**How is the face formed and when it goes wrong: In relation to Cleft lip/Palate**

**Lay Abstract**

The face is a very important aesthetic but complex part of the body. Therefore, when the face does not form normally it results in something abnormal- a deformity. One of the consequences of abnormal facial development is a cleft lip and/or palate. This is when the upper lip and/or the palate of a child does not form properly and has a gap or split in it. Normally, before the baby is born the lip and palate join as usual but when something goes wrong it doesn’t form typically and so this gap/split is present at birth. This report will look at how the normal face forms and then look at how and why it goes wrong and what causes these gaps of the lip/palate. The report will also show the different types of this abnormality of the lip/palate. As this is a very common problem present in a lot of children from birth, this report will demonstrate the challenges in understanding this abnormality of facial development and show the importance of understanding the normal process of facial morphogenesis in the first instance.

**Scientific Abstract**

The normal development of the face relies upon the correct morphogenesis of structures in utero that usually occurs within the first trimester of embryonic life. The face is a very complex structure involving many genes and factors and with it being such a crucial part of life, both physically and aesthetically and therefore mentally, its important for everything to be just right. However, when the normal process doesn’t go to plan this results in dysmorphogenesis, which cleft lip and palate (CLP) is an example of as the lip/palate doesn’t fuse together and the infant is left with a gap. Although the exact cause of CLP is unknown, it is thought to be a mixture of genetics, environment and the teratogens the mothers are exposed to within the environment. This report will demonstrate the normal development of the face for the purpose of understanding how it goes wrong, resulting in CLP. Since there is still a lot to be understood about CLP it will also shed light on recent advances in relating SHH and certain genes as a possible cause for this dysmorphogenesis. The report will also briefly look at the relation of CLP with the genes associated with syndromic and non-syndromic diseases and the different types of CLP. There are many other facial defects that are a result of dysmorphogenesis, however as CLP is one of the most common yet poorly understood facial defect, it will be the main focus of this report.

**1. Introduction**

Cleft lip and/or palate (CLP) in the UK is one of the most widely seen facial birth defects in the UK with about 1 in 7001 babies suffering from this consequence of dysmorphogenesis. However, despite this facial malformation being so common and well managed , CLP is still relatively poorly understood in regard to what causes it, with there only being speculation on certain growth factors such as TGFA, various genes and environmental factors elicited potential causes. CLP is something that requires long term treatment with several surgical interventions needed for a number of years2 and thus with the longevity of this disease it is important to know how it goes wrong in the first instance. The correct morphogenesis of the face occurs upon the basis of harmonised events that result in the correct migration, proliferation and fusion and so the normal formation of the face3. In relation to this report, the lip and palate formation occurs in the first trimester of embryonic life and so marks a critical period of formation as it must be around this time where the dysmorphogenesis occurs, resulting in this facial birth defect4. The formation of the lip and palate occurs so early on that this process is sensitive with its interaction with the genetic and environmental factors during this first trimester, and so these factors are assumed to be a cause of CLP4. Of particular importance is the genetic syndromic cause of CLP- Van der Woude syndrome as it accounts for 2% of all cases of CLP and is thus the most common cause of CLP as a result of a genetic mutation.5 Following on from this, the report will explore both the syndromic and non-syndromic causes of CLP. It is important to understand how the normal facial development occurs in order to know where it goes wrong and results in facial defects. This is why in this report, I aim to explore a brief overview of how the face and palate are formed so I can provide a basic understanding of how normal morphogenesis occurs. The report will then focus on the consequence of dysmorphogenesis- CLP and demonstrate what is known about this ailment in terms of types and causes and possible genetic relations in syndromic Vs non-syndromic CLP.

**2. Overview of facial and palate development**

**Facial development: Weeks 4-6:**

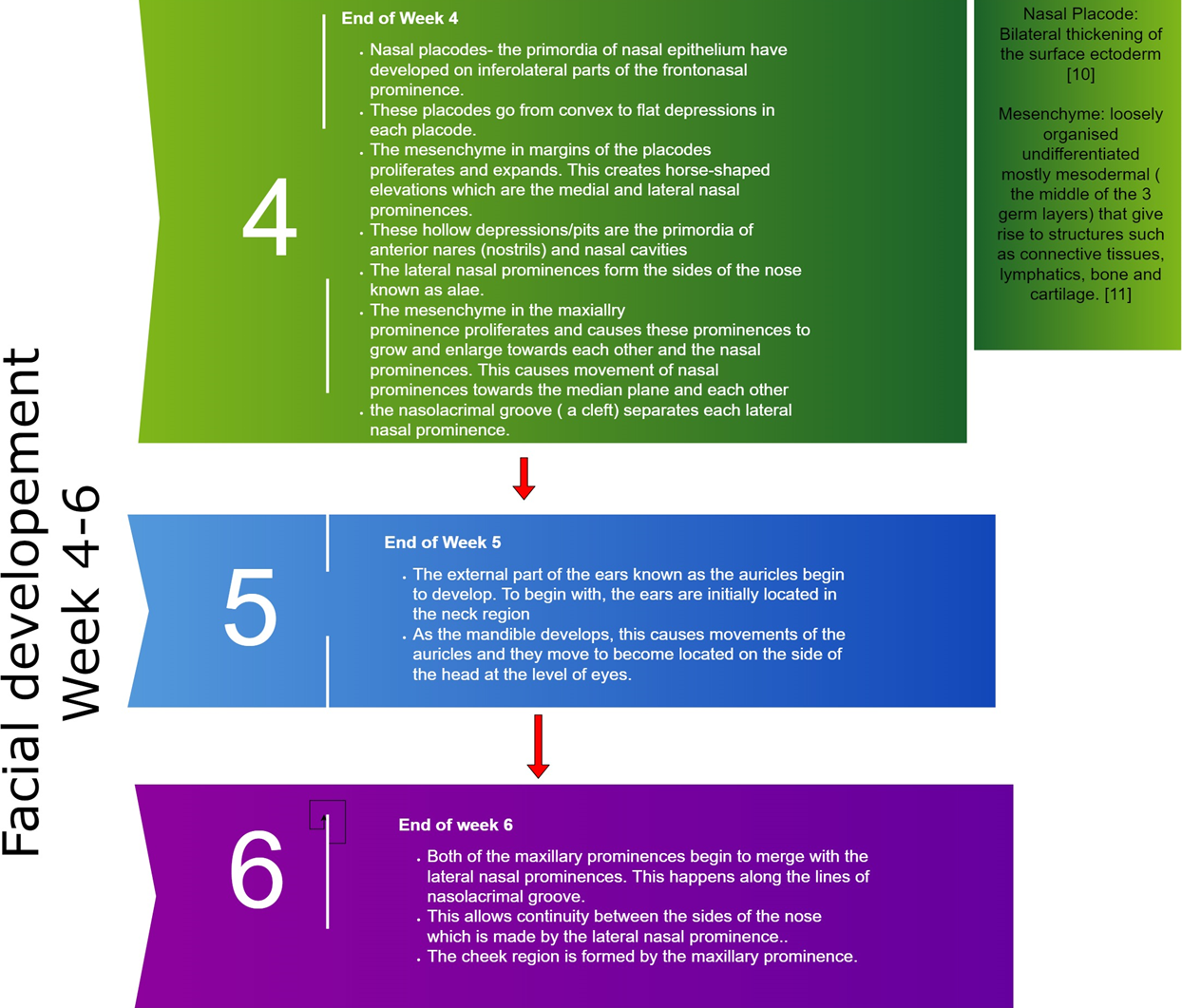
Once the anteroposterior axis of the embryo has been well established, the craniofacial development begins6. During the 4th and 6th weeks of embryonic development the external human face as we know it is formed7. The face is formed by the fusion of prominences that can be described as ‘facial swellings’ that surround the stomodeum which is an ectodermal depression8 (one of the 3 and most external primary germ layers in an embryo)9. These ‘facial swellings’ are known as facial primordia and there are 5 of them: a single frontonasal prominence followed by 2 paired prominences; a maxillary and mandibular prominence respectively.

Figure 1: Flow chart showing the formation of the face from weeks 4-6. Information adapted from [10]

The expansion of neural crest populations in the 4th week is what allows the growth of these prominences as they are the main source of connective tissue elements such as the bone, cartilage and ligaments of the face and oral region 10 . Essentially the prominences are mesenchyme (derived from the neural crest) that grow and continue to proliferate. The first parts of the face to form are the lower lip and jaw as they are the result of the unity of the medial ends of the mandibular prominences in the median plane10. Table 1 above, is a flowchart showing how the face is formed from weeks 4-6. As the face and mesenchyme expands, it pushes the prominences from a lateral aspect to a more medial one and makes the structures more continuous and begins to form the shape of the face.

Although the formation of these prominences is well known, the molecular regulation concerned with patterning and movement of the mid and upper-facial regions is not yet fully understood. The signals SHH and FGF8 are known to have a major influence in patterning this area of the face but the exact genetic interactions these signals have in regulating the movement of these prominences, are not yet known12. Thus, this could be another reason as to why this less understood area contributes to the poorly understood aetiology behind CLP.

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| *Prominence* | *Develops/forms in to:* |
| Frontonasal prominence | Forehead, dorsum and the apex of the nose |
| Lateral nasal prominence | Alae (sides of the nose) |
| Medial nasal prominence | Nasal septum, ethmoid bone and cribriform plate |
| Maxillary prominence | Upper cheek regions and the upper lip |
| Mandibular prominence | Chin, lower lip and the lower cheek region |

Table 1 shows a summary of the facial development from the difference prominences.

Table 1: Shows a summary of what the different facial prominences develop into. Information adapted from 10 (pp186)

**Palate development: Weeks 6-8**

Though the face is almost formed by the end of the 6th week, the completion of the palate formation is what marks the nearing of the end of facial development, as the basic shape of the face comes together between weeks 4 and 1013. The palate separates the developing oral and nasal cavities and the primary palate itself is made by the 2 maxillary and 2 medial nasal processes14. The median process of the primary palate begins its formation by beginning of the 6th week and once formed, the primary palate marks the anterior/midline aspect of the premaxillary part of the maxilla. This part of the palate is only a small portion of the adult hard palate as we know it10.

However, the main hard palate in adults is formed by the secondary palate. By the 7th and 8th weeks , palatine shelves are produced by the medial wall of the maxillary prominences. These palatine shelves face medially and to begin with they grow downward , alongside the lateral surface of the tongue. Eventually as the 7th week comes to an end there is a sudden change in direction of these palatine shelves as they begin to grow upward, in a horizontal direction13. This directional change is brought about by the mesenchyme of the palatine processes releasing hyaluronic acid10. Around the same time, the lower jaw drops and grows in a downward and forward direction allowing the tongue to descend to make space14 , thereby permitting the palatine shelves to join with each other. On the anterior surface, these palatine shelves fuse with the triangular shaped palate12. This is what forms the secondary palate13. Together, the secondary palate with the primary palate, form the adult hard palate14. The incisive foramen (the hole at the front of the hard palate) marks the midline between the two primary and secondary palates12.

**3. What is Cleft lip and palate (CLP) and Epidemiology?**

A facial cleft is the consequence of the complete or partial failure of fusion between any of the facial prominences discussed above. The 2 most common types of facial cleft are cleft lip and/or cleft palate. When the maxillary prominence doesn’t fuse correctly with the intermaxillary process (tissue formed by the merging of the nasal processes that become the philtrum (area between the nose and lip) of the upper lip)14, the resulting formation is a cleft lip13. If the 2 palatine processes don’t join together in the midline of the palate, then this results in cleft palate13.

CLP is one of the most prominent facial birth defects in the UK1. Cleft lip and cleft palate usually occur together but their incidence in terms of occurring together or in isolation varies- one of the factors responsible for this variation is race/geographic origin13. Globally, the figures are similar with highest risk group being Asians at 14:10,000 then Caucasians having a risk of 10:10,000 births15. Although there is much evidence in many studies that show that CLP is most common in the Asian population, closely followed by Caucasians, with the lowest incidence being amongst the African descent, it is important to point out that this data could be very outdated- up to 30 years old and underepresentative16. It is also pertinent to take into account that many of the CLP cases that happen in developing countries are in rural areas and so will not be registered. This is probably why some studies suggest the idea of developing complete and effective registries so that the records can reflect a more accurate representation of CLP globally17.

Moreover, another factor that is involved in the difference of distribution of CLP is gender. Amongst females there is a higher occurrence of cleft palate compared to males who have a higher rate of cleft lips13. This disparity of CLP occurrence between gender is of substantial importance as the figures are quite high; cleft lip is higher in males by 65% whereas cleft palate is higher in females by 55%12. Out of infants affected with CLP, a huge 60%-70% are male10. Furthermore, a clinical statistical study carried out on Japanese patients with CLP found that the levels of CLP severity was also wide-ranging due to gender patterns. The study concluded that patients of the male gender only had “milder cleft lip, cleft lip and palate or cleft palate, whereas the percentage of females tended to be greater as cleft severity increased”18. Although this study is relatively recent and so could be deemed reliable, the method of investigation used- chi-squared test still has its limitations. The method of chi-squared testing is good for the large sample used in this case, but it is also important to consider that this type of investigation doesn’t do anything other than show the existence of a relationship between the two factors (in this case the relation of gender and CLP) and thus it doesn’t add any strength to the relationship. It simply identifies a positive correlation/link. Additionally, despite the sample size of 782 patients18 being quite large it could be argued that since the incidence of CLP in Japan is higher at 1 in 500 babies being affected19, that this sample size might be too small and thus doesn’t produce an accurate result. However, from the above studies it is permissible to assume that there is in fact a relationship between gender and the different types of CLP the different genders suffer from as well as the difference in prevalence amongst the 2 genders.

**3.1 Types of CLP**

Since CLP can either be cleft lip or palate alone or cleft lip and cleft palate together, there are many different types. Firstly, cleft lip and palate can be organised into to 2 main groups; anterior cleft anomalies and posterior cleft anomalies. When there is a lack of mesenchyme in the maxillary prominences as well as in the median palatine processes, there is the formation of an anterior cleft anomaly. These can include a cleft lip with or without the alveolar section of the maxilla- this section is where holes are present where the teeth normally lie. If the cleft separates both the front and back of the parts of the palate and goes from the lip and alveolar part of the maxilla to the incisive foramen then this is described as a complete anterior cleft anomaly. A posterior cleft anomaly is similar to the anterior one in that it also separates the anterior and posterior parts of the palate. However, the differentiating factor between the two is that the posterior cleft anomaly includes the clefts of the secondary palate which extend through both the soft and hard parts of the palate, again to the incisive foramen. It is thought that these anomalies are a consequence of the defecting development of the secondary palate resulting in the abnormality in the growth of the lateral palatine processes. This results in the inability of palatine shelves to fuse and thus results in an incomplete fusion and cleft. The width of the stomodeum, the ability of the lateral palatine processes to move and changed degeneration sites on the palatal epithelium are other aspects thought to influence and contribute to these abnormalities10.

Figure 3 explains some of the most common types of cleft lip and the anatomical defects that have caused them.

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| Type of cleft lip | Anatomical defect |
| Unilateral cleft lip | The mesenchyme does not grow and smooth out the epithelium on top🡪results in the failure of fusion between the maxillary prominence on affected part to join with the already merged nasal prominences. This forms a labial groove. The lip is also split into medial and lateral parts due to the epithelium of the labial groove being overextended and the floor of the groove to degenerate. Simonart band- a bridge of tissue sometimes joins the unilateral cleft lip that has failed to reach completion. |
| Bilateral cleft lip | The mesenchymal masses in both maxillary prominences don’t meet to be able to fuse with the merged medial nasal prominences🡪 both of the labial groove’s epithelium becomes overextended and breaks down.  Complete bilateral cleft of the lip and alveolar part of the maxillary occurs when the median palatal process faces anteriorly and hangs free. The orbicularis oris muscle controls the pursing of the lips and the opening of the mouth. Damage to the continuity of this muscle makes this abnormality especially deforming. |
| Median cleft lip | The medial nasal prominences completely or partially fail to fuse together to form the median palatal process due to a mesenchymal deficit.  The incomplete process of mesenchymal masses in the mandibular prominences to fuse together and smooth out the embryonic cleft between them, results in a median cleft of the lower lip. |

Table 2: shows the most common types of clefts and the anatomical defects that have caused them. Information adapted from [10]

1 in 2500 infants have cleft palates and as previously mentioned it is more common in females. Here the cleft can cross the soft and hard palate or be a cleft uvula which has a fishtail appearance. When cleft palates are associated with cleft lips- as most people know them as, the cleft pierces through the alveolar part of the maxilla and the lips bilaterally- this happens in severe cases. The differential mark that distinguishes between an anterior and posterior cleft is the incisive foramen and when a cleft passes through the soft palate and is anterior to the incisive foramen, it is known as a complete cleft of the posterior palate10.

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| --- | --- |
| Type of cleft palate | Anatomical defect |
| Clefts of the primary or anterior palate | These are clefts in front of the incisive foramen. The clefts form due to the mesenchymal masses in the lateral palatal processes not joining and fusing with the primary palates’ mesenchyme. |
| Clefts of the secondary or posterior palate | These are clefts behind the incisive foramen. The clefts form due to the mesenchymal masses in the lateral palatal process not joining to fuse with each other and the nasal septum. |
| Clefts of the primary and secondary parts of the palate | These are clefts of the anterior and posterior palates. These occur when the mesenchymal masses in the lateral palatine processes don’t join and fuse with the mesenchyme in the primary plate, each other or the nasal septum. |

3 groups categorise the unilateral and bilateral clefts of the palate and table 3 summaries them by showing the type of cleft and the anatomical defect associated with it10.

Table 3 shows the 3 categories of unilateral and bilateral clefts and the anatomical defects associated with them. Information adapted from [10]

**3.2 Causes of CLP**

**3.2 (1) Syndromic and Non-syndromic CLP**

Depending on whether a patient suffers from syndromic or non-syndromic cleft lip or palate (NSCLP), the aetiology of CLP varies accordingly. The most common cause for CLP is NSCLP as it accounts for 70% of all the cases; with NSCLP accounting for such a large proportion of cases it is no surprise that NSCLP is the best studied20. NSCLP can be described as a disease resulting from the interaction of genes and the environment21 and because of this NSCLP has a multifactorial aeitiolgy22. Alternatively, syndromic forms of CLP are usually the result of chromosomal abnormalities or monogenic diseases and thus have a strong genetic link21 accounting for the remaining 30% of infants born with congenital deformities in a syndromic form20. CLP here is usually present as part of a syndrome.

There are have been many genes that have been identified within syndromes that also cause CLP as an abnormality. As such, Van der Woude syndrome (VWS) prevails as one of the commonest forms of syndromic CLP held accountable for about 2% of all cases of CLP23. Clinically, the differentiating factor between VWS and CLP is that patients with VWS have pits present in their lower lips which is not a characteristic of CLP24. However, since VWS accounts for a substantial amount of CLP cases, its causative genetic findings are very relevant to the syndromic causes of CLP. Recent studies found that mutations in the IRF6 (interferon regulatory factor 6) gene is the cause of VWS – a transcription factor which is expressed in the palatal shelf epithelium and on the border of where the fusion of the palatine shelves takes place13. A mutation in this gene would then affect the process of palate formation. Furthermore, another study has found that there is a direct 12% increase in risk of developing all common forms of CLP if there is a mutation in a well-known haplotype which is linked to the gene IRF624. Trisomy 13/Patau syndrome, Edwards syndrome and DiGeorge syndrome are a few other syndromic causes of CLP25.

As previously mentioned, non-syndromic causes of CLP account for the majority of CLP causes and are a result of the interaction between the gene and environment21. Table 4 below demonstrates the different causes of NSCLP.

Table 4- Shows the reported causes of NSCLP. Information adapted from [20,26,27]

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| --- | --- |
| Genetics | * IRF6 * Ch8q24 * VAX1 * FGFR2 * BMP4 |
| Maternal risk factors | * Smoking * Alcoholism * Pregestational diabetes * Gestational diabetes * Age>40 years * Folate deficiency * Zinc deficiency |
| Teratogens | * Valproic acid * Phenytoin * Retinoic acid * Chemical solvents * Pesticides * Occupation related: leather, shoemaking, healthcare |

One of the causes illustrated in the table above is highlighted as genetics (mutations in these genes would lead to the disease). Apart from the genes listed in the table which are thought to have a causative role behind CLP, studies have also suggested variants of the gene for growth factor TGFA (transforming growth factor alpha) increasing the risk of CLP28. TGFA is protein that is secreted and thus binds to the epidermal growth factor (EGFR) which is conveniently located on the palate epithelium when the palate closes29. Studies also propose that epidermal growth factor (EGF) and TGFA along in conjunction with glucocorticoids allows the epithelial cells of the palate to multiply and differentiate30. With such a vital role in palate formation it is easy to see the biological reasoning behind suggesting mutations in the TFGA gene being seen as a relative risk in developing CLP. In fact, a meta-analysis carried out between the relationship between a polymorphism of TGFA Taq 1 and CLP concludes that there is indeed a possibility that TGFA Taq 1 polymorphism is a risk associated with CLP31. With so much evidence favouring the link between CLP and TGF, it is only fair to consider other studies that claim the contrary. Several studies admit that the TFGA gene has been explored and considered well but have concluded that association between this gene and CLP is insufficient as the evidence is varying. Opposing opinions express there has never been a study conducted on whether a TFGA gene mutation affects the relative risk of CLP via a parent-of-origin effect32. Nevertheless, it is vital to consider the validity and reliability of a meta-analysis as it combines so many studies, effectively increasing the sample size and adding to the power of the study. Therefore, the proposed association of TGFA polymorphisms increasing the risk of CLP can be seen as true.

Aside from the environmental factors listed in the table as NSCLP aetiologies such as teratogens and maternal risk factors, ironically the gene IRF6 listed as a syndromic cause is also a cause for NSCLP- it has the strongest assoctaion21. Mutations in this gene is what makes it a causative agent behind CLP. Dissimilarities in the IRF6 gene are responsible for 12% of the genetic input towards CLP33. This again signifies the importance of the IRF6 gene in both syndromic and non-syndromic causes of CLP as well as being responsible for VWS.

Additionally, it is also important to mention the genes and environmental interactions that lead to the pathogenesis of CLP and so below table 5 shows the possibility for some of the genes and environmental agents interacting. Again, it is important to stress that these are just speculated possibilities of how some of the genes and environmental factors interact as well as mentioning that this is only valid for some of the environmental agents21. Despite many studies being carried on the aetiologies behind syndromic and non-syndromic CLP, the underlying variants that are the source behind the disease are still relatively unknown6.

Table 5: shows how some environmental risk factors could interact with genes and cause mutations . Adapted from [21]

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| --- | --- |
| TGFA | Smoke, vitamin deficiency |
| TGFB3 | Smoke, alcohol |
| MSX1 | Smoke, alcohol |
| ADH1C | alcohol |
| EPHX1, GSTM1, GSTT1, NAT1, NAT2, CYP1A1 | Smoke, drug abuse, occupational exposure |
| RARA | Vitamin A intake |
| MTHFR, RFC1 | Folic acid intake |

The discovery of how specific genetic changes in certain genes leads to craniofacial defects is only based on relatively new data. Thus, it is pertinent to signify that despite the presence of a genetic factor being found in both syndromic and non-syndromic CLP, this information has only been recently discovered and the relatively new genes involved have only been identified in a small number of cases21. Thus the cause of CLP is still very unclear.

**3.2 (2) SHH and CLP**

Interference in the signalling pathway of Shh and genes associated with the expression of Shh is another proposed cause behind cleft palate. The protein sonic hedgehog (Shh) is a secreted substance that is present and has effects on different embryonic tissues12; its part of the Hedgehog pathway which is significant as it “provides inductive signals critical for developmental patterning of the brain and face”34. Studies suggest that Shh has many roles directly involved early on with the development of the face such as ensuring the midline of the neural plate is patterned well35. Furthermore, it is proposed that in order for some neural crest cells to survive and then go on to differentiate, they need to rely on the hedgehog signal6. Elsewhere, research illustrates that to bring about the expression of hedgehog signalling in the face- to allow the growth of the frontonasal prominence, Shh expression in the neuroectoderm is vital- this was shown in chicks36. The relation of Shh and facial formation is very clear and so a mutation in Shh expression could be a cause of CLP. Although these studies show how important Shh is for the development of the face, the most striking factor to point out is that most of these studies are based on results from avian embryos, mice and as last-mentioned chicks- not humans.

A lot of the research conducted in the pursuit of understanding the pathogenesis behind cleft palate has been based on an animal which is a favourite in also understanding how the initial normal palatal development occurs- a mouse. Obviously, humans and mice are different so it could be deemed unreliable to use research in such studies done for mice to reflect the relationship between Shh and CLP and relate it back to humans since there are anatomical differences present. Further evidence making the results from mice studies slightly more redundant is that during the palate closure in mice and humans, the palatal shelves behave differently in relation to the primary palate and nasal septum in the hard palate in both37. Regardless of this, animal studies have enabled insightful knowledge to be gained that has later been translated to humans, especially since the developmental process amongst the mammalian species is so similar 37. Therefore, it is important to not completely disregard all the findings from such studies as it does aid research allowing better understanding of the pathogenesis of disease.

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**4. Conclusion**

As mentioned earlier, the exact causes behind why infants suffer from CLP is unclear. Despite there being evidence for a genetic basis (since a family history of CLP increases the changes of another child being born with CLP), environmental factors such as a lack of folic acid, intake of valproic acid and steroid medication, smoking etc the cause of disease cannot be pinpointed. Research based on animals such as avians and have mice have enabled the progression into understanding the normal morphogenesis of facial formation as well as knowing more about CLP pathogenesis. For example, the study of mice has allowed the recognition of the teratogen Cylcopamine inhibiting the hedgehog pathway resulting in lateral CLP in mice and this has now been linked to embryonic defects in the midline and lower medial nasal prominences34. However, to continue future research, recognition of the morphological differences between us humans and for example mice is important to be able to translate and apply more of the findings to human disease pathogenesis. Eventually once these differences are established, we can continue to use such studies to further our understanding of palate formations and disease pathogenesis of CLP in humans37.

To conclude, while we have grasped the process of facial formation there is still a lot to be learnt behind the mechanism of control of cell patterning in facial and palate formation. Knowing what signals, transcription and growth factors etc are involved in driving the process of facial morphogenesis will then help us identify which signalling pathways go wrong and result in dysmorphogenesis. Obtaining knowledge of specific signals that control different parts of embryonic tissue patterning how genes interact as part of this process, will allow us to identify perhaps the genes that are responsible for these signals and thus help us identify specific gene mutations that could result in CLP. Following on from this, recent progression in discovering the genetics behind CLP has allowed us to identify some specific genes involved in the pathogenesis of CLP. If such research continues, with the aid of animal models, then this could allow us to have better protective and preventive measures in place for expecting mothers. As a future direction, with help of new research the ability of being able to recognise genes that put mothers at risk could be greatly beneficial as this could help them avoid potential environmental factors/agents that could interact with the genes and result in their infants suffering from CLP. Furthermore, identifying genes such as IRF6 in mothers pre pregnancy could be helpful by allowing them genetic counselling and implement protective measures again like taking vitamin supplements21. As we can see, even this new but limited information can help us take preventive measures for at risk mothers which is why more research into the genetics behind CLP in both syndromic and non-syndromic forms is needed. Understanding the mechanism of control behind facial morphogenesis with further research using animal models is also crucial so that together with identifying genotypes that are a risk factor for CLP, we can understand the aetiology and pathogenesis of CLP more accurately. To deal with craniofacial defects, identifying and treating defective genes is at present is a vital part of the clinical agenda38 and hopefully in the future this will help with the management of CLP.

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