Prenatal Genetic Counseling for a Novel Genetic Etiology identified in a Fetus with Compound Heterozygous Alterations in *MYH6*

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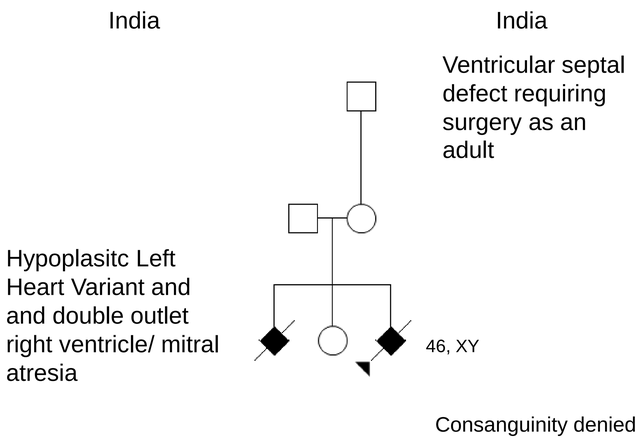
BACKGROUND

- Fetal and neonatal Diagnostic Exome Sequencing (DES) is being used more frequently in cases of infant demise, with or without congenital anomalies.
- Several studies have shown the clinical utility of DES for prenatal and neonatal patients due to increasing ease of DES and knowledge of the human genome (1-3).
- The increasing use of prenatal DES results in unique counseling challenges when diagnoses are made prior to the onset of certain symptoms, especially when novel genetic findings are identified.

PEDIGREE



CLINICAL PRESENTATION

- The proband presented at 19 2/7 weeks gestation for fetal anatomic survey where congenital heart defect was suspected.
- Serum screening showed multiple analytes out of the normal range.
- Follow-up echocardiogram confirmed hypoplastic left heart (HLH), severe coarctation of the aorta, aortic stenosis, perimembranous ventricular septal defect (VSD), and multiple small to moderate VSDs.
- No additional anomalies were reported in the fetus.

METHODS

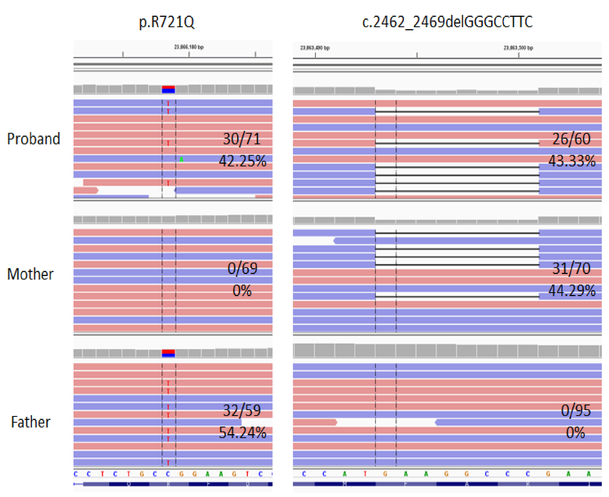
- Diagnostic exome sequencing and analysis of the proband, and parents, including novel genetic analysis was performed on cultured amniocytes as previously described (4, 5).
- Maternal cell contamination analysis was performed and maternal cell contamination was not detected in this fetal sample.
- Co-segregation analysis and Sanger confirmation was performed on the unaffected sister to the proband.

RESULTS

- Amniocentesis revealed a normal male karyotype (46, XY).
- DES revealed compound heterozygous alterations in *MYH6* c.2162G>A (p.R721Q) and c.2462_2469delGGGCCTTC (p.R821Hfs*65) , secondary findings analysis was not performed for the proband.
- Co-segregation analysis revealed that the alterations were inherited from asymptomatic heterozygous parents.

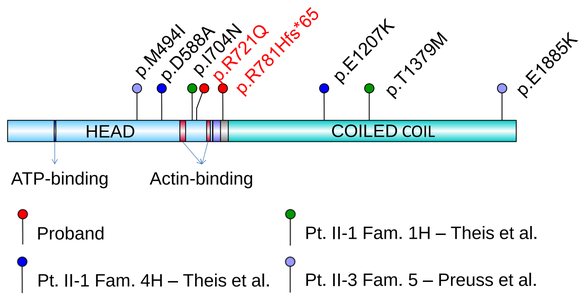
CO-SEGREGATION ANALYSIS

Alteration	Proband	Father	Mother	Unaffected Sister
c.2162G>A (p.R721Q)	+/-	+/-	-/-	+/-
c.2462_2469delGGGCCTTC (p.R821Hfs*65)	+/-	-/-	+/-	-/-



MYH6 (OMIM 160710) GENE INFORMATION

- Encodes the cardiac alpha myosin heavy chain (a-MyHC), a subunit of cardiac muscle myosin protein, a major component of the sarcomeres, essential for generation of the mechanical force required for muscle contraction, myofibrillar assembly and proper heart development (reviewed in England, 2013).
- a-MyHC forms homodimers or heterodimers with beta myosin heavy chain (b-MyHC) encoded by the MYH7 gene (OMIM: 160760).
- *In vivo* animal models:
  - Homozygous *Myh6* knock-out mice displayed in utero lethality between day 11 and day 12, possibly due to observed gross heart defects (6).
  - Myh6+/- mice show cardiac myofibrillar disarray, cardiac dysfunction and fibrosis.
  - Zebrafish deficient in *myh6* (atrial myosin heavy chain; amhc) have revealed disruption of atrial myofibrillar organization and weak atrial contractility which indirectly causes ventricular wall thickening and narrowing of the ventricular lumen and influences atrioventricular valve formation (7).
- Heterozygous alterations in *MYH6* have been associated with:
  - autosomal dominant hypertrophic cardiomyopathy (HCM; OMIM: 613251),
  - dilated cardiomyopathy (DCM; OMIM: 613252), and
  - atrial septal defects (ASDs; OMIM: 614089).
- Biallelic alterations in *MYH6* n reported in just three affected individuals with complex heart defects (8), (9).



MYH6 c.2161C>T (p.R721W) ALTERATION

- Review of the literature revealed that the p.R721Q variant affects a highly conserved arginine that had previously been reported to be altered to a tryptophan (p.R721W) in a patient with sick sinus syndrome. Ventricular cardiomyocytes expressing this *MYH6* p.R721W variant have been shown to have disrupted patterns of myofibrils and disintegrated sarcomeric structure in rats. In addition, a p.R721W *MYH7* alteration, homologous to p.R721W of *MYH6*, has been shown to cause malignant hypertrophic cardiomyopathy, frequently associated with conduction abnormalities.
- a-MyHC consists of a head, neck, and tail domains (10). Coiled coils are formed by tail domains of two a-MyHC molecules to stabilize the interaction of the head domains with actin for generation of mechanical force required for muscle contraction. The p.R721 amino acid is located in the actin-binding converter domain of a-MyHC.

GENETIC COUNSELING

- Counseling of the proband's parents included discussion of the possible 25% recurrence risk, options for testing in future pregnancies, and limitations of such testing.
- Prenatal and preconception testing for future pregnancies through CVS, amniocentesis, or pre-implantation genetic diagnosis (PGD) was explored with the couple based on the apparent recessive inheritance pattern.
- Limitations of diagnostic testing was reviewed in depth, including the novel genetic etiology of this inheritance pattern in *MYH6.*
- The couple returned for a subsequent pregnancy and found to only have the maternally inherited alteration. At 24 weeks, two fetal echocardiograms were normal.
- Cardiac workup for the couple's healthy daughter and themselves was recommended.

CONCLUSION

- The *MYH6* c.2162G>A (p.R721Q) alteration had some considerable pathogenicity information, although was functionally difficult to characterize. As it is uncertain that p.R721Q will have a similar effect as p.R721W.
- The phenotypes reported in individuals with heterozygous *MYH6* alterations had a similar presentation to the proband.
- Generally gain of function mutations lead to an autosomal dominant phenotype, it is uncertain if a homozygous loss of function have the same phenotype of if the phenotype in this family due to the amplification of the gain of function mutation with loss of the wild type copy.
- Additional studies of heterozygous *MYH6* alterations supported by further investigations of the molecular mechanism of the c.2162G>A (p.R721Q) alteration are needed to aid to elucidate the phenotype and pathogenicity.
- This case not only presents a novel genetic inheritance for *MYH6* but also the complexities of genetic counseling for prenatal DES.

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