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Identification of a *CHRNA1* c.2T>C homozygous variant in three fetuses with features of lethal multiple pterygium syndrome in an Ashkenazi Jewish family

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1. Introduction

Lethal multiple pterygium syndrome (LMPS, OMIM: 253290) is a rare autosomal recessive disorder characterized prenatally by intrauterine growth retardation, multiple pterygia, fetal akinesia, severe arthrogryposis, hypoplastic heart and lungs, cystic hygroma, edema, and mid-trimester fetal demise.[1,2] Non-specific features, such as cystic hygroma and fetal edema, may be visualized by ultrasound in the first trimester of pregnancy, but the hallmark features of the disorder often are not appreciated until the second trimester or later. While both environmental and genetic factors can trigger LMPS, in recurrent cases it is essential to identify any underlying genetic cause in order to provide early diagnosis and medical intervention in affected pregnancies. Toward this end, several causal pathogenic variants have been identified and reported in acetylcholine receptor subunits encoded by the *CHRNA1*, *CHRND*, and *CHRNG* genes[3,4], as well other genes, such as those encoding nebulin (*NEB*)[5], and the ryanodine receptor (*RYR1*)[6].

We report a comprehensive genetic study performed for an Ashkenazi Jewish (AJ) family with a history of ultrasound findings consistent with LMPS and fetal demise, at the time of their fourth pregnancy.The couple was non-consanguineous, had two mid-trimester losses and one healthy child, and their fourth pregnancy had a cystic hygroma at 11 weeks gestation. We utilized whole exome sequencing (WES) and mutation specific sequencing (MSS) to identify and confirm the novel genetic variant in the *CHRNA1* gene which likely causes LMPS in this family.

2. Methods

- DNA was isolated from blood and chorionic villus specimens. Archived DNA was available from the first two affected pregnancies.
- WES was performed for the parents and first pregnancy. Sonicated DNA was enriched using custom Agilent SureSelect® XT and sequenced on an Illumina HiSeq™ 2500 system. Sequence data was mapped and aligned to Human Genome Build GRCh37/hg19 using CLC Bio software.
- MSS was performed for the healthy son and the third and fourth pregnancies. The targeted gene region was PCR amplified, Sanger sequenced, and assessed using SeqScape® Software v3.0 (Thermo Fisher Scientific, Waltham, MA).
- Marker analysis was performed with a multiplex STR system (PowerPlex® 16 System, Promega, Madison, WI) to rule out maternal cell contamination in prenatal specimens.
- Positive results were reported using the numbering and nomenclature recommended by the Human Genome Variation Society (HGVS, http://www.hgvs.org).

3. Results

We performed a comprehensive molecular genetic study of an AJ family with a history of three pregnancies with clinical findings consistent with LMPS (**Figure 1**). First, we utilized WES and identified a homozygous c.2T>C (p.Met1?, legacy nomenclature p.Met1Thr) variant in the *CHRNA1* gene in archived DNA from the first affected fetus and heterozygosity for the variant in each of the parents (**Figure 2**). Subsequently, we performed MSS to demonstrate that the LMPS phenotype segregated with the *CHRNA1* c.2T>C variant in this family. The only healthy child did not harbor the variant. We identified homozygosity for the variant in the third and fourth pregnancies, which were both affected (**Figure 3**).

The *CHRNA1* c.2T>C variant alters the initiation codon and is predicted to result in the absence or truncation of the encoded cholinergic receptor nicotinic alpha 1 subunit. This novel variant has not, to our knowledge, been reported in individuals affected with LMPS. However, other loss of function/null *CHRNA1* variants, present in a homozygous state, have been reported to cause this disorder[4]. These reported similar cases, combined with our segregation data, present a strong argument that the *CHRNA1* c.2T>C variant is the cause of recurrent pregnancies affected with LMPS in this family.

Also of note, there is evidence to suggest that the *CHRNA1* c.2T>C variant is an AJ founder mutation. There are ten occurrences of this variant in healthy individuals listed in the Genome Aggregation Database (https://gnomad.broadinstitute.org). Eight of these individuals are AJ and two are non-Finnish European, a population that may include individuals of AJ ancestry.

4. Conclusions

- This genetic study of an AJ couple with three affected fetuses and one healthy child demonstrated that homozygosity for the *CHRNA1* c.2T>C variant segregated with clinical findings consistent with LMPS.
- The variant is likely an AJ founder mutation: eight of ten individuals with this variant in gnomAD are AJ.
- To our knowledge, this is the first case report which provides evidence that the *CHRNA1* c.2T>C (p.Met1?) variant is likely pathogenic and causes LMPS.
- This finding should be considered in the differential for similar cases in other AJ families.

References

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Figures

Figure 1. Pedigree of the family showing genotypes for the *CHRNA1* c.2T>C variant. Solid filled symbols indicate a clinical phenotype consistent with lethal multiple pterygium syndrome.

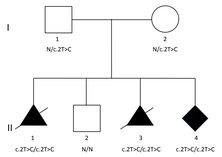


Figure 2. Confirmatory Sanger sequence traces for whole exome sequencing showing homozygosity for the *CHRNA1* c.2T>C variant in the first fetus and heterozygosity for the variant in the parents. Green checkmark denotes nucleotide of interest.

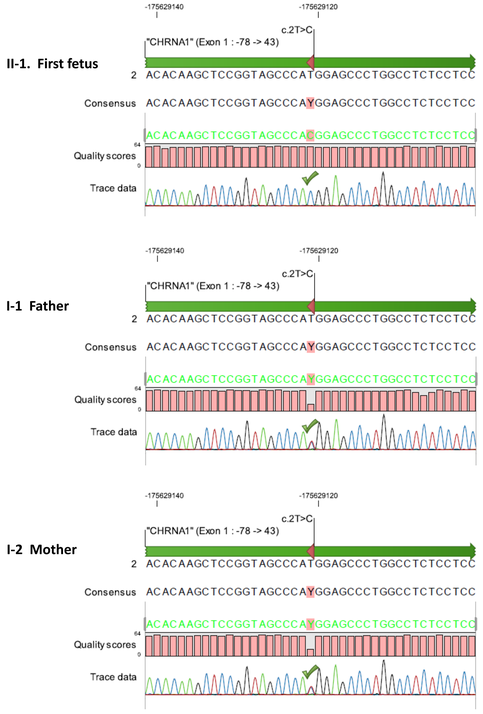
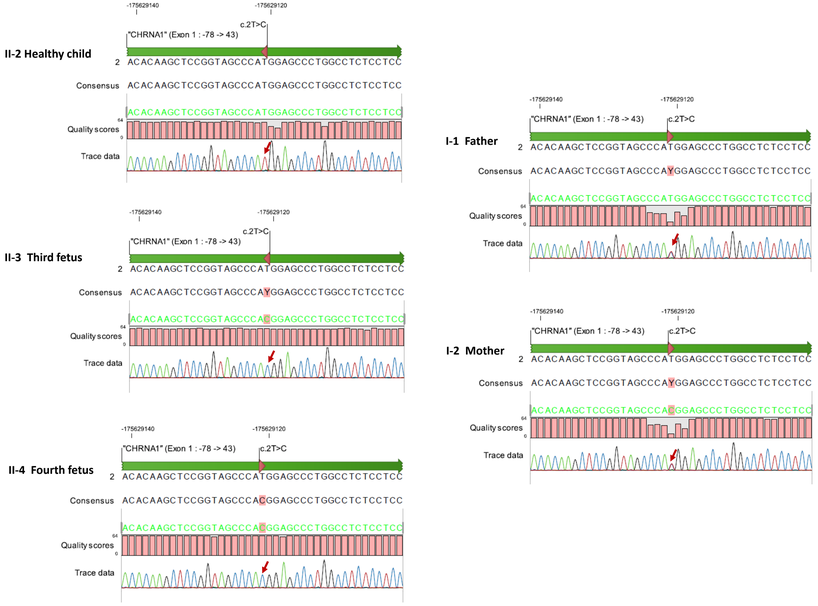


Figure 3. Sanger sequence traces for mutation specific sequencing showing normal sequence in the healthy child, homozygosity for the *CHRNA1* c.2T>C variant in the third and fourth fetuses, and heterozygosity for the variant in the parents. Red arrow denotes nucleotide of interest.



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