Clinical Case Report

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Lethal neonatal mitochondrial phenotype caused by a novel polymerase subunit gamma mutation

A case report

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Abstract

Rationale: Polymerase subunit gamma (POLG) is a gene that codes for the catalytic subunit of the mitochondrial DNA polymerase, which is involved in the replication of mitochondrial DNA. Mutations in these genes are associated with a range of clinical syndromes characterized by secondary mtDNA defect including mtDNA mutation and mtDNA depletion which may culminate in complete failure of energy production (respiratory changes complex 1 defect) as in this case.

Patient concerns: We herein report a full term Saudi female neonate born to consanguineous parents, who was noticed immediately after birth to have severe hypotonia, poor respiratory effort, and dysmorphic features. She had 3 siblings who died with same clinical scenario in neonatal period.

Diagnoses: Molecular genetic testing revealed a novel compound heterozygous mutation of POLG gene c.680G>A (p.Arg227Gin) and c.3098C>T (p.Ala1033Val).

Interventions: The patient remained in neonatal intensive care unit with multidisciplinary team management and was ventilator dependent until she passed away.

Outcomes: The detected mutation had led to complete failure of energy production (respiratory changes complex 1 defect) until she died at the age of 5 months.

Lessons: Mitochondrial respiratory chain defect should be considered in patients with severe neonatal hypotonia,encephalopathy, and respiratory failure especially in highly consanguineous population.

Abbreviations: MDS = mitochondrial DNA depletion syndromes, MNGIE = mitochondrial neurogastrointestinal encephalopathy disease, mtDNA = mitochondrial DNA, NICU = neonatal intensive care unit, POLG = polymerase subunit gamma.

Keywords: mitochondrial, neonatal, novel, polymerase subunit gamma mutation, Saudi

1. Introduction

Mitochondrial diseases are chronic, genetically determined disorders caused by dysfunction of mitochondrial DNA (mtDNA) either due to deletion or replication.[1]

9pt?>Isolated complex I deficiency is the most commonly identified biochemical defect in mitochondrial oxidative-phosphorylation disorders,[2] but it is probably under-diagnosed, since both lactate levels and muscle morphology may be normal[3,4] and the clinical features are extremely variable. The most common clinical phenotype in childhood is probably Leigh syndrome (LS, MIM 25600).[5,6] Other common phenotypes include Alpers syndrome.[2–4,6]

The mitochondrial DNA depletion syndromes (MDS) are autosomal recessive disorders with genetic and clinical problems due to defect in the proteins involved in mtDNA replication resulting in decrease in the amount of mtDNA in the tissues and organs.[7,8]

The nuclear-encoded mtDNA polymerase gamma (POLGI) gene provides instructions for making the active piece, called the alpha subunit, of a protein called polymerase gamma .Polγ is the only DNA polymerase that is active in mitochondria and it is very important for replication of mtDNA.[9]*POLGI* is associated with mtDNA depletion syndromes.[10,11]

Nuclear mutations are probably involved in 90% to 95% of children with complex I deficiency[12] but it was not until 1998 that the first mutations were identified in the NDUFS4 and NDUFS8 genes by the group in Nijmegen.[13,14] An increasing

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Consent for publication: Written informed consent was obtained from the patient's parents for publication of details of the case report. Personal information was not mentioned in a way, which can lead to identification of the patient or his family.

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number of pathogenic mutations in genes encoding different structural subunits of complex I have since then been identified. However, the genetic cause remains unclear in about 60% of patients with complex I deficiency.[12]

The clinical phenotypes of POLG related disorders include the autosomal recessive and dominant adult onset progressive external ophthalmoplegia,[15,16] myoclonic epilepsy myopathy sensory ataxia syndrome,[17,18] ataxia neuropathy spectrum including mitochondrial recessive ataxia syndrome, and sensory ataxia neuropathy dysarthria ophthalmoplegia syndrome[19,20] and the hepatocerebral MDS (Alpers-Huttenlocher syndrome).[21,22]

POLG mutations were identified in individuals with clinical features of mitochondrial neurogastrointestinal encephalopathy disease (MNGIE).[23]

In our report we describe a Saudi female neonate with a lethal mitochondrial depletion syndrome due to a novel POLG compound heterozygous mutation with severe hypotonia, very poor respiratory effort and gross dysmorphism. Her 3 siblings had died at another hospital with the same clinical scenario, hence genetic testing could not be performed.

2. Case presentation

A full term Saudi ±37 weeks gestational age female neonate delivered by caesarian section due to previous caesarian section with Apgar score 5 and 6 at 1 and 5 minutes, respectively, was noticed immediately after birth to have severe hypotonia and very poor respiratory effort compromising her ventilation for which prompt intubation in delivery room was done and the baby was immediately shifted to neonatal intensive care unit (NICU) and connected to mechanical ventilation. She had dysmorphic features in the form of low set ears, micrognathia, bilateral clubfeet, and cleft palate.

The baby was born to first-degree cousin parents to gravida 5 para 4 mother with 1 living 6 years old boy and 3 early neonatal deaths; 1 male and 2 females; all of them died with severe hypotonia and poor respiratory effort and all had the same dysmorphic features. They died in different hospitals with no specific final diagnosis in all of them. Maternal polyhydramnious and weak fetal movement were consistent features in the pregnancies of our patient and all her siblings who died.

Thorough examination of the patient in NICU revealed, birth weight of 2330 g (<10th percentile), length of 46 cm (25th percentile), and head circumference of 33 cm (25th percentile) with dysmorphic features typical to those found in her dead siblings.

Neurologically, she had severe hypotonia compromising her ventilation and feeding, absent deep tendon reflexes in all limbs with intact cranial nerves. Extensive work up was carried out including; complete blood count, renal functions, liver functions, bone profile, blood glucose, congenital infections screen, cerebrospinal fluid analysis for cell count, glucose, protein, lactate, pyruvate and amino acids, tandem mass spectrometry for metabolic diseases, biotinidase level, very long chain fatty acids, abdominal ultrasonography, and karyotyping which all came out to be normal.

Magnetic resonance imaging (MRI) brain showed elements of volume loss that includes prominent Sylvian fissures and extra-axial spaces, marked alteration in the signal intensity affecting bilateral corpus striatum in both T2 and T1 (Fig. 1A and B). Remarkable lactate peak was also noted (Fig. 1C). Furthermore, MRI spectroscopy with Voxel on Basal Ganglia demonstrated decreased N-acetylaspartate (NAA) peak and increased choline peak (Fig. 1D).

Electroencephalogram confirmed abnormal electrical discharge coinciding with partial seizures with secondary generalization.

Initial suspicion of Prader Willi was excluded by negative FISH genetic testing of PWS gene and possibility of spinal muscular atrophy was also excluded by normal SMN1 gene.

The patient was revisited and multidisciplinary team discussion including neonatologist, genetic consultant, pediatric neurologist, pediatric gastroenterologist, and ophthalmologist with a final agreement about the possibility of mitochondrial disease.

Parents were counseled about the clinical condition of their baby and the expected terminal event due to severe hypotonia and ventilator dependency. They were counseled about the high possibility of a running mitochondrial disorder in their family and they accepted to start genetic work up for them, their baby, and the living apparently normal child. Written informed consent was obtained.

2.1. Molecular genetic analysis of the POLG gene for the family

The study was approved by the research and ethical committee of Alhada Armed Forces Hospital, Taif, Saudi Arabia. Written informed consent was obtained from the patient's parents for contribution of their child in the study.

3. Methods

Exons 3 and 19 of the POLG gene (OMIM174763 chromosome 15q26.1) were amplified from genomic DNA by polymerase chain reaction and sequenced directly. The resulting sequence data were compared with the reference sequence NM_002693.2

4. Results

The baby carried the possibly pathogenic heterozygous variant c.680G>A (p.Arg227Gin) in exon 3 on paternal allele as well as the variant c.3098C>T (P.Ala1033Val) which is of unclear pathological significance on the maternal allele of the POLG gene (Fig. 2).

The possibly pathogenic POLG variant c.680G>A (p.Arg227Gin) in exon 3 was carried by the father in heterozygous state while the c.3098C>T (p.Ala1033Val) in exon 19 of the POLG gene was not detected (Fig. 3).

The reverse was true for the mother as she carried the c.3098C>T (p.Ala1033Val) variant in exon 19 of the POLG gene in heterozygous state while the c.680G>A (p.Arg227Gin) variant in exon 3 was not detected (Fig. 4).

Genetic counseling of the parents was carried out to avoid recurrence of the condition in their offspring.

The baby died at the age of 5 months after a prolonged stay in NICU on mechanical ventilation and total parenteral nutrition with failure of all attempts of weaning from mechanical ventilation and all trials of feeding.

5. Discussion

Mitochondrial diseases form a highly diverse group with variable clinical symptoms and different ages of onset. They are predominantly monogenic disorders and recent advances in sequencing technologies have revolutionized the diagnosis of

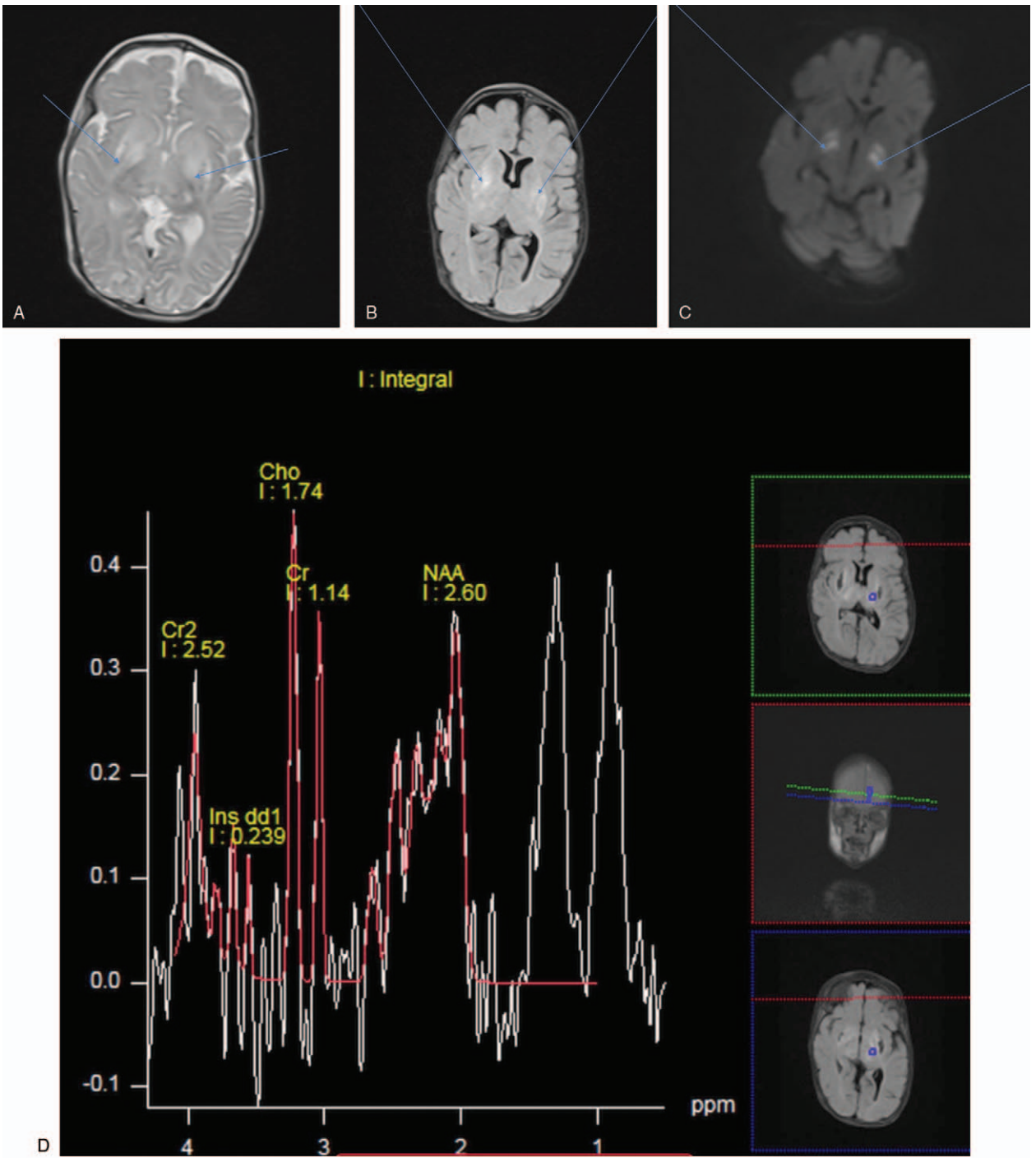

Figure 1. A: Axial T2-weighted MRI images at level of basal ganglia shows hyperintensity within bilateral lentiform nuclei. B: Axial FLAIR MRI images at level of basal ganglia shows hyperintensity within bilateral lentiform nuclei. C: Axial diffusion-weighted image shows areas of diffusion hyperintensity involving bilateral lentiform nuclei. D: Single—voxel MRI spectroscopy from the VOI containing the left affected basal ganglia demonstrated decreased NAA peak and increased choline peak. A discernible doublet is seen at 1.33 ppm suggestive of lactate peak. FLAIR = fluid attenuation inversion recovery, MRI = magnetic resonance imaging, NAA = N-acetylaspartate, VOI = volume of interest.

patients with mitochondrial diseases. Despite these advances, the genotype–phenotype correlations remain difficult to predict. The large heterogeneity of the human genome makes functional validation of novel disease variants essential.[24,25]

Clearly common disease alleles in mitochondrial disease are due to mutation in nuclear genes, which are important to maintain our mitochondrial DNA[26] with an estimated prevalence of pathogenic nuclear DNA mutations in a northeast England study on adults with symptomatic mitochondrial disease of 2.9 per 100,000 compared with 20 per 100,000 for the pathogenic mtDNA mutations.[27] A Spanish study found an estimated prevalence of 5.7 per 100,000 for mitochondrial diseases in a population over 14 years of age.[28]

Emmanuele et al[29] studied neonatal cord blood samples for 10 common mtDNA point mutations and a prevalence of 1 in 200 was detected. On the other hand, an Australian study

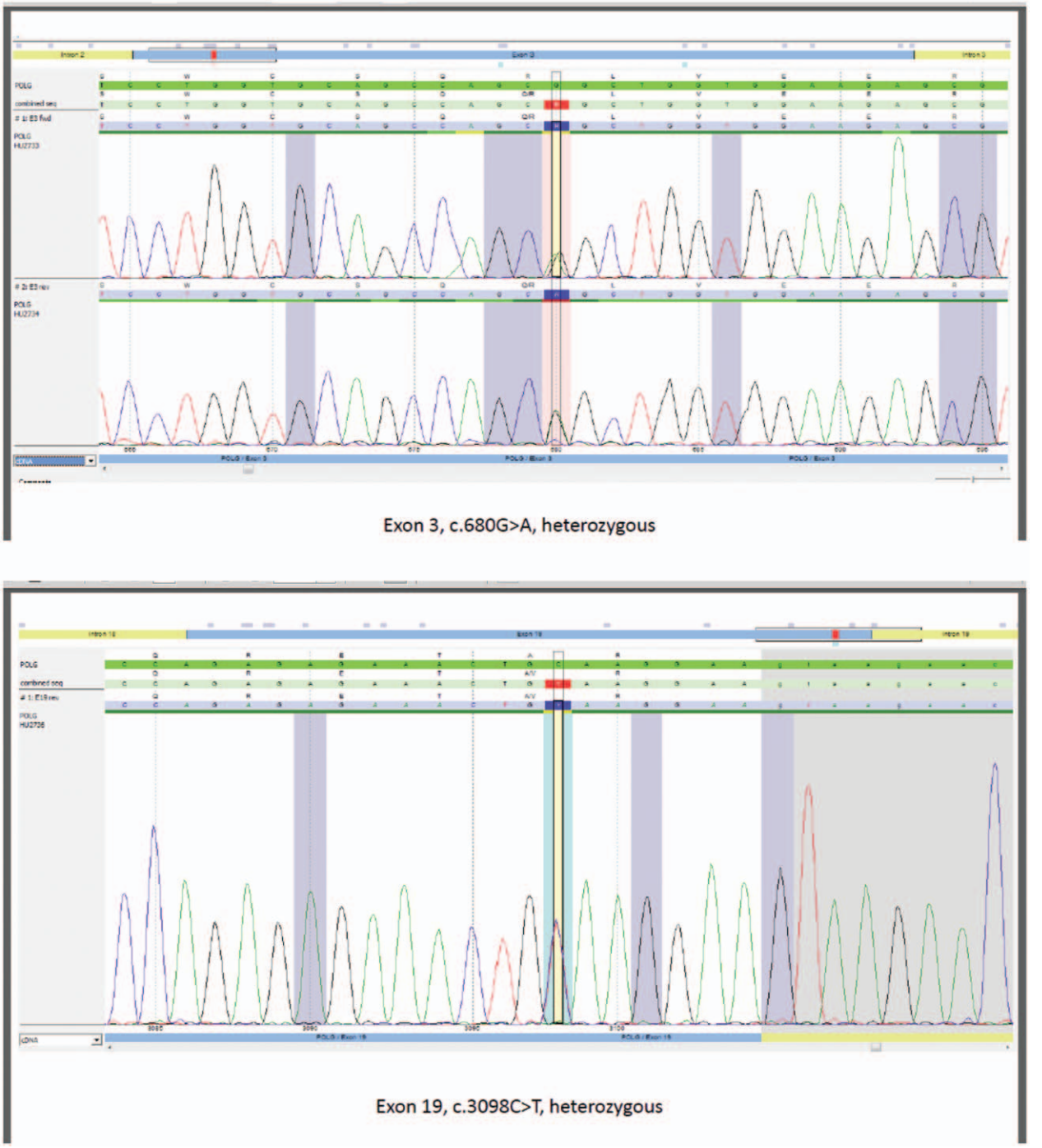

Figure 2. Chromatogram of the patient.

estimated the minimal birth prevalence of primary mitochondrial disorders to be 6.2 per 100,000 births.[30]

To date, there are >230 reported mutations in the human POLG gene alone.[31] The number of individuals harboring a recessive pathogenic mutation in *POLG* has been estimated to approach 2% of the population.[26]

There is one recently diagnosed infant with MNGIE syndrome who had severe hypotonia and generalized muscle weakness requiring ventilator assistance and severe abdominal distention with hypoactive bowel and congenital anomalies in the form of low set ears and bilateral clubfeet, with compound heterozygous missense mutation in c.679c>t predicting p.R227W and c.2542G>A predicting p.G848s. Sequencing of parental samples confirmed recessive inheritance.[23]

Our patient is similar to this report in having severe hypotonia and poor respiratory effort with low set ears and bilateral clubfeet with a novel compound heterozygous mutation; c.680G>A (p. Arg227Gin) and c.3098C>T (P.Ala1033Val); in POLG gene. Segregation analysis revealed that the baby inherited the POLG variant c.680G>A (p. Arg227Gin) from the father while the POLG variant c.3098C>T (P.Ala10033Val) was inherited maternally.

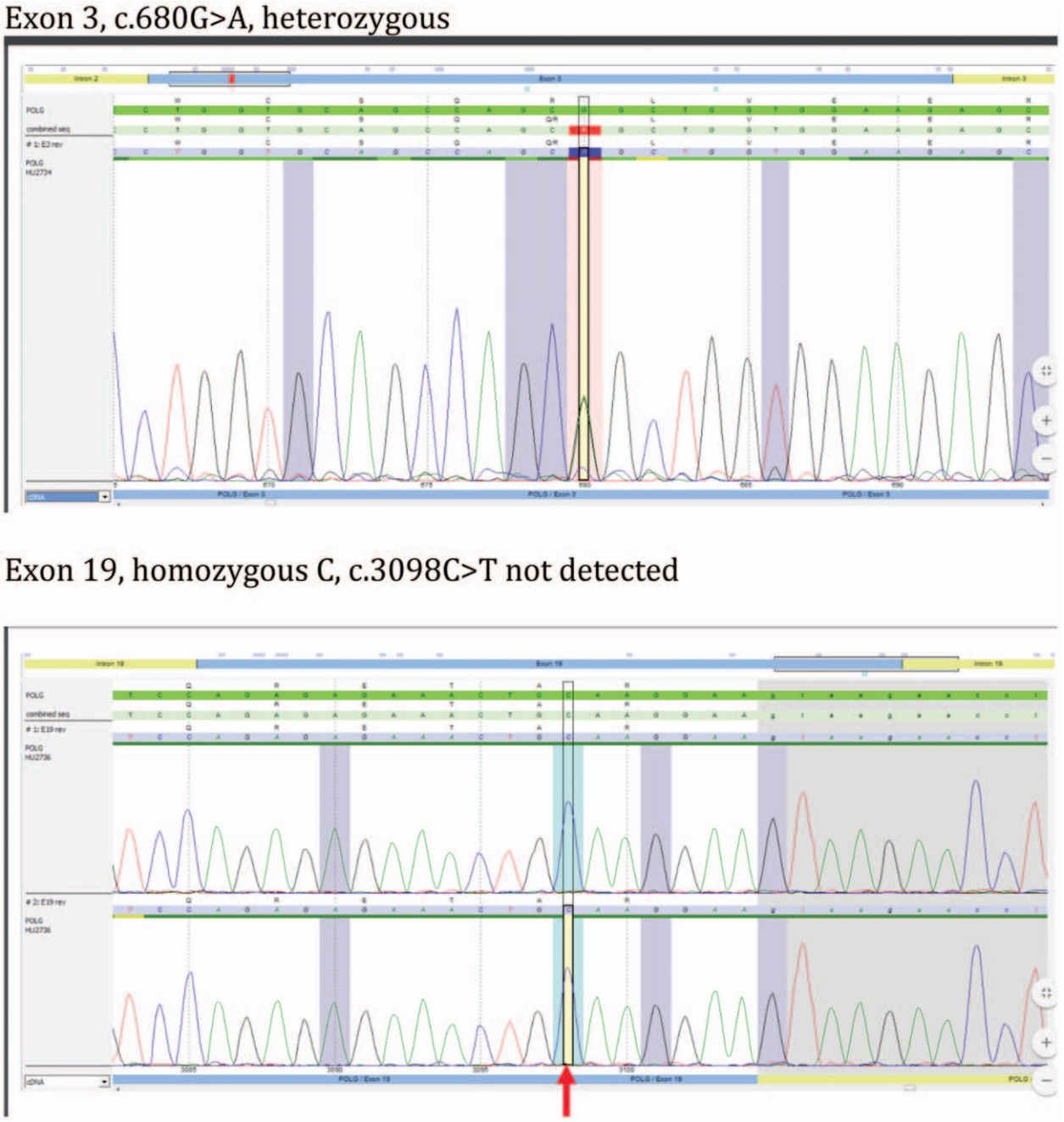

Figure 3. Chromatogram of the father.

To the best of our knowledge the POLG variant c.680G>A (p. Arg227Gin) has not been described in literature or databases so far and there is no information regarding its allele frequency in the general population (ExAC database). Six out of 10 bioinformatic analysis tools used, predicted a significant effect on protein function by the variant p.Arg227G confirming its pathogenic effect.

The mutation described by Giordano et al[32] is affecting the same protein position pArg227Trp in compound heterozygosity in his male patient with MNGIE syndrome.

The POLG variant c.3098C>T (P.Ala1033Val) has been annotated as a rare polymorphism (rs551708243) and its allele frequency in the general population is 0.02% (ExAC databases) which would be compatible with a potential pathogenicity. To the best of our knowledge the variant has not been described in the literature so far. A significant effect on protein function was predicted by 2 out of 10 bioinformatic analysis tools used.

Three siblings of our patient died with same clinical scenario. All of them had severe floppiness, respiratory failure, and were ventilator dependent which started immediately after birth. They all died in the neonatal period.

These similarities in clinical findings emphasize the hypothesis that all of them carried the same disease and hypothetically the same mutation causing defective respiratory chain energy production like their currently reported sibling especially after genetic confirmation of carrier status of their parents.

Lack of genetic testing of the other 3 siblings who were delivered and died in another hospital might remain a limitation of our report.

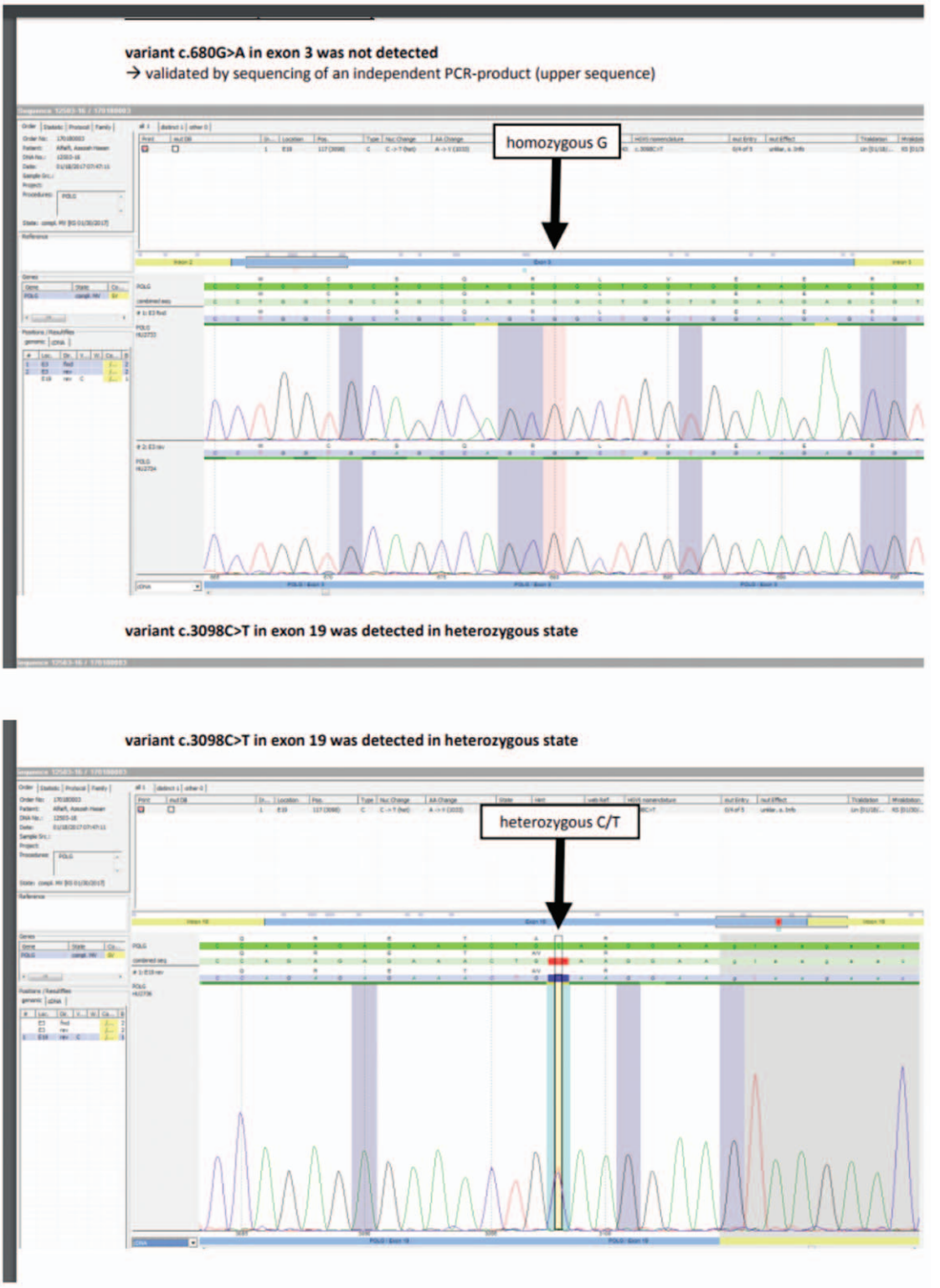

Figure 4. Chromatogram of the mother.

6. Conclusion

Mitochondrial respiratory chain defect is not an uncommon cause of severe neonatal hypotonia (paralysis), with absent deep tendon reflexes culminating in respiratory failure or intrauterine deaths. The main clue to diagnosis is the encephalopathy and the characteristics MRI findings in highly consanguineous population.

Author contributions

MA and NK: planned, conducted and reported the work.

HK and AH: shared in conducting the work
YA: conducted the radiological part of the work
Conceptualization: Mohamed AlJabri, Naglaa M. Kamal.
Data curation: Mohamed AlJabri, Haifa Korbi, Abdulrahman Halabi, Mashour Alsayyali.
Investigation: Mohamed AlJabri, Mashour Alsayyali.
Methodology: Mohamed AlJabri, Abdulrahman Halabi, Yahea Alzahrani.
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Supervision: Mohamed AlJabri.
Software: Naglaa M. Kamal.
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Writing – original draft: Naglaa M. Kamal, Haifa Korbi.
Writing – review & editing: Naglaa M. Kamal.
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