intracellular host defense. On the subject of early miscarriage (EM), it seems that imbalance in Th1/Th2/Th17 immune cells may be one of the most important immunogenetic factor involved in the rejection of fetus. IL-17a is a secretory cytokine from Th17 cells and helps in recruitment of inflammatory cells. Therefore, we hypothesized that differential IL-17a gene level due to variants may result in susceptibility to recurrent miscarriage. **Aims and Objectives:** The aim of present study was to assess the association of the IL17A (rs4711998, rs8193036) gene polymorphisms in EM cases versus controls (viable intrauterine foetus and no prior miscarriage.). **Material and Methods:** 85 cases with a history of three or more EM and 100 controls were recruited. Isolation

of DNA was performed using peripheral blood and genotyping was done through PCR-RFLP. **Results:** Out of two IL-17a SNPs (rs4711998, rs8193036), rs8193036 variant showed to be a risk factor in EM cases ($P = 0.0015$, $df = 2$, $OR = 0.8682$ and $95\% \text{ CI} = 0.4138 \text{ to } 1.8218$ by genotype). **Conclusion:** The results showed a significant association of rs8193036 variant with early miscarriage. This might suggest a protective effect of this variant towards early miscarriage by modulating the effect of inflammatory cytokine IL-17a.

P82

A Novel Mutation in *FRMD7* Results in Putative Disruption of the FERM Domain and Causes X-Linked Idiopathic Congenital Nystagmus in a North Indian Family

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Background/Objectives: Idiopathic congenital nystagmus (ICN) is the most common form of oculomotor disorder characterized by involuntary bilateral ocular oscillations. A three generation North Indian family affected with X-linked idiopathic congenital nystagmus (XLICN) was recruited in the current study. Our aim was to identify the causal mutation for ICN in the recruited family by screening the candidate gene, FERM domain containing-7 (*FRMD7*). **Materials and Methods:** Complete protein coding region including 5' UTR, 3' UTR, and splice junctions of *FRMD7* was screened by PCR-Sanger sequencing. A cohort of healthy individuals was checked for presence of the putative causal variant by allele specific PCR. In addition to detailed bioinformatics analysis, protein modeling was carried out to evaluate the plausible deleterious effect of the variant on the three dimensional structure of the protein. **Results:** Targeted sequencing revealed a novel A to G transition in exon seven of *FRMD7*, resulting in a conservative substitution of methionine by valine at codon 186. All the affected males and carriers in the family shared this variant; however, this was absent in the unaffected males as well as 100 unrelated healthy individuals from the same population. Protein modeling revealed that the M186V variant was present in a loop structure connecting an α -helix of FERM-M and a β -strand of FERM-C domain. Also, M186 residue resided in a hydrophobic pocket at the interface of the two sub-domains; therefore, the change p.M186V might destabilize the interaction between the FERM-M and FERM-C domains. **Conclusions:** We report a novel and rare mutation c.556A>G (p.M186V) in *FRMD7* gene; this mutation is predicted to disrupt intra-domain interactions within the FERM domain and thus causes XLICN. We believe that the mutation identified in this study can be used for prenatal diagnosis in the future generations of the family studied.

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A case of Lenz Majewski syndrome: report of the sixth case in literature with *PTDSS1* gene mutation

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Lenz Majewski syndrome is an extremely rare syndrome characterized by osteosclerosis, intellectual disability, characteristic facies and distinct craniofacial, dental, cutaneous and distal – limb anomalies. Till date only eight typical cases have been published in medical literature. Recently, mutations in *PTDSS1* gene have been identified as causative in five unrelated individuals. We report the first case of LMS from India and the sixth mutation proven case in the world.

P84

Heritable / genetic microcytic hypochromic anemia: Four unusual case vignettes

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Background: Anemias with hypochromic microcytosis commonly occur due to iron-deficiency or are related to the β -thalassemia syndromes. We present four such cases with unusual genetic/heritable etiologies. **Case Studies:** **Case 1:** A 22-year-old male with anemia, unconjugated hyperbilirubinemia and spleen span 19 cm showed marked anisopoikilocytosis with hypochromic microcytic anemia. Hb HPLC suggested HbH disease. HbH preparation showed Heinz bodies with 2-3% RBCs showing golf-ball inclusions. Heat stability test revealed an unstable hemoglobin. α -globin gene sequencing showed heterozygosity for Hb Zurich-Albisrieden [$\alpha 59(\text{E8})\text{Gly}>\text{Arg}(\alpha 2)$] and Hb Sallanches [$\alpha 104(\text{G11})\text{Cys}>\text{Tyr}(\alpha 1)$]. **Case 2:** Hb HPLC in 3 members of a family with congenital cyanosis revealed a variant hemoglobin with retention time of 4.8 minutes. α -globin gene sequencing revealed Hb M-Iwate ([$\alpha 87(\text{F8})\text{His}>\text{Tyr}$]) that was confirmed on PCR-RFLP. The microcytic hypochromia in the proband was explained by iron deficiency. **Case 3:** Bone marrow in a 7- and 5-year-old sister-brother duo with anemia, splenomegaly, and growth failure revealed erythroid hyperplasia with 36% dyserythropoiesis. HEMPAS test showed erythrocyte lysis in 4/5 control sera. Sequencing of SEC23B exon 12 revealed homozygous c.1385A→G;Y462C mutations in both siblings, confirming congenital dyserythropoietic anemia, type II. **Case 4:** A 20-year-old male with mild jaundice, microcytic hypochromic anemia and short stature since age 5 years had splenomegaly 3 cm below costal margin. Tests for hemolytic anemias and endocrinopathies were negative. Bone marrow aspiration performed one year earlier was reported outside as erythroid hyperplasia with moderate dyserythropoiesis. RBC

histogram displayed dimorphic RBCs. Bone marrow review showed 51% ringed sideroblasts, suggesting congenital sideroblastic anemia. **Conclusions:** Microcytic hypochromic anemia occasionally requires more extensive work-up beyond hemogram, blood film, HPLC and iron profile. A judicious step-wise approach based on the clinical background, blood findings and exclusion of common etiologies (like coexistent iron deficiency in case 2) is essential before specialized genetic testing to yield the correct, often unusual diagnoses in these patients.

P85

Evaluation of A Monoclonal Antibody as A biomarker for Prostate Cancer

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Background: Prostate cancer is the commonest solid organ malignancy diagnosed in men. Prostate cancer is the second most common cancer in men. The current screenings method relies on a combination of Prostate Specific Antigen (PSA) and Digital Rectal Examination (DRE) with subsequent TransRectal Ultrasonography (TRUS) and biopsy. It is well established that PSA is not a very sensitive assay as more than 30% of cancers cannot be identified while the other two are invasive procedures. Hence, there is a need to develop new screening and diagnostic markers for prostate cancer to reduce the morbidity and mortality associated with this pathology. **Aim:** To evaluate a monoclonal antibody (PC-164) raised against prostate neoplastic tissue for subsequently using it for screening, diagnosis and managing prostate cancer. **Objectives:** To characterize and validate a hybridoma raised antibody (PC 164) as a potential biomarker, for prostate cancer. **Material and Methods:** Immunoblotting, immunocytochemistry and immunohistochemistry was performed by established methods on cells and tissues using the PC-164 monoclonal antibody. **Results:** Immunoblotting with purified IgG antibodies from clone 164, revealed two bands with Mr of 49,000 and 120,000 daltons when assessed with prostate cancer tissue. Immunocytochemistry results also showed positive reactivity with prostate cancer tissue and cell lines. Immunohistochemistry performed with 1:2000 dilution showed positive protein expression in prostate cancer tissue. Benign hyperplasia of the prostate also showed positivity in some areas while no expression was seen in tissues like spermatogenic hyperplasia, ovarian cancer, adenocarcinoma of brain, colon and stomach cancer. **Conclusion:** Immunoblotting, Immunocytochemistry and Immunohistochemistry to detect prostate specific proteins. Hence, it can prove to be a promising biomarker for screening, diagnosing and treating prostate cancer in the future.

P86

Cytogenetic Studies in Chromosome Instability Syndromes

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Background: Chromosome instability syndromes or chromosome breakage disorders are a group of inherited human diseases unified by the abnormal behavior of their chromosomes. These single gene disorders mainly have an autosomal recessive mode of inheritance. They show hypersensitivity to specific chromosome damaging agents and manifest as high levels of breaks, radials or sister chromatid exchanges(SCE). The affected individuals exhibit a variety of clinical features as seen in Fanconi anemia, ataxia telangiectasia (AT), Bloom syndrome and Cockayne syndrome.

Aims and Objectives: To report our experience of cytogenetic studies on 125 clinically suspected cases. **Material and methods:** There were 76 cases of aplastic anemia referred for the chromosome stress test with Mitomycin C (MMC) to differentiate cases of Fanconi anemia. Ataxia telangiectasia was suspected in 38 cases, where the cultures were exposed to Bleomycin and G2 cobalt-60 irradiation. Blood cultures of 11 cases suspected to have Bloom, Cockayne or other breakage disorders were exposed to UV irradiation and treated with BrdU to check for sister chromatid exchanges. Age or sex matched controls and internal controls were used in all cases.

Results: Fanconi anemia was detected in 7/76(9.2%) cases while 4 cases were borderline positive. Ataxia telangiectasia was detected in 4/38(10.5%) cases, while another 16 cases were suspected to be mildly affected. There was no frank positive case which showed a very high frequency of SCE, also known as harlequin chromosomes. **Conclusion:** Fanconi anemia can exhibit mosaicism, where a few metaphases show a large number of radials, exchange figures and breaks, while a majority of metaphases are normal. Revertant mosaicism is also known to occur, where a positive case can revert back to normal. This can pose a problem in interpretation or a discrepancy of results when tested after a period of time. Hence correlation of laboratory results with clinical findings is important.

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Fragile-X, CNVs and single gene disorders in selected cases from western India

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Background: Fragile X Syndrome(FXS) is one of the common causes of the autism spectrum disorder, where the full mutation occurs mainly in males. Some women with premature ovarian failure(POF), besides mothers of affected males have the Fragile

X premutation and need prenatal diagnosis or preimplantation genetic diagnosis (PGD). Copy number variations (CNV) detected on chromosome microarray (CMA) are occasional causes of autism. **Aims and Objectives:** A retrospective analysis of selected cases referred to our clinic for genetic counseling and co-ordination for specialized genetic testing at other centres, for over a decade. **Material and Methods:** A total of 958 samples were sent out for fragile-X testing by southern hybridization or Amplidex PCR. Another 174 samples mainly of children with autism were tested for CMA/aCGH. For single gene disorders, 150 samples were sent. **Results:** The CGG repeats in the FMR1 gene were in the full-mutation range in 55/676 (8.1%) suspected males while a premutation was present in 5/676 (0.7%) males. Among the suspected female children, full-mutation was seen in 4/99 (4.0%) and premutation in another 4% cases. In suspected carriers including mothers, sisters and aunts, premutation was detected in 32/79 (40.5%) cases studied. Among 102 females with POF, 6 (5.9%) were premutation positive. From samples sent for CMA/aCGH, 40/172 (23.3%) showed pathogenic CNVs, 29/172 (16.9%) had benign variants, 27/172 (15.7%) cases had CNVs of uncertain clinical significance and 49/172 (28.5%) showed only loss of heterozygosity (LOH) mainly due to consanguinity. Single gene disorders detected included SMA, DMD, SCA, CAH, CF, Rett, Thalassemia, Tay Sachs, Myotonic Dystrophy, Krabbe, Huntington, CMT1A, Canavan, LMBB, Kennedy and Lesch-Nyhan disease. **Conclusion:** The awareness of the need for genetic testing for counseling, management, prenatal and preimplantation genetic diagnosis has increased. It is important to check women with POF for a premutation in the FMR1 gene prior to IVF. PGD for chromosomal and single gene disorders is gaining importance in India.

P88

Association of Proinflammatory Cytokine Genes (IL-6, TNF- α And IL-1 β) Polymorphisms With Disease Susceptibility of Indian SLE

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Background: Systemic lupus erythematosus (SLE) is an inflammatory, multi-factorial autoimmune disease characterized by production of auto-antibodies and organs damage. The single nucleotide polymorphisms (SNPs) in candidate cytokine genes have been implicated as genetic risk factors for SLE in some populations. **Aims and objective:** To investigate possible associations of the SNPs in proinflammatory cytokine genes (IL-6, TNF- α and IL-1 β) with susceptibility and phenotypic presentation of the disease. **Material and Methods:** A cohort of 200 Indian SLE patients and 201 healthy controls were studied. Serum levels of the proinflammatory cytokines were detected by bead based multiplex assay. SNPs of IL-6, TNF- α and IL-1 β were determined by PCR-RFLP and direct DNA sequencing methods. **Results:** IL-6 (-174G) allele was found to be significantly increased in patients as compared to controls ($p=0.0102$). IL-6 (-174G/G) genotype was significantly associated with malar/

discoid rash, oral ulcers, arthritis, renal disease, haematologic disorders and Immunologic disorders ($p < 0.007$). TNF- α (-308A) allele frequency was found to be more in patients as compared to controls ($p < 0.0001$). TNF- α (-308GA+AA) genotype frequencies were found to be significantly associated with oral ulcers, arthritis, renal disease and haematologic manifestations ($p < 0.05$). TNF- α (-238G/A) polymorphism in SLE did not show a statistically significant difference when compared with controls ($p > 0.05$). IL-1 β (+3953T) allele frequency was significantly increased in patients as compared to controls, however, it was not found to be associated significantly with clinical manifestations of the disease. Serum levels of these cytokines were found to be significantly higher in patients as compared to healthy controls ($p < 0.01$). **Conclusions:** The IL-6 (-174G/C) and TNF- α (-308G/A) SNPs appear to be associated with the disease susceptibility and distinct clinical features in Indian SLE patients.

P89

FISH in Multiple Myeloma: Comparison between FISH results on unsorted bone marrow and isolated plasma cells.

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Background: Multiple myeloma (MM), is a cancer of the plasma cells (PCs) which are rare in bone marrow (BM). Therefore the abnormal cell line may be missed if only unsorted BM is analyzed by fluorescence in situ hybridization (FISH). **Aims and Objectives:** The objective of this study was to compare the FISH test results on unsorted BM aspirates and on isolated PCs from MM patients. **Material and Method:** Heparinized BM was collected from 108 MM patients and PCs were isolated by immunomagnetic separation with the EasySep Human whole blood CD138 positive selection kit using manufacturer's protocol. FISH was set-up with Abbott (Vysis) probes on the isolated PCs and unsorted BM to check for deletions 13q14.3, ATM, p53, and IGH rearrangements. FGFR3-IGH and/or IGH-MAF translocations were tested in cases positive for IGH rearrangements. **Results:** The percentage of cells positive for various abnormalities was 3-5 times higher in isolated PCs compared to unsorted BM. On an average in BM cells v/s isolated PCs, deletion 13q14.3 was seen in 23.6% v/s 85.4%, deletion p53 in 29.9% v/s 84.2%, deletion ATM in 8.5% v/s 37.5% and rearrangement at IGH locus in 13.8% v/s 82.2%. Hyperdiploidy and hypodiploidy were also detected with 3-5 fold increase in PCs than BM. In 6 cases, the abnormality was detected only in PCs and not in BM. Also, in 4 cases the abnormality was seen on unsorted BM (<5%) but was not seen in isolated PCs. In 32 cases, isolated PCs showed the presence of abnormalities in majority of the cells, while in unsorted BM the abnormality was seen in <10% cells. **Conclusion:** The percentage of abnormal cells detected by FISH on isolated PCs was significantly higher compared to unsorted BM aspirates. Hence in MM cases, FISH on isolated PCs is now routinely carried out.

P90

Cytogenetic study of Klinefelter Syndrome

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Introduction: Klinefelter syndrome (KS) is a sex chromosomal disorder characterized by the presence of an additional X chromosome in a phenotypic male (47, XXY). Less commonly, variant karyotypes with additional X or Y chromosomes or mosaic karyotypes with two or more differing chromosome complements may be seen. Most patients present in adulthood / after puberty with poorly developed secondary sexual characteristics / infertility. Boys with KS are identified either by prenatal diagnosis or when investigated for developmental delay. However, the phenotype is variable; therefore many patients remain undiagnosed in the absence of cytogenetic analysis which is essential for the definitive diagnosis of KS. **Patients and Methods:** This is a retrospective study of all patients seen at the Christian Medical College, Vellore, between 2003 to 2013, whose karyotypes showed KS. The clinical and laboratory findings were obtained from patient records. **Results:** There were 90 patients with KS which was clinically diagnosed in only 58 patients (64%). The median age of patients was 27 years (range 1 - 51). Sixteen were below 18 years of age. Common presenting features in adults were gynaecomastia (37%), small testes (31%), infertility (28%), azoospermia (24%), obesity, tall stature and poorly developed secondary sexual characteristics (~10% each) and developmental delay (63%) and dysmorphism (75%) in boys below 10 years. Laboratory findings showed low testosterone (45/54, 83%) and elevated luteinizing hormone (32/38, 84%), follicle stimulating hormone (61/64, 95%), total cholesterol (48%), LDL (56%) and triglycerides (28%). Chromosome analysis revealed three groups of karyotypes: 47XXY in 77 (86%) patients, variant karyotypes (48,XXXY, 49,XXXXY, 48,XXYY) in 7 (8%) and mosaic 47XXY/46XY in 6 (7%). Deletion of the azoospermia factor region on the Y chromosome was seen in 1/14 adults. **Conclusion:** This series of KS shows the role of cytogenetic analysis which provided a definitive diagnosis in 36% of adults and 16 children in whom the features of KS were not apparent. An accurate and early diagnosis enables timely interventions and optimal management so that these patients can have a better outcome.

P91

Somatic Genomic Change of Rs9344 SNP of CCND1 in Gingio Buccal Squamous Cell Carcinoma of Indian Patient Population

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Introduction: Amplification of chromosomal region 11q13

containing so many genes including TPCN2, MYEOV, CCND1, ORAOV1, and FGF19 is frequently found in oral squamous cell carcinoma (OSCC) and other malignancies. 11q13 spans almost 14 Mb and contains more than 200 genes and is affected by various patterns of copy number gains, suggesting a complex mechanisms and selective pressure during tumor progression. **Objectives:** To study the expression and copy number variations of few genes in 11q13 regions of precancerous and cancer patient samples. To identify the somatic genomic changes in rs9433 SNP in gingio buccal squamous cell carcinoma patient samples **Methods:** In this study, Gene expressions and copy number variations were measured by TaqMan methods for precancerous and cancer samples. In this study, genetic changes of CCND1 A870G polymorphism were analyzed by RFLP method for gingio buccal squamous cell carcinoma. **Results:** In lichen planus, precancerous lesion, only MYEOV (mean $\Delta\Delta Ct$ 1.26, paired t-test p value 0.01) and CCND1 (mean $\Delta\Delta Ct$ 0.68, paired t-test p value 0.002) were significantly down regulated. Polymorphism at G/A870 (rs9433) was studied in 84 of gingio buccal tumor patient samples. A/G < G/G, A/A < A/G, G/G < A/G, A/G < A/A conversions from normal to tumor tissues were observed in 7 gingio buccal SCC patient samples. **Conclusion:** Precancerous lesions lichen planus, leukoplakia and gingio buccal squamous cell carcinoma are not following same pathway. First time in our study, we reported several types of genomic changes of SNP rs9344 (A870G) of CCND1 in gingio buccal tumor with respect to their normal origin.

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The Role of Meiotic Gene Abnormality in the Onset of Aneuploidy and Pregnancy Loss.

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Background: Spontaneous abortion accounts for 10-15% of all recognised pregnancies, around 60% of which exhibit chromosomal abnormalities. Improper separation of chromosomes result in chromosomal instability (CIN) and onset of aneuploidy. Onset of aneuploidy in meiotic cells may lead to birth defects and pregnancy loss. Key pathways leading to aneuploidy include defects in spindle assembly checkpoint, centrosomal abnormalities, alterations in kinetochore-microtubule dynamics and faulty chromosome cohesions. Literature study reveals an increasing repertoire of genes being involved in the pathways mentioned above. **Aims and Objectives:** To study of probable deregulated expression of 43 selected genes (reportedly involved in pathways leading to aneuploidy) in several aneuploidic abortuses. Investigate possible correlation between pregnancy loss and defects in pathways leading to aneuploidy. Obtain expression

signatures for first trimester and second trimester miscarriage. **Material and Methods:** Primary fetal fibroblasts obtained from abortuses and amniotic fluids of pregnant women were cultured and karyotyped. RNA was isolated from aneuploid abortuses (test) and euploid amniotic fluid samples (control). RNA quality was analysed using AGILENT BIOANALYZER platform. RT-PCR analysis of selected genes was performed in both aneuploid samples and several euploid amniotic fluid samples. Expression data was statistically analyzed using R bioconductor package (Limma). **Results:** Gene expression analysis show deregulation of key meiotic genes in a majority of aneuploid abortuses. LATS2, MAD1L1 and CDC27 are among a few highly upregulated genes. NEK2, BUB1B, CDC20 and UBE2C have been found to be down regulated maximally across the abortuses. Spindle assembly checkpoint, spindle dynamics and cell cycle progression are the major pathways affected. Aneuploid abortuses hierarchically clustered into two discrete groups according to trimester of pregnancy termination. Abortuses in each trimester showed significant differential gene expression (p. value <0.05) when compared to euploid amniotic fluid samples (controls) and also with respect to each other. Interestingly abortuses of both trimesters show common and unique repertoire of deregulated genes. **Conclusion:** Deregulated expression of meiotic genes lead to possible fetal aneuploidy thus triggering spontaneous abortions. Multiple affected pathways can lead to aneuploidisation. Distinct gene expression signatures seem to control fate of conception in different trimesters of pregnancy. Existence of common and unique expression signature might be indicative of them playing the central and associated roles respectively towards aneuploidy mediated miscarriage.

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A Study to Genotype the Primary and Secondary Modifiers of Beta Thalassaemia Among Patients from Pune, India

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Background: Severity of beta thalassaemia is modified by primary and secondary modifiers. Primary modifiers are mutations in the HBB gene. Secondary modifiers are genes involved in the regulation of alpha and non-alpha globin chains, which include mutations in the HBA gene and polymorphisms in the HBG gene. **Aims and objectives:** To genotype hundred patients with beta thalassaemia and characterize the types of mutations in the HBB gene and in secondary modifiers, HBA and HBG genes. **Material and Methods:** Blood samples of hundred patients with a clinical diagnosis of beta thalassaemia major were obtained after informed consent. DNA was isolated and analysis for primary mutations was done using Amplification Refractory Mutation System Polymerase Chain Reaction (ARMS PCR) for five common Indian mutations. Analysis for secondary modifiers that is alpha globin deletions and alpha globin triplications was done using Gap PCR.

Presence or absence of the Xmn1 polymorphism in the HBG gene was by Restriction Fragment Length Polymorphism (RFLP). **Results:** HBB mutations could be identified in all but 3 patients. Among 97 patients, 36% were homozygous, 25% were heterozygous, 38% were compound heterozygous for the beta globin mutations. The allele frequency of the IVS1-5 (G/C) mutation was 62%, Cd 8/9 (+G) mutation was 20%, 619 bp del was 12%, Cd 41/42 (-TCTT) mutation was 4% and the IVS1-1 (G/T) mutation was 1%. The structural variant HbS was not detected in any patient and the allele frequency for the Cd 26 (G/A) mutation (HbE variant) was 19%. HBA gene analysis revealed that allele frequencies of the α -3.7 deletion mutation was 9%, for the α -4.2 deletion mutation was 2%, and for the $\alpha\alpha\alpha$ anti3.7 triplicated allele was 2.5% (1/40 patients). The Xmn1 polymorphism analysis identified the frequency of the wildtype C allele was 86%, whereas of the ameliorating T allele was 18%. Two ameliorating genotypes were obtained; IVS1-5/IVS1-5/ α -3.7/ $\alpha\alpha$ /T/T and IVS1-5/+Cd8/9/+ $\alpha\alpha$ / α -4.2/C/T. **Conclusion:** These different genotypes indicate a variable severity of the disorder amongst patients clinically classified as beta thalassaemia major.

P94

Survey and Cytogenetic Analysis of Mentally Retarded Individuals in Human Population of Himachal Pradesh.

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Background: Mental retardation is a generalized disorder appearing before adulthood, characterized by significantly impaired cognitive functioning and deficits in two or more adaptive behaviors. Due to large geographic and ethnic diversity in India, there is a need to undertake studies on mental retardation. The present study is a step towards this direction and gives etiological basis of mental retardation in North Indian population. **Objectives:** To study pattern of inheritance, frequencies of mentally retarded individuals and frequency of mental disability in each genetic type. To study birth order in MR children. To study the MR on the basis of region, age, religion and sex. To study maternal age effect on MR. To evaluate the IQ levels in mentally retarded children by Intelligence Quotient Test (IQ tests). To study parental age effect in Down's syndrome patients. To examine all MR children clinically. **Materials and Methods:** 150 mentally retarded and 150 normal individuals from population of Himachal Pradesh. Fifty suspected to be Down's syndrome were analyzed separately to study parental age effect on mongolism. Among 150 MR individuals, 30 MR patients were analyzed cytogenetically to find chromosomal abnormalities in them. Peripheral lymphocytes of the MR probands were cultured in RPMI 1640 with 10% fetal calf serum and phytohaemagglutinin. Studies were made using standard techniques and G-banding using trypsin with slight modifications. **Results:** Pedigree analysis of 150 mentally retarded individuals resulted that 6 (4%) mentally retarded children were familial and 144 (96%) were of sporadic type. The incidence of MR was found to be higher in males than

in females with male female (M: F) sex ratio, 1.9:1. Sex ratio was found to be 2.5:1 in 50 Down's syndrome individuals. In 150 MR and 50 Down's syndrome individuals no maternal and paternal age effect was seen.. Among 150 MR and 50 Down's syndrome individuals, cases with mild mental retardation were greater. By clinical analysis, it was found that MR is associated with morphological (mouth, eye etc.) and behavioral abnormalities (Obesity, neonatal jaundice, pneumonia etc.). Among 30 MR patients which were selected for cytogenetic analysis, frequency of chromosomal abnormalities was found in 18(60%) cases. Most common autosomal abnormality was found to be trisomy 21 (Down's syndrome) and other abnormalities were found to be deletions, ring chromosome, dicentric chromosome and marker chromosomes. **Conclusion:** Present investigation came to the conclusion that MR can be caused by both genetic and non-genetic factors or MR is a heterogeneous disorder. (Abstract truncated).

P95

Genetic Evaluation of Polymorphism of Candidate Genes in Patients with Type 2 Diabetes Mellitus of Jammu Region (J&K State).

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Background: Diabetes is a disease known since antiquity. Type 2 Diabetes Mellitus (T2DM) is a non-autoimmune, complex, heterogeneous and polygenic metabolic disease condition in which the body fails to produce enough insulin, characterized by abnormal glucose homeostasis. T2DM is genetically heterogeneous disease caused by the complex interplay of several environmental factors and susceptibility genes. Several candidate genes are involved in the manifestation of disease. **Aims and Objectives:** To investigate the role of SNP polymorphism of 4 candidate genes PGC 1 Alpha (Gly482Ser), IRS 1 (G972R), PI3K (Met326Ile) & KCNJ11 (E23K) in individuals predisposed to type 2 diabetes mellitus in three diversified ethnic groups (Brahmins, Khatri and Guptas) of Jammu region of J&K state. **Material and Methods:** Blood samples of 800 individuals (400 cases and 400 controls) were collected from different hospitals and private clinics. DNA was extracted using Phenol Chloroform method and was further analyzed for the presence of mutation. The mutation was detected by Polymerase Chain Reaction (PCR) and restriction fragment length polymorphism (RFLP) was done by using restriction enzyme. **Results:** Frequency of wild type and mutant genotype was calculated by using Chi square test and Hardy Weinberg Law. In addition to molecular genetic analysis, clinical features of patients were also compared with controls to ascertain their role with disease and its progression. Details regarding: mode of treatment (insulin and exercise), pattern of diet (Veg /Non Veg) family history, alcohol or smoking abuse was also recorded. The details of clinical characteristics and other features of the individuals belonging to different endogamous groups (Brahmins, Khatri and Guptas) were also

compared and summarized. **Conclusion:** Our study concluded that there lies a strong association of PGC1 Alpha and PI3 K with type 2 diabetes mellitus.

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Frequency of Incidence of JAK2 V617F Mutation in Myeloproliferative Neoplasms (MPNs)

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Background: Myeloproliferative neoplasms (MPNs) are heterogeneous group of clonal stem cell disorders, defined by distinct clinical and cytomorphological phenotypes and, to some extent, known genetic features. According to the World Health Organization (WHO) 2008 criteria, MPNs are classified as chronic myelogenous leukemia, polycythemia vera (PV), primary myelofibrosis (PMF) and essential thrombocythemia (ET). The molecular analysis of MPNs includes detection of a specific somatic point mutation in JAK2 gene on 9p24 (JAK2V617F). This mutation has been reported in 80–97% PV, in >50% PMF and in 40–60% ET cases. **Aims and Objectives:** The aim of the study was to detect incidence of JAK2V617F mutation in BCR/ABL-negative MPN cases, namely PV, PMF and ET. **Material and Methods:** A prospective study of MPN cases was undertaken from August 2012 to November 2014. Presence of JAK2V617F mutation was detected by ARMS-PCR. Positivity for the mutation was further sub-typed as homozygous or heterozygous. **Results:** Out of 93 suspected MPN cases, 83 were diagnosed as MPN according to WHO 2008 criteria. Out of these 83 cases, JAK2V617F mutation was detected in 93.33% of 30 PV (32.14% being homozygous and 67.86% heterozygous), in 48.57% of 35 PMF (11.76% being homozygous and 88.24% heterozygous) and in 33.33% of 18 ET (100% heterozygous) cases. Homozygous JAK2 mutations were found to be more frequent in PV and PMF than in ET. **Conclusions:** The results of our study are comparable to those shown by other similar studies emphasizing the significance of JAK2V617F mutation analysis in the diagnostic algorithm of suspicious or proven BCR/ABL-negative MPN. Determination of the mutation was found to be specifically useful for diagnosis of PV, as nearly all cases were found to be positive.

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Differential Diagnosis to Molecular Diagnosis of Urea Cycle Disorder by Newborn Screening Using Urine GC-MS and Blood MS/MS

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Background: This two year pilot study investigated the screening, diagnosis and outcomes of Inborn Errors of Metabolism (IEM) in symptomatic infants and children referred

to Navigene Genetic Science Pvt Ltd. **Methods:** All symptomatic children suspicious for IEM from NICU/PICU were referred to Navigene. Urine samples collected on specially designed filter papers were analysed on GC-MS using modified MILS method for 110 IEMs. Dried Blood spots collected on Guthrie cards were analyzed by MS/MS. The diagnoses were further confirmed with molecular study wherever required. **Results:** Total 330 high-risk samples were analysed from Jan13 till Nov 14. Age range was from 2 days to 18 years. Thirty cases (9 %) were screened positive. The disorder profile consisted of methylmalonic aciduria (6), Propionic aciduria (5), 3-hydroxy-3-methyl glutaryl Co-A lyase deficiency (2), Alkaptonuria (2), Glutaric Aciduria Type I (1), Glutaric aciduria Type II (1), Isovaleric acidemia (1), Pyroglutamic Aciduria (1) and Urea Cycle disorder (1) and others. **Case Study:** Three days female with h/o death of previous 3 siblings, presented with vomiting, seizures and hyperammonemia, Urine GC-MS analysis showed elevation of uracil and orotate confirming it as urea cycle disorder. Blood MS/MS study showed elevation of citrulline. Considering both results Citrullinemia was suspected in this case. Molecular study by direct sequencing of all coding exons of ASS1 gene was carried on patient and parents. Index case showed splice-site mutation c773+ 2dupT in intron 11 of ASS1 gene in homozygous state confirming Citrullinemia. Parents showed heterozygosity. **Conclusions:** The patients of IEM generally present with metabolic decomposition and other non specific symptoms, which delay the referral to the IEM screening and in turn results in complications and leads to high mortality. Routine NBS and genetic counseling is thus imperative for ensuring early diagnosis and preventing mortality and morbidity associated with IEM. Considering difficulties in implementation at national level, we suggest implementing routine Metabolic Screening Program for all NICU/PICU admissions. GC-MS analysis using MILS modified method allows simultaneous and accurate screening of wide range of IEMs and hence has the potential to tremendously improve the diagnostic capabilities.

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Functional characterization of exonic mutations causing splicing defects in autosomal recessive genetic disorders

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Background: Mutations in Phenylalanine hydroxylase (PAH) and N-Acylsphingosine amidohydrolase (ASA1) cause Phenylketonuria (PKU) and Farber lipogranulomatosis (FL), respectively. The effect of missense and nonsense mutations is traditionally believed to be due to altered structure/function of the expressed protein. However, role of these mutations in perturbing processing/stability of the respective gene transcript is not well studied. **Aims and objectives:** In the current study, we assessed the effect of exonic missense/nonsense mutations causing PKU and FL on mRNA splicing. **Material and methods:** Nine (5 missense and 4 nonsense) PAH and eleven (all missense) ASA1 mutations were investigated

for their effect on splicing efficiency of the respective gene transcript. Minigene constructs incorporating the mutant region for each mutation were generated and the resultant effect on splicing was investigated by transient transfection in HeLa cells. **Results:** The p.E368G (c.1103A>G) PAH missense mutation resulted in skipping of exon 11 though no splice regulatory elements were predicted in the affected region, based on computational analysis, suggesting the possible presence of an uncharacterized/ novel exonic splicing regulatory element (ESR). Similarly, two ASA1 missense mutations viz. p.W169R (c.505T>C) and p.E180K (c.538G>A) exhibited a deleterious effect on splicing by causing skipping of exon 8. An ESR sequence was computationally predicted (ESEFinder) to be inactivated by c.505T>C. In contrast, c.538G>A appeared to generate a new ESR element. **Conclusions:** Recent evidences including our own have indicated that missense and nonsense mutations could also manifest as splicing defects due to disruption of SREs. The current study identified 3 missense mutations severely affecting splicing by disrupting SREs, including a possible novel SRE in the PAH exon 11. The results also help in predicting potential 'weak' exons in genes. Overall, our studies indicate the need to include transcript analysis while predicting genotype-phenotype correlations in autosomal recessive genetic disorders.

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Effect of Heme Oxygenase Gene Polymorphism on Methemoglobin Levels in NADH-Cytochrome b5 Reductase Deficiency.

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Background: Recessive congenital methemoglobinemia is a rare disorder caused by NADH-cytochrome b5 reductase (NADH-CYB5R) deficiency with an autosomal recessive inheritance. Heme Oxygenase converts heme to biliverdin which is reduced by biliverdin reductase A to bilirubin. Heme Oxygenase-1 (HMOX-1) is an inducible cytoprotective enzyme involved in the generation of the endogenous antioxidant bilirubin and vasodilating carbon monoxide. HMOX-1 gene promoter contains a polymorphic dinucleotide (GT) repeats which influence the modulation of HMOX-1 gene expression in response to oxidative stress. Although a dinucleotide (GT) repeat polymorphism in the HMOX-1 promoter is thought to be involved in the pathogenesis of disorders by modulating the expression of HMOX-1 gene, however the association of this polymorphism with methemoglobin levels is not well understood. **Aim and Objective:** The aim and objective is to study to elucidate the association between the length of (GT)_n repeats polymorphism and methemoglobin levels in 25 cases of NADH-CYB5R deficiency. **Material and Method:** We studied for HMOX-1 (GT)_n repeats polymorphism by Gene Scan analysis on an ABI PRISM 310 genetic analyzer in 25 cases of

NADH-CYB5R deficient cases along with 25 normal controls. **Results:** Previously diagnosed 25 cases methemoglobinemia with a history of cyanosis, bluish discoloration due to NADH-CYB5R deficiency were investigated. The number of (GT) n repeats in the human HOMX-1 gene promoter showed a distribution of 20-32 repeats in the NADH-CYB5R deficient cases whereas number of (GT) n repeats in the 25 normal individuals showed a distribution of 32-39 repeats. **Conclusion:** The present study demonstrated that polymorphisms like short (GT) n repeats which are a hallmark of methemoglobinemia could be the major contributing factor for cyanosis in NADH-CYB5R deficient patients. This data could also be valuable marker to the clinicians, mainly hematologist, when attempting a definitive diagnosis for the cause of methemoglobinemia due to NADH-CYB5R deficiency that will help clinical decisions for treatment.

P100

Genetic Polymorphisms and Risk Of Ischemic Stroke and Acute Myocardial Infarction: A Hospital Based Case Control Study

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Background: The patho-physiology of acute myocardial infarction (AMI) and ischemic stroke (IS) is similar. Understanding the association of polymorphic variants of candidate genes with these diseases is necessary to identify high risk population for better prevention. **Aims and objectives:** The present study was aimed to find out association of genetic polymorphism of angiotensin converting enzyme (ACE) insertion/deletion and endothelial nitric oxide synthase (eNOS-4) ab 27 base pair tandem repeat gene with acute myocardial infarction and ischemic stroke. **Materials and Method:** A hospital based case control study was carried out in Assam Medical College (AMC), Dibrugarh to recruit the study subjects. All the consecutive AMI and IS cases were recruited from the Cardiology and Neurology department of AMC. Apparently healthy, without any history of AMI and IS, age and gender matched control subjects which are unrelated to the cases were also recruited. Relevant socio-demographic and clinical information including blood samples were taken from each subjects after obtaining informed consent. Blood samples were processed for biochemical investigations, DNA extraction and PCR analysis for ACE and eNOS-4 genes. **Results:** A total of 232 acute myocardial infarction & 129 Ischemic Stroke cases and equal number of age and gender matched control subjects were recruited. The present study revealed significant association of Del/Del genotype of ACE gene & eNOS-4AA genotype of eNOS-4 gene with AMI. The ischemic stroke subjects revealed significant association with eNOS-4AA genotype. Significant interaction between genotypes and various environmental factors have been observed and the risk for development of either AMI or IS was enhanced. **Conclusion:** The present study describes the possibility in reducing the incidence of acute myocardial infarction and ischemic stroke by controlling modifiable environmental factors in a genetically predisposed population.

P101

Angiotensin II type 1 receptor (AT1R) gene polymorphisms and Essential Hypertension

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Background: Hypertension affects around 1 billion people worldwide and despite advancement in medical facilities and studies related to it, its percentage is still increasing, making it one of the main reasons for increased mortality. The genetic basis of hypertension has been established as 33% but only few forms show Mendelian inheritance pattern. SNPs in various genes have been reported to affect gene expression, binding efficiency or catalytic activity, therefore SNP studies in regulatory regions seems to be critical to detect its role on overall functionality of a gene. Though association studies in complex disorders have mostly been contradictory but this could be due to complex interplay of environmental, genetic and dietary patterns which are known to influence complex disorders like Hypertension. **Aims and Objectives:** To study association of Angiotensin II Type 1 receptor (AT1R) gene polymorphisms with Essential Hypertension. **Material and Methods:** Blood sample of recruited subjects was collected after getting ethical approval and individual consent. Total of 111 samples including both cases and controls were collected in collaboration with PGIMER, Chandigarh and Health Centre of Panjab University, Chandigarh. Genomic DNA was isolated from collected blood samples using Phenol-Chloroform method. rs5186 and -214 polymorphisms of AT1R were selected as both included binding sites of miRNA and transcription factor respectively. Targeted DNA fragment was PCR amplified and the genotyping of SNP was done through PCR-RFLP. Chi square test was further performed to look for the significance of these SNPs. **Results:** Minor allele frequency for rs5186 in cases was found to be 0.075 in comparison to already reported frequency of 0.38 in Indian study and for -214 it came out to be 0.17. **Conclusion:** Difference in allele frequency between different studies might be due to sample variation (samples from different regions of India) and sample sizes which differ in each study.

P102

Correlation of Arsenic Toxicity with Oral/Oropharyngeal Malignancy in West Bengal.

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Background: In India, the age standardized incidence rate of oral cancer is 12.6 per 100,000 populations. The effect of heavy metals in the development of this carcinoma has been correlated in countries. Since West Bengal is an arsenic prone area, we have chosen this metal in our study. Its effect can be detected through the cytogenetic damage in the form of micronuclei, chromosomal aberrations, sister chromatid exchanges and aneuploidy. **Aims and Objectives:** The objective of this study is to find out any possible correlation between arsenic toxicity and the development of oral carcinoma in this state. **Material**

and Methods- Patients attending ENT Head & Neck Surgery, Oral & Maxillo Facial Surgery departments of our hospital were screened for the presence of oral/oropharyngeal carcinoma. 65 cases and 10 control individuals were selected for the study. The hair and buccal smear samples were obtained on their proper consent and questionnaire. 20 case and 10 control hair samples were analyzed by the method of flow injection-hydride generation-atomic absorption spectrometry for arsenic count and their buccal smears were Pap stained and examined for the presence of micronuclei and apoptosis. **Results:** Out of 20 cases, 16 showed their arsenic count above the safe limit, whereas, all the controls' arsenic count were within the safe limit. The study showed a significant difference of the micronuclei and apoptosis frequency between the cases and controls. **Conclusion:** The micronuclei and apoptosis frequency proved to be significant. Although a higher percentage of cases showed elevated arsenic count, if compared to controls, yet a larger sample size is required to come to any proper correlation with the other parameters as well.

P103

Influence of Vitamin D Receptor Gene Variants on Vitamin D3 Modulated Granulysin and Perforin Positive Cells in Pulmonary Tuberculosis

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Background: 1,25-dihydroxyvitamin D3 [1,25(OH)₂D₃] is the active form of vitamin D, acts through vitamin D receptor (VDR). Variant genotypes of VDR gene have been shown to influence immune response to tuberculosis. Granulysin and perforin are cytolytic effector molecules expressed by cytotoxic T lymphocytes and natural killer cells. They mediate the lysis of Mycobacterium tuberculosis (Mtb) infected cells and play an important role in immunity to tuberculosis. **Aims and objectives:** The present study was aimed to explore the regulatory role of VDR 3'UTR BsmI and TaqI polymorphic variants on 1,25(OH)₂D₃ modulated granulysin and perforin positive cells induced by culture filtrate antigen (CFA) of Mtb. **Material and Methods:** BsmI and TaqI genotyping was done by polymerase chain reaction (PCR) based restriction fragment length polymorphism method. Peripheral blood mononuclear cells isolated from 81 healthy controls (HCs) and 79 pulmonary tuberculosis (PTB) patients were cultured with CFA with and without 1,25-dihydroxyvitamin D3 at 1X10⁻⁷ M concentration for 72 hrs. The percentage intracellular granulysin and perforin positive cells were determined by flow cytometry and data were correlated with VDR gene variants. **Results:** 1,25-dihydroxyvitamin D3 significantly decreased the percentage granulysin and perforin producing cells in CFA stimulated cultures as compared to cultures stimulated with CFA alone in HCs and PTB patients (p<0.05). Significantly decreased percentage of granulysin and perforin positive cells were found in HCs and PTB patients with TaqI tt genotype (p<0.05). No significant difference was observed among the BsmI genotypes in both study groups. **Conclusion:** The present study results suggest that TaqI tt genotype of VDR gene

may be associated with decreased percentage of granulysin and perforin positive cells, which could impair immunity to tuberculosis.

P104

Short Stature - The Only Morphological Stigmata in Isochromosome Xq

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Background: Isochromosome is defined as the structurally abnormal chromosome consisting of either two short or long arms, because of the abnormal transverse misdivision of the centromere (centric fission), resulting in unbalanced chromosomal constitution, monosomy for the missing and trisomy for the duplicated arms (Young 2005). The formation may also be because of the more complex U-type exchange resulting in acentric or dicentric products. In iso-chromosome of long arm of X chromosome clinical features are like Turner syndrome but in milder form. **Aims and Objectives:** To study the prevalence of IsochromosomeXq in Eastern Indian Population. **Material and methods:** The case records of female patients who visited our hospital (Ramakrishna Mission SevaPratishthan, Vivekananda Institute of Medical Sciences; RKMS, VIMS) with primary amenorrheawere analyzed in our institute. All these patients underwent karyotyping as a part of evaluation of primary amenorrhea along with either of the following gonadal dysgenesis, 2,3 Thyroid function tests (Free T4, TSH), ultrasound examination of genitourinary system. Serum FSH and LH were analyzed for patients who were 12 yr of age and above in our biochemistry department. The technique of karyotype analysis was performed according to guidelines from the International System for Human Cytogenetic Nomenclature (ISCN, 2005). **Result:** Out of 70 primary amenorrheapatient 5 patients were found to have isochromosomeXq. Two of them were mosaic Turner patients and three of them were with pure isochromosomeXq. **Conclusion:** A female with short stature but without typical clinical findings of TS with or without having hyper-gonadotropic hypo-gonadism should be evaluated for Karyotyping. Early diagnosis will influence the initiation of treatment thus improving the quality of life in these patients.

P105

Regulatory Role of Vitamin D Receptor Gene Variants on Vitamin D3 Modulated MIG and IP-10 Production in Pulmonary Tuberculosis

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Background: 1,25dihydroxyvitamin D3 (1,25(OH)₂D₃) evokes its immunomodulatory effect by binding to vitamin D receptor (VDR) in the immune cells. We have shown the regulatory role of VDR gene variants on immune response in tuberculosis. The monokine induced by interferon gamma (MIG/CXCL9) and interferon gamma inducible protein-10 (IP-10/CXCL10) are involved in the recruitment of T-cells

at the site of infection and granuloma formation. **Aim and Objectives:** To find out the regulatory role of VDR promoter Cdx2(-1739G/A) and 3'UTR TaqI polymorphic variants on 1,25(OH)2D3 modulated culture filtrate antigen (CFA) induced MIG and IP-10 production. **Material and Methods:** Cdx2 polymorphism was studied with allele-specific primers by polymerase chain reaction (PCR), and TaqI genotyping was done by PCR-based restriction fragment length polymorphism method. Peripheral blood mononuclear cells separated from 46 healthy controls (HCs) and 47 pulmonary tuberculosis (PTB) patients were cultured with CFA of Mycobacterium tuberculosis with or without 1,25(OH)2D3 at 1×10^{-7} M concentration for 72 hrs. The MIG and IP-10 levels were estimated in the culture supernatants by ELISA and correlated with VDR gene variants. **Results:** In HCs and PTB patients, 1,25(OH)2D3 significantly decreased the CFA stimulated MIG and IP-10 production compared to cells stimulated with CFA alone ($p < 0.05$). In PTB patients, Cdx2 AA and TaqI tt genotypes significantly increased the MIG production in CFA with 1,25(OH)2D3 treated cells. In both the study groups, TaqI tt genotype significantly increased IP-10 production in CFA with or without 1,25(OH)2D3 treated cells ($p < 0.05$). **Conclusions:** The results suggest that Cdx2 AA and TaqI tt genotypes are associated with increased MIG and IP-10 production. The higher production may be due to the association of Cdx2 AA and TaqI tt genotypes with higher expression of IFN- γ . 1,25(OH)2D3 may downregulate the inflammatory response through Cdx2 GG and TaqI TT genotypes at the site of infection.

P106

Ameliorative Effects of S-Allyl-L-Cysteine (SAC) Against Lead Induced Toxicity

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Background: Lead is a toxic metal that has been known to mankind for thousands of years and its toxic effects can cause a number of adverse human health effects. S-Allyl-L-cysteine (SAC), a natural abundant constituent of Garlic is well-known for its beneficial properties and used against several chemical induced toxic effects. **Aim And Objectives:** Our aim was to verify the hypothesis that lead toxicity resulted in the oxidative stress and there may be a link between oxidative stress with inhibition of lymphocytes proliferation and genotoxicity. Further the protective effects of SAC against oxidative stress and genotoxicity in Peripheral Blood Lymphocytes Cultures was also studied. **Materials And Methods:** Lymphocytes were cultured in vitro and experiments were carried out with administration of low and high doses (10^{-6} M and 10^{-4} M respectively), in two time durations (24 and 69 hours) of lead acetate and/or 10 μ M of SAC in cultured lymphocytes (Macro and Micro Cultures) of healthy individuals. Oxidative stress markers were estimated from the macrocultured

lymphocytes. Various parameters such as Sister Chromatid Exchanges (SCEs), Cell Cycle Proliferative Index (CCPI), Average Generation Time and Population Doubling Time were studied to evaluate the genotoxic effects of lead acetate from microcultured lymphocytes. **Results:** The results indicate a significant increase in the frequency of SCEs with a concomitant decrease in CCPI suggesting that the lead acetate modulate the cytogenetic mechanism in a dose and duration dependent manner. Significant elevated MDA level was observed along with reduction in Glutathione levels in low and high doses in both time durations. Lowered activity of various key antioxidant enzymes such as G-Px, G-Rd, GST, SOD and Catalase was also observed in a dose and duration dependent manner. **Conclusion:** Lead acetate has an immediate impact, causing an increase in MDA levels and suppressing lymphocyte proliferation. Beside this it also increases the frequency of SCE which may responsible for genomic damage. (Abstract truncated).

P107

Association between MTHFR C677T polymorphism and Migraine risk: a meta-analysis

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Background: Migraine is a debilitating neurovascular disease, which is characterized by nausea, vomiting, photophobia, neurological disturbances and severe recurrent headache. Strong familial aggregation of migraine indicates a significant genetic component for the disease. Several evidences suggest that endothelial dysfunction plays an important role in migraine and that genes affecting vascular endothelial function could contribute to the etiology of the migraine. Methylenetetrahydrofolate reductase gene variant (MTHFR C677T, rs1801133) plays important roles in the vascular oxidative stress response, are associated with migraine. **Aim and Objective:** A number of studies have examined the association of MTHFR C677T polymorphism with migraine susceptibility; however, the conclusions were contradictory. The aim of this study was to quantitatively summarize the evidence for the MTHFR C677T polymorphism and migraine risk. **Methods:** Electronic searches of PubMed, Springer Link, Elsevier and Google Scholar databases were conducted to select case-control studies and odds ratio (OR) with 95% confidence interval (CI) was used to assess the strength of association. Statistical analyses were performed using software open MetaAnalyst. **Results:** In present meta-analysis, total 19 studies, including 9,299 cases and 27,857 controls were included. The overall analysis showed that MTHFR C677T was associated with a significant increase in the risk of migraine in the allele contrast (T vs C: OR=1.2; 95% CI=1.0-1.36; $p=0.03$), homozygote model (TT vs CC: OR = 1.27; 95%CI = 1.0-1.6; $p=0.03$) and in the recessive model (TT vs CT+CC: OR = 2.1; 95%CI = 1.3-3.4; $p=0.002$). The meta-analysis stratified by ethnicity showed that individuals with the mutant T allele had increased risk of migraine (OR= 1.33; 95% CI: 0.99-1.78; $p=0.05$) in Asians but not in Caucasians

(OR= 0.97, 95% CI: 0.93-1.01; p= 0.19). **Conclusions:** The present meta-analysis suggested that MTHFR C677T polymorphism might influence migraine in overall and Asian but not in Caucasian populations.

P108

Association Between MTHFR C677T Polymorphism and Breast Cancer Risk

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Background: Breast cancer (BC) is one of the most frequent diseases in women that influenced by environmental, genetic and epigenetic factors. Dietary folate as well as polymorphic variants in one-carbon metabolism genes may modulate risk of breast cancer through aberrant DNA methylation and altered nucleotide synthesis and repair. Methylenetetrahydrofolate (MTHFR) enzyme plays an important role in folate metabolism which is involved in DNA methylation, repair, and synthesis. **Aims and objective:** Several studies have reported the association between methylenetetrahydrofolate reductase (MTHFR) C677T and breast cancer. However, findings have been conflicting. In order to investigate the association, a meta-analysis was performed. **Methods:** A literature search of PubMed, Google Scholar, Springer link and Elsevier databases was conducted on articles published before July, 2014. Crude odds ratios with 95% confidence intervals were calculated. Statistical analyses were performed using software open MetaAnalyst. **Result:** Sixty seven case-control studies including 26,206 cases and 30,208 healthy controls were identified. Meta- analysis showed a marginal significant effect in the allele contrast model (T vs. C: OR= 1.1, 95% CI=1.02-1.12, p=0.002), Homozygote model (TT vs. CC: OR= 1.14, 95% CI=1.04-1.25, p=0.005), and dominant model (TT+CT vs. CC: OR = 1.1, 95% CI = 1.01–1.21, P=0.01). In subgroup analysis stratified by ethnicity, MTHFR C677T variant was statistically significantly relevant to BC risk in Asian population (T vs. C: OR= 1.11, 95% CI=1.02-1.21, p=0.01; TT vs. CC: OR = 1.25, 95% CI = 1.04–1.49, P=0.01) but not in Caucasian population (T vs. C: OR= 1.03, 95% CI= 0.99 -1.06, p=0.05; TT+CT vs. CC: OR = 1.06, 95% CI = 0.98–1.13, P = 0.10). **Conclusion:** The present meta-analysis indicated that MTHFR C677T polymorphism is significantly associated with BC in overall and Asian population but not in Caucasians.

P109

On testing of gene-gene interaction based on case-control data using genotype similarity between individuals

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Background: Some possible explanations for the limited success of genome wide association study (GWAS) are that the current biostatistical analysis paradigm ignores all prior

knowledge about disease pathobiology and/or the linear modeling framework of GWAS considers only one marker at a time thus failing to exploit their full genomic context and giving rise to multiple comparison problems. Also gene-gene (G-G) or marker based interactions might be another cause for this. This led to the development of multilocus association that combines information from multiple markers at a time. In spite of some already available multilocus association methods for testing association, no study is done to see its effectiveness in detecting G-G interaction. **Aims and objectives:** We adopt a more holistic approach that explores G-G interaction, combining information from multiple markers of two genes. This new approach is promising in identifying the variants along with relatively potent causal markers in gene interaction models. It has the flexibility to use knowledge from other sources (biological pathways, databases etc) and helps to prioritize genetic variations to be analyzed for G-G interactions to interpret genetic association studies in a biologically meaningful manner. **Material and Methods:** Our method is based on case-control data and the information contained at each marker is captured through a kernel function based on genotypes of two individuals. We use this genotype similarity score or kernel score to develop a novel statistical method avoiding the huge burden of multiple comparisons to a great extent. The newly proposed statistic for testing any such G-G interaction is easy to compute but the calculation of p-value poses another challenge. However, we are able to derive the asymptotic distribution of the statistic under the null hypothesis of no interaction. **Results:** The asymptotic distribution of the test statistic helps in the fast calculation of pvalue even in presence of thousands of markers spread over different genes. Extensive simulations studies show that our method is very powerful in detecting any such interaction under various genetic models and is very robust. **Conclusions:** This method will have immense importance to the geneticists because of its robustness, powerful detection of interaction that may occur with or without main effects, ability to fast calculation of p-value with less multiple comparison burdens.

P110

Haplotype Analysis of Myotonic Dystrophy Type 1 Locus in India: Molecular Genetic Approach

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Background: Myotonic dystrophy type 1 (DM1) is the most common form of muscular dystrophy affecting adults and is due to trinucleotide sequence (CTG) in the 3' UTR region of DMPK gene located at 19q13.3 chromosome. Several neighbouring genes (markers) located on the same chromosome, that are statistically associated and transmitted together (haplotype), influence the disease pathogenesis as caused by mutated DMPK. **Aims and Objectives:** The intention of the present study is to analyse

the genetic characteristics of DM1 locus in India, for which four polymorphic markers (HhaI, HinfI, Bpm1, CKMM) were tested. **Material and Methods:** Clinically diagnosed and molecularly confirmed 26 DM1 patients and their family members were included in the study. PCR-RFLP analysis was performed for intron 5 (C/T)/HhaI, DMPK (G/T) intron 9/HinfI, Bpm1 and CKMM genetic polymorphism. The SNP Stat Online Software was used to construct haplotype group and for Linkage-Disequilibrium (LD) analysis. **Results:** In all DM chromosomes: Allele 2 had higher frequency in HhaI and HinfI while allele 1 had higher frequency in Bpm1 and CKMM. Total 10, 6, 8 and 10 haplotype group had been formed in Proband (patients), Proband's father, Proband's mother and in combined group respectively. Haplotype combination, 2 (HhaI), 2 (HinfI), 1 (Bpm1), 1 (CKMM Taq1) and 1 (CKMM Nco1) are more susceptible (Prone) for DM1. The HhaI & HinfI; HinfI & Bpm1; and HinfI & Taq1 show significant LD only. **Conclusion:** The haplotype analysis in the DM1 population of India supports the linkage disequilibrium between the DM mutation and HhaI, intron 9/HinfI, Bpm1 and CKMM Taq1 polymorphisms as reported worldwide. The results of the haplotype analysis suggest a common origin of the mutation in Indian population.

P111

The Distribution of Six DNA Markers of Selected Pesticide Metabolizing and Detoxification Genes in Rural Population of Punjab, North West India

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Background: The North-West border Indian state of Punjab is an agriculture dominating state and pesticides have become integral part of its rural life; the agriculture workers are highly exposed to several types of pesticides of different chemical compositions. The harmful effects of pesticides on human health have been documented worldwide. Enzymes such as cytochrome P450 super family (CYPs) and glutathione-S-transferases (GSTs) are usually involved in the initial metabolism of pesticides. Similarly, the P-glycoprotein (P-gp), encoded by the human multidrug resistance protein 1 (MDR1) gene, plays critical role in the detoxification of pesticides. No data are available in literature on the distribution of the markers of these pesticide metabolizing and detoxification genes in the people of Punjab and it is therefore that the present study was conducted. **Aims and Objectives:** The present study aims at investigating the distribution of six DNA markers of three genes involved in initial metabolism and detoxification of pesticides in rural population of Punjab. **Material and Methods:** Three hundred subjects inhabiting the rural areas in different districts of Punjab were enrolled for this work. After informed consent of the subjects, the blood samples were collected and the genomic DNA was extracted using the inorganic method. For genotyping of four SNPs viz. rs1045642 (MDR1), rs3892097 (CYP2D6*4), rs16947 (CYP2D6*2) and rs1695 (GSTP1) PCR-RFLP technique

was used, and for typing GSTM1 and GSTT1 multiplex PCR was performed followed by agarose gel electrophoresis. **Results:** The present study has provided baseline data on the studied six DNA markers in the rural people of Punjab. The results have been compared with similar studies reported for the Indian and world populations. **Conclusion:** Further investigations are desirable from Punjab and the neighbouring states to appreciate the pattern of the distribution of the studied polymorphisms of the pesticide metabolizing and detoxification genes in North India.

P112

Genetic Polymorphism of Dopamine Receptor Genes and Nicotine Dependence in Smokers of North West Indian Region

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Background: Cigarette smoking, like many other addictive behaviours, has a genetic component, and the dopamine D1 and D2 like receptors genes (DRD1, DRD2, DRD3) are candidate genes for contributing to these behaviours. Estimation of genetic susceptibility to tobacco smoking would be valuable in early identification and prevention of nicotine dependence. **Aims and Objectives:** The present study was undertaken to ascertain the association, if any, of dopaminergic gene polymorphisms amongst smokers by case control study. **Material and Methods :** DRD1, DRD2 (TaqIA, TaqIB, TaqID) and DRD3 polymorphisms were genotyped using PCR-RFLP method in smokers (n=173) and non-smokers (n=188) inhabiting North-West region of India. Written informed consent was obtained before collection of intravenous blood samples for this study. DNA was extracted using standard salting out method. **Results:** All five SNPs studied, were found to be in Hardy-Weinberg equilibrium. DRD2 TaqID (C>T, rs1800498) polymorphism showed statistically significant differences between smokers and non-smokers ($\chi^2=9.93$, d.f =2, $0.007 < p < 0.05$). On association analysis of dopamine receptor gene polymorphisms with nicotine dependence (ND) in smokers and non-smokers, only DRD3 gene showed significant association. Individuals carrying predominant homozygous genotype CC(Ser/Ser) showed a 2 folds risk of ND when compared with those carrying mutant TT(Gly/Gly) genotypes, with an Odds Ratio of 2.08, 95% CI 1.102-3.933, ($0.023 < p < 0.05$). In dominant inheritance model, the minor (T) allele containing genotypes (TT+CT) in DRD3 gene polymorphism (rs6280, C>T), were overrepresented in smokers (19.65%+52.02%) when compared to the non-smokers (12.76%+48.93%) suggesting a genetic effect of T allele on nicotine dependence showing > 1 fold increased risk (Odds Ratio 1.57, 95% CI 1.009-2.445, $0.045 < p < 0.05$). **Conclusion:** The present study suggests that individuals carrying minor T allele of DRD3 gene may be relatively more susceptible to develop nicotine dependence in their lifetime than others. Further studies are desirable to confirm this observation.

P113

Non-synonymous SNPs in the Catestatin/Parastatin region of Chromogranin A: profound associations with plasma glucose, blood pressure and risk for type 2 diabetes/hypertension

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Chromogranin A (CHGA) is a 48-kDa protein involved in the sorting/packaging of catecholamines and neuropeptides into secretory granules of endocrine/neuro-endocrine tissues. CHGA is also a pro-hormone that gives rise to several important bioactive peptides including two overlapping peptides: Catestatin (CST: human CHGA352-372), a catecholamine release-inhibitory peptide and Parastatin (PRST: human CHGA356-428), a parathyroid hormone release-inhibitory peptide. To identify naturally-occurring polymorphisms within CST/PRST domains, we re-sequenced this region in a Chennai population (n=2700). We discovered two novel polymorphisms (viz. Gly367Val and Arg377Ser) and two previously reported polymorphisms (viz. Gly364Ser and Arg381Trp). We further genotyped the Gly364Ser and Arg381Trp SNPs in a Chandigarh population (n=800) using Taqman assay. In the Chennai population, individuals with 364S or 381W allele displayed ~2 mm Hg higher Mean Arterial Pressure (MAP)/Systolic Blood Pressure (SBP) (p=0.04 for 364S) and ~8 mg/dl higher plasma glucose (p<0.05) levels as compared to G364 or R381 carriers. In the Chandigarh population as well, 364S variant was found to be associated with elevated blood pressure levels showing ~8 mm Hg higher SBP (p=0.004), ~6 mm Hg higher Diastolic Blood Pressure (DBP; p=0.001) and ~7 mm Hg higher MAP (p=0.001) levels. Of note, these SNPs showed drastic differences in their frequencies between the Chennai (Ser364: 6.28%, Trp381: 9.10%) and Chandigarh (Ser364: 3.48%, Trp381: 12.39%) populations. In both populations, the variant alleles were found to occur in significantly higher frequencies in diabetic/hypertensive cases than controls. Analysis of secondary structures of PRST wild-type and variant peptides using molecular modelling and molecular dynamics simulation studies revealed that the presence of 364S or 381W mutation introduces instability and causes dramatic changes in the PRST peptide structures providing insights into the molecular basis for their differential physiological effects. Thus, common genetic variations in CST/PRST appear to alter the risk for metabolic syndrome in Indian populations.

P114

BCL2 -938C>A and BAX -248G>A promoter polymorphisms in the development of AML

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Background: BCL2 (B-cell lymphoma 2) and BAX (BCL2-associated X protein) proteins are anti-apoptotic and pro-apoptotic determinants of Mitochondrial mediated apoptosis, their relative expression determines the cell fate. The promoter polymorphisms in these genes were shown to alter the protein function or expression and exert an impact on apoptosis regulation. Deregulation in the expression of any of these genes leads to disruption of cellular homeostasis and malignant transformation. **Aims and Objectives:** The present study was aimed to determine the association of BCL2-938C>A and BAX-248G>A promoter polymorphisms with origin and progression of acute myeloid leukemia (AML). We also have performed combined genotype analysis to evaluate the cumulative effect of risk genotypes in the AML development. **Material and Methods:** These polymorphisms were genotyped by polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) in 221 AML patients and 305 age and sex matched healthy controls. **Results:** Our study revealed that BCL2-938CA (p=0.018) and BAX-248GG (0.043) genotypes were significantly associated with increased risk for AML occurrence. BAX-248A allele had shown decreased risk of AML. The combined analysis had shown that BCL2-938CA+AA-BAX-248GG group had a 1.63 fold (95% CI: 1.08-2.45, p=0.02) increased risk for AML. None of the clinical variables had shown any significant association with both polymorphisms. With respect to complete remission (CR) rate, BAX-248GG genotype (p=0.002) and G allele (p=0.009) had conferred significant risk for complete remission failure. Although the log rank test was not significant, survival analysis had shown a trend where BCL2-938CA genotype and BAX-248GG had reduced median disease free survival (DFS) of 9 months and 10 months, respectively. **Conclusion:** BCL2-938C>A and BAX-248G>A gene polymorphisms might contribute to the origin of AML. Moreover, influence of BAX-248GG genotype on CR and DFS rate suggest that the BAX-248G>A polymorphism can serve as marker for poor prognosis in AML.

P115

Nucleophosmin Mutation in De-Novo Acute Myeloid Leukemia

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Background: Acute myeloid leukemia (AML) with mutated

nucleophosmin gene (NPM1) has distinctive clinical, hematological and molecular features, and is included as a provisional entity in 2008 World Health Organization classification. In this study, we analyzed the frequency and features of AML with mutated NPM1 in Indian patients. **Methods:** One hundred consecutive patients of de-novo AML were evaluated for NPM1 mutation and their features were compared with unmutated NPM1 patients. **Results:** AML with mutated NPM1 was seen in 21% cases. There was female preponderance with median age of 51 years. Distinguished features in mutated group were less bleeding manifestations and bone pains; more lymphadenopathy; higher median total leucocyte and platelet count; less frequency of pancytopenia and more preserved megakaryocytes. Morphologically, cup-shaped nuclei in blasts correlated with NPM1 mutation to statistical significance. Amongst the FAB subtypes, NPM1 mutation was seen in M1, M4 and M2 subtypes with none present in M0 and M3. Immunophenotypically, there was statistically significant negativity for CD34, strong association with monocytic markers (especially CD11c), CD123 was seen at higher frequency and higher mean fluorescence intensity values (MFI) for CD33 were observed in mutated cases. **Conclusions:** Important findings in this study which have not been highlighted in detail in previous studies in NPM1 mutated cases include less bleeding manifestations and bone pains, lower frequency of pancytopenia and more preserved megakaryocytes, higher CD123 expression and higher MFI values for CD33. In addition, blasts with cup-shaped nuclei were found to be independent predictors of NPM1 mutations.

P116

Vascular Endothelial Growth Factor (VEGF) Polymorphisms in Gall Bladder Cancer Progression and Survival

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Background: Angiogenesis plays an important role in growth, progression, and metastasis of tumors. The most important regulator of angiogenesis is vascular endothelial growth factor (VEGF). VEGF has been associated with advanced stage and poor survival of several cancers. **Aims and Objective:** In the present study, we evaluated the association of functional polymorphisms in the VEGF gene with gall bladder cancer (GBC) progression and survival. **Material and Method:** VEGF polymorphisms were studied using ARMS PCR for -1154G>A and -2578C>A, PCR - RFLP for +936C>T and -2549I>D amplified by PCR in DNA extracted from venous blood from 63 GBC patients who were operated before 2007 (to ensure at least 5 years survival) and in whom follow up data is available. **Results:** No significant association was found for all these polymorphisms with age, sex, presence or absence of gall stones (GS), tumor depth and distant metastasis. +936CT

genotype was significantly associated and risk protective with tumor stage ($P=0.017$, $OR=0.15$). -1154GA and -2578CA genotype showed significant association with regional lymph node metastasis ($P=0.036$ and 0.021) and showed protective association ($OR=0.19$ and 0.12). In survival analysis, patients with -2578CA genotype showed significantly longer survival (chi-square=7.017 and $P=0.030$). **Conclusion:** VEGF gene polymorphisms are important in progression and survival of GBC.

P117

Preimplantation Genetic Diagnosis: A perspective

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Background: Preimplantation genetic diagnosis (PGD) is an alternative to prenatal diagnosis wherein genetic testing is performed on embryos before a clinical pregnancy is established. PGD has been applied to patients carrying chromosomal rearrangements, single gene disorders and to also increase the implantation rates and reduce the incidence of spontaneous abortions. **Aims and objectives:** Our aim is to review the progress of PGD since its inception in terms of its applications, methodologies used and outcomes. We will also discuss the ethical issues of PGD to produce “designer babies” and further explore the role of newer technologies as alternatives to PGD. **Materials and methods:** Nowadays trophoctodermal biopsy is preferred to obtain PGD sample as against polar body biopsy or cleavage stage biopsy which were done in the past. With improvements in genetic techniques there is no longer a requirement to depend upon techniques such as FISH and PCR for chromosomal and single gene disorder detection. Furthermore, the genetic material from embryos can also be analyzed by subjecting them to array CGH or by karyomapping. However work is going on to develop novel systems or holistic approaches that incorporate computational and mathematical tools to analyze genotypic and phenotypic data. **Results & Conclusions:** The biggest ethical debate over PGD is the possibility of genesis of a eugenic population, in the future, wherein the children may get regarded as made-to-order consumer products. The second possibility that poses an ethical dilemma is that of discarding the not used embryos. Though PGD is certainly very reassuring for children and parents with known Mendelian disorder, new uses of PGD have raised questions about their ethical acceptability and the adequacy of regulatory structures to review new uses.

P118

Chromosomal Abnormalities Arising Due To Maternal/Paternal Meiotic Errors: An Important Cause For Recurrent Pregnancy Loss.

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Background: A miscarriage or spontaneous abortion is the natural death of an embryo or fetus in the womb. Occurrence of

miscarriage ranges between 15-30% of all known pregnancies (Stovall et al., 2002). Studies performed on abortuses have consistently shown that approximately 50% are associated with fetal chromosome abnormalities. The sex chromosomal abnormalities arise due to maternal/ paternal meiotic errors. The maternal versus paternal meiotic error vary substantially among the different chromosomes with nearly 50% of XXY pregnancies being the result of paternal non-disjunction whereas less than 10% of other trisomies arise by paternal meiotic error. Monosomy X, another large category (~7% of spontaneous abortions), is generally caused by loss of the paternal sex chromosome (Hassold T et al., 1992), which is consistent with the lack of maternal age effect on the incidence of 45X. **Objective:** Study of chromosomal abnormalities due to meiotic errors in recurrent spontaneous abortions. **Methods:** Chromosomal study is done by tissue culture technique, to rule out the abnormality. In cases where no chromosome preparation was possible, multiplex Interphase FISH probe set was used. Results: We report findings of 48 spontaneous abortion cases (POC samples including fetal and placental tissue). Tissue culture was performed on all 48 specimens. Successful culture and Karyotype was obtained in 14 out of 48 cases, in the remaining cases FISH was performed. We report six cases with chromosomal abnormality which includes autosomal trisomy, monosomy X, XYY, and XXY cell line in the aborted fetus. **Conclusion:** This study shows 6.25% of cases showing autosomal trisomy for chromosome 13, 18, and 21; and 8.33% of cases have sex chromosomal anomalies. Identification of genetic cause can have implications for proper genetic counselling and future prenatal diagnosis. Karyotype can unravel hitherto unknown microscopic anomalies; whereas FISH can unravel sub-microscopic anomalies in non-dividing cells also, but requires prior knowledge of possible chromosomal region and is also limited in terms of number of target regions. The present report emphasizes on need for including more sensitive methods like chromosomal microarray in order to increase the scope of identifying cryptic genetic aberrations.

P119

Turner Syndrome and a variant of Turner Syndrome

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Background: Turner syndrome is the most common female sex chromosomal abnormality. The incidence is 1 in 2000 to 1 in 5000 live births. The most common karyotype of Turner syndrome is 45,X0 in 80% of affected females and remaining may have some variants. The main characteristics of this disorder are short stature, gonadal dysgenesis, primary amenorrhea, a webbed neck, widely spaced nipples. **Aims and objectives:** To describe the underlying importance of chromosomal analysis. Case description: Newborn baby (case 1) was referred with webbing of neck, wide spaced nipples and long philtrum and a

14-year-old girl (case 2) with short stature, low set ears, webbed neck and no secondary sexual characteristics for chromosomal analysis. **Materials and Methods:** Chromosomal analysis of these cases were carried out by using standard GTG banding technique. **Results:** Cytogenetic analysis revealed 45, X[20] in case 1 and mos45,X[17]/46,X,del(Xp)[3] in case 2. FISH analysis was performed for case 2 on the same peripheral blood sample using CEP X/Y probe (Vysis, USA) showed 81% of the cells with single green signal for X chromosome and 19% of the cells shown two green signals, which authenticate the presence of second abnormal cell line 46,X,del(Xp). In addition, FISH study confirms there is no Y chromosome. **Conclusion:** Early recognition of Turner syndrome and timely investigations should be helpful. Karyotyping is definitely helpful in the evaluation of short stature and FISH study is essential to detect low level mosaicism with normal X chromosomes in such patients for the appropriate counseling.

P120

Prevalence of Malaria in A Tertiary Care Hospital in Navi Mumbai, India

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Background: Malaria is a major cause of morbidity in the tropics. Disease is of global importance, results in 300–500 million cases yearly and 1.5–2.7 million deaths annually. Approximately 2.48 million malarial cases are reported annually from South Asia, of which 75% cases are from India alone. **Aims and Objectives:** To find the prevalence of malarial parasites at tertiary care hospital, Navi Mumbai, India. **Material and Methods:** This prospective study was carried out at Department of Microbiology, MGM Medical College and Hospital, Navi Mumbai, India, over a period of one year from January 2013 to December 2013. After obtaining informed consent, 3-5 ml blood specimens were collected from antecubital vein of all patients by taking sterile precaution. Thick and thin smear were prepared and stained with Field stain and examined under light microscope using an oil-immersion lens (100x magnification) after putting a drop of paraffin oil. Positive result of malaria given if at least one asexual form of parasite was detected in 100 microscopic fields in thick blood film otherwise the report was given as negative. Blood parasite density was determined from the thin films by counting the number of parasites in 200 white blood cells (WBCs). **Results:** Total 4878 blood smear were studied, 809 (16.58%) were positive for malarial parasites. July to November, with peak in October (Rainy and winter) season was found to malaria season. Plasmodium vivax species was more predominant than Plasmodium falciparum (65.51% Vs 6.55%). Mixed species were 27.93%. Malarial infections occur more in males than in females with ratio of 3:1. Malarial infections occurred most in the age group 11-50 years with peak in age group 21-30 years. **Conclusions:** The prevalence of malarial infection was 16.58%, Rainy season was found to malaria season. Plasmodium vivax was predominant, Male

- female ratio was 3:1, young working age group found more prone to infection.

P121

Characterization of *Aspergillus* Species and its Antifungal Drug Sensitivity Testing

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Background: Aspergillosis is the most common fungal infection of the respiratory tract; it is usually caused by *Aspergillus fumigatus* and less commonly by *A. flavous* or *A. niger*. *Aspergillus* is a very large genus containing over 185 species to which humans are constantly exposed. At least 30 *Aspergillus* species have been associated with human diseases, and *A. fumigatus* remains the most frequent cause of invasive aspergillosis. **Aims and Objectives:** To identify the *Aspergillus* species and its antifungal drug sensitivity testing. **Material and Methods:** This prospective study was carried out at Department of Microbiology, MGM Medical College and Hospital, Navi Mumbai, India, over a period of six months from May 2014 to October 2014. Identification of *Aspergillus* species was done by standard methods. Antifungal drug susceptibility testing was done using disc diffusion method. **Results:** Eleven isolates were included in this study *Aspergillus niger* (55%), *Aspergillus fumigatus* (27%) and *Aspergillus flavous* (18%). *Aspergillus niger* showed maximum sensitive to Miconazole (MIC) 100%, Nystatin (NS) 100%, Amphotericin-B (AP) 50%, and minimal sensitive to Ketoconazole (KT) 33%, Itraconazole (IT) 17%, Fluconazole (FLU) 0%. *Aspergillus fumigatus* showed maximum sensitive to Miconazole (MIC) 100%, Nystatin (NS) 100% and minimal sensitive to Ketoconazole (KT) 33%, Itraconazole (IT) 0%, Fluconazole (FLU) 0% and Amphotericin-B (AP) 0%. *Aspergillus flavous* showed maximum sensitive to Miconazole (MIC) 100%, Nystatin (NS) 100%. **Conclusion:** *Aspergillus niger* was the major fungal isolates. The antifungal drug susceptibility test showed maximum sensitivity to Miconazole and Nystatin and minimal sensitive to Amphotericin-B where as the resistant to Itraconazole, Fluconazole and Ketoconazole. This study supports the use of Miconazole and Nystatin as a choice of drugs for treatment of Aspergillosis.

P122

Identification of Mutations in the *NODAL* Gene in Congenital Heart Disease Patients in Indian population

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Background: The TGF- β superfamily member *NODAL* is known to play a critical role in vertebrate embryonic development by

inducing mesoderm & endoderm formation, cardiomyocyte differentiation and determining left-right axis. Defects in *NODAL* signaling are known to be involved in diverged pathological conditions, including heterotaxy syndrome and several other forms of isolated cardiovascular malformations. **Aim and Objectives:** The aim of present study is to understand the role of *NODAL* in human cardiac development. Therefore, in this study we have investigated whether genetic variants in *NODAL* gene are involved in isolated forms of congenital heart diseases (CHDs). **Material and methods:** We have screened 300 unrelated probands with congenital heart disease using PCR amplification of coding region of the gene followed by direct DNA sequencing method. We have also analyzed 100 age and ethnic matched controls. **Results:** We identified three missense (P51L, R165H and D327E) mutations and 5 synonymous (L6L, A67A, Q133Q, K317K, T318T) changes in the *NODAL* gene. With an exception of R165H, all other changes are not found at least in 200 control chromosomes. All these variants are conserved across the species. Phenotype analysis of the patients carrying these heterozygous mutations revealed a high incidence of septal defects. In addition to this, three intronic variations (c.235+12 C>T; c.933+20G>A; c.933+63bp G>A) have also been revealed. In silico analysis of the above mentioned non-synonymous variations reveals that these mutations have impact on the protein secondary structure and may debilitate the function of protein. **Conclusion:** The genotype-phenotype analysis as well as in silico studies, together suggests that the sequence variants observed in the *NODAL* are possibly causal for CHD. Further in vitro functional studies are in progress that would confirm the involvement of these mutations in CHD.

P123

NAT2 Gene Polymorphism as A Predisposing Factor for Phenytoin Intoxication In Tuberculous Meningitis or Tuberculoma Patients Having Seizures- A Pilot Study.

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Background: Simultaneous administration of phenytoin and isoniazid (INH) in tuberculous meningitis or tuberculoma patients with seizures results in higher plasma phenytoin level and thus phenytoin intoxication. Genotypic status that influences response to drug treatment is of increasing importance. **Aim & objective:** Present study observes the effect of allelic variants of N-acetyltransferase 2 (*NAT2*) gene on the prevalence of phenytoin toxicity. **Material & Methods:** Sixty patients with tuberculous meningitis or tuberculoma with seizures and taking INH and phenytoin simultaneously for minimum period of seven days were included in study. Plasma phenytoin was measured by high performance liquid chromatography. *NAT2* gene polymorphism was

studied using restriction fragment length polymorphism and allele specific PCR. **Results:** Sixty patients were grouped into those having phenytoin intoxication and those with normal phenytoin level and also classified as rapid or slow acetylators by NAT2 genotyping. Genotypic analysis showed that out of seven SNPs of NAT2 gene studied, six mutations were found to be associated with phenytoin intoxication. For rs1041983 (C282T), rs1799929 (C481T), rs1799931 (G857A), rs1799930 (G590A), rs1208 (A803G) and rs1801280 (T341C) allelic variants, the proportion of homozygous mutant was higher in phenytoin intoxicated group than in phenytoin nonintoxicated group ($\chi^2 = 3.93$, $P = 0.006$; $\chi^2 = 21.2$, $P = 0.0001$; $\chi^2 = 10.1$, $P = 0.002$; $\chi^2 = 21.0$, $P = 0.0001$; $\chi^2 = 15.8$, $P = 0.01$; $\chi^2 = 5.76$, $P = 0.01$ respectively). **Conclusion:** Homozygous mutant allele of NAT2 gene at 481site can act as predisposing factor for phenytoin intoxication among tuberculous meningitis or tuberculoma patients having seizures.

P124

Identification and Characterization of Novel Non-synonymous Variation in Paired Domain of PAX9: Probable Cause of Congenital Tooth Agenesis in an Indian Family

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Background: Congenital tooth agenesis (CTA) is a common developmental anomaly in human. About 20% of world population lack at least one 3rd molar (Wisdom teeth) by birth which is presently considered as an evolutionary consequence by scientific communities. Excluding 3rd molar agenesis about 2.5 to 6.9% of the population suffers from CTA affecting other tooth type. CTA is classified into 3 types; Hypodontia (<6 missing teeth), Oligodontia (≥ 6 missing teeth) and Anodontia (all teeth missing). CTA could be of familial or sporadic in origin and found either in syndromic or non-syndromic condition. So far 5 major genes are associated with CTA viz. PAX9, MSX1, EDA, AXIN2 & WNT10A and among them PAX9 is mainly associated with non-syndromic familial tooth agenesis while other genes might be associated with syndromic tooth agenesis. **Aims and objectives:** Identification of genetic cause of non-syndromic tooth agenesis in an Indian family. **Objectives:** Mutational screening of candidate PAX9 in familial non-syndromic hypodontia and in vitro functional validation of potentially pathogenic mutant underlying CTA. **Materials and methods:** Orthopantomograph (OPG) and peripheral blood samples (for genomic DNA) were collected from three affected and one unaffected members of the family (BANG1). All coding exons including exon-intron boundaries and putative promoter region of PAX9 were amplified by PCR followed by Sanger sequencing. In vitro mutagenesis was performed to create c.336C>G change, observed in BANG1

family, in PAX9 cDNA cloned in pcDNA 3.1/myc-His(A). Wild type and mutant PAX9 were expressed in COS7 cell line and analyzed for their nuclear-cytoplasmic distribution through Western blot analysis and confocal microscopy. **Results:** Autosomal dominant mode of inheritance was evident in this BANG1 family segregating CTA affecting all types of tooth with variable expressivity. A novel transition variation NM_006194.3:c.336C>G in second coding exon of PAX9 causing a non-synonymous NP_006185.1:p.Cys112Trp was observed in all the affected individuals except in unaffected member of the family. In silico homology modeling indicates that this variation resides in DNA binding paired domain region. Nuclear-cytoplasmic distribution analysis indicates that the cytoplasmic retention of mutant protein is relatively higher than wild type. **Conclusions:** In vitro functional study of the mutant protein and in silico analysis indicates that loss of function leading to haploinsufficiency of PAX9 could be one of the probable causes for CTA in this family.

P125

Standardization of IGK Rs 121918591 by PCR-AFLP

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Background: Immunoglobulins (antibodies) are glycoproteins present in serum gamma globulins produced by B-lymphocytes (B cells) or plasma cells in response to exposure to antigen. The basic structural unit (monomer) of immunoglobulin (Ig) molecules consists of four polypeptide chains (2 Light and 2 Heavy) covalently linked by disulphide bonds. The polypeptide chains of immunoglobulins are encoded by three non-linked clusters of autosomal genes, one cluster coding for heavy chains (H), a second one for kappa light chains (κ) and a third one for lambda light chains (λ). Separate identity exists for each chain since they are coded for on separate chromosomes, the heavy chain (H) gene family is on chromosome 14, the kappa chain (κ) gene family is on chromosome 2 and the lambda chain (λ) gene family is on chromosome 22. The human IGK locus is located at band 2p11.2 of the short arm of chromosome 2. A nsSNP in the N terminal region C $\rightarrow$ T resulting in Ala 135Val was found to be related to a newly detected inborn error metabolic disorder. **Aims and Objectives:** In present study the methodology for analysis of nsSNP rs 121918591 in the N terminal region of IGK using a heather to unreported PCR-RFLP method is attempted and standardized. **Material and methods:** Blood samples were collected with their informed consent under ethical clearance no ICEC/4/2011. Primer designing was undertaken using gene sequence downloaded from NCBI. Genomic DNA was isolated from blood and subjected to amplification. **Results:** The PCR reaction conditions were standardized vis-à-vis salt concentration and annealing temperature. The amplified fragment was digested with FaeI and the products loaded on 12% PAGE. The visualization methodology for the fragment pattern on PAGE was standardized using different concentrations of gels, presence or absence of glycerol and voltage required for obtaining best band pattern, intensity and sharpness.

P126

Role of Intronic SNP and Its Association with Oral Squamous Cell Carcinoma in the Populations of Western Part of India.

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Background: Oral Squamous Cell Carcinoma (OSCC) – a subset of Head and Neck Cancer is the sixth most common cancer worldwide and leading cancer in India. Gujarat, western part of India is a prime tobacco growing belt, henceforth high consumption of tobacco among the population of this region led to high occurrence of OSCC, includes deregulation of genes involved in PI3K/AKT(Phosphatidylinositol 3- kinase – Akt) pathway, such as PIK3CA (Phosphoinositide-3- kinase catalytic α) and PTEN (Phosphatase and Tensin homologue).

Aims & Objective: The present study was aimed to find the genetic association of various polymorphisms in intron 9 of PIK3CA gene and intron 5 of PTEN gene with primary OSCC tumors; since these are the polymorphic regions studied in various human cancers. **Materials & Methodology:** 50 OSCC patients for PIK3CA gene, 60 OSCC patients for PTEN gene and 36 healthy individuals were included in the study. The polymorphisms in Intron 9 of PIK3CA and intron 5 of PTEN gene were analyzed by PCR-direct sequencing. **Result:** We have observed rs114587137 (C>T) and rs17849071 (T>G) SNPs in intron 9 of PIK3CA gene and rs35560700 (C>T) SNP in intron 5 of PTEN gene. T/T genotype of rs17849071 (T>G) has found the risk for developing OSCC; while T/G genotype has seemed protective effect against OSCC ($P=0.01$). We have found significant association of rs35560700 (C>T) with OSCC; where frequency of C/T genotype was found significantly higher in OSCC patients when compared to healthy individuals ($P=0.0003$). Conclusion We found significant inverse association of rs17849071 (T>G)SNP in PIK3CA gene with OSCC pathogenesis, suggesting that presence of G allele in an individual would greatly protect against the development of OSCC. Moreover, T allele of rs35560700(C>T) SNP in PTEN gene has shown significant risk for developing OSCC. As this is a population base study, larger sample size would be helpful to confirm our findings globally.

P127

Analysis of Obesity Related Gene with Special Reference to Solute Carrier Family 6 (Amino Acid Transporter), Member 14 (Slc6a14): A Computational Approach

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Obesity is an epidemic, natural consequence of over nutrition and sedentary lifestyle. It is a condition characterized by an excess of body fats with globally projected around 1.12 billion obese individuals by 2030. Single nucleotide polymorphism (SNP) suggests genome-wide screens to identify common genetic variants associated with obesity. In this context, non-

synonymous single nucleotide polymorphism (nsSNPs) used as a potential biomarker to identify deleterious and neutral effect on protein function. The main objective of this work to analyze the functional impact of SLC6A14 gene present on chromosome Xq23 in relation with X-linked obesity by using computational tools and strategies. For this purpose firstly, SNPs were retrieved from dbSNP database. The computational algorithms namely, PolyPhen, SIFT and I-Mutant 2.0 were used to identify the potentially deleterious nsSNPs. Herein, one SNP (rs182839907) was found which gave the positive results with all three above mentioned computational tool. The gene network analysis was carried out with STRING 9.1 tool showed that the following genes, SLC16A2, SLC22A1, SLC22A6, SLC23A1, SLC29A1, SLC29A2, SLC38A1, SLC38A2, SLC38A5, SLC39A6 are functionally associated with SLC6A14 gene. The homology models of the SLC6A14 proteins having the nsSNPs were predicted using SWISS-MODEL software. Ramachandran plot using PROCHECK algorithm was done to check the stereochemical properties of the predicted models. Root Mean Squared Deviation (RMSD) was calculated by superimposing with native model. The free energy values for the mutant models were high as compared to their native structure. The present study may suggest that nsSNP (rs182839907) would be a potential biomarker to screen the obesity among individuals.

P128

Fat Mass Obesity Associated (Fto) Gene: Computational Analysis of Non-Synonymous Single Nucleotide Polymorphisms (Nssnps)

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Obesity is condition in which excess body fat is accumulated to the body extents and lead to adverse effect on health by reducing life expectancy and increasing health problems. It is associated with various diseases like cardiovascular, diabetes type 2, obstructive sleep apnea, certain types of cancer and osteoarthritis and accounts for the leading preventable cause of death worldwide. It is the result of interplay between genetic and environmental factor. Polymorphism in various gene controlling appetite and metabolism plays important role for the development of obesity. Non-synonymous single nucleotide polymorphisms (nsSNPs) are used as biomarkers to disease susceptibility. In this study, non synonymous SNPs in Fat Mass Obesity Associated (FTO) gene were screened for its functional impact on the protein. Firstly, SNPs were retrieved from dbSNP database. The computational algorithms namely, PolyPhen and PANTHER, were used to identify the potentially deleterious nsSNPs. The following four SNPs , rs139000284, rs139577103, rs368490949 and rs373076420 are found to be most significant SNPs affecting the protein function. The gene network analysis using STRING algorithm showed that the following genes, NPY, IGF2BP2, CDKAL1, TCF7L2, SLC30A8, TMEM18, HHEX, TCF7 and GNPDA2 are functionally associated with FTO gene. The

homology models of the FTO proteins having the snSNPs were predicted using SWISS-MODELLER software. The stereochemical properties of the models were checked by Ramachandran plot using PROCHECK algorithm. Root Mean Squared Deviation (RMSD) were calculated by superimposing with native model. The free energy values for these mutant models were high as compared to their native structure. The present study suggests that these four nsSNPs can be used as biomarkers to screen obesity susceptibility.

P129

Effects of Gender on The Relationship Between IL6 Promoter Polymorphism and Lymphocyte Gene Expression in Schizophrenia

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Background: Gender differences in the pathogenesis and clinical manifestations of schizophrenia are postulated to be secondary to putative influence of sex hormones especially estrogen. Estrogen has significant immunomodulatory effects and neuroimmunopathogenesis is one of the overarching explanatory paradigms to understand schizophrenia. Among several immune factors, interleukin-6 (IL6) plays a significant role; increased IL6 expression both in the periphery and brain is an established observation in this disorder. **Aim:** To examine the potential effect of gender on the relationship between IL6 promoter polymorphism and gene expression. **Methods:** Antipsychotic-naïve schizophrenia patients (DSM-IV) [N=53] were examined in this study in comparison with healthy controls [N=73]. DNA and RNA was extracted from lymphocytes that were isolated from peripheral blood. The IL6 174 G/C polymorphism (rs1800795) was genotyped by TaqMan 5' nuclease assay. IL6 gene expression was quantified using reverse transcription followed by TaqMan gene expression assay. **Results:** There was a significant impact of gender-by-genotype interaction on the lymphocyte gene expression level in schizophrenia patients [M:F=33:20; GG:GC=37:16; F=5.3; p=0.026]. Male schizophrenia patients carrying GC genotype had significantly greater expression of IL-6 than those with GG, however, this was not observed in healthy controls [M:F=49:24; GG:GC=52:21; F=0.3; p=0.561]. **Conclusion:** Our preliminary findings suggest potential gender-specific influence of IL6 promoter variant on its expression in the lymphocytes of schizophrenia patients. The lower levels of estrogen, which is found to have a modulating effect on IL-6 expression, could be a reason for enhanced expression of IL-6 in male schizophrenia patients in comparison to female patients. However, the lack of such gender-specific interaction in the healthy control group needs further evaluation.

P130

A Novel and Recurrent Lynch Syndrome Associated Indian Founder Mutation in Mismatch Repair (MMR) Gene MLH1

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Background: Lynch syndrome (LS) is an autosomal dominant inherited colorectal cancer syndrome due to germline mutations in MMR genes, mostly MLH1 and MSH2. MMR gene mutation may occur at high frequency in certain geo-ethnic groups due to founder effect. Founder mutations facilitates genetic testing and counselling but are yet to be reported in MMR gene in South Asians. **Aims and Objectives:** To identify recurrent MMR gene mutation and establish its founder effect. **Material and Methods:** Germline mutation in MMR genes was analyzed in our cohort of 70 Indian LS families. In the initial analysis, a novel germline MLH1 mutation c.156delA was identified in 2 unrelated families who hailed from the same community (Shia Momin) and region (Patan, Gujarat), indicating a possible founder mutation. This mutation was screened in 68 LS probands in our cohort and in samples from 400 healthy individuals or cancer affected cases from Shia Momin community. Following PCR-CSGE analysis of MLH1 Exon 2, mutation was confirmed by Sanger sequencing. Founder effect was established by haplotyping with a panel of 6 highly polymorphic microsatellite markers flanking the mutation site. **Results:** The c.156delA mutation was identified in 16 individuals from 6 families hailing from Gujarat (5 Shia Momin & 1 Hindu family) with some mutation carriers being unaffected so far. This mutation was absent in probands who were not from Gujarat. Haplotype analysis revealed a conserved region of approximately 2.5 Mb in all mutation carriers, providing an evidence for a common ancestor between these extended families. **Conclusion:** This study on the largest LS cohort identified the first Indian founder mutation in MLH1 gene. As this founder mutation is recurrent in the Gujarati Geoethnic group and detected by simple PCR-CSGE method, its screening could be a cost-effective strategy for genetic testing of Indian LS suspects, especially those originating from Gujarat.

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Genetic Counseling an Essential Component of Thalassemia Management for Mitigating Disease Burden

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Background: Beta-thalassemia is a type of hemoglobinopathy wherein the body fails to produce enough hemoglobin leading to decreased oxygen supply to the organs causing life threatening

problems. It follows autosomal recessive mode of inheritance requiring both the copies of the gene to be nonfunctional to cause the disease. Thalassemia and Sickle Cell Society (TSCS) in Hyderabad is a referral center where the patients from different parts of Telangana, Andhra and Maharashtra-Karnataka border come for subsidized transfusion and chelating drug therapy. **Aims & Objectives:** To evaluate the problems faced by families with a child affected with thalassemia and the role genetic counseling plays in creating awareness to mitigate the burden of this disease both at the family and community level. **Materials & Methods:** 151 Families at TSCS were randomly interviewed during the transfusion session of their child. Details were recorded in a well designed proforma about the clinical, financial and social factors associated with diagnosis and treatment of their child. **Results:** In only 39% of the cases the primary health care provider diagnosed Beta-thalassemia, while in the majority of the cases, especially from smaller districts, the families had to travel to Hyderabad for diagnosis and still travel for transfusions monthly once/twice.. Most of the cases were diagnosed between the ages of 3months to 5 years. Contrary to popular belief 46% of the cases were born to non-consanguineous couples. 14% of the patients had an older sib with thalassemia. **Conclusions** The study revealed that primary care physicians require training to diagnose thalassemia. TSCS can train personnel so that sub-centres can be set-up in districts for management of the disease. Appropriate genetic counseling should be given for preventing recurrence of the disease in the same family especially by promoting extended family screening and appropriate pre-natal testing. Effective Government policies need to be introduced to mitigate the burden of this disease in our population.

P132

A Case Report of Breast and Ovarian Cancer Family

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Introduction: Breast cancer is a type of cancer originating from the breast tissues. Both men and women can be affected by it. Detecting it early can significantly reduce the death risk. Hereditary Breast and Ovarian cancer (HBOC), is an inherited genetic condition and an autosomal dominant inheritance pattern. About 6-8% of breast and ovarian cancers are hereditary. Women who carry BRCA1 or BRCA2 mutations have an estimated lifetime risk between 60% and 85% for developing breast cancer. **Case Study:** A 82 year old woman was diagnosed with Ovarian carcinoma during her treatment her granddaughter approached the department of genetics to know her risk of getting the neoplasm. A detailed pedigree indicated that two of her daughters and one granddaughter had already succumbed to a breast malignancy. Hence molecular testing for BRCA genes was advised. Next Generation Sequencing found a pathogenic heterozygous mutation in Chr 17: 41276044 del CT in exon 2 of BRCA1 gene

with a predicted amino acid change p.Glu23Valfs*17, which was reported as a pathogenic mutation. **Genetic Counselling :** Based on the pedigree different members of the family with a risk of inheriting the disease were advised targeted mutation analysis for the pathogenic mutation. Four family members were found to be positive for that mutation. These individuals were provided genetic counseling and placed in the surveillance programme, which included regular monitoring for ovarian cancer by CA 125 and ultrasound and mammogram for breast cancer screening. Other family members were offered genetic counseling based on the BRCA mutation results for appropriate screening and management. **Conclusion:** Genetic counseling in this family was tricky as the same pathogenic mutation was responsible for early onset breast cancer and late onset ovarian cancer leading us to hypothesize that, variants of unknown significance in the same gene maybe responsible for the differences in the age of onset or other minor genes maybe playing a role.

P133

Genetic Variability of 15 Autosomal STR Loci in Central Indian Populations

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Background: India is a country known for its biological and human diversity. Central Indian region of India is also very much diverse and has been centre of attraction for studying genetic variations. Studies using various markers have been helpful in understanding the relationship between ancestry, demographic and migration history of humans.

Aims and objectives: The study was planned with the aim to determine the genetic variation and genetic relationship at 15 Autosomal STRs loci (TH01, D3S1358, vWA, D21S11, TPOX, D7S820, D19S433, D5S818, D2S1338, D16S539, CSF1PO, D13S317, FGA, D18S51, D8S1179) in four Castes and two Tribal populations of Central India for forensic purposes. **Material and Methods:** DNA was extracted from blood samples collected from 485 unrelated individuals using phenol-chloroform extraction procedure. Real Time PCR ABI 7000 was used for quantification of DNA. Amplification was done using AmpflSTR identifiier Kit (ABI) and Investigator® IDplex Plus PCR Amplification Kit (Qiagen). **Results:** Statistical analysis of the data revealed all the studied STRs loci were highly informative and discriminating with Combined Power of Discrimination >0.99999. All these loci fall under Hardy-Weinberg equilibrium after Bonferroni correction (p>0.003) except loci D18S51, D7S820 and CSF1PO. Genetic affinity of these populations was also analysed using pair wise genetic distance (Fst) based method including neighbour joining tree and PCA plot. **Conclusion:** The present study reveals DNA profiling based on STRs in combination with appropriate reference population database is a useful tool for genetic characterization and forensic investigation.

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Cytogenetic survey of Primary Amenorrhea - A study of 818 cases from Department of Cytogenetics, Metropolis Healthcare Limited.

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Background: Amenorrhea is the absence or cessation of menstruation and seen normally in prepubertal, pregnant and post menopausal females and leads to 20% of patients with infertility. Genetic disorders pose considerable health and economic burden on family. Though most of the genetic diseases cannot be cured, early detection and medical intervention is helpful. Hence, laboratory diagnosis is crucial for confirmation of clinical diagnosis. **Aim:** To estimate the type and related incidence of sex chromosomal abnormalities in referred cases with history of primary amenorrhea (PA). **Method** Total of 818 cases were referred for Conventional Cytogenetic evaluation in which clinical indication of PA was common and was associated with other findings like USG abnormality, short stature, genital abnormalities, poor development of secondary sexual characters and infertility. 72 hrs Blood Lymphocyte Cultures were setup and chromosomal study was done by GTG banding. Further confirmation of diagnosis by targeted FISH using centromeric probes for chromosomes X and Y and locus specific probe for SRY gene wherever applicable was done. **Results :** Out of 818 cases, 461 were referred with indications of PA and 357 for PA and related findings. Out of 461 cases, 377 (81.77%) showed normal chromosomal constitution (46,XX) and 84 (18.22%) showed abnormal chromosomal constitution. Out of 357 cases, 239(66.94%) showed normal chromosomal constitution (46,XX) and 118(33.05%) showed abnormal chromosomal constitution. Percentage-wise distributions of abnormal cases are as tabulated below:

Abnormal chromosomal constitution	Percentage-wise distribution of 461 cases with PA (%)	Percentage-wise distribution of 357 cases with PA and associated findings (%)
Numerical aberrations	12.58% (n=58)	19.60% (n=70)
Structural aberrations	3.47% (n=16)	9.52% (n=34)
Translocations	0.86% (n=4)	1.12% (n=4)
Normal variants	1.30% (n=6)	2.80% (n=10)

Conclusion: Our studies revealed that majority of cases with clinical manifestations have sex chromosomal abnormalities. Timely cytogenetic evaluation and Genetic Counseling in such cases may be essential for management and can improve the quality of life.

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Genetic Polymorphism of Glutathione S-Transferase Genes *GSTT1*, *GSTM1*, *GSTP1* and Susceptibility to Breast Cancer.

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Background – Glutathione S- transferase (GSTs) play an important role in the detoxification of xenobiotics by catalyzing nucleophilic attack by glutathione on electrophilic compounds. Earlier studies have indicated an association between genetic variants of *GSTT1*, *GSTM1* and *GSTP1* genes and an increased risk of developing various types of cancer .In the present study an attempt has been made to determine the association of these variants with susceptibility to breast cancer, which is the most common cancer among women in India and second most common cancer world-wide. The incidence of breast cancer in India in 2012 was 27 % of all the cancers. **Aims and Objectives:** To investigate the role of genetic polymorphisms of *GSTT1*, *GSTM1* and *GSTP1* genes in susceptibility to breast cancer. **Material and Methods:** A total of 300 subjects comprising of 200 breast cancer women and 100 normal healthy women from Punjab were genotyped for *GSTT1* ,*GSTM1* and *GSTP1*. Genotyping of *GSTT1* and *GSTM1* was done by allele specific PCR and *GSTP1* was done by PCR-RFLP method. **Results:** The distribution of genotypic frequencies of *GSTT1*null, *GSTM1* null and *GSTP1* variants (Ile105Val, Val105Val) were 36.5%, 51%, 47.5% and 14.5% in patients and 34% ,41% ,49% and 12% were in controls respectively. Comparison between patients and controls revealed a significant increase in the genotypic frequencies of *GSTT1* null (OR=1.841), *GSTM1* null (OR=1.498) and *GSTP1* variant Ile105Val (OR=1.0051) among patients as compared to controls. The study also indicated that individuals carrying both *GSTT1* and *GSTM1* null genotypes had a greater risk of developing breast cancer (OR=2.23). **Conclusion:** Results support an involvement of GST gene polymorphisms, both independently and in combination, in susceptibility to breast cancer.

P136

Association of Cholesteryl Ester Transfer Protein (-629C/A) Gene Polymorphism with Non Diabetic Atherosclerosis.

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Background: Cholesterol ester transfer protein (CETP) plays a central role in HDL metabolism .It facilitates RCT and acts as anti atherogenic factor while by transferring CE and receiving TG it increases small dense LDL and decreases HDL which is a pro atherogenic effect. The activity of CETP has been shown to be genetically determined and CETP(-629C/A) is a functional gene polymorphism in the promoter region. **Objective:** To evaluate the role of new biomarkers LCAT, CETP along with CETP gene (-629C/A) polymorphism in patients of non diabetic atherosclerosis. **Material & Methods:** Study group consisted of 100 subjects (50 with positive angiography as cases, 50 with negative angiography as controls). LCAT (Biovender) and CETP(Bmassy) were estimated by ELISA. For genetic polymorphism ,extracted DNA from blood sample was amplified by PCR, which was digested overnight with Aval restriction

enzyme. Digested products obtained were run on 3% agarose gel and RFLP was studied by gel documentation system. **Results:** CETP levels was significantly higher in cases than control group ($p = 0.035$) while LCAT levels were not significantly different between cases and controls ($p = 0.764$). Frequency of the AA genotype was more (72%) in cases compared to controls (64%) while CA genotype was found in 26% cases and 34% controls with one CC genotype in each group. Mean CETP Level was significantly higher in cases with AA genotype compared to controls ($p < 0.05$) while CETP in cases with CA genotype was higher although not significant ($p > 0.05$) compared to controls. **Conclusion:** We found significantly higher levels of CETP in cases who had positive angiography for atherosclerosis as compared to controls which suggest an association of CETP with atherosclerosis. Also AA was more frequently present in cases (72%) compared to controls (64%). However, ours being a small study, more studies with larger sample size are needed to confirm genotypic risk associated with CETP (-629C/A) gene polymorphism especially AA homozygosity.

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A Rare Trisomy 18p Syndrome Characterised by Microarray Analysis and Metaphase FISH

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Trisomy 18p is a rare chromosomal aberration, in which there are three copies of the short arm p of chromosome 18. Only 27 such cases have been documented in medical literature. We report Trisomy 18p syndrome in a 4 year old male child with developmental delay, dysmorphism, and dolicocephaly and club foot. Microarray analysis was performed as a first tier test, using Agilent 4x180K(ACGH+SNP array), which revealed duplications of 15Mb genomic region on 18p11.32 - p11.21 (64847 bp to 15165737 bp) and 340kb on 18p11.21 (14196900bp to 14537567bp) of chromosome 18p. This aberration was further validated by karyotyping and fluorescence in situ hybridisation (FISH). It was found to be present in all metaphases of cultured lymphocytes in the form of an extra structurally abnormal chromosome (ESAC) which showed the presence of an aqua signal when hybridised with Vysis DNA probe for CEP18/spectrum aqua. Karyotyping and FISH for CEP 18 were done in both parents who did not show any evidence of the ESAC. Therefore the occurrence of ESAC in the proband was de novo. Some reports suggest that in Trisomy 18p syndrome, the phenotype is found to be variable and varies from normal to mild. Molecular characterization of the ESAC is important for understanding the phenotype-genotype correlation. The recurrence risk in the subsequent offsprings is dependent on the inheritance, size, presence of euchromatin, level of mosaicism and presence of uniparental disomy(UPD). Microarray is a powerful tool for molecular characterisation and it detects the exact region and involved genes in any ESACs, thus contributing to personalised genetic counselling.

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Negative Epistatic Interaction of Sick Cell Trait and Alpha-Thalassemia Against Severe *P. falciparum* Malaria.

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Background: Malaria is the most important public health problem in India. Alpha-thalassemia and sickle cell trait hypothesized to protect against severity of *P. falciparum* malaria. Population based study showed the negative epistatic between these two inherited hemoglobin disorders. **Aim and objective:** To study the influence of alpha-thalassemia and sickle cell gene on the severity of *P. falciparum* malaria and its epistatic interaction when co-inherited together in the individual. **Material and Methods:** In this case-control study, 232 adult patients with *P. falciparum* malaria admitted in the Department of Medicine, were analyzed for sickle cell gene and alpha-thalassemia at Sickle Cell Clinic and Molecular Biology Laboratory, V.S.S. Medical College, Burla, Odisha. Age, gender and ethnic matched 232 individuals with no history of malaria since 5 years were taken as control. Sickle cell was confirmed by ARMS-PCR and alpha-thalassemia was analyzed by Multiplex-PCR. **Results:** Out of 232 patients, 184 were HbAA, 36 were HbAS and 12 were HbSS where as in control 198 were HbAA and 34 were HbAS. In both the patients and controls with HbAA, the incidence of alpha-thalassemia was 39.1% in patients compared to 51.0% in control (adjusted odd ratio [OR], 1.62; 95% CI, 1.08-2.43; $p = 0.023$). Similarly the incidence of alpha-thalassemia was 22.2% in patients with HbAS compared to 52.9% in control with HbAS (adjusted [OR], 0.25; 95% CI, 0.09-0.71; $p = 0.012$). The incidence of ARF, Jaundice, cerebral malaria and death were significantly lower in patients (HbAA) with alpha-thalassemia compared to patients (HbAA) without alpha-thalassemia. Patients co-inherited with both HbAS and alpha-thalassemia had a greater HbA/HbS ratio compared to patients with HbAS without alpha-thalassemia ($p < 0.01$) leading to more clinical severity. **Conclusion:** Both sickle cell trait and alpha-thalassemia enjoy a selective advantage separately against *P. falciparum* malaria. These protective effects of both hemoglobin disorders become cancel when co-inherited together in the individual.

P139

A Novel Symptomatic B0-Thalassaemia Mutation Cd-15(-T) in Compound Heterozygote First Time with Sickle Cell Gene from Eastern India & Response to Hydroxyurea

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Background The HbS and β -thalassaemia genes are common haemoglobinopathies with variable prevalence in India. Like HbS homozygotes, HbS- β -thalassaemia also shows severe clinical presentation. Odisha state is having a high prevalence of these genes. This is the first report of two cases with a novel β^0 -thalassaemia mutation (codon 15, TGG>_GG;del T) co-inherited with HbS with severe clinical symptoms of repeated VOC, BT, Hosp. **Aim and objective** Molecular characterizations of HbS β^0 thalassaemia and response to HU therapy. **Material & method** Standard laboratory investigations confirming HbS and β -thalassaemia were adopted. CE-HPLC was done to quantify hemoglobin fractions. PCR-ARMS was adopted to confirm known mutations and direct β globin gene sequencing was done to identify rare mutations. CBC and Biochemical investigations, Radiological examination of shoulder and Hip joints, USG of abdomen were done to correlate clinical findings. Hydroxyurea (HU) was administered at a low and fixed dose (10 mg/kg/day) based on indications. **Result** Both the 2 cases of HbS- β -thalassaemia with codon 15 (-T) mutation had repeated VOC, BT, Hosp as the presenting symptom at 2 and 3 yr of age respectively. CBC agreed to typical thalassaemic red cell picture (MCV 66.9 ± 1.8 , MCH 19.7 ± 0.42) with high HbA2 (6.75 ± 1.4) in CE-HPLC. The cases were confirmed to be HbS- β^0 -thalassaemia with a novel β -thalassaemia mutation at codon 15, TGG>_GG; del T by direct globin gene sequencing. The patients were treated with Hydroxyurea (HU) and are under regular follow up since 2 yr with 100% response. **Conclusion** All HbS- β -thalassaemia are not symptomatic but this novel β^0 thalassaemia when co-inherited with HbS showed clinical manifestation. Hydroxyurea has been a drug of choice for the SCD patients. It responds well and effective even at a low dose of 10 mg/kg/day and can be administered this drugs to cases of HbS- β^0 Thalassaemia patients.

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Genotype-Phenotype Correlation in Patients with HbS β -Thalassaemia in Eastern India.

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Background: HbS β -thalassaemia is a variant form of sickle cell disease (SCD), resulting from the inheritance of HbS and β -thalassaemia genes. In the state of Odisha, the frequency of sickle cell and β -thalassaemia cases are 21.0% and 3.75% respectively. **Aim and Objective:** To study the genotype-phenotype correlation of β -thalassaemia mutation in the clinical severity of HbS β -thalassaemia. **Material And Methods:** Out of 7625 screened, 2049 SCD cases with HbA2 >3.5 were considered for confirmation of HbS β -thalassaemia. Cases were diagnosed by CE-HPLC, parents study and confirmation was done by ARMS-PCR and direct DNA sequencing. Four hundred confirmed cases with HbS β -thalassaemia were studied in our centre. XmnI polymorphism and α -thalassaemia were

studied by RE digestion and GAP-PCR. Cases with severe clinical features were treated with hydroxyurea at a low dose of 10 mg/kg/day. **Results:** Four prevalent Indian β -thalassaemia mutations, viz. IVS1-5(G $\rightarrow$ C), cd15(G $\rightarrow$ A), FS41/42(-CTTT), cd 30(G $\rightarrow$ C) accounted for 96.25% (385), 1.0% (4), 0.75% (3), 0.5% (2) respectively. Two rare mutations viz. cd15(-T) and cd126-131(-17bp deletion) reported for the first time from this part were found in 1.0% and 0.5% respectively. Of 385 cases with IVS1-5(G $\rightarrow$ C) mutation, 252 (65.5%) were symptomatic, while rest were asymptomatic. All the cases carrying other 5 mutations had presented with severe clinical manifestations. 2/3rd of cases with HbS β -thalassaemia had severe clinical manifestation, although XmnI polymorphism [96.5% (301/312)] and α -thalassaemia [51.95% (80/154)] were found in majority of cases. **Conclusion:** In our study, clinical variability of patients with HbS β -thalassaemia could not be essentially explained by the possible association of XmnI polymorphism and α -thalassaemia. Additionally, IVS1-5(G $\rightarrow$ C), the common mutation in our study was found to be frequent in both the symptomatic and asymptomatic groups. Hence we feel strongly a need to assess the possible role of other genetic modifiers linked to the β -thalassaemia mutations especially with IVS1-5(G $\rightarrow$ C) in explaining clinical variability of HbS β -thalassaemia.

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De-Novo Compound Heterozygous Mutations in MYH7 Gene in Patient with Restrictive Cardiomyopathy

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Background Restrictive cardiomyopathy is a myocardial disorder with impaired ventricular filling, with either normal or decreased ventricular volume, and normal or increased ventricular mural thickness and normal systolic function. The sarcomeric genes are known to be associated with restrictive cardiomyopathy. Many recent studies showed the association between the variable expressivity and age of onset of disease to be associated with different factors making this disease a complex phenotype. Disease complexity with double heterozygous and compound heterozygous is known to be associated with the severity of disease phenotype in recent reports. **Aims and Objectives** The aim of study is to evaluate the clinical phenotype of proband and screening of candidate gene to find the genetic abnormality of the patient for understanding of interrelationship between the phenotype and genotype. **Materials and Method** Diagnosis was based on ECG, 2D-echocardiography, cardiac catheterisation. 5ml of blood sample was collected and DNA was isolated using phenol-chloroform method. Sequencing of the hotspot region of MYH7 gene i.e. exon 23 was done by Sanger method (ABI 3730). Screening of family members available was also done clinically as well as genetically. The study was ethically approved by Institutional committee and informed written consent was taken from all participants. **Results** On the basis of clinical assessment of ECG, 2D-echocardiography and

cardiac catheterisation, patient was diagnosed with restrictive cardiomyopathy without hypertrophy. On genetic testing, de-novo compound heterozygous mutation was found in the exon 23 in MYH7 gene i.e. p.E902k and p.D906N. These variants were absent in 85 other cardiomyopathy patients. Screening of parents and siblings was also done clinically as well as genetically and found to be normal. Both mutations on bioinformatic analysis found to affect protein function. Presence of compound heterozygous mutation may explain the early age at onset and the severity of condition. **Conclusion** This report of compound heterozygous will further provide basis for the complexity and variable expressivity of phenotypes in patients in such complex diseases. This also provides basis that expression of disease phenotype may be modified by different mutations in same gene or different genes or by different environmental factors.

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Sickle Cell Disorder- A Family Study with total Ten Children (8 Homozygous and 2 Heterozygous) from Satpuda Hilly Ranges of Maharashtra

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Sickle Cell Disorder is a common blood genetic disorder found among tribal population groups from Maharashtra State. Highest Prevalence (about 20%) have been reported among tribal population groups Madia, Gond Pardhan, Halba from Gadchiroli Dist. and Bhil & Pawara from Nandurbar District. Taking into consideration the need of tribal groups residing in the Satpuda hilly ranges of Nandurbar Dist. We established Community Control Program Centre with help of local tribal youths. The Centre is located between 3rd and 4th Hilly ranges of Satpuda at Roshmal Bk. Tal. Dhadgaon Dist. Nandurbar, Popularly known as **Sickle Cell Dawakhana**. The centre provides diagnostic facilities, Possible Treatment (Ayurvedic mostly), and counselling for last 16 years. We also conduct extended family studies of the index patient. While screening the families we came across one family wherein parents are Heterozygote's, having total ten children, 8 Homozygote's and 2 Heterozygote's. We collected all information from the family and carried out haematological and other investigations. To our information, this is the first largest family report with 8 Homozygote children in world medical literature. We will present family pedigree analysis along with Haematological investigations.

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Analysis of A/u Insertion/Deletion Polymorphisms in Four Endogamous Groups from North West India

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Background: Due to its geographical location, Northwest India acted as a major route for human migration to Indian subcontinent since prehistoric and historic times. Therefore, it possesses an exclusive genetic profile primarily due to

various migratory events in this region. **Aim:** The present study assessed the population genetic structure and diversity in four ethnically different caste groups from, North West India (Punjab). **Methods:** A total of 646 unrelated samples belonging to four endogamous groups (Bania, Brahmins, Jat Sikh and Scheduled Caste) were genotyped for four human-specific insertion/deletion polymorphic loci (*ACE*, *APO*, *PLAT*, *FXIII*) using polymerase chain reaction (PCR). **Results:** All the loci were found to be in Hardy Weinberg equilibrium (HWE) except *FXIII* in all the ethnic groups. The average heterozygosity for all the loci in these ethnic groups ranged from 0.221±0.252 to 0.359±0.237. Overall gene diversity (F_{st}) was found to be 8.6%, probably indicating ancestral commonalities of the populations. The phylogenetic analysis revealed that Brahmin, Jat Sikh and Bania were closer to each other than Scheduled Caste group. **Conclusion:** The picture emerging from our analysis revealed lesser genetic heterogeneity among Brahmin, Jat Sikh and Bania groups as compared to Scheduled Caste group despite of their similar geographical proximity.

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Improved Multiplex Ligation Dependent Probe Amplification Technique for Studying Copy Number Variations

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Background: Extensive research around the world lead to identification of copy number variations (CNVs) in several candidate genes as leading causes of many complex disorders including Schizophrenia, Autism etc. Multiplexed Ligation dependent Probe Amplification (MLPA) is a popular downstream technology to analyse the identified CNV prone region in a sample. Conventional MLPA (MRC Holland) needs about two days' time for complete analysis and around 1500.00 INR per reaction per sample. The reference probes (P300, P200) available for analysis were found to lie in genomic regions containing single nucleotide polymorphisms (SNPs) and/or CNVs. There is a need for improvising the technique to avoid false CNV calls at lesser cost and time. **Aims and Objectives:** Design new reference probes for more reliable results. Reduce total cost of technique facilitating more sample's analysis. Reduce total MLPA run time for quicker results. **Materials and methods:** P300 probe mixes studied using Database of Genomic Variants identified SNPs and CNVs. New reference probes are designed against SNP and CNV free regions using MAPD software as described by Zhi *et al.*, 2010. *i-MLPA* (Improved MLPA) uses proprietary reagents to reduce the total cost and turnaround time by reducing hybridization time from 16-20hrs to 30mins. Each end product mixed with LIZ500 marker, formamide is electrophoresed on automated DNA sequencer and results were analysed using GeneMarker software. Statistical analysis of 16hr and 30mins data of same samples is done using chi-square and two tailed *t*-test. **Results and conclusion:** No ambiguous calls were seen using new probe mix unlike with p300 mix. Chi-square analysis of peak ratios of 16hr and 30mins data shows high

similarity with expected ratios. No significant difference in signal intensities and peak ratios were observed. This projects *i-MLPA* as genuine method providing more promising results in less duration at a cost of around 300.00INR per reaction per sample.

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Molecular Testing for Inherited Genetic Diseases: A Metropolis Laboratory Experience

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Background: There is a high prevalence of genetic disorders in India which is attributed to the lack of awareness, consanguineous marriage favored in many communities and non-availability of good genetic counselors. Also, there is a dearth of good genetic testing labs in all the parts of India that offers wide range of genetic testing menu including the prenatal testing. **Aims and Objectives:** The purpose of our study was to develop reliable assays for molecular detection of common genetic disorders prevalent in Indian population. **Materials and Methods:** Seven Hundred and Seventy Three clinical samples were referred to Metropolis healthcare limited in the last 2 years with clinical indication for different genetic disorders. Molecular assays were developed and used for clinical genetic testing for the below mentioned disorders. N=152 Cases - Duchene Muscular Dystrophy, N=112 Cases- Spinal Muscular Atrophy, N=176 Cases- Spinocerebellar Ataxia (SCA) Panel, N=25 Cases Friedreich Ataxia, N=149 Cases- Huntington Disease, N=140 Cases - Y-Chromosome Microdeletion N=20 Cases - Rare Beta-Globin gene variants. Pre- and Post-Test Genetic counseling was offered to the patient or family members. **Results:** Out of 152 cases with suspicion of DMD, 97 (63.8%) cases were positive for one or more of the 18 exons (Begg's and Chamberlain's) set. The remaining cases with strong clinical suspicion were counseled for additional testing for 79 exon deletion/duplication and point mutation detection by sequencing. In case of SMN1 exon 7 deletion for SMA, 45.53% (51 cases) positivity was reported. Complete beta globin gene sequencing was performed for identification of rare hemoglobin variants and beta thalassemia mutations as indicated on hemoglobin electrophoresis..... **Conclusion:** Molecular genetic testing is essential for confirmation of clinical diagnosis as well as for screening of family members. The carrier parents can be further counseled for pre-natal diagnosis for future pregnancies. Thus, lab diagnosis and counseling are very important to control birth of children inheriting the genetic disorders. (Abstract truncated).

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Association of 14bp Insertion/Deletion (indel) Polymorphism of HLA-G Gene in Women with Recurrent Miscarriages from Amritsar Region of Punjab, India.

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Background: Recurrent miscarriage (RM) is classically defined

as the loss of three or more consecutive pregnancies before 20th gestational week. The occurrence of RM has been estimated in 1–3% of couples attempting to bear children. Human leukocyte antigen (*HLA*)–G gene is a non-classical HLA class Ib gene located within the major histocompatibility complex (MHC) at 6p21.3 position. HLA-G is thought to contribute to maternal acceptance of the semi-allogenic foetus, modulating the maternal immune system during pregnancy. The 14-bp insertion/deletion polymorphism may influence both the HLA-G isoform splicing patterns and HLA-G mRNA stability. **Aims and Objectives:** The present study is a case-control study designed to study the association of 14bp indel polymorphism of HLA-G gene in women with RM and their comparison with age matched healthy control women. **Material and Methods:** This study included 44 women with at least two or more consecutive miscarriages before 20th gestational week and 40 healthy control women with at least one healthy live born. Genotyping was done by Polymerase Chain Reaction (PCR) followed by Agarose Gel Electrophoresis. **Result:** Out of 44 women with recurrent miscarriages, 11 (25%) were found as homozygous (+14bp/+14bp) for insertion allele, 9 (20.5%) as homozygous (-14bp/-14bp) for deletion allele and 24 (54.5%) as heterozygous (+14bp/-14bp) for insertion/deletion allele. Out of 40 control women, 13 (32.5%) were found as homozygous (+14bp/+14bp) for insertion allele, 11 (27.5%) as homozygous (-14bp/-14bp) for deletion allele and 16 (40.0%) as heterozygous (+14bp/-14bp) for insertion/deletion allele. **Conclusion:** Our pilot study demonstrated that 14bp indel polymorphism of HLA-G gene has no significant association with RM in women from Amritsar region of Punjab. However it is an ongoing study and statistical analysis of more samples is needed to get a conclusive result.

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DNA repair gene defects and its association with myelodysplastic syndrome

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Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic stem cell disorders characterized by dysplastic changes in the bone marrow, ineffective hematopoiesis resulting in cytopenias, and an increased risk of developing acute myeloid leukemia (AML). The progression of disease is a consequence of the accumulation of mutations, probably due to an increased DNA damage burden and/or reduced ability to deal with the damage. **Aim & Objective:** To study the expression of DNA repair genes and cell cycle checkpoints in MDS to understand the association of DNA repair defects with MDS. **Material and Methods:** The study was carried out in 44 MDS patients. Expression analysis of DNA repair genes was performed using Real-Time PCR system. Relative quantification was carried out with the $\Delta\Delta CT$ method using the endogenous control genes (GAPDH). Single nucleotide polymorphisms (SNPs) of DNA repair genes (XRCC1, hOGG1, XRCC3, XPD and RAD) were carried out by PCR-RFLP. Expression of cell cycle checkpoint (Chk1 and Chk2) was carried out by western blotting. **Results:**

Among 44 MDS patients the MDS subtype frequency was 50% (RA), 11% (RAEB I), 13% (RAEB II), 5% (RARS), 18% (RCMD) and 3% (MDS-U) respectively. In our study, the frequency of AA (XRCC1 Arg280His) and CT (XRCC3 Thr241Met) variant allele was higher in MDS-RAEB II and RAEB I patients as compared to other subtypes. The DNA repair genes Lig4, Ku70, Ku80 and XRCC3 expression was significantly down-regulated in MDS patients compared to controls. The significant difference was not observed in cell cycle checkpoint protein expression in MDS. **Conclusion:** The study suggests that the DNA repair genes are associated with high risk MDS. However, a large number of samples need to be studied to confirm the association of DNA repair gene with MDS pathogenesis.

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Glucose-6-Phosphate Dehydrogenase Deficiency-Who,When,Why And How.

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Background: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common human enzymopathy affecting 400 million people, globally. G6PD deficiencies being X-linked genetic syndrome, females are more affected than males. Heterozygous females go undetected in a commonly used method. G6PD deficiencies in adults are missed due to skewing of X- chromosomes. **Aim and Objective:** The aim of the study was to identify who is entitled for G6PD deficiency. Different methods for detections of G6PD deficiency were rationalized. When and Why G6PD deficiency detection is so important was targeted. **Material and Methods:** Cross section retrospective study was conducted on 2568 Saudis inclusive of 800 adult males, 784 adult females and 984 neonates (448 male and 536 females) were studied from King Abdulaziz University Hospital (KAUH) in Jeddah, Western Saudi Arabia. Blood samples were screened for G6PD activity by fluorescence spot test, semi quantitative color reduction test, spectrometric quantitative evaluation and DNA molecular typing. Hemoglobin (Hb) was measured on the same sample by BC-3200 Auto hematology Analyser. **Results:** The prevalence of G6PD deficiency identified by fluorescence spot test was 4.6% and all were deficient male. By semi quantitative method, the prevalence rate was 3.2% and again all were male deficit patients. However, when quantitative spectrometric method was used, the prevalence was found in 5.7%, where in 4.6% deficient patients were males and 1.1% were females. **Conclusion:** G6PD deficiency test should be performed all neonates. It was concluded that many undiagnosed partially G6PD deficient female are missed by commonly used screening method. There is no reliable biochemical screening assay to detect G6PD heterozygous, since standard methods test both red cell populations in single sample. Only DNA analysis meets the requirement. In view of the cost effectiveness, we suggest to undertake the DNA analysis in samples, identified with revised cutoffs.

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Genetic Association of ISL1 Gene Polymorphism with Chronic Otitis Media with Effusion

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Background: Otitis Media (OM) is a most common childhood disease characterized by middle ear infection. Chronic otitis media with effusion (COME) is a condition lasting at least for three months arises due to interaction between environmental and genetic factors. Previous studies have shown high eosinophil count in patients with otitis media. Recently, mutations in ISL1 gene were found to be associated with otitis media development. **Aims and Objectives:** The aim of the study was to identify the genetic associations between ISL1 gene variants with COME and to evaluate their functional role in disease manifestation. **Materials and Methods:** We have conducted a case control study to identify the genetic associations between ISL1 gene polymorphisms and COME. To determine the eosinophil percentage in blood plasma, total blood count was estimated in cases and controls. **Results:** Screening of coding region and exon-intron boundaries of ISL1 gene identified seven known polymorphisms (c.-492A>G, c.-240G>A, c.28+17C>T, c.504A>G, c.513G>A, c.567C>T and c.*651A>T) and four novel variations (c.-405G>A, c.498G>C, c.*504T>A and c.*733G>A). Association testing for these variants have identified the sex-specific association between c.504A>G polymorphism (P=0.0322) in females with COME. The decreased minor allele 'G' frequency in cases (0.227) compared to controls (0.352) indicates its protective role in disease process. Family based study ensured the absence of transmission of protective genotype 'GG' to the affected individuals which confirmed the protective role of the minor allele 'G' in disease development. Further, total blood count analysis of COME subjects showed abnormally high eosinophils in 40.7% of cases. Patients with GG genotype had significantly lower peripheral eosinophil counts than with the AG and AA genotype (P< 0.05) suggesting the protective role of this variant in OM development. **Conclusions:** This study demonstrated the sex specific association of c.504A>G variant with COME and also significant association of different genotypes for this variant with high eosinophil count in COME cases.

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Role of GSK3 β and Cyclin D1 in The Etiology of Esophageal Cancer

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Background Esophageal Cancer, regardless of the histological subtype, has the worst survival statistics among all malignancies.

Risk of malignant transformation is associated with both external factors (tobacco, infectious organisms etc) and internal factors (inherited mutations, immune conditions, and mutations etc). GSK3 β has been shown to be a crucial enzymatic regulator of a diverse number of cellular functions including cell structure, metabolism, and survival. GSK3 β dependent threonine phosphorylation triggers nuclear export of cyclinD1 and subsequent cytoplasmic degradation. Inactivation of GSK3 β stabilizes the cyclinD1 which can lead to increased proliferative activity of the cell and aid malignant transformation. There is also evidence for a pathologic role of GSK3 β in promoting tumor cell survival, proliferation and invasion. The expression studies of these two proteins will give an insight on their etiological role in cancer of esophagus. **Aims and Objective** Understanding the role of GSK3 β and CyclinD1 in the etiology of esophageal carcinogenesis by evaluating expression levels of GSK3 β and cyclinD1 in esophageal pathologies including cancer. **Material and Methods** A total of 132 esophageal pathologies (FFPE archival samples) were evaluated for GSK3 β (cytoplasmic, Tyr216 active form) and CyclinD1(nuclear) expression levels by immunohistochemistry method. Screening was performed by three independent observers and data was analyzed. Data was categorized based on the histology/pathological type and statistical analysis was performed. **Results** 132 esophageal pathologies included Squamous cell carcinoma (n=66), Adenocarcinoma (n=41) and other pathologies (n=25). A total of 48% of cancer samples showed elevated/active GSK3 β expression compared to 40% of other pathologies (Hyperplastic cells) absence/poor expression in normal tissue. 18% of these esophageal cancer cases showed elevated nuclear expression of CyclinD1 as compared to none of the controls or other pathologies. **Conclusion** Active form of GSK3 β (Tyr216) was found to be expressed in 48% and 40% of cancer and hyperplastic cell population indicating its possible cell proliferative and anti-apoptotic role, influencing esophageal carcinogenesis. Overall 18% of cancer samples showed cyclinD1 overexpression. These may be the cases where mutation in cyclinD1 may lead to escape from GSK3 β dependent phosphorylation and nuclear export into cytoplasm for degradation. Overexpression of Cyclin D1 in 18% of the cancer cases may lead to re-replication of DNA thereby leading to malignant transformation. Studies on cyclinD1 and GSK3 β inhibitors may give insight towards targeting therapies for esophageal cancer.

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Screening of Cervical Smears: Promoter Methylation of Hmlh1 & Rar β 2 Genes and Prevalence of HR-HPV Sub-Types in Cervical Pathologies

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Background Though persistent infection with oncogenic

Human Papillomavirus (HPV) types is considered the most important risk factor for cervical cancer development, less than 5% of women with HPV will eventually develop cervical cancer supporting that other molecular event, like gene methylation may co-contribute to cervical carcinogenesis. In India, due to lack of awareness of screening and non-availability of appropriate infrastructure, cervical cancers are detected at advanced stages where cure is difficult. **Aims & Objectives** Identifying women who are sexually active for more than 3 years, with an abnormal pappanicolaou (Pap) smear or cervical lesions seen on visual inspection. Testing PAP DNA samples for the presence of high-risk (12) HPV subtypes to see their prevalence in the population. Evaluate promoter methylation status of hMLH1 & RAR β 2 genes to establish the association of epigenetic changes with pathologies of the cervix. **Materials and Methods** Organized screening programs were conducted in different rural and urban areas to identify women with abnormal PAP cytology. Complete information about the screening program was given to the women participants. Pap samples were collected along with the personal and medical history. **Results** Pap smears of 530 women were obtained via screening programs. 66% of cases were found to show an abnormal Pap cytology, mostly inflammatory smears. Upon exclusion of inflammatory smears, 32% were found to be abnormal smears. Of all abnormal cases 35.8%, 31.8% cases showed hMLH1 & RAR β 2 methylation respectively. Of the cases detected with presence of HPV, 14.1% showed HR-HPV subtypes. Of these 81.8%, 54.5% cases showed hMLH1 & RAR β 2 methylation respectively. **Conclusion** hMLH1 gene methylation seen in cases may be responsible for the genomic instability in these patients. RAR β 2 gene product inhibits transcription of viral oncogenes (E6 & E7) in HR-HPV immortalized cells. RAR β 2 promoter methylation, resulting in loss of expression, may affect this function, giving scope for viral oncogene transcription & carcinogenesis. hMLH1 methylation testing along with screening for HR-HPV subtypes could prove to be biomarkers for potential cancer risk in these patients. It is also important that the prevalence of specific HR-HPV subtypes has to be determined in our population before we go ahead with administering the cervical vaccines.

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Exome sequencing of a selected gene panel in patients with Autism

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Background ~1 in 88 individuals or 10 to 15/10,000 children are known to be autistic; while many researchers are working on the possible genes that affect autism, little is known about the role of these genes and their expressed proteins in brain dysfunction generating autistic phenotype. An alternative and effective approach to candidate gene analysis for association

with disease risk is the exome-sequencing method. Targeted sequencing of a panel of genes based on data from current research, to detect variants associated with a spectrum disorder such as autism, represents a cost-effective approach, which will help identify and understand the role of causative mutations.

Aim & Objectives We have used the exome sequencing approach to identify risk variants that may contribute to etiology of autism in patients with and without a family history of autistic features. **Materials and methods** Participants were recruited as part of the project designed to identify genes which may be related to the pathophysiology of autism. Here, we present analysis of exome seq reports from 10 subjects (5 female/5 male, average age = 12.1, SD =4.01). DNA isolated from blood was used to perform exome capture using Agilent SureSelectXT Human All exon V5 kit. **Results** Different gene mutations predicted to be damaging by SIFT and Polyphen tools were identified. These genes were involved brain development, survival and differentiation of dopaminergic neurons. abnormalities in serotonin levels etc. which are commonly related with autism. Only non-synonymous variants found in the sample were used for clinical interpretation. **Discussion** In this presentation, we discuss the genetic variants that are associated with autism in patients with and without a family history. A combination of different gene mutations seem to be responsible for the varied symptoms seen in autistic patients. In such a scenario, targeted exome sequencing is the best approach to find pathogenic variants associated with disease. Targeting multiple genes by exome sequencing gives scope for personalized treatment; genetic counseling is mandatory for interpreting the possible consequences of the variants seen in the sequencing report.

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MMP9 polymorphism, prevalence and effect on cardiac iron overload in patients with beta thalassemia major.

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Beta thalassemia patients have iron overload due to regular transfusions, which leads to left ventricular dysfunction. Left ventricular dysfunction (LVD) is a condition which reduces the ability of the ventricle to fill with or eject blood. The impaired ventricular function can be attributed to unfavorable ventricular remodeling. Among the pathways that contribute to remodeling process, matrix metalloproteinases (MMPs) appear to be of particular interest. The aim of the study was to evaluate the association of 1562 C>T variant with the echocardiographic features in beta thalassemia patients. One hundred and thirty beta thalassemia patients who were above ten years of age were selected for the study. DNA was extracted from blood samples in EDTA and genotyped for MMP9 1562 C>T variant by PCR-RFLP (Sph1 restriction enzyme) method. Out of these one hundred and thirty patients, thirty patients underwent echocardiography for the cardiac dysfunction. Mean age and

height of thalassemia patients in the study were 14.3years (± 5.5) and 136.6cm (± 15.3) respectively. Genotype frequency of MMP9 1562 C>T variant among study subjects were CC, CT and TT were 54%, 42% and 3% respectively and allele frequency of C type was 75% and T type was 25%. Among the conventional echocardiography parameters LVEDD (mean of CC was 34.6 and CT+TT was 41.9, p-value=0.007), left atrium (mean of CC was 25.4 and CT+TT was 31.1, p-value=.015), EDT(mean of CC was 103.4 milliseconds and CT+TT was 148.8 milliseconds, p-value=0.023) and lv mass (mean of CC was 96413.04mg and CT+TT was 131477mg, p-value=0.046) were found to be associated with the MMP9 variant. **Conclusion:** Present study shows that 1562 C>T variant of MMP9 is associated with susceptibility of the left ventricular dysfunctions may be due to iron overload.

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Are MTHFR and MTRR polymorphisms considered to be as risk factor in mothers of Down syndrome cases?

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Background: Down syndrome, a trisomy of chromosome 21, is one of the commonest disorders, with huge medical and social burden integrated with high cost management. Maternal folic acid supplementation has been associated with a reduced risk for DS and the literature available proved the role of folate metabolism in preventing the risk factor for DS in high risk pregnancies. Most of the studies reported from India well documented the role of MTHFR gene polymorphism, however, MTRR gene, as a maternal risk factor for DS has not yet been studied. **Aims and objectives** The present study aimed at evaluation of MTRR (C524T and A66G) along with MTHFR(C677T and A1298C) gene polymorphisms as maternal risk factors for DS. **Material and methods** The genomic DNA was extracted and frequency of polymorphisms was evaluated in 45 mothers of clinically confirmed DS and 54 control mothers with normal children. **Results:** The frequency of T,C,T and G allele of MTHFR C677T, MTHFR A1298C, MTRR C524T and MTRR A66G polymorphisms respectively was found to be higher among mothers of case (23.33%, 55.56%, 33.3% and 52.22% respectively) compared to controls (12.96%, 3.33%, 23.15% and 3.64% respectively). Genotype frequencies of CT and CC of MTHFR C677T and MTHFR A1298C respectively were higher among mothers of case than control (controls (42.2% versus 25.9% and 22.2% versus 3% respectively) with odds ratio 4.76(95% CI 0.1868-121.51; P value=0.033) and 20(3.282-121.84; P=0.0012) respectively. Genotype frequencies of CT and TT (MTRR C524T) and of GG (MTRR A66G) was found to be significantly higher among mothers of case than controls (64% versus 35.1%; 17.7% versus 5% and 95.5% versus 3%) with odds ratio 6.105(2.3216-16.0556; P value=0.0002), 10(2.29-49.584; P value=0.0025) and 2.359(CI 0.0005-0.4965, P=0.0183) respectively. **Conclusions** This study

clearly reveals the genetic association of folate metabolism genes MTRR along with MTHFR as maternal risk factors for having DS.

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TP- PCR: Advancement in Diagnosis of Fragile X Syndrome

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Fragile X Syndrome (FXS) is second most common types of inherited intellectual disorder. It is mostly caused due to hyper expansion and hypermethylation of CGG repeat within the first exon of FMR1 (Fragile X Mental Retardation) gene at Xq27.3 chromosome, thus silencing FMR1. FXS is characterized with mental retardation, behavioral impairment, and autism. Full mutation is less common but the carrier status is more widespread in the general population prevalence being 1 in 113–259 females and 1 in 260–810 males. Premutation expands to full mutation in subsequent generations. Present study aims to do molecular characterization of FMR1 gene in clinical suspects of FXS by using TP PCR technique. Family screening is offered to positive subjects to find individuals with high risk for expansion. Option of prenatal diagnosis is offered if required. Genomic DNA was extracted from 30 clinical suspects of FXS (both male and female). Bisulphite treated samples were subjected for methylation studies. TP- PCR amplicons were subjected to fragment analyses and results were documented. Out of 30, males (9) with full mutations and female (1) with premutation were detected. Family screening in the premutation female and in one of the full mutation male determined that both got permutation allele due to maternal transmission. Mother's permutation allele has expanded to full mutation in the FXS male subject. **Conclusions:** Present study emphasized the need of TP PCR as reliable, rapid, and cost effective diagnostic method to be established, against negatively affirmation of PCR and traditional southern blot. Extended family screening could be offered to the FXS positive subjects along with supportive counselling and prenatal diagnosis options.

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Association Analysis of Various Anthropometric and Clinical Factors in Diabetic Retinopathy (DR) in Population of Punjab

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Background: Diabetic retinopathy (DR) is one of the major microvascular complications of associated with both Type 1 diabetes (T1D) and Type-2 diabetes (T2D). The risk of developing DR depends on both the duration and the severity of hyperglycemia. Global prevalence of DR in patients with diabetes was recently estimated to be 34.6%. It is the leading

cause of blindness worldwide. **Aim and Objective:** To find the correlation between various anthropometric and clinical factors in the progression of DR in the population of Punjab. **Material and Methods:** The present study investigates the association of clinical variables with DR. Total of 401 samples including 101 DR cases, 150 T2D and 150 unrelated healthy controls above the age of 50 years were enrolled in the present study. **Results:** Out of 101 cases, 44.34% DR cases were having non-proliferative diabetic retinopathy (NPDR) and 55.66% cases were having proliferative diabetic retinopathy (PDR). Anthropometric and clinical data analysis revealed that waist circumference (WC), hip circumference (HC), High-density Lipoproteins (HDL), Triglyceride and duration of diabetes were found to be significantly different among DR and T2D cases. When DR cases were compared with healthy controls HC, waist-hip ratio (WHR), Random blood sugar (RBS), cholesterol, HDL were found to be statistically significant. Principal component analysis extracted 4 variables [WC, Cholesterol, triglycerides (TG), and WHR] that explained 77.1% of cumulative variance suggesting obesity as a strong indicator of DR development. However, high loadings of TG and LDL cholesterol explained nearly 83.85% of variance indicating dyslipidemia as main risk factor in T2D progression. **Conclusion:** Obesity and dyslipidemia are strong indicators in the development of DR and T2D among population of Punjab.

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Fanconi Chromosomal Breakage Analysis Report of 98 Patients

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Background: Fanconi Anemia is a rare genetic disorder of high phenotypic variability with diverse congenital anomalies, bone marrow failure; solid tumors and developmental abnormalities. Fanconi patients are hypersensitive to cross-linking agents and exhibit high frequency of chromosomal breakage. Thus, Mitomycin C or diepoxybutane were utilized for cytogenetic diagnosis of Fanconi. Occasionally, despite of strong suspicion of FA in patients, chromosomal breakage test can be negative because of somatic mosaicism. To date, 15 complementation groups and 16 fanconi associated genes have been identified. Complementary DNA testing helps to resolve such ambiguous cases. However, chromosomal instability analysis is the first line gold standard cost effective procedure with less turnaround time for fanconi diagnosis. **Aims and Objectives:** To report the observed frequency of Fanconi anemia from our referral centre. **Subjects and Methods:** Chromosomal Instability analysis was done on 98 patients referred to cytogenetics section between 2005 to 2011. The lymphocytes were Phytohemagglutinin stimulated for 24 hours and further incubated with (20ng/ml) and without Mitomycin C for next 48 hours. In both patients and controls chromosomal breaks and radials were scored on 50 metaphases each with & without Mitomycin C. **Results:** Among 98 patients 35 revealed a high frequency of breaks & radials, 34.7%, this is very high compared to most of the respective frequency reports available from India. The affected male:

female ratio is 2:1 and mean age of the diagnosis is 9.2 and 5 respectively. Out of 3 familial cases 2 were positive for fanconi.

Conclusions: With high frequency report of Fanconi 34.7% among index cases and considering the diversity, endogamy and consanguinity in Indian population - early diagnosis, early intervention, and screening for sibs by chromosomal instability assay is advised.

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Spectrum of Hemoglobinopathies in Santhal's Tribal Community of Jamui District, Bihar

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Background: A few studies done earlier have reported the cases of hemoglobinopathies in Bihar. However, there is not much information on distribution of hemoglobinopathies in tribal population of Bihar. After Bihar partition, most of the tribal regions are located in the state of Jharkhand and according to census 2011, the scheduled tribe (ST) population in Bihar is 0.9% of total population of Bihar. **Aim and Objectives:** To determine the spectrum of hemoglobinopathies in the tribal population of jamui district, specifically in santhal's tribes because santhal's tribal community is the most populous, out of the total reported 28 schedule tribe's population. **Material and Methods:** A community based pilot study was conducted at Khaira and Sono block of Jamui district. A total of 153 santal tribe's, age >14 of both gender were screened for hemoglobinopathies using hemoglobin testing system (VARIANT β -Thalassemia short program, Bio-Rad laboratories, California, USA) after taking written informed consent. Value Hb A2 < 3.5 % and HbF < 1% were considered as normal, which were provided by manufacturer. **Result:** Out of 153 Santhal's, 143 (93.5%) were normal, 5 (3.3%) had β -Thalassemia Trait, 4 (2.6%) had HbS trait and 1(0.6) had HbE trait. **Conclusion:** The present study provides the status of hemoglobinopathies in this region of Bihar. On screening using HPLC (VARIANT β -Thalassemia short program), about 6.54% of of tribal population had hemoglobinopathies. In this prospective, we emphasize that a large population based study and molecular analysis needs to be done, to further evaluate the status of hemoglobinopathies in tribal and non-tribal people of Bihar to prevent thalassemia major in coming generations.

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Study of Risk Factors in Oral Carcinoma and the relationship between Tumor Thickness and Regional Nodal Involvement

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Background: Oral cancer is a subtype of head & neck cancer which most commonly involves the tongue but may also occur on the floor of the mouth, gingiva, lip and palate. It is now accepted that thickness is a more accurate predictor of nodal

metastasis, local recurrence, and survival than tumor size/diameter. **Aims and Objectives:** To correlate tumor thickness with nodal metastasis in all the cases. To also record the clinical T-stage, pathological T-stage, vascular invasion, and perineural infiltration and to establish a correlation, if any with tumor thickness. **Material and Methods:** This was a retrospective as well as a prospective study. A total of 25 cases of oral carcinoma with radical neck dissection were evaluated. Histologically proven cases of oral carcinoma which had already undergone radical surgery were included in this study. **Results :** Most of the patients in this study were young adults who had a long term history of tobacco usage in its various forms. A detailed history was obtained from these patients regarding the type of tobacco used & the duration of usage. We found that the tongue was the most common site of oral carcinoma involvement (7 cases) in cases of smokeless tobacco usage whereas the retromolar trigone area, gingivo-buccal sulcus and buccal mucosa were more or less equally involved in those cases who were tobacco smokers. Table I depicts the distribution of cases according to site of the oral carcinoma lesion and type of tobacco abuse among these 25 patients. **Conclusion:** Tobacco addiction, either as a smoking habit or the chewing of smokeless tobacco over a long period of time was associated with the development of Oral Carcinoma. The buccal mucosa, gingivo-buccal sulcus & the retromolar trigone were the common sites of development of squamous carcinoma among the smokers. The tumor thickness of Oral Squamous Cell Carcinoma was found to be an important prognostic indicator for the occurrence of metastases to the regional cervical lymph nodes. In this study of 25 cases, a tumor thickness of 0.6 cm or more was found to be significantly associated with regional lymph involvement by the oral squamous cell carcinoma.

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Genetic Diversity in Reasi and Udhampur Districts of Jammu and Kashmir, India.

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Indian subcontinent has special position in human evolutionary history due to populations' divergence, many migrations, and cultural evolution since the first emigration of man "Out of Africa" (Wells et al., 2001). India has served as a major corridor in the dispersal of modern humans as it is positioned at the tri junction of Africa, Northern Eurasia and oriental realms (Cann, 2001). It is agglomerated with 4635 anthropologically well defined populations having 532 tribes, 72 primitive tribes (36 hunters and gatherers) (Tamang et al. 2012). Majority of non-tribal population belongs to the Hindu religion. Several other religious communities contribute a fraction of Indian population. Jammu and Kashmir state of India possess an excellent geographical location in the Himalayan region, divided into three regions Jammu (having 65% of Hindus, 31% of Muslims and 4% Sikhs), Kashmir (97% are the Muslims and rest belongs to other communities) and Ladak (46% Buddhists, 47% Muslims and 6% Hindus). The population is highly endogamous and have various sub-endogamous groups. Further, Reasi and

Udhampur are adjacent districts of Jammu division, represent very interesting location and mountain ranges in the regions that separate Jammu and Kashmir divisions. Both the districts encompass pleasant valleys as well as extremely difficult terrains to inhabit and might have acted as doorways to Central Asia from Indian Subcontinent plains. These districts have various Hindu and Muslim ethnic groups -Rajput, Brahmin, Mahajan, Khaytriya Gujjar, Bakarwal and the tribes: Watal, Chura, Saryara, Megh, Ratal, Kabirpanti, Jolaha, Dumna, Doom, Dhyar, Balmiki, Bangi, Gardi, Ramdasia, Batwal, Basith, Barwala etc. We have undertaken a research project to exploit Y chromosomal and mtDNA markers to understand the origin and ancestral history of ethnically diverse contemporary population groups of Reasi and Udhampur districts, to workout genetic relationship among themselves and explore their relationship with rest of the studied Indian and world population groups. It is anticipated that this study will shed light on various perspectives of human migration history. We may come across some untold evolutionary history stories and most likely shall discover some novel maternal and paternal lineages, isolated and restricted to these regions.

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Detection and Evaluation of BCR/ABL Gene mutations in CML Patients Treated with Imatinib Mesylate

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Background:Chronic myeloid leukemia (CML) is associated with a characteristic chromosomal translocation called the Philadelphia chromosome (Ph). This abnormally is the result of a reciprocal translocation between the long arms of chromosomes 9 and 22, which generates the BCR-ABL fusion oncogene. The BCR-ABL p210 fusion protein is found in more than 95% of patients with CML. This oncoprotein is an activated tyrosine kinase stimulating several pathways transducing intercellular signals leading to abnormal cellular functions. The first generation of tyrosine kinase inhibitors called Imatinib mesylate was approved by FDA for treatment of CML patients. Unfortunately, BCR-ABL tyrosine kinase domain (TKD) mutations are the most important factor contributing to imatinib-resistance and poor prognosis in CML in all phases. In this study we evaluated mutation analysis and clinical findings of TKD mutations in patients with CML.**Material and Methods:** We have used European Leukaemia Net (ELN) guideline for diagnosis and management of CML patients. It has been used a semi-nested RT-PCR followed by bi directional sequencing to detect mutations in a cohort of 166 Iranian CML patients. Molecular response was evaluated through quantitative assessment of BCR-ABL fusion gene expression by real-time RT-PCR (Reverse transcriptase polymerase chain

reaction). Participants were grouped into three categories: those with imatinib resistance or suboptimal response (n=110), those with showing favorable response (n=26), and those diagnosed before introduction of imatinib (n=30). This study was approved by the ethical committee of Iran University of Medical Sciences. **Results:** In total, 34 mutations in 19 distinct codons were identified in 32 patients of the first group, of which two were novel. The most frequent mutations were G250E (x5), followed by T315I (x4) mutations, M244V and F359C (X3 each) mutations, and mutations of E255K, M351T, F359V and E459G (2x each). Two imatinib-resistant mutations involving D276N and E279A were identified for the first time in this study. The most commonly mutated region was drug binding site (29%) followed by p-loop region (26%) and C-terminal (13%). The frequency of mutations in chronic phase (CP), accelerated phase (AP) and blastic phase (BP) were 23%, 75% and 62% respectively. **Conclusion:** Approximately, 30% of imatinib-resistant patients demonstrated mutations with high frequency of G250E and T315I. This report expands the spectrum of ABL mutations and stresses the use of mutation testing in imatinib-resistant patients for continuation of treatment procedure and selection of second-generation tyrosine kinase inhibitors or bone marrow transplantation.

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Clinical and Molecular Study of NPC in Iran: Report of 5 Novel mMutations

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Niemann–Pick disease type C (NPC) is a rare autosomal, recessive, neurovisceral disorder caused by mutations in the NPC1 (95%) or NPC2 (5%) genes. RT-PCR and sequencing methods were used for molecular investigation for NPC1 and NPC2 genes in 5 patients. **Case I:** 15 years old boy who had hepatosplenomegaly and jaundice in neonatal period. Liver biopsy at that time was compatible with a lipid storage disease. (c.1069C>T) p.S357L was found. **Case II:** 3.5 years old boy, splenomegaly was detected at 6 month of age. He had psychomotor developmental delay. (c.1180C>T) p.Y394H **Case III. IV :**Two sister, aged 13,17 years old ,product of consanguineous marriage;13 years old sister was presented with chief complaint of progressive ataxia and dysarthria since 9th years of age. Since 11th.years of age progressive dysphagia started but now dysphagia is in a plateau state. Cognitive state remains unchanged. She has no seizure nor gelastic cataplexia. (c.1433A>C) p.N 478 T **Case V:** 13-year-old boy, who presented primarily with neurologic symptoms. He started to develop ataxia and dysarthria at the age of eight years. Dementia, dysphagia, d and seizures, in that sequence, followed within a couple of years. He was anarthric and

bedridden four years after onset. : (c.1192C>T) p.H398Y]. All of the mutations were software analyzed and were missense mutations.

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Identification of Genetic Loci for Uric Acid in North Indians using Genome Wide Association Study

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Background: Serum uric acid (SUA) levels has been associated as a risk factor for multiple complex diseases like gout, hypertension and renal diseases. Understanding the control of uric acid homeostasis may help to improve patient's conditions with hyperuricemia and other related complications. Hence it is necessary to understand the genetic factors controlling SUA level in different population. **Aims and objectives:** Current study aims to identify genetic variants associated with uric acid level using GWAS in North Indians of Indo-European ethnicity. **Material and Methods:** Discovery phase of GWAS was performed in 2,280 individuals. Genotype information of 5,39,662 single nucleotide polymorphisms (SNPs) was analyzed to establish the association with serum uric acid level. SNPs with p-value $<10^{-4}$ were replicated among 5,000 different individuals in the second phase of study. We also analyzed the variance in uric acid levels explained by SNPs using GCTA. **Results:** The analysis of first phase GWAS data identified novel genetic loci in 34 genes significantly associated with serum uric acid levels among North Indians. We replicated genetic variants in *SLC2A9* and *ABCG2* gene that has been previously shown to be associated with uric acid in other population. We further observed that based on genetic information of all the SNPs used in the present study, 7% of variance in uric acid levels can be explained in North Indian population that is in good agreement with earlier published results in Europeans, where 7.7% of the variance was reported. However, SNPs with significance level $<10^{-4}$, explained only 2% of the variance in uric acid level among North Indians. **Conclusion:** This study will help in identifying novel genetic markers associated with serum uric acid and will provide new insights into the genetics of uric acid homeostasis.

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Role of Fumarate Hydratase Gene Mutation in the Etiology of Uterine Fibroids

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Background: Uterine leiomyomas or fibroids are the most frequent solid pelvic tumors in female genital tract. It is estimated that one in four women during reproductive period develop this kind of benign neoplasia. Fumarate hydratase (FH)

has been shown to play a crucial role as a tumor suppressor with its lowered activity in tumors. The FH gene is localized on 1q42.3–43 with ten exons that encodes for fumarate hydratase which catalyzes conversion of fumarate to malate in TCA cycle. So far, twenty different mutations in the FH gene were identified which are associated with uterine fibroids. **Aims and Objectives:** The aim of the present study is to determine the role of FH (exon1) mutations with uterine fibroids in South Indian population from Andhra Pradesh. The objective of the study is to identify the mutations associated with disease phenotype. **Material and Methods:** A case-control study was conducted with a total of 40 clinically, ultrasonographically confirmed uterine fibroid patients and 50 healthy women without the history of any gynec problems were included in the present study. Demographic details and blood samples were collected from all the subjects along with written informed consent. DNA was extracted by standard Sucrose method and analyzed for FH gene (exon 1) mutation by PCR-amplification followed by single stranded conformation polymorphism. **Results:** The demographical features like, age p value=0.031 OR (CI) = 2.84(1.18-6.82), BMI p value=0.003 OR (CI) = 0.16 (0.04-0.54) revealed a significant difference between the two groups. The results obtained in the study showed similar band pattern in all the control and patient samples analyzed so far. **Conclusion:** The study revealed no band variation in the control subjects and patients with uterine leiomyoma. However, large number of samples and the remaining exonic regions of the FH gene have to be analyzed to confirm the results.

P165

Genetic Correlation of XmnI, MTHFR (C677T), ACE (I/D) Polymorphism with The Clinical Severity of Beta Thalassemia in Delhi-NCR Region.

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Background: - Beta thalassemia is a disease of clinical heterogeneity. The patient's phenotype is determined by a/b-globin imbalance, and is influenced by a variety of genetic factors linked or unlinked to the b-globin gene cluster. The high production of HbF during the neonatal period protects the newborn from the clinical manifestations of b-thalassemia. The increased HbF is an ameliorating factor in thalassemia patients, which is known to reduce the severity. The homozygous state for XmnI polymorphism, which is associated with increased expression of Gg gene, plays an important role in ameliorating the clinical features of homozygous b-thalassemia and its clinical presentation. There is an increased risk of thrombosis in patients with beta thalassemia major because of the hypercoagulability which is due to genetic defect in MTHFR gene. The MTHFR C677T polymorphism is responsible for thrombotic events in beta thalassemia. Deaths in 80% cases of beta thalassemia occur due to heart failure, arrhythmias and cardiomyopathies. And individuals with metabolic syndrome are at high risk of CVD. ACE plays an important role in heart failure. **Aims and objectives:** The study aims to elucidate the genotype phenotype correlation of XmnI, MTHFR (C677T),

and ACE (I/D) with the clinical severity of beta thalassemia individuals. **Materials and methods:** A total of 100 individuals with beta thalassemia major and intermedia were included for analysis. Genotyping was done by PCR-RFLP method. And statistical analysis was done for association between clinical severity and genotypes. **Results:** 40% of the beta thalassemia patients were found to be heterozygote for XmnI polymorphism, 27% were carriers for MTHFR (C677T) polymorphism. There was high frequency of ACE (DD) genotype in the cohort of beta thalassemia patients. **Conclusion:** The subjects with XmnI, MTHFR(C677T) and ACE(I/D) polymorphisms have been evaluated with beta thal disease severity and found that they are important biomarkers.

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Variation in Exon-Intron Architecture of Autosomal and X-linked Genes in Dipteran Species

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Background: *Drosophila* represents an attractive model system to understand classical as well as molecular genetics and genomics and more than 70% genome similarities with human make it a popular human disease model. *Drosophila* contributes immensely in understanding the function of various genes and their possible structural and functional variation through time and space by comparative genomics studies among different species. Recent studies have found introns as regulatory component of gene-expression rather than junk DNA and play a major role in expressing multiple proteins from a single gene. Therefore, to understand the gene and protein evolution, study of both exons and introns in term of their size, position, number and GC content is equally important. There are earlier reports about the exon and intron size variation across eukaryotic genomes at inter-species level, however, it is still under debate. **Aims and Objectives:** The primary objective of this study aims at the pattern of variation in exon-intron architecture across the genome (autosomes and X-chromosome) of various Dipteran species and their possible reasons. **Material and Methods:** The chromosomal map of autosomes and X- chromosomes of studied species were accessed from Fly base. Total number of genes present were ascertained using the Map Viewer, NCBI database (<http://www.ncbi.nlm.nih.gov>). The chromosomes were divided according to their high and low recombination region and equal number of genes were randomly selected from each division/group. The details of the genes included in this study e.g. ID, locus, length and functions were retrieved from NCBI and Fly base databases. The number of exons and introns present in each gene were analysed using the computational tool Genscan. **Results:** The results clearly show that a strong relationship exists between the intron size variations with respect to high/ low recombination region along X-chromosome. However, no such relation is observed in case of exons of X-linked genes. In addition, differential pattern of variations is observed in exon-intron architecture of autosomes and X-chromosomes in

Drosophila as well as in its closely related species. **Conclusion:** The overall findings of this study may provide some clue to understand the gene/protein evolution and the underlying mechanism in higher eukaryotes including human.

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Association of Apolipoprotein E, A5 And Endothelial Nitric Oxide Synthase Gene Polymorphisms in Myocardial Infarction: A Hospital Based Study in North East India

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Background: Apolipoprotein E, Apolipoprotein A5 and Endothelial nitric oxide synthase (eNOS) gene plays a crucial role in regulation of vascular tone and vaso-relaxation in cardio vascular homeostasis through cholesterol and triglyceride metabolism. However, genetic polymorphism controlling these proteins have not been fully explored. We studied genetic polymorphism of ApoE, ApoA5 and eNOS gene and their association with Myocardial Infarction (MI) in a population of North East India. **Aims & Objectives:** To find the association of Apolipoprotein E, Apolipoprotein A5 and Endothelial nitric oxide synthase (eNOS) gene polymorphism with MI. **Methods:** A hospital based case-control study in 400 homogenous volunteers (200 cases and 200 matched controls) was conducted. Gene polymorphisms were determined by polymerase chain reaction based methods. **Results:** Significant association was observed for eNOSa (OR=4.49; p=0.001), T-786C rs2070744C allele with MI (OR=2.11; p=0.02). Besides these gene polymorphisms, risk of MI significantly increases with hypertension (OR= 2.96; p=0.001), hypercholesterolemia (OR= 3.31; p=0.002) and smoking (OR=4.22; p=0.001). We have also observed that the individuals carrying Apo E3/E4 genotype significantly increase the risk of MI (p=0.002) **Conclusions:** Subjects carrying Apo E3/E4 genotype, eNOS4a and rs2070744C allele has higher risk for development of MI in addition to conventional risk factors including smoking, hypertension and dyslipidemia. Further the impact of these alleles seems to be the highest among the middle aged individual (41 years to 50 years).

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Non-Familial Sporadic Heritable Retinoblastoma and it's Correlation with Paternal Sperm DNA Damage

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Background: Poor sperm DNA quality observed by raised DNA Fragmentation Index (DFI) may be the common etiological factor in infertility, recurrent spontaneous abortions, congenital malformations and cancers. Unlike oocyte, male germ cells divide continuously and therefore a source of new mutations. Sperm is highly vulnerable to oxidative damage to both nuclear

and mitochondrial DNA. **Aims and Objectives:** Retinoblastoma (RB) is the most common childhood intraocular malignancy but the causative factors are not known. This study was planned with an aim to analyze the sperm DNA quality by DFI calculation in fathers of children with non-familial sporadic heritable RB. **Material and Methods:** PCR was conducted to exclude cases where parents of RB patients were positive for RB1 mutations. 20 fathers of children with sporadic RB and 15 controls i.e. fathers of healthy children were enrolled. Semen & blood samples were collected and analyzed in accordance with WHO 1999 guidelines. DFI by Sperm Chromatin Structural Assay, Reactive oxygen species (ROS) by Chemiluminescence Assay and 8-OHdG by ELISA were calculated. Study was initiated after ethical clearance and informed consent was obtained from cases and controls. **Results:** Mean ages of cases and controls were 33.17 ± 11.2 yrs and 28.5 ± 4.54 yrs respectively. Semen analysis of cases and controls showed no remarkable differences. Seminal mean ROS levels were significantly higher [45.78 ± 38.4 vs 22.75 ± 8.18 RLU/s/million; $p=0.0143$] in cases as compared to controls. The 8-OHdG levels were significantly higher in cases [$72.5(12.8-631.1)$ vs. $32.7(5.6-89)$ pg/mL; $p=0.006$] compared to controls. Mean DFI levels were higher in cases as compared to controls [29.31 ± 5.8 vs 23.27 ± 11.22 ; $p=0.156$] but were not significant. **Conclusion:** This study suggests the role of supra-physiological ROS levels in oxidative DNA damage. The results show that paternal sperm DNA damage may be a cause of childhood retinoblastoma and paternal factors may play a critical role in embryonic development.

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Deregulation of Mirnas in Cervical Cancer: Control by DNA Methylation

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Background: In recent years, miRNAs have emerged as key players to regulate tumor progression in humans. However, these are also subjected to control, by intricate genomic architecture, that regulates its expression and little is known about miRNA regulation by epigenetic mechanisms in cervical cancer. **Aims and Objectives:** We hypothesize that several miRNA genes may gain intrinsic epigenetic modifications in order to maintain a neoplastic phenotype. Our research study focuses on (i) identification of methylation regulated miRNAs during cervical carcinogenesis, (ii) understanding the functional role of altered miRNAs in cervical cancer development and progression and (iii) the clinical utility of the miRNAs as a diagnostic and prognostic marker. **Material and Methods:** Methylation microarray and next generation sequencing was performed in 15 each of normal, premalignant, and tumor samples to identify the miRNA regulated by DNA methylation and its impact on miRNA expression. The microarray data was normalized and analyzed using Feature extraction V9.5.3 and Gene Spring V10.1. NGS data was analyzed by mapping to

human genome (hg19) which was further aligned to miRBase19 and analyzed using R based bioinformatic tools (SHRIMP and SAMtools). **Results:** Microarray analysis revealed 63, 62, and 61 hypermethylated and 62, 62, and 64 hypomethylated miRNA promoters (tumor vs normal, pre-malignant vs normal and tumor vs pre-malignant). NGS data analysis revealed that a total of 455, 503, and 454 miRNAs were altered in normal, premalignant, and tumor samples respectively. We are also investigating the function and fate of specific cellular targets involved in proliferation, adhesion, migration and apoptosis; and their role in progression of cervical cancers. **Conclusion:** Our findings show epigenetic changes at miRNA promoter region are an early and frequent occurrence during cervical carcinogenesis leading to silencing of key miRNAs involved in regulation of oncogenic transformational processes. This will help facilitate development of new diagnostic and therapeutic strategies.

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Amyotrophic Lateral Sclerosis (ALS) – An Overview

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Amyotrophic lateral sclerosis (ALS) is a progressive, fatal neuromuscular disease involving both upper and lower motor neurons. The causes of ALS (also known as Lou Gehrig's disease) are not fully understood. For the vast majority of people with ALS, the mind and senses remain intact and unaffected. Clinical symptoms include muscle weakness, with atrophy; twitching and cramping of muscles, impairment of the use of the arms and legs, difficulty in projecting the voice; and in more advanced stages, shortness of breath and difficulty in breathing and swallowing (ALS Association 2000). However, its symptoms make it difficult to distinguish from other muscular atrophies and related forms of the disease, particularly for epidemiological studies (Roman 1996). Most people who develop ALS are of the age groups of 40 and 75, with the majority after age of 60. The disease is relatively rare and occurs throughout the world with no obvious racial, ethnic or socioeconomic boundaries. According to the Amyotrophic Lateral Sclerosis online genetic database, there has been 117 genes reported till date which are responsible for the ALS. In a report of U.S. Library of Medicines genes like *c9orf72*, *SOD1*, *TARDBP*, *FUS*, *ALS2* are 30-40% responsible for the FALS in USA and other European countries and contribute to the development of the sporadic ALS (SALS) and 20% worldwide. Among all of the genes responsible for the ALS, *SOD1* has the highest frequency of mutations and the patients diagnosed with ALS shows the highest frequency with the same. The whole data whether it is about genes, prevalence, factors responsible for ALS in European and Western countries are available in databases like ALSOD (ALS online genetic database). However, such information is scanty in India to differentiate the ALS from various others motor neuron disorders due to lack of appropriate clinical resources. It is important to carry out various clinical and genetic studies with respect to the disorder and

design a database, which contains all this information about ALS in India. Such databases will help Indian researchers to find the ascertain epidemiology of disease and helps them to differentiate the ALS from other neuro degenerative disease which mimics like ALS.

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Genetic and Environmental factors predisposing to Depression and Suicidal behaviour in Idu Mishmi Tribe of Arunachal Pradesh.

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Background-Complex association of suicidal behavior and depression in suicidal patients has been observed and it may be independent of the risk of suicide associated to depressive disorders. Heterogeneity and hereditary complexity of suicidal behaviour make it more composite and need of demonstrable intermediate phenotypes have been advised.

Aim and Objective- Objective of the study is to find the candidate endo-phenotypes of suicidal behavior and role of risk gene polymorphisms in it. **Method-** Suicidal behaviour was assessed using Columbia suicide severity rating scale, PHQ-9 and open ended questions in Idu Mishmi tribe of Arunachal Pradesh, India. Depression diagnosis was done using Patient Health Questionnaire-9 (PHQ-9) whereas trait of Impulsivity, Aggression and risk genetic markers (5-HTTLPR and sin2, BDNF, COMT) were evaluated to know the diathesis status among Tribesmen's and women whereas Alcoholism, social support and food pattern were recorded to see them as possible risk factors. T-test, correlation regression were performed as statistical methods to know the significance of the risk factors in suicidal Behaviour. **Result-** Rate of suicide ideation and attempt were recorded high as indicator of Suicidal behaviour in Idu stock of Mishmi Tribe. Aggression, Impulsivity score were also found high in persons with suicidal behaviour compared to non suicidal persons. Genetic analysis of serotonin transporter gene (5-HTT) shows the high frequency of 5-HTTLPR, SS allele and sin2, 12 allele in the Idu Mishmi Population. **Conclusion-** Complex association of genetic and environmental risk factor found to play a role in the development of depression leading to suicidal behavior among Idu Mishmi tribe.

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Molecular characterization of tetherin/BST-2 gene promoter in Indian HIV infected long term non progressors

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Background: Tetherin/BST-2 is a recently identified host

restriction factor which tethers newly formed HIV-1 virions to the surface of HIV infected cells and prevents HIV egress and further infection. Long term non progressors (LTNPs) are rare unique individuals who are infected with HIV but control the infection without antiretroviral therapy (ART). It is not known whether polymorphisms of tetherin/BST-2 are associated with natural control of HIV-1 infection. Till date, no variations in tetherin/BST-2 gene have been reported in Indian HIV positive individuals. **Aim:** The aim of this exploratory study is to characterize tetherin/BST-2 gene promoter in Indian HIV infected LTNPs. **Methods:** Three groups of patients; 34 HIV infected LTNPs with CD4 counts >500 cells/cumm, 17 clinical progressors with CD4 counts <200 cells/cumm, and 77 healthy controls were selected for study. DNA was extracted from PBMCs followed by BST-2 promoter amplification, sequencing and docking studies of AP2 repressor protein against BST-2 promoter. **Results:** BST-2 promoter was found to be highly polymorphic. Three novel insertions namely -17 Ins CTTTCAGC, -203 Ins A and -443 Ins CGCCCCCAGACCCAGGCC were found in Indian population. Four novel substitutions at positions -58 G>A, -236 G>A, -260 G/A, -268 C>T and three reported substitutions at positions -245C>T, -305A>G and -380 G>A were also found to be present. -443 Ins was present in 12% HIV clinical progressors and 9% controls while this insertion was absent in LTNPs. Bioinformatic analysis through protein-DNA interaction showed lower AP2 repressor binding affinity in LTNPs. **Conclusion:** -443 Ins corresponds to additional AP2 repressor binding site in BST-2 promoter. Absence of -443 Ins in LTNPs indicates that BST-2 expression might be one of the contributory factors for slow disease progression in LTNPs. This is first genomic study on HIV infected LTNPs from India which highlights importance of BST-2 gene in control of HIV infection.

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Epidemiology of Inbreeding Depression on Physical, Reproductive and Cognitive Behavior

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Background: Inbreeding increases the level of homozygotes for autosomal recessive genetic disorders and generally leads to decline in fitness of a population known as inbreeding depression which provides a major focus for clinical studies. **Aims and Objectives:** We aimed to estimate the effect of inbreeding on physical, reproductive and cognitive behaviors among different populations of Jammu region. **Material and Methods:** The physical parameters (n = 1270, aged 5-15 years) were categorized into height, weight and body mass index (BMI) and the BMI categories were employed as adapted by World Health Organization (WHO). The Wechsler Intelligence Scales for Children (WISC) was used to measure cognitive behavior (n = 408, aged 6-15 years) domains: the verbal IQ (VIQ), performance IQ (PIQ) and full scale IQ (FSIQ). Reproductive behavior of the mothers (n = 999, grouped 25-45 and 46-65 years of age) were based on fertility, mortality,

secondary sex ratio and selection intensity. Family pedigrees were drawn to access the family history and children's inbred status in terms of coefficient of inbreeding (F). **Results:** We found significant decline in children's cognitive abilities due to inbreeding and high frequency of mental retardation among offspring from inbred families ($p < 0.001$). Children of inbred families showed decline in mean value for height, weight and BMI ($p < 0.0001$). The frequency of underweight children was found higher among inbred ($< 18.5 \text{ kg/m}^2 = 47.31\%$) category as compared to non-inbred ones ($< 18.5 \text{ kg/m}^2 = 13.41\%$) and subsequent depression was found among the inbred children due to increase of inbreeding coefficient. We found significant difference in selection intensity, secondary sex ratio and mortality. The total mortality among inbred was higher (10.93%) than in non-inbred (7.48%) families, while fertility parameters remained unaffected. **Conclusion:** Our comprehensive assessment provides the evidence for inbreeding depression on physical, reproductive and cognitive behaviors in comparison with different environmental factors.

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Demographic and Clinical Profile of Dilated Cardiomyopathy Patients from North India

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Background: Dilated Cardiomyopathy is a disease of the heart muscle, primarily affecting the left ventricle. It is a disorder involving a heterogeneous group of cardiac-muscle and is characterized by ventricular dilatation, impaired systolic function, reduced myocardial contractility and with a left ventricular ejection fraction of less than 40%. Of all the various cardiomyopathies, DCM is two to threefold more common cause of heart failure. Some studies suggest that 30 to 35% of idiopathic DCM may have familial history. **Method:** 74 idiopathic DCM patients were recruited from Department of Cardiology, AIIMS. Written consent was taken from all the patients along with their family members for clinical screening (ECG and echocardiography). The inclusion criteria for the patients was Left ventricular ejection fraction (LVEF) $< 40\%$ and absence of past history of myocardial infarction or coronary artery disease. **Results:** Out of 74 DCM patients 27 (36.4%) were familial and 47 (62.2%) were sporadic. 5 individuals from different families who were asymptomatic were also detected to have DCM after the screening. The prevalence was higher in males 46 (62.2%) compared to females 28 (37.8%) and 58 (78.37%) of these patients belongs to NYHAI and has an ejection fraction ranging between 21-30%. The mean age of onset in males was 34.54 yrs, where as in females it was 39.57yrs. Atrial fibrillation was present in 23 (31.0%) of the patients out of which 17 (73.9%) were males and 6 (26.1%) were females. Most of the patients were literate and married. **Conclusion:** There is marked variation in age of onset, disease penetrance or clinical severity. Therefore the patient with Idiopathic DCM must be recommended for the screening of first degree relatives to rule out Familial DCM and those diagnosed with familial DCM

should rescreen unaffected but at risk family members every three to five years.

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Genetics and Epigenetics Variations Can Influence Malaria Susceptibility

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Malaria is a mosquito-borne infectious disease, caused by the *Plasmodium* species, mainly *Plasmodium falciparum* (Pf) and *Plasmodium vivax* (Pv), endemic in many parts of the world. Genetic and epigenetic variations are suggested as an important host factors for determining outcome in malarial infection. Genetic polymorphisms and methylation difference at CpG sites of lipid raft and drug transporter genes, which encode enzymes essential for malaria parasites invasion and antimalarial drug response, could be associated with the pathogenesis of malaria. Hence we tested for variations in genes such as *ABCB1* and *ADRB2* in a case-control study. We also tested the global methylation status of different malaria patient groups. PCR-RFLP, DNA sequencing, Bisulfite DNA sequencing and HPLC methods were performed to check variations. Significant SNP and DNA methylation differences were noticed in our study. Results of our study provides evidence for the proposed role of *ABCB1* and *ADRB2* mediated mechanisms in etiology of malaria susceptibility. Global methylation analysis revealed an indirect relationship between host DNA methylation and parasitemia levels.

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Aberrant DNA methylation in early oral carcinogenesis - A review

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Background: Despite well-established pathways for pre-malignant disease to oral carcinoma, there are no reliable biomarkers predicting progression. Aberrant DNA methylation, an early landmark event in carcinogenesis, may act as a biomarker for lesions progressing to cancer. **Aims and Objectives:** We conducted a review of studies to summarize current evidence on DNA methylation in oral pre-cancer as a potential biomarker of disease progression, and to ascertain knowledge gaps to inform future research. **Methods:** We identified all relevant studies till date using combined key search against PubMed, Web-of- Science and Embase databases. Inclusion criteria were studies of human subjects that examined DNA methylation in oral pre-cancer published in English language peer-reviewed

journals. **Results:** Twenty studies on DNA methylation in oral pre-cancer (3 cohort; 6 case-control; 11 cross-sectional) were reviewed; one-third of these were conducted in India. Sample sizes ranged from 4 to 284 affected cases. Nineteen studies examined promoter regions of tumor suppressor genes primarily involved in cell cycle control (n=15 studies), DNA repair (n=8 studies) and apoptotic pathways (n=4 studies), and reported hyper-methylated frequencies. The hypermethylation of commonly examined genes varied among cases (p16=17.5-87.5%, MGMT=4-72.7% and DAPK=0-35.3%). Two cohort studies observed a greater proportion of p16 hyper-methylation in lesions transforming to malignancy compared to lesions that regressed (57-63.6% vs. 8-32.1%; p<0.01). Study that explored genome-wide methylation patterns reported three novel frequently hyper-methylated genes (TRHDE;ZNF454;KCNAB3). Major limitations were small sample sizes for detecting associations, poor definition of controls and cross-sectional studies that could not track longitudinal changes. Strengths included use of biopsy-confirmed samples and standard and validated analytical methods for methylation patterns. **Conclusion:** Although evidence is inconclusive, emerging data suggest that methylation patterns may be a marker in early oral carcinogenesis, and future genome wide methylation studies can help identify 'driver' loci of disease progression to guide early diagnosis at critical windows.

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Genomics of Chronic Obstructive Pulmonary Disease and Lung Function: A Review

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Background: Chronic Obstructive Pulmonary Disease (COPD) is characterized by irreversible airflow obstruction and measured through lung function. As per GOLD guidelines 2013, forced expiratory volume in one second (FEV₁) and the ratio of FEV₁ to forced vital capacity (FEV₁/FVC) are two important diagnostic measures of COPD. Genome-wide association studies (GWASs) in last five years have identified several genetic variants related to lung function and COPD in European populations. These polymorphisms need to be validated in different population groups to establish universal biomarkers influencing lung function and COPD. **Aim and Objective:** The present review aims to gather current information related to genetic variants associated with COPD and lung function identified through GWASs along with their validation in different population groups. **Material and Methods:** All GWASs and their related meta-analyses, Indian candidate and validation studies available at PubMed (10th December, 2014) were reviewed. **Results:** This review article identifies significant association of a total of 40 loci: *CHRNA3/5*, *HHIP*, *GPR126*, *ADAM19*, *AGER*, *INTS12-GSTCD-NPNT*, *HTR4*, *THSD4*, *FMA13A*, *IREB2*, Chromosome 10 (SNP rs10761571 and rs7896712), Chromosome 14 (SNP rs1214725), *CDH2*, *MYBPC1*, *CTNNA3*, *DNER*, *HLA-DQB1*, *HLA-DQA2*, *KCNJ2*,

SOX9, *GSTM1*, *XRCC*, *CYP2A6*, *SERPINA1*, *IL37*, *ASXL3*, *C10orf11*, *IL16/STARD5/TMC3*, *ME3*, *ADCY2*, *TGF-β1*, *CDC97*, *CRP*, *MFAP2*, *RARB*, *CDC123*, *MMP15*, *RAB4B*, *EGLN2*, *DBH* with COPD and Lung function at or near genome-wide significant level (P<10⁻⁸) in different population groups. **Conclusion:** The findings of GWASs provides biological information on normal physiology of lung function and COPD pathogenesis. The validation of GWAS loci is important for countries like India which has unaddressed and high epidemiological burden of COPD.

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Family History of Cancer and Risk of Squamous Cell Carcinoma of Esophagus - An Outcome of a Case Control Study

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Background: A number of environmental factors are associated with esophageal squamous cell carcinoma (ESCC) risk, however the familial factors in modifying the risk of ESCC are not studied well. We conducted a case-control study to evaluate the relationship between family history of cancer (FHC) and ESCC risk. **Material and Methods:** We recruited 703 histopathologically confirmed ESCC cases and 1664 matched controls. Detailed information was obtained from participants on FHC. Conditional logistic regression models were used to calculate odds ratios (ORs), 95% confidence intervals (95% CIs) and interactions. **Results:** In general, FHC showed a strong association with ESCC risk which got strengthened in participants who had cancer in first degree relatives (OR 6.35; 95% CI, 4.32 - 9.32). Participants with cancer history in blood relation were at a higher risk of ESCC (siblings FHC group had OR = 10.80; 95% CI, 6.03 - 19.34) except in participants whose children had cancer. Association with ESCC risk was found also in participants who had history of cancer in their spouses. With increase in number of relatives with cancer history, the ESCC risk increased. On analysis the risk estimates in subjects who had same organ FHC, the overall association with ESCC across various relation groups was strengthened. In presence of FHC, the ESCC risk was very strong in subject who harbored certain variant genotypes of cytochrome P450 (*CYP 2C19*, *CYP2D6* and wild genotype of *CYP2E1*). The FHC has an additive effect on various ESCC risk factors reported from the population. An interaction of FHC with adobe house was found in participants who lived in such houses. **Conclusion:** The study shows that FHC as a proxy of genetic and shared environmental risk factors could be responsible for the high incidence of ESCC in Kashmir.

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Sickle Cell Anaemia- Community Control Program for Tribal Population Groups of Satpuda Hilly Ranges from Nandurbar, Dist Maharashtra.

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Background: During last 10 years of 20th Century we screened most of the tribal population groups from Maharashtra State and found that Madia, Pardhan, Otkar and other tribal groups from Gadchiroli Dist. and Bhil & Pawara tribal population groups from Nandurbar District have higher prevalence of sickle cell disorder. (Heterozygous >20%). We identified 3 voluntary health organization i.e. SEARCH of Dr. Abhay Bang, LOKBIRADARI project of Dr. Prakash Amte and AMHI AMCHYA AROGYASATHI of Dr. Satish Gogulwar, trained their staff members & encourage them to undertake work on Sickle Cell Anaemia. There was no any NGO working on this problem from Nandurbar District, hence in 1998 Maharashtra Arogya Mandal established Community Control Program Centre in Satpuda hilly ranges (Nandurbar district) with help of local tribal youths. **Aim:** To provide facilities and create awareness for sickle cell disorder in high prevalent tribal area. **Objectives:** Screening, Diagnosis, Possible Treatment, Counselling etc. **Material and Methods:** The centre is popularly known as **Sickle Cell Dawakhana** (Roshmal Budurk, Taluka Dhadgaon, Dist. Nandurbar.) We provide all the following facilities -

- Accurate diagnosis
- Possible Treatment and follow up
- Population genetic surveys
- Health education –
- Improvement in the quality of life (QOL)
- Genetic counselling
- Marriage counselling
- Guidance for Prenatal diagnosis & Family Planning
- Research
- Training

Results: We are working in this area for last sixteen years. We screened more than 1.5 lakhs tribal people and >2000 patients are under our medical supervision. **Conclusion:** Patients and parents are happy with our medical and social treatment and we have good response. Ten point program will be presented.

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Genome Wide Association Study of Glycemic Related Traits in Indians

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Background: Genome wide association study (GWAS) is a robust and reliable method to identify genomic segments associated with complex diseases like type 2 diabetes (T2D). T2D is defined on the basis of clinical measurements of fasting plasma glucose, post prandial glucose and glycosylated hemoglobin (HbA1C). Because of diverse nature of diabetes,

several robust markers associated with contributing glycemic traits may lose their significance in the GWAS of T2D. **Aims and objectives:** We aimed to perform GWAS of several glycemic traits including fasting plasma glucose, fasting plasma insulin, HbA1C and post prandial glucose in >7,000 individuals of Indo-European ethnicity. **Material and Methods:** GWAS of glycemic traits were performed in two phases: discovery phase and replication phase. A total 2,280 individuals were genotyped using Illumina Human 610-Quad Bead chip. After stringent quality control 5,39,662 single nucleotide polymorphisms (SNPs) were tested for association with inverse normal transformed values of above mentioned traits. Signals with P-value <10⁻⁴ were taken for further validation in replication phase in another 5,000 individuals using Golden Gate assay. **Results:** The discovery phase results identified various novel genetic loci significantly associated with glycemic related traits. We replicated several genetic variants (*ADRA2A* and *CSMD1*) associated with fasting plasma glucose and plasma insulin. Further, our analysis revealed that genetic variants associated with plasma glucose (*CDKN2A*, *TP53INP1* and *PRC1*) post glucose load (*TP53INP1*, *LPIN2*, *TCF7L2*) and HbA1c (*WISP1*, *CDKAL1*, *RASGRP1*, *HMG20A*) were also found to be associated with published T2D GWAS. We further compared the allele frequencies of susceptible SNPs with that of total SNPs studied, and observed that 65% of susceptible SNPs had higher MAF, while 31% of variants were of intermediate frequency and 4% were low frequency variants. **Conclusion:** These results suggested that variants of glycemic related traits with higher MAF contributed significantly to the epidemic of T2D in Indians. This study will help in better understanding of complex etiology of T2D.

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Study of Bacterial Diversity in Beta-Thalassemia Patients of North Maharashtra Region by tRFLP Analysis.

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Background: Beta-thalassemia is autosomal recessive disorders caused by the reduced (β^+) or absent (β^0) synthesis of beta globin chains of the hemoglobin(Hb) tetramer (Weatherall et al.). Thalassemia is prevalent in the Mediterranean, Middle-East, Central Asia, Indian subcontinent, and Far East (Antonio et al.). T-RFLP analysis is rapid, convenient, sensitive, high-throughput, and highly reproducible method in microbial ecology studies and thus has been often used for analyzing large numbers of samples. **Aims and Objectives:** The primary goal of the t-RFLP analysis is to test the environmental samples using 16S rDNA as well as 18S rDNA amplification from community and restriction enzyme digestion (TaqI, HaeIII) and to perform T-RF analysis (presence/absence of peaks and area of peaks) on the determination of bacterial species richness, diversity index, community structure and subsequent comparison of profiles between environmental samples. **Material and Methods:** Blood samples were collected from transfusion dependent thalassemia population from North Maharashtra. DNA isolation from Blood samples,

PCR amplification of the 16S gene, Restriction digestion and desalting of digested products, GeneMapper data analysis. **Results:** Numbers of T-RF developed in this technique have been used as indicators of microbial species richness. As a result of a T-RFLP profiling a graph called Electropherogram is formed, which is an intensity plot representation of an electrophoresis experiment. In the present study, in the thalassemia blood samples bacterial richness ranged from 2 to 34 with an average of 17.57. **Conclusion:** This result states that various kinds of bacteria are present in the blood of thalassemia patients. The thalassemia patients are prone to many types of infections. Bacterial population in the blood of thalassemia is the fact of concern and should be taken seriously.

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Effect of Parental stress, Coping Strategy and XmnI- G γ (HBG2) Polymorphism in Beta Thalassemia Disease Severity: An Evaluation of Gene- Environment Interaction.

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Background: The life expectancy of a child born with the Thalassemia disease depends upon regular blood transfusion. There is high variability in the severity of the disease (even between Thalassemia Major and Intermedia), depending upon the mutations causing the disease and linked XmnI- G γ (HBG2) gene promoter polymorphism. XmnI-G γ (HBG2) gene promoter polymorphism has an important role in the regulation of HbF level and will help in amelioration of the severity of Beta- Thalassemia disease. These factors are population specific. Besides genetic factors, Social, Psychological, nutritional, physical activity and economic background of the patients and their family also have immense influence on the quality of life and further on morbidity and mortality of the suffering Children. In view of the enormous heterogeneity in the clinical manifestation of the disease, it is important to evaluate genetic and environmental factors for better management of the affected individuals. **Aims and Objectives:** To understand the relation and effect of parental stress, coping strategy and XmnI- G γ (HBG2) polymorphism and their interrelation the on health related quality of life associated with Beta thalassemia Patients. **Material and Methods:** Detection of XmnI- G γ (HBG2) polymorphisms was done by PCR-RFLP and Genotyping method related to it. The Interview method was implemented on 85 patients and their parents for the collection of Parental stress, coping strategy and other disease related information through respective already validated Questionnaire schedules. **Result:** 51.67% of Thalassemia patients are heterozygous for XmnI- G γ (HBG2) (C/T) risk allele. Parental stress and coping strategy was to be important in disease severity among studied patients. **Conclusion:** Evaluation and understanding of interaction of genetic modifiers with social factors in the amelioration Beta thalassemia disease is important, besides therapies available.

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Oxidative DNA Damage Assessment in *in situ* COPD Cases

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Background: Chronic Obstructive Pulmonary Disease (COPD) is defined by airflow- limitation and is quantified by the lung function test. Stone-crushing activities of drilling and dressing cause respiratory distress which may lead to COPD from oxidative stress via inflammation and generation of reactive oxygen species. Oxidative stress can cause oxidation of cellular macromolecules including DNA. As oxidative DNA damage in COPD cases defined *in situ* have not come to attention, in the present study the comet assay in peripheral blood leukocytes of COPD cases identified from stone-crushing units and in the healthy non-exposed controls was carried out. **Aims and Objectives:** Association of basal and oxidative DNA damage in peripheral blood leukocytes of *in situ* defined COPD cases on the basis of the pulmonary function test. **Material and Methods:** After Institutional Ethics clearance and informed written voluntary consent, the COPD cases were identified by spirometry. The peripheral blood leukocytes from all the study participants was assessed for genetic damage using the alkaline single cell gel electrophoresis assay and the modified comet assay using lesion-specific enzymes viz. endonuclease III(Endo III) and formamidopyrimidine glycosylase(Fpg). Nucleoids (100/individual) were assessed for DNA damage using an image analysis system. The observations were subjected to statistical analysis. **Results:** The study group (males) comprised COPD cases (n=30; 38.53 $\pm$ 1.21y) working at the stone-crushing units for 5-14y (10.36 $\pm$ 0.50y) with a daily work-shift of 7-13h/day (9.60 $\pm$ 0.32h), and the controls (n=30; 36.73 $\pm$ 0.68y) who all smoked and chewed tobacco. COPD severity among the workers included moderate (40.00%), severe (26.66%) and very severe (33.33%) cases based on the expiratory lung volume. Genetic damage was significantly elevated in COPD cases (1.20 fold of %DNA in tail 48.02 $\pm$ 1.32 vs. 39.91 $\pm$ 1.02, p=0.000) with 1.6 fold increase in oxidized pyrimidines (2.30 $\pm$ 0.14 vs. 1.45 $\pm$ 0.17, p=0.001) and oxidized purines (1.47 $\pm$ 0.13 vs. 0.92 $\pm$ 0.09, p=0.002) compared to the controls. However, genetic damage did not vary for severity of COPD. **Conclusion:** The stone crushing activities hence have shown development of COPD and increased genetic damage in workers.

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Genomic Affinities Among Different Population Groups of Jammu Region of J&K State

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Background: Human genomic diversity is the result of differential accumulation of genetic variations in individuals

and populations throughout the evolution. The identification of such distinctive characteristics in the DNA represents the basis of human identification, genetic diversity and population genetics. The state of Jammu and Kashmir harbors heterogeneous population groups inhabiting the different geographical regions. A little work is being carried out regarding the study of genetic diversity of the people of the state. **Aims and Objectives:** To study genomic diversity study in six of the prominent population groups (Brahmins, Rajputs, Bhagats, Chamar, Gujjars and Jatt Sikhs). **Material and Methods:** Nine autosomal DNA markers belonging to seven *Alu* insertion/deletion polymorphisms namely *Alu* ACE, *Alu* APO, *Alu* PV-92, *Alu* PLAT, *Alu* FXIIB, *Alu* D1, *Alu* CD4; LPL *PvuII* and ESR *PvuII* polymorphism was studied. Blood samples were collected randomly from 600 unrelated healthy individuals after prior consent. DNA was extracted and amplified by PCR using target specific oligonucleotide primers and finally subjected to agarose gel electrophoresis. Further, for LPL *PvuII* and ESR *PvuII*, the PCR product was subjected to restriction digestion using *PvuII* restriction enzyme. Allele frequencies were used to calculate average heterozygosity. **Results:** All the markers except *Alu* CD4 were found to be highly polymorphic with high heterozygosity values in almost all the population groups of the state. It was observed that most of the genomic diversity was attributed to individuals within the population. **Conclusion:** The study may help in future work on the genetic heterogeneity in other population groups of the state which may give a genetic insight and genetic basis underlying the different genetic diseases prevailing in the state.

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Prevalence of various cardiac disorders in Pune population and associated physiological complications

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Background: Cardio vascular disease (CVD) contributes about 16.7 million of global deaths and is among top five causes of deaths in Indian population, having a share of about 31% in total disease burden. CVD is one of leading cause of mortality in developed countries and accounts for 30% of all disability cases. **Aim and objectives:** The main objective of current study is to unravel prevalence of various cardiac disorders and associated complications in Pune population. **Methods:** The present study is approved by Institutional Human Ethics Committee of Bharti Hospital Katraj and Savitribai Phule Pune University, Pune. Hospital statistics are followed and all the entries are monitored. For classification of various types of CVD, published criteria are followed. All patients have gone through ECG, 2-D Echo cardiography, biochemical estimations and pathological analysis in the hospitals. **Results:** Prevalence percent of rheumatic valve heart disease is 57%. Prevalence percent

of other CVD is comparatively less such as Ischemic Heart disease (16.82%), Cardiomyopathy (3.73%), Coronary Heart disease (3.73%) and Congenital Heart disease (0.93%). Total serum cholesterol level is higher in females (160.66±42.66) than males (138.64±45.87). Serum alkaline phosphatase is elevated in females (73.33±25.98) than in males (67.47±23.65). Left ventricular ejection fraction (LVEF) % is slightly higher in females (55.93±8.94) than males (55.63±8.34). Males (n=25) shows higher respiratory rate 18.03±4.72 than females (n=82) 17.60±2.33. **Conclusion:** In Pune population prevalence of rheumatic valve heart disease is found to be more as compared to other CVDs. Physiological complications are more severe in females.

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Role of various variants of phase-1 xenobiotic metabolising genes in modifying esophageal squamous cell carcinoma risk in Kashmir- a high risk area.

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Background: Esophageal squamous cell carcinoma (ESCC) is the most common cancer and a great public health concern in Kashmir. Though some environmental risk factors are associated with its risk, the role of genetic markers is still unclear. **Aims:** The current study, was undertaken with the aim to analyse the relationship between gene variants of various Phase I xenobiotic metabolising enzymes (XMEs) like cytochromes P450 (*CYP*) 2A6a, 2A6b, 2A6c, 2A13, 2C19, 2D6, 2E1 and *CYP1A1* and ESCC. **Methods:** We recruited 492 histopathologically confirmed ESCC cases and equal number of matched controls. Conditional logistic regression models were used to assess the association of various genotypes, gene-gene (GxG) and gene environment (GxE) interactions towards ESCC development. **Results:** Individual inverse association towards ESCC risk among subjects carrying variant genotypes of *CYP2A6c* (OR = 0.60; 95% CI = 0.36 – 0.96) and *CYP2A13* (OR = 0.53; 95% CI = 0.31 – 0.89) was retained in their combination as well (OR = 0.31; 95% CI = 0.11 – 0.87). However, an increased risk was observed in subjects harboring variant genotype of *CYP2C19* (OR = 3.33; 95 % CI = 1.98 – 5.61) and *CYP2D6* (OR = 2.12; 95 % CI = 1.11 – 4.25). The higher risk in participants with *CYP1A1* variant genotype (OR = 2.87; 95% CI = 1.00 - 8.44) was further increased when *CYP2E1* wild genotype was also present (OR = 5.68; 95% CI = 1.09 - 29.52). Synergistically, a significant GxG interactions turned out between *CYP2A6a* and *CYP2A6b* as well as between *CYP1A1* and *CYP2E1* genotypes ($P = <0.001$) in modifying ESCC risk. Similarly, positive GxE interactions were observed between *CYP2A6b*, *CYP2A13*, and *CYP1A1* with smoking ($P = <0.05$). **Conclusion:** Harboring certain genotypes of the phase-1 XMEs seems to play a role in the ESCC etiology in Kashmiri population.

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Association Of TP53 Expression And GST & CYP1A1 Polymorphism In Oral Squamous Cell Carcinoma

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Background: Oral squamous cell carcinoma (OSCC), a malignancy of the lip, mouth or tongue is one of the most common cancers, with a global incidence of 75,000 to 80,000 new cases per year. Environmental etiologic agents like tobacco, alcohol, polyaromatic hydrocarbon (PAH) etc. are associated with the oral carcinogenesis. Cytochrome P450 1A1 (CYP1A1) gene producing Phase I enzymes are required to activate PAH which get detoxified by metabolism using Phase II enzymes Glutathione-S-Transferases (GST). Polymorphic CYP1A1 and GST enzymes involved in activation and detoxification deficiency of tobacco related procarcinogens. TP53 is a key regulator of cell integrity with impact on cell cycling, growth, DNA repair, cell cycle arrest or apoptosis. If TP53 is not optimally expressed, the damaged DNA remains unrepaired and mutation becomes fixed in the dividing cells. Deregulated expression of TP53 plays an important role in carcinogenesis. **Aims and Objectives:** Comparison of CYP1A1, GST polymorphism with TP53 expression status interaction in diagnosed OSCC patients. **Methods:** A study on OSCC patients (n=20) were analyzed. GST polymorphism was detected by PCR amplification using specific primers and CYP1A1 polymorphism was analyzed using PCR-RFLP with total DNA isolated from peripheral blood. TP53 expression status was determined from the tumor tissues using quantitative Real-Time PCR (qRT-PCR). **Results:** The median age of the OSCC patients were 49.5 years. 65% of diagnosed OSCC patients were comprised of the polymorphic CYP1A1 out of which 84.62% have under-regulated TP53 expression. Even 60% of patients have polymorphic GST gene with 66.67% down-regulated TP53 expression cases among them. Again there were 30% cases with both polymorphic GST and CYP1A1 genes of which 83.33% TP53 under-expressed incidence. **Conclusions:** It may be stated from the study that Phase I (CYP1A1) and II (GST) enzyme polymorphisms and down-regulation of TP53 gene expression are associated with squamous cell carcinoma formation.

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Mutation in PAX3 associated with Neural Tube Defect

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Background: Neural tube defect is a complex congenital

malformation which occurs due to both genetic and environmental factors. Pax3 gene belongs to the class of transcription factor having a paired domain, homeodomain, tranactivation domain and an octapeptide chain is also present. PAX3 gene is known to play important role in embryonic neural tube development.

Aims and Objective: To check novel mutation in PAX3 gene associated with neural tube defect in familial cases. **Material and Method:** The study sample included 167 patients and 284 controls out of which six familial cases having more than one affected cases were chosen for further sequencing study in exonic region of PAX3 gene. **Result:** A missense mutation is observed in exon6 at position 222221238 with T>A change of aliphatic non polar amino acid to aromatic non polar amino acid I317Y. Another mutation was observed which showed insertion of T at position 222221368 in exon6 leading to frame shift mutation. The effect of this insertion was analyzed using mutation taster. The deleterious and damaging effect of mutation was analyzed using Polyphen. **Conclusion:** Our results support the hypothesis that mutations in the gene for PAX3 can lead to neural tube defect. The frame shift mutation at 313 position leads to further change in amino acid sequence downstream. This change is observed in homeodomain region of PAX3 gene which has a DNA binding activity and may play important role in neural crest migration and further neural tube closure.

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Study of Abacavir hypersensitivity in Indian Subpopulation and its association with HLA-B*5701 and HCP5 rs2395029

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Background- A cost cutting approach towards development of newer screening cum confirmatory test would save a lot of waiting anxiety for the patients simultaneously making the diagnostics economical and faster. The HLA complex protein 5 gene (hcp5) rs2395029(G) allele is in linkage disequilibrium with HLA B*57:01. This has been studied and reported in various global populations and is a proved pharmacogenomic biomarker. The presence of HLA B*57:01 has been associated with hypersensitivity reaction (HSR) to the drug abacavir in HIV positive individuals. **Aims and Objectives-** In the present study we aim to report the HLAB*57:01 associated HSR in a subpopulation of Indian subcontinent using simple screening test. Our major objective is to screen the patients for hcp5 polymorphism and purge the economically challenging and time taking step of confirmatory test. In the present study a positive and negative screening was further backed up with a confirmatory test to check the validity of the screening test. This is further followed by advice to individuals on their drug sensitivity status. **Materials and Methods** – In the present study we assess the risk of hypersensitivity to the drug abacavir in HIV positive individuals. We have attempted to detect the presence of the hcp5 gene polymorphism as a screening test, and in case that is positive or negative we proceed to HLA B typing using SSP-PCR or SBT, as confirmatory test. If the

confirmatory test is also positive, the final result is declared as positive and the patient is advised against abacavir. **Result** – We carried out a total screening of 137 patients for the presence of hcp5 polymorphism. The negative screening results of 124 individuals further confirmed them to be negative. A positive screening showed one isolated negative confirmatory while all others were found to be positive. This suggest that a screening test itself in case of negative results could be used as final confirmatory for abacavir HSR and the patients could be advised as per. **Conclusions** – We conclude that any early negative screening of hcp5 polymorphism at the given locus could play an important role by purging the need to go through the confirmatory studies for abacavir HSR and thus be more economical on the patients with a minimal time of reporting.

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Structural and Functional analysis of Protein-Protein interaction network in neurodegenerative disorders

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Background: Proteins are the structural and functional workhouse in the cell that takes part in virtually every event within and between the cells and ultimately determine the behavior of biological system. Recently network centered approaches have been increasingly used to comprehend the fundamentals of biology. There are different databases documenting the interactions of proteins but none of them have been able to reveal the molecular mechanism behind the binding process occurring between molecules. One plausible way to address this problem is the inclusion of structural details of the complexes comprising of 3-D structures of proteins, interface as well as topological properties. **Aims and objectives:** Based on this premise, the present study is focused on studying the interface properties of neurodegenerative disorder related proteins to classify the neurodegenerative disorder associated proteins and non-associated proteins. It aims to find novel genetic markers that might increase the individual's susceptibility to neurodegenerative disorders. **Materials and Methods:** The study takes into consideration- Alzheimer's Disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis, Schizophrenia, Epilepsy, Huntington Disease, Friedreich Ataxia, Prion Disease and Progressive Supranuclear Palsy. The protein-protein interactions involved in these disorders were supplemented by interface properties coming from known complexes. Further, machine learning algorithm was used to generate a classifier to predict additional gene products that may be associated with these neurodegenerative disorders. **Results:** Among several classifying algorithms applied to generate machine learning models, best performance was achieved using Rotation Forest with 71.7% accuracy, 69.6% specificity and 72.7% sensitivity. A list 50 genes was obtained which are predicted to be associated with neurodegenerative disorders. **Conclusions:** The results from structural and functional analysis of neurological protein-protein interaction network can be used for further analysis and validation.

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A Preliminary Study on Human Spermiation Defect

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Background: Spermiation is the release of mature sperms from Sertoli Cells into the lumen of the seminiferous tubules. Spermiation defect is an unrecognized entity in andrology practice. Spermiation defects cases were never suspected and investigated in andrology clinic and no attempt was made to understand spermiation defect in human. Most of information on spermiation defect so far is from animal experiments. Research on spermiation can help to understand some of the cases of male infertility. We assume some cases of non obstructive azoospermia with normal testicular histology/cytology could be due to spermiation defect. **Aim and Objective:** In order to understand the etiologic factors of spermiation defect, present study was proposed to explore sertoli cell maturation status, sex chromosomal aneuploidy, retinoic acid deficiency and heavy metals toxicity in spermiation defect. **Material & Methods:** We enrolled 13 cases of spermiation defect and 15 normal fertile men for this preliminary study. Hormone assays like FSH, LH, Prolactin, Testosterone and Estrogen, etc were estimated through Abbot Axym automated system. AMH & Inhibin B were estimated through commercially available ELISA kits. Sex chromosomal aneuploidy was investigated by interphase FISH using X & Y probes. The Yq microdeletion was investigated by PCR method. Presences of heavy metals (Lead, Cadmium, and Mercury, etc) were investigated by Elemental Electron Microscope scanning using Energy-dispersive X-ray spectroscopy (EDXS) in seminal testicular cells & Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) in serum. We have also measured vitamin A concentration in serum according to Kubler and Lorenz (1963) method. **Results:** This study found normal value of AMH in all cases of spermiation defects (i.e mature sertoli cell) and no sex chromosomal aneuploidy. The study found high level of estrogen (53% cases) and no differences in vitamin A level between normal fertile control & spermiation defects cases. The study also observed high platinum in 4 cases (along with high iron in 2 cases) in seminal cells and high level of lead, cadmium, chromium, manganese and nickel in serum. **Conclusions:** This finding indicates high serum concentration of heavy metals like lead, cadmium and high platinum & iron in seminal cells along with high estrogen may be important underlying etiologic factors for spermiation defect in human.

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Genetic variant analysis in three Indian ethnically diverse human genome

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Background: India with about one-sixth of the world's

population, is mainly constituted of Indo European (IE), Austro-Asiatic (AA), Dravidian (DR) and Tibeto-Burman (TB) linguistic lineages with varied cultural and social framework. India has been underrepresented in genome-wide surveys of human variation. Understanding genetic diversity and risk allele identification by massively parallel sequencing approaches have been facilitated by Next Generation Sequencing (NGS) technologies. **Objective:** Creation of a catalogue of genetic variants from genome of three Indian ethnically diverse individuals for understanding the genetic architecture. **Method:** We sequenced the whole genome from three individuals belonging to Tibeto-Burman, Indo European and Dravidian (DR) families by Next Generation Sequencing Technology. Genomic DNA was sheared and used in the preparation of the whole genome shotgun libraries as per Illumina's library preparation protocols (Illumina, CA). The libraries were then sequenced on a HiSeq 2000 sequencing machine (Illumina, CA) to obtain around 15X sequence data. **Result:** Approximately 2.72 million SNPs and 0.26 million Indels (insertion deletion) in TB, 1.98 million SNPs and 0.16 million Indels in IE and 2.55 million SNPs and 0.23 million Indels SNPs in DR individual were obtained. **Conclusion:** Present study reports genetic variants in three ethnically diverse individuals of India. Availability of this information will aid studies aimed at understanding genetic diversity, performance markers and clinically relevant changes in context of heterogenous Indian population.

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Genetic Variants of APOBEC3G-90C/G and -571G/C Polymorphism and Risk of acquiring HIV infection

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Background: APOBEC3G is a member of Apolipoprotein B-mRNA Editing Catalytic Polypeptide (APOBEC) family and is known as a host viral restriction factor against HIV-1. APOBEC3G protein activity blocks retrovirus infection by inducing mutations of guanines to adenines (G>A) in the viral cDNA. Natural sequence variations in the promoters region of APOBEC3G gene may result in variable expression of APOBEC3G in different individuals. **Aims and Objectives:** We aimed to investigate the association of APOBEC3G-90C/G and -571G/C gene polymorphism with risk of acquiring HIV infection. **Materials and Methods:** In the present case control study, we enrolled a total of 156 HIV infected patients confirmed by ELISA test and 156 unrelated healthy individuals. Polymorphism for APOBEC3G-90C/G and -571G/C gene were genotyped by polymerase chain reaction and restriction enzyme length polymorphism. **Results:** Frequency of APOBEC3G-90C/G

genotype did not significantly differ between HIV infected patients and healthy controls. HIV infected patients with -571GC genotype were at significantly higher risk of acquiring HIV infection ($P=0.018$, OR=3.46; 95%CI: 1.39-8.61) as compared with healthy controls. Patients with haplotype CC of APOBEC3G-90C/G and APOBEC3G-571G/C conferred significantly higher risk of acquiring HIV infection ($P=0.041$; =3.68; 95% CI: 1.06 - 12.79). In dominant models, individuals with -571GC+CC genotype have a significantly higher risk of acquiring HIV infection ($P=0.01$; OR=2.73; 95% CI: 1.12-6.02). **Conclusion:** Individuals with APOBEC3G-571GC genotype and haplotype CC of APOBEC3G-90C/G and APOBEC3G-571G/C may have higher susceptibility to acquiring HIV infection. Limitation of the study is that the healthy controls may not have been exposed to similar risky behavior and hence this association should be studied in a larger comprehensive study.

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Clinical and molecular characterization of Fragile X Syndrome

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Fragile X syndrome (FXS) is the most common form of inherited intellectual impairment and known genetic cause of autism. It is one of the first human diseases to be linked to an expansion of triplet nucleotide repeats. FXS is caused by expansions of the CGG repeat sequence located in the 5'untranslated region of the *FMR1* (fragile X mental retardation-1) gene. Estimates report that FXS affects approximately 1 in 4000 males and 1 in 6000 females. The syndrome is transmitted as an X-linked dominant trait and with reduced penetrance (80% in males and 30% in females) It is characterized by mild to severe mental retardation, with IQ between 20 and 60, mildly abnormal facial features of a prominent jaw and large ears, mainly in males, and macro-orchidism in post-pubescent males. In this study we evaluated 125 idiopathic mental retardates using CGG RP PCR. *FMR1* exon 1 specific primer is used for the experiment. The primers were *FMR1* Forward (TCA GGC GCT CAG CTC CGT TTC GGT TTC A) and *FMR1* Reverse 6-carboxyfluorescein (FAM)-labeled (FAM-AAG CGC CAT TGG AGC CCC GCA CTTCC). This PCR is primarily distinguished from a more conventional two-primer, gene-specific PCR by the addition of a third PCR primer that is complementary to the *FMR1* triplet repeat region. In this method, primers hybridize randomly across the CGG repeat tract and at the 3' junction of the CGG tract and downstream sequences. After PCR, samples were stored at -15 to -30°C and either protected from light before analysis or analyzed immediately by CE Automated Sequence Analysis. PCR products detected by CE were analyzed using Peak-Scanner 1.0 (Applied Biosystems). The peak size in base pairs was converted to the number of CGG repeats by referencing the base pair size of the process control alleles to the base pair size of the sample's product peaks.

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CYP1A1 MspI gene polymorphism and breast cancer susceptibility in females of Jammu region

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The cytochrome P450 1A1 gene codes for the phase I metabolic enzyme. This gene is considered as a possible candidate for breast cancer risk. The product of the gene is involved in the phase I detoxification of polycyclic aromatic hydrocarbons (PAHs) and 2-hydroxylation of estrogens and mammary carcinogens into 2-hydroxy catechol metabolites. Several studies have been carried out to detect the role of

CYP1A1 polymorphisms in breast cancer risk but results are inconsistent. In the present work, a population based case-control study was designed to clarify their importance of *CYP1A1*MspI polymorphism in determining breast cancer susceptibility in females of Jammu region. A total of 52 cases and 65 age matched controls were genotyped for *CYP1A1*MspI polymorphism by using PCR , restriction digestion and electrophoresis. Potential non genetic risk factors for breast cancer were also considered for the said study. the results suggest that there is no significant correlation between *CYP1A1* Msp I polymorphism and occurrence of breast cancer in females with breast cancer of Jammu region. Small sample size is the limitation of the present study.

