

CASE REPORT

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Exome Sequencing Reveals Cause of Hypomyelinating Leukodystrophy

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Abstract: Here we present a case in which exome sequencing rapidly produced a molecular diagnosis for a patient with hypomyelinating leukodystrophy, for whom years of costly conventional testing had not identified a molecular diagnosis. The patient had severe developmental delay, ataxia, nystagmus, external ophthalmoplegia, hypometric saccades, and high myopia. Magnetic resonance imaging of the brain demonstrated severe failure of myelination of the corona radiata and centrum semi ovale. Through exome sequencing, two novel heterozygous missense variants were identified in the *POLR3A* gene (c.1160C > G; p.Ala387Gly and c.3781G > A; p.Glu1261 Lys). We propose the use of exome sequencing for the diagnosis of genetically heterogeneous conditions such as hypomyelinating leukodystrophy as a cost-effective primary diagnostic tool.

Keywords: hypomyelination, leukodystrophy, exome, next-generation sequencing, *POLR3A*, clinical diagnostics

Journal of Genomes and Exomes 2012:1 7–14

doi: [10.4137/JGE.S10127](https://doi.org/10.4137/JGE.S10127)

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Introduction

Hypomyelinating leukodystrophies are a heterogeneous group of neurodegenerative disorders that feature abnormal myelination of the central nervous system. The classic hypomyelinating leukodystrophy is Pelizaeus-Merzbacher disease (PMD, MIM # 312080), characterized by nystagmus, dystonia, cerebellar ataxia, spastic paraplegia, and psychomotor developmental delay. PMD is X-linked and is caused by mutations in or duplications of the proteolipid protein 1 (*PLP1*, MIM # 300401) gene. When strict clinical criteria are used for diagnosing PMD, ~25% have point mutations of *PLP1*, 60%–70% have duplications, a very small percentage have complete deletion or triplication, and remaining cases are undiagnosed at the molecular level.^{1,2} A significant percentage of patients with overlapping clinical features of PMD do not have detectable *PLP1* mutations or gene duplications and have been historically referred to as having Pelizaeus-Merzbacher-like disease (PMLD).² Recently, the molecular cause of many of these patients has been elucidated. Mutations in *GJC2/GJA12* have been identified as the cause of approximately 8% of autosomal recessive PMLD cases.³ PMLD is also thought to be caused by mutations in genes that affect *PLP1* transcription. Two known regulators of *PLP1* are the homeodomain proteins *Nkx2.2* and *SOX10*. However, to date, only one mutation in *SOX10* has been described in a patient with features of PMD.⁴ Furthermore, genes associated with other leukodystrophies have recently been identified but cannot explain all PMLD cases. For example, *LMNB1* duplications have been shown to be a cause of adult-onset autosomal dominant leukodystrophy,⁵ MCT8 mutations were found in 11% of hypomyelinating leukodystrophies of unknown etiology in one study⁶ and mutations in the polymerase (RNA) 3 (DNA directed) polypeptide A and B (*POLR3A* and *POLR3B*) have also been linked to rare hypomyelinating leukodystrophies.^{7,8,26} It is estimated that the molecular basis of hypomyelinating leukodystrophies remains unidentified in 30% of patients.⁹

The American College of Medical Genetics and Genomics recently released a policy statement regarding at what point whole exome and genome sequencing should be considered in the clinical diagnostic assessment of a phenotypically affected

individual. One indication for whole exome or genome sequencing is in the diagnosis of genetic disorders with a high degree of genetic heterogeneity.¹⁰ The hypomyelinating leukodystrophies fit in this category. Additional indications include situations where the patient is suspected to have a genetic disorder for which no testing is clinically available or a patient presents with a likely genetic disorder but genetic testing available for that phenotype has failed to arrive at a diagnosis.¹⁰

Case Report

Medical history

A male patient was delivered by caesarean section for cephalo-pelvic disproportion at term following a pregnancy complicated by preeclampsia. His birth weight was eight pounds and he had an Apgar score of 9 at one minute. He was discharged from the nursery at four days of life. The first of multiple hospital admissions for dehydration associated with infections, such as pneumonia, was at six months of age. While delayed early development was noted, he walked at one year. Past surgical history includes orchidopexy and herniorrhaphy. He was evaluated by a speech pathologist for dysarthria. At age 12, he was evaluated by a pediatric neurologist and ophthalmologist for development concerns. Magnetic resonance neuroimaging (MRI) demonstrated severe failure of myelination of the corona radiata and centrum semi ovale. Eye examination revealed mild external ophthalmoplegia, hypometric saccades, and high myopia. A follow up examination at age 18 by a pediatric neurologist noted an ataxic gait, particularly on attempting to walk in tandem, backwards and forwards, as well as unsteadiness when turning rapidly. Hearing was normal. Extraocular movements were normal, but a tendency to thrust his head in the direction of outer gaze was noticed. Fundoscopy was normal. Deep tendon reflexes were difficult to obtain in the arms, and were 3+ in the legs. Symmetric and plantar responses were equivocal. Mild impairment in fine motor tasks was noted. A repeat MRI scan was similar to his previous evaluation, consistent with a disorder of myelination of the brain, particularly affecting the supratentorial structures.

Negative genetic testing

Genetic testing for duplication of the proteolipid protein 1 (*PLP1*, MIM # 300401) associated with



Pelizaeus-Merzbacher disease (MIM # 312080) and lamin B1 (*LMNB1*, MIM # 150340), and also associated with adult-onset autosomal-dominant leukodystrophy (MIM # 169500) were negative. Additionally, sequence analysis of *PLP1*, gap junction protein gamma-2 (*GJC2*, MIM # 608803; hypomyelinating leukodystrophy 2, MIM # 608804), family with sequence similarity 126 member A (*FAM126A*, MIM # 610531; hypomyelinating leukodystrophy 5, MIM # 610532), early growth response 2 (*EGR2*, MIM # 129010; congenital hypomyelinating neuropathy 1, MIM # 605253), and SRY-box 10 (*SOX10*, MIM # 602229; peripheral demyelinating neuropathy, central dysmyelination, MIM # 609136) were all negative (Table 1).

Results

A total of 124,493 variants were found, of which 2,743 were given a classification of 1–3 by the Center for Pediatric Genomic Medicine's (CPGM) Rapid Understanding of Nucleotide variant Effect Software (RUNES v1.0) (Supplement Table 1). Eleven known disease causing variants with an allele frequency of 1% or less were found, but were not noted in genes associated with a phenotype consistent with this patient's presentation. Thirty-nine genes had homozygous variants, and 43 genes had 2 or more heterozygous variants consistent with an autosomal recessive inheritance pattern. Of these genes, five genes had both a homozygous variant and two or more heterozygous variants, leaving a total of 77 candidate genes. Analysis of these genes found only one gene relevant to the clinical history, the polymerase (RNA) 3 (DNA directed) polypeptide A (*POLR3A*, MIM # 614258). The patient was found to be heterozygous for two novel missense variants in the *POLR3A*, gene, c.1160C > G (p.Ala387Gly) found in 28/49 (57%) reads and c.3781G > A (p.Glu1261Lys)

found in 20/43 (47%) reads (Table 2, Supplemental Figs. 1 and 2). Both variants were confirmed by Sanger sequencing (Supplemental Figs. 3 and 4). Two *in silico* tools, SIFT and PolyPhen2, predict these variants to be deleterious and probably damaging, respectively (Table 2). Neither variant has been seen in 527 patients sequenced at Children's Mercy or in the Washington University Exome Variant Server, which at the time of analysis contained sequence data from 10,757 alleles.¹¹

Loss of function mutations in *POLR3A* have been reported to cause hypomyelinating leukodystrophy-7,^{7,8,26} (MIM # 607694), an autosomal recessive neurodegenerative disorder characterized by the onset in childhood of progressive motor decline that manifests as spasticity, ataxia, tremor, and cerebellar signs, as well as mild cognitive regression. Other features may include hypodontia or oligodontia and hypogonadotropic hypogonadism; however, the authors report that there is considerable inter- and intrafamilial variability.⁷ We detected two novel, rare variants (c.1160C > G, c.3781G > A) in highly conserved regions of *POLR3A* in a patient with clinical features consistent with previously reported cases of hypomyelinating leukodystrophy-7. Although both variants are novel, they are predicted to be damaging and deleterious and are found in regions conserved through *Xenopus*. Taken together, the clinical presentation and genotype data are consistent with a diagnosis of hypomyelinating leukodystrophy-7.

Discussion

Genomic medicine, empowered by whole exome and whole genome sequencing, has been widely heralded as transformational for medical practice.^{12–15} Herein we present a case where exome sequencing was able to rapidly produce a molecular diagnosis for a patient

Table 1. Results of previous clinical testing.

Gene	MIM #	Disease	MIM #	Result
PLP1-dup	300401	Pelizaeus-Merzbacher disease	312080	Neg
LMNB1-dup	150340	Autosomal dominant leukodystrophy	169500	Neg
PLP1	300401	Pelizaeus-Merzbacher disease	312080	Neg
GJC2	608803	Hypomyelinating leukodystrophy 2	608804	Neg
FAM126A	610531	Hypomyelinating leukodystrophy 5	610532	Neg
EGR2	129010	Congenital hypomyelinating neuropathy 1	609136	Neg
SOX10	602229	Peripheral demyelinating neuropathy, central dysmyelination, Waardenburg syndrome, and hirschsprung disease (PCWH)	609136	Neg

**Table 2.** Clinically relevant variants identified by exome sequencing.

Variant	Gene	cDNA	Protein	Reads	SIFT	PolyPhen2
1	POLR3A	c.1160C > G	p.Ala387Gly	28/49 (57%)	Deleterious (0.0)	Probably damaging (1.0)
2	POLR3A	c.3781G > A	p.Glu1261Lys	20/43 (47%)	Deleterious (0.0)	Probably damaging (0.999)

presenting with a hypomyelinating leukodystrophy in which years of costly conventional testing had not disclosed a molecular diagnosis. Even though in this case we found variants within a gene recently linked to hypomyelinating leukodystrophy, an additional benefit of whole exome sequencing is the ability to discover novel disease causing genes. Indeed, for heterogenous diseases, such as the hypomyelinating leukodystrophies, for which approximately 30% of patients never receive definitive molecular diagnoses, exome sequencing is particularly powerful.

As more cases of patients with POLR3A mutations are reported in the literature it will be important to see if genotype-phenotype correlations can be elucidated. Clearly hypomyelinating leukodystrophy-7 has a heterogeneous clinical presentation as patients both with and without oligodontia and hypogonadotropic hypogonadism have been reported.^{7,8,26} Furthermore, it is still unclear if nonsense mutations cause more severe disease or earlier onset. Lastly, it will be crucial to determine if there are any correlations between mutation and prognosis, which ultimately may direct treatment choices.

Methods

Targeted exome sequencing

The CPGM at Children's Mercy Hospital performed exome sequencing on a research basis. Isolated genomic DNA was prepared for sequencing using the Kapa Biosystems library preparation kit and eight cycles of PCR (polymerase chain reaction) amplification. Exome enrichment was conducted with the Illumina TruSeq Exome v1 kit (62.2 megabase) following a slightly modified version of the manufacturer's recommended protocol. The enrichment protocol was modified to

Table 3. Statistics of exome sequencing.

Total bp sequenced	12,639,330,284
Total bp aligned	12,142,401,437
Mean target coverage	104.16
Median target coverage	90
Target with 0X coverage	1.86%
Target with 16X coverage	92.30%

use the Kapa Biosystems PCR amplification kit for the post-enrichment amplification step to limit polymerase induced GC-bias.¹⁶ Successful enrichment was verified by qPCR of four targeted loci and two non targeted loci of the sequencing library pre- and post-enrichment prior to sequencing.¹⁷ The enriched library was sequenced on an Illumina HiSeq 2000 using v3 reagents and 1 × 101 bp sequencing reads.

Sequence analysis

Sequence data was generated with Illumina RTA 1.12.4.2 and CASAVA-1.8.2, aligned to the human reference NCBI 37 using GSNAP, and variants were detected and genotyped using GATK. Sequence analysis employed FASTQ files, the compressed binary version of the Sequence Alignment/Map format (bam, a representation of nucleotide sequence alignments) and Variant Call Format (VCF, a format for nucleotide variants). A total of 12.6 gigabasepairs (Gbp) of sequence was generated, of which 96.1% was aligned. 94.7% of aligned reads had a mapping quality (MapQ) of 2 or greater, which resulted in a mean and median target coverage of 104X and 90X, respectively. 53.43% of nucleotides were on target, with the target defined with a 0 bp extension (Table 1). Variants were characterized with the CPGM's Rapid Understanding of Nucleotide variant Effect Software (RUNES v1.0).¹⁸ RUNES incorporates data from the Variant Effect Predictor (VEP) software,¹⁹ and produces comparisons to NCBI dbSNP, known disease mutations from the Human Gene Mutation Database,²⁰ and performs additional *in silico* prediction of variant consequences using ENSEMBL and UCSC gene annotations^{21,22} (Fig. 2). RUNES categorizes each variant according to the American College of Medical Genetics (ACMG's) recommendations for reporting sequence variation,²³⁻²⁵ as well as reporting an allele frequency derived from CPGM's Variant Warehouse database.¹⁸

Author Contributions

Conceived and designed the experiments: DLD. Analyzed the data: DLD, NAM, CJS, and SES. Wrote



the first draft of the manuscript: DLD. Contributed to the writing of the manuscript: CJS, SES, GMH, EGF and SFK. Agree with manuscript results and conclusions: DLD, NAM, CJS, SES, GMH, EGF and SFK. Made critical revisions and approved final version: DLD, NAM, CJS, SES, GMH, and SFK. All authors reviewed and approved of the final manuscript.

Funding

This work was funded by the Marion Merrell Dow Foundation and Children's Mercy Hospital.

Competing Interests

NAM is a stock holder in Illumina whose sequencing technology was used in the study. Other authors disclose no potential conflict of interest.

Disclosures and Ethics

As a requirement of publication author(s) have provided to the publisher signed confirmation of compliance with legal and ethical obligations including but not limited to the following: authorship and contributorship, conflicts of interest, privacy and confidentiality and (where applicable) protection of human and animal research subjects. The authors have read and confirmed their agreement with the ICMJE authorship and conflict of interest criteria. The authors have also confirmed that this article is unique and not under consideration or published in any other publication, and that they have permission from rights holders to reproduce any copyrighted material. Any disclosures are made in this section. The external blind peer reviewers report no conflicts of interest.

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Supplementary Data

Table S1.

Total variants	Cat. 1–3	MAF < 0.01	Homozygous	Comp. het	Clinically relevant
124,493	2,743	759	39	43	1 (POLR3A)

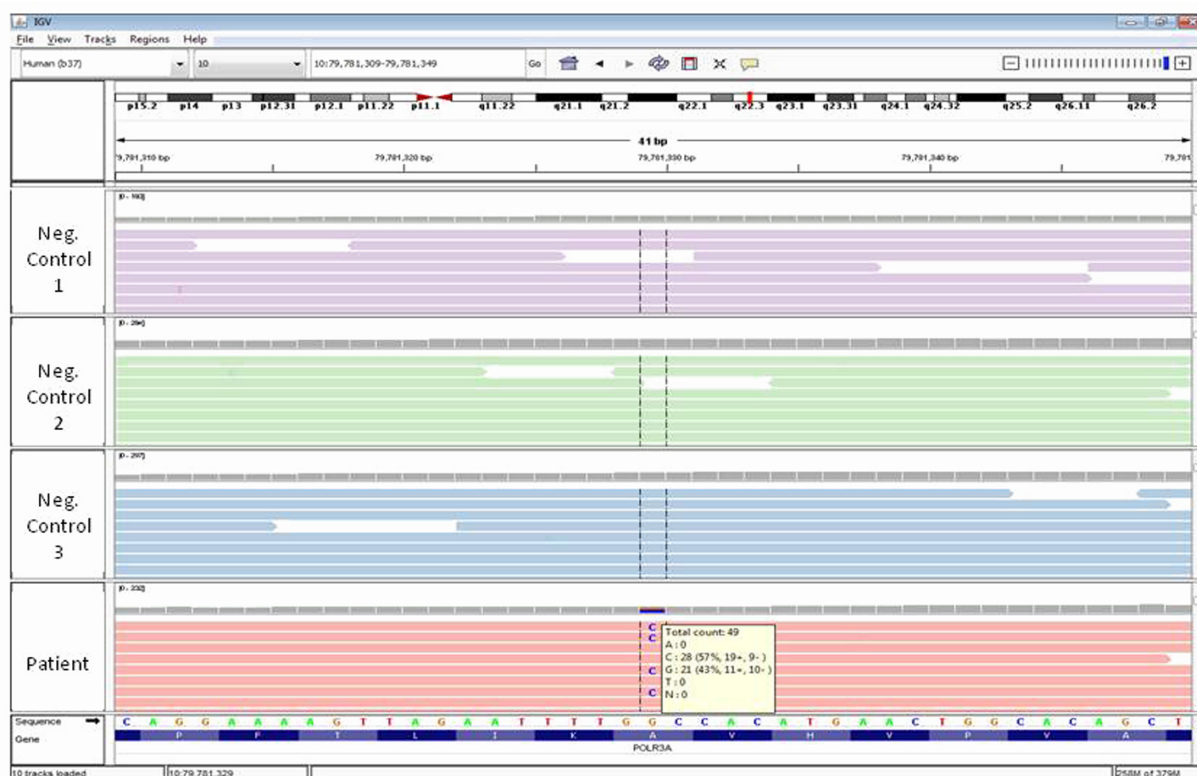


Figure S1. Variant POLR3A c.1160 C > G (p.Ala387Gly) shown in integrative genomics viewer with 3 unrelated controls.

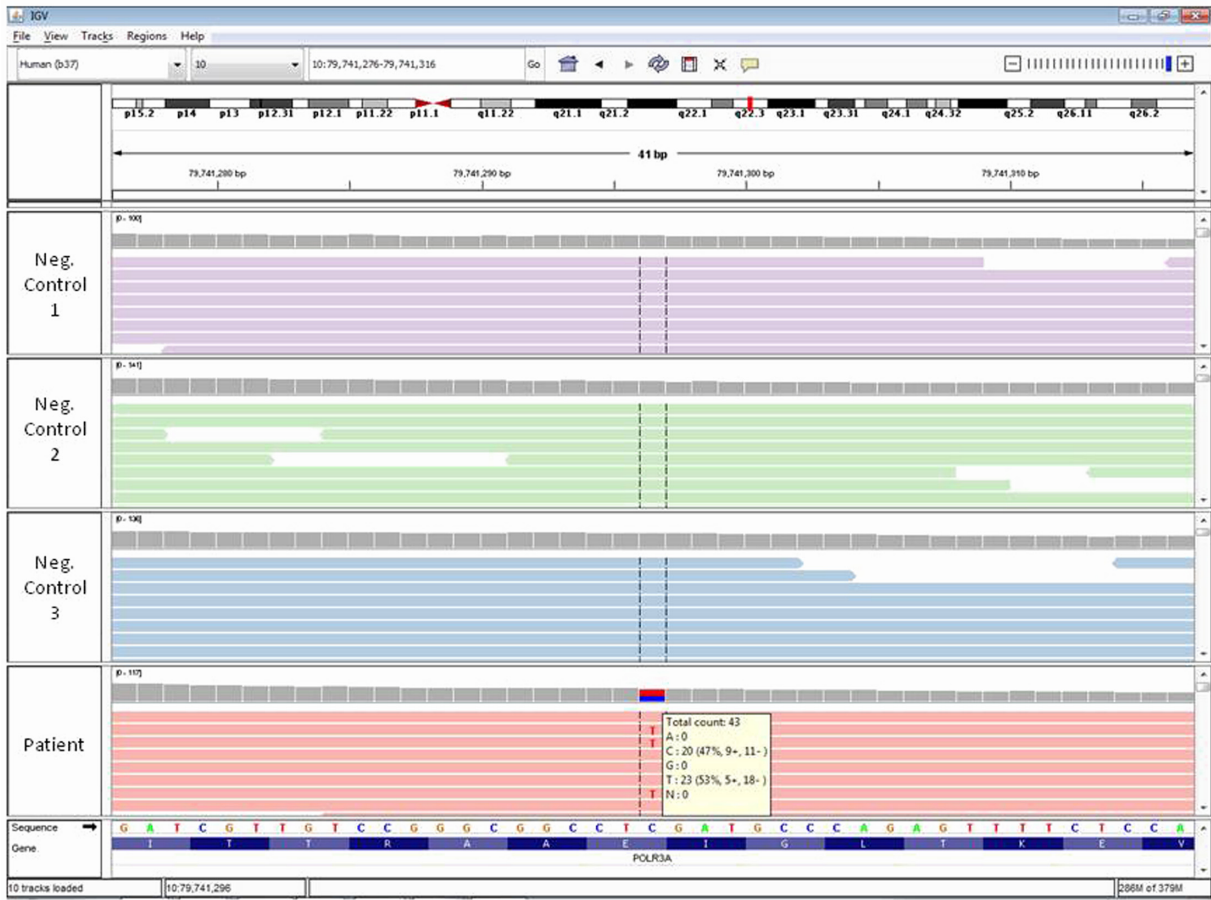


Figure S2. Variant POLR3A c.3781 G > A (p.Glu1261Lys) shown in integrative genomics viewer with 3 unrelated controls.

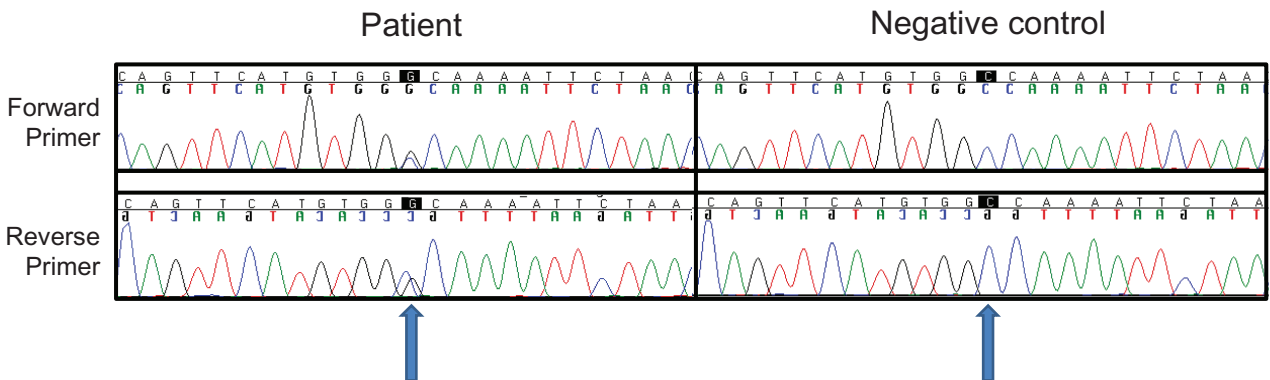


Figure S3. Sanger sequencing conformation of variant POLR3A c.1160 C > G (p.Ala387Gly).

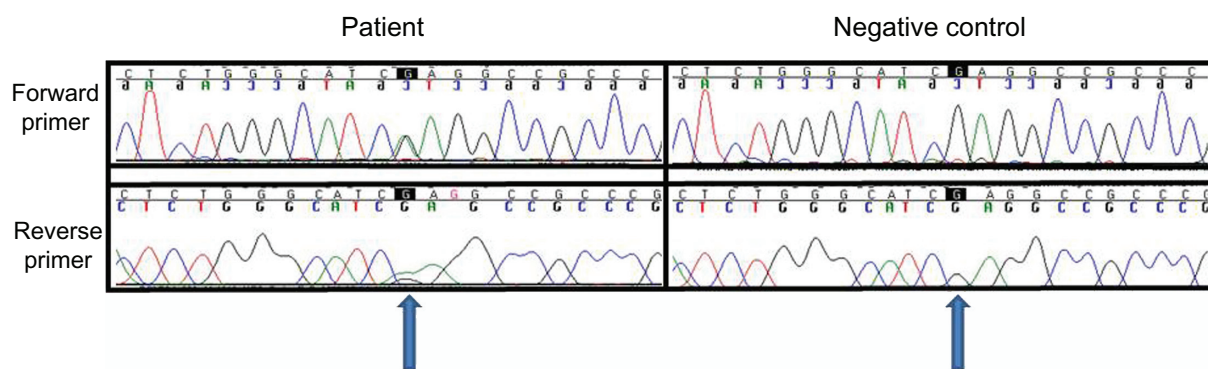


Figure S4. Sanger sequencing conformation of variant POLR3A c.3781 G > A (p.Glu1261Lys).