Introducing Mitrail Valve Prolapse

Mitrail valve prolapse (MVP) is a common condition that sometimes leads to serious complications such as regurgitation, endocarditis, and heart failure. Determining the molecular etiology of MVP could aid in risk stratification of patients, facilitate counseling of patients and family members, and may identify novel treatment targets.

Genetic testing of patients with Ehlers-Danlos, Loefy-Dietz, and Marfan syndrome, who often have MVP as part of their clinical picture, has demonstrated the importance of mutations in collagen genes, TGFβ genes, as well as the FBN1 gene. The FLNA gene has also been linked to genetic valvular disease. Comprehensive gene sequencing in select individuals may uncover as yet unrecognized genetic contributions to familial MVP. We hypothesize that rare, deleterious variants in genes related to heart development, connective tissue, or known MVP genes may underlie some of the currently unexplained cases of genetic MVP.

Methods

Due to the clinical heterogeneity of MVP, we used whole exome sequencing (WES) to identify genetic causes in 3 patients suspected of having a genetic form of MVP. Patients were enrolled in the North Carolina Genomic Evaluation by Next Generation Exome Sequencing clinical trial, NCGENES. We are using bioinformatics-based as well as biological tools to evaluate candidate MVP genes and variants.

We examined three patients with varying presentations of MVP who were suspected of having a genetic cause based on age of onset, severity, and family history.

- **NCG_00261**: 15 year old female Dx with MVP at age 3 necessitating repair; multiple affected individuals in the family (see pedigree); no apparent connective tissue problems, but some family members with MVP also have A fib.

- **NCG_00157**: 42 yr. old male Dx with Thoracic Aortic Aneurysm and MVP; working diagnosis of Loefy-Dietz, but no TGFβR mutations identified by clinical testing.

- **NCG_00354**: Male with early-onset MVP necessitating repair; also has patellar subluxation and loose skin; Mother is similarly affected.

WES workflow and Variant Calling Pipeline

Break up DNA and keep exonic regions

Sequence end to end

Align to Human Genome

Knowledge database

Known Disease Causation?

Yes

No

[Diagram of WES workflow and Variant Calling Pipeline]

A linkage analysis was done using an snr chip to genotype NCG_00261 and her family members. rs4663726 obtained significant LOD score in all 4 family schemes we analyzed.

- **rs4663726**: Falls within COL6A3 gene on 2p73.3
- **WES** did not identify any variants in COL6A3 with MAF < 0.48 for NCG_00261.

We looked for evidence of a micro deletion by applying a statistical outlier algorithm to exonic coverage data in NCG_00261 compared to 145 other NCGENES participants. We found no evidence of an exonic deletion in COL6A3.

We are now sequencing regions of the COL6A3 promoter, paying special attention to genomic regions with identified ENCODE marks.

Because the proband in this family, NCG_00261, is the most severely affected, we are focusing on identifying candidate MVP variants from her WES data (see summary of candidate variants below)

**NCG_00261 Summary of Linkage Analysis and WES-identified candidate variants**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>Variant</th>
<th>In Silk Predictions &amp; Databases Affected</th>
<th>Codon Score</th>
<th>Genotype</th>
<th>LOD</th>
<th>MAF</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>COL6A3</td>
<td>Collagen type 6, variant</td>
<td>rs4663726</td>
<td>rs4663726</td>
<td>2.14</td>
<td>Rare</td>
<td>0.21</td>
<td>0.001</td>
<td>Rare variant</td>
</tr>
</tbody>
</table>

**Conclusion & Future Directions**

- NCGENES provides a mechanism to identify potential genetic causes in undiagnosed patients presenting with clinically heterogeneous cardiac disorders.
- Rare, predicted deleterious variants were identified in genes that make interesting MVP candidate genes based on their apparent biological context.
- Many of these genes are expressed in the developing heart, and some may be developmentally regulated.
- We plan to determine whether WES-identified candidate variants segregate with disease in affected family members.
- We will validate candidate genes by doing functional studies in animals or cell lines - ex. Knockdown of NUP43 in zebrafish or cultured cardiac cells.
- We plan to directly examine MVP patient tissue to determine pathophysiological characteristics associated with specific genotypes.

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