Biallelic, and not Monoallelic, Loss of Function Variants in *TRIM63* Most Likely Cause Cardiomyopathy

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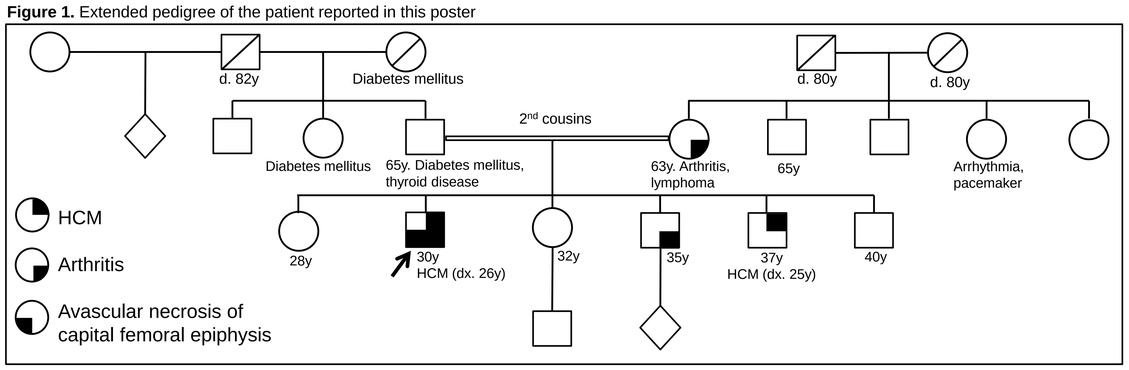
BACKGROUND

- *Tripartite motif-containing 63* (*TRIM63*), also known as *Muscle RING finger protein 1* (*MURF1*), encodes a skeletal and cardiac muscle specific E3 ubiquitin ligase that maintains the stability of the sarcomere.
- Heterozygous loss of function variants in *TRIM63* have been reported to cause hypertrophic cardiomyopathy (HCM)[1]. However, the reported families were small, the role of one of the variants in the report, p.Q247*, was questioned by another study[2], and two out of the three variants in the initial report (p.Q247* and p.A48V) have high allele frequencies in the Genome Aggregation Database (gnomAD) incompatible with the estimated prevalence of HCM.
- Studies in mice[3], *in vitro* cell culture[4], and one published family with HCM[5], support the role of biallelic loss of function variants in *TRIM63* as the likely cause of HCM.

CASE REPORT

- 30-year-old man with HCM, tachycardia, bilateral avascular necrosis of the hips, arthritis, and ataxic gait.
- Previous panel sequencing of 27 known cardiomyopathy genes at Ambry Genetics was negative.
- The family history is significant for a brother with hypertrophic cardiomyopathy. Parents are 2nd cousins.

Figure 1. Extended pedigree of the patient reported in this poster



METHODS

- Diagnostic whole exome sequencing was performed on the proband and parents as previously described[6] with the exception of the capture reagent used which was IDT xGen Exome Research Panel V1.0. Approximately 97% of the proband's exome was covered at 20x or higher.
- Population frequency cutoffs for variant filtering included 0.1% for dominant, 1% and 0.2% for recessive characterized and uncharacterized genes, respectively, and 0.2% for incomplete penetrance models. The latter model included variants that are found in the Human Gene Mutation Database (HGMD), those that cause truncations such as frameshift, nonsense, and canonical splice, and those found in known imprinted disorder-causing genes. Population databases used were 1000 Genomes Project, Exome Sequencing Project (ESP), and Exome and Genome Aggregation Consortia (ExAC and gnomAD, respectively). Candidate gene analysis was done as previously described[7].

RESULTS

- Filtering of variants in characterized disease genes resulted in 14 individual variants in 13 genes, none of which were clinically relevant for the proband's presentation.
- Filtering of variants in uncharacterized genes (i.e. candidate genes that currently are not strongly linked to any human disease) resulted in 12 individual variants in 12 genes, of which a homozygous nonsense variant p.Q247* in *TRIM63* (NM_032588 c.739C>T) was considered relevant to the patient's HCM phenotype based on available literature data (see Background and Interpretation sections)

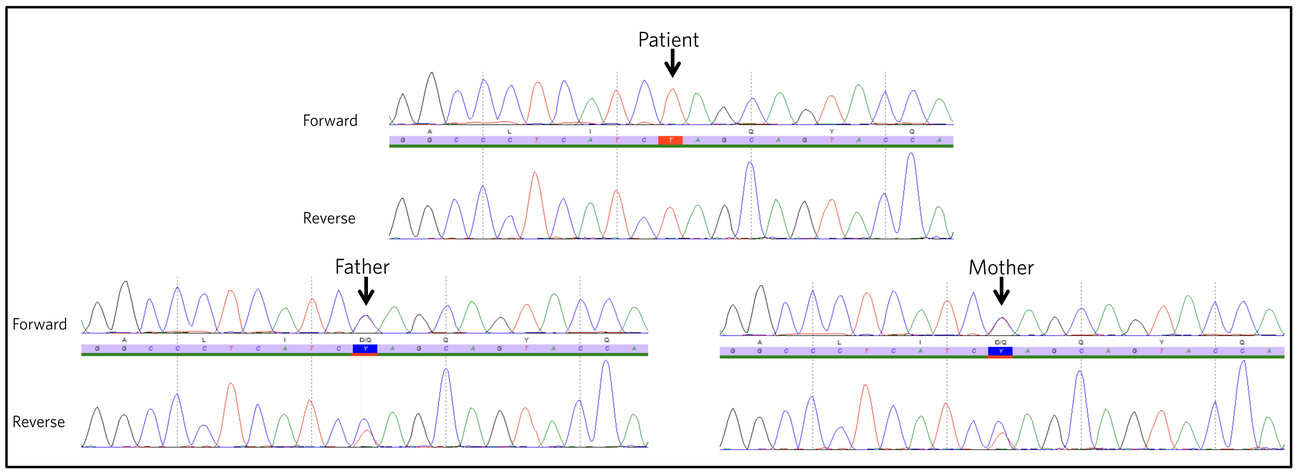
Figure 2. Integrated Genome Browser (IGV) view showing the NM_032588 c.739C>T (p.Q247*) in *TRIM63* in the patient who appeared to be homozygous, and the two parents who appeared to be heterozygous. The number of reads with the variant allele and reference allele in each individual are shown on the left of this figure.

Patient
#variant reads: 55
#reference reads: 1
Ratio: 98.21%

Father
#variant reads: 33
#reference reads: 28
Ratio: 54.10%

Mother
#variant reads: 48
#reference reads: 30
Ratio: 61.54%

Figure 3. Sanger confirmation of the NM_032588 c.739C>T (p.Q247*) variant in *TRIM63* (arrows) in the patient and the parents. The sequence and colors of the nucleotides of the reverse strand have been changed to match those of the forward strand.



INTERPRETATION

- We have identified a patient with HCM homozygous for a truncating nonsense variant p.Q247* in *TRIM63* (NM_032588 c.739C>T) expected to cause nonsense mediated decay of the mutant transcripts and a complete loss of full length protein.
- Several lines of evidence suggest that biallelic, rather than monoallelic, loss of function of *TRIM63* most likely causes HCM:

1. The initial report of heterozygous p.Q247* in *TRIM63* in 2012 involved small families with HCM which hindered genotype-phenotype correlation[1].
2. A report published in 2014 questioned the pathogenicity of the heterozygous p.Q247* variant because it was found in a 22-year-old patient suspected of long-QT syndrome as well as in his asymptomatic 47-year-old mother. Both individuals lacked HCM[2].
3. Two of the three heterozygous variants in the initial report that were thought to cause HCM have high allele frequencies in gnomAD which are incompatible with the known prevalence of HCM of ~ 0.2%:
   - ❖ p.Q247* = 79/10,152 i.e. 0.7782% in Ashkenazi Jews
   - ❖ p.A48V = 277/126,248 i.e. 0.2194% with 2 homozygotes in non-Finnish Europeans
4. Lack of homozygous loss of function variants in *TRIM63* in gnomAD (and also in the Exome Aggregation Consortium or ExAC).
5. Animal model shows that unlike wild-type mice, homozygous *Trim63* (aka *Murf1*) knockout mice develop an exaggerated cardiac hypertrophic response after pressure overload[3].
6. *In vitro* studies have also shown that depleting Murf1 in neonatal rat ventricular myocytes by short interfering RNAs causes a significant increase in cell size and upregulation of markers of hypertrophy[4].
7. There is a report of one adult patient from a consanguineous family with HCM who was homozygous for the p.Q247* loss of function variant but whose heterozygous siblings were asymptomatic[5].
   - ❖ This patient also carried a heterozygous missense variant in another cardiac related gene, *TRIM54*. However, the contribution of this missense variant to HCM is likely minimal because of the observation that introduction of wild-type *TRIM63* in the patient's cultured myocytes was able to rescue the disrupted organization of the microtubule network.
   - ❖ The patient's older half-sister with HCM was also homozygous for p.Q247*. However, she had a history of hypertension which could also have contributed to HCM.

TAKE-HOME POINTS

- Heterozygous variants in *TRIM63* had previously been implicated in cardiomyopathy in humans. However, with the release of large control databases (ExAC and gnomAD) these previously-identified variants were deemed unlikely to be disease-causing dominant alleles due to their high population frequencies.
- By diagnostic exome sequencing (DES) we identified a patient with cardiomyopathy harboring biallelic loss of function variants in *TRIM63*, similar to another previously published patient. These patients, combined with evidence from *in vivo* studies of knockout mice as well as *in vitro* cellular studies, make a strong case to support the notion that biallelic loss of function variants in *TRIM63*, rather than heterozygous variants, are the cause of cardiomyopathy in humans.
- DES is a useful tool for identifying not only candidate human disease genes but also for clarifying the mutational mechanism of disease.

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