Identifying Genetic Determinants of Mitral Valve Prolapse

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INTRODUCTION

Mitral 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, Loeys-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.

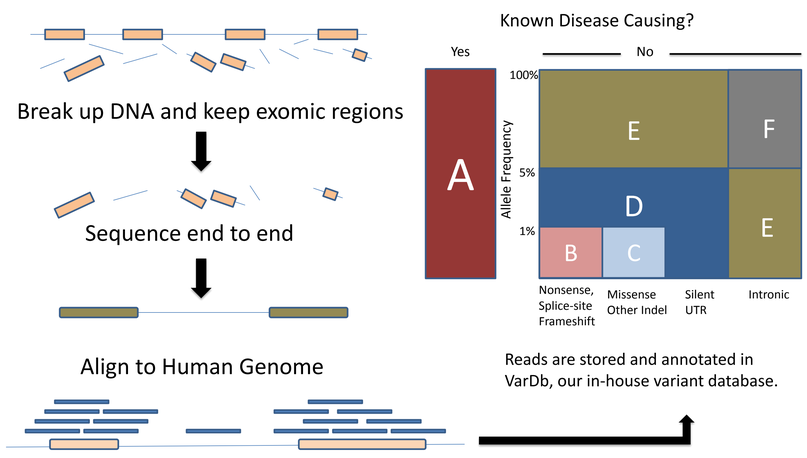
METHODS

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 ***N***orth ***C***arolina ***G***enomic ***E***valuation by ***N***ext Generation ***E***xome ***S***equencing clinical trial, ***NCGENES***. We are using bioinformatics-based as well as biological tools to evaluate candidate MVP genes and variants.

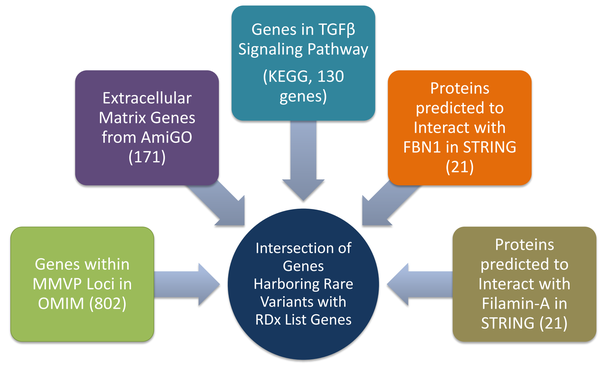
We examined three patients with varying presentations of MVP who were suspected of having a genetic cause based on age of onset, severity, and family history.

- **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 Loeys-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.

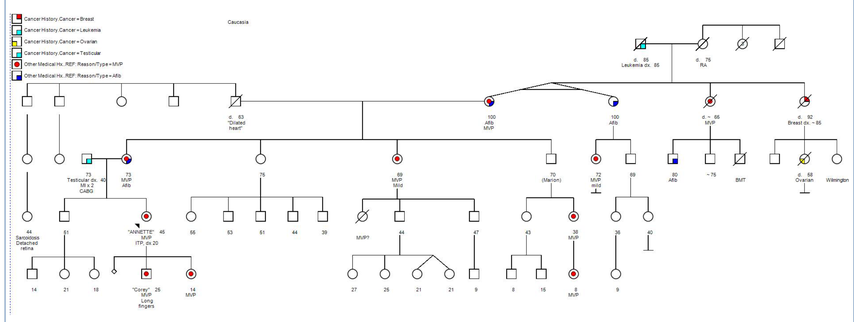
WES workflow and Variant Calling Pipeline



- Automated custom workflow created by RENCI returns all variants in known disease genes, A-F
- No variants with MAF <.001 that could explain the patients' phenotypes were found in 7 genes known to be associated with MVP: *ACTA2, CBS, COL3A1, FBN1, FBN2, MYHL1, and MYLK*
- We queried the variant database using Python to return all potentially deleterious variants with MAF <.001 in the entire exome. These variants were manually curated.
- Relevant parameters include MAF in 1000 genomes and Exome Variant Server, ConDel score, published gene information, Gene Ontology and Function, and Protein Domains Affected
- We generated Research Diagnostic (RDx) Lists of Genes from *in silico* databases in order to systematically evaluate variants in genes that could be associated with MVP.



NCG_00261 Summary of Linkage Analysis and WES-identified candidate variants



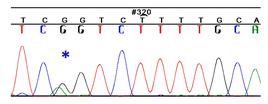
A linkage analysis was done using an snp chip to genotype NCG_00261 and her family members. **rs4663726** obtained significant LOD score in all 4 family schemes we analyzed.

- rs4663726 Falls within *COL6A3* gene on 2q37.3
- 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)

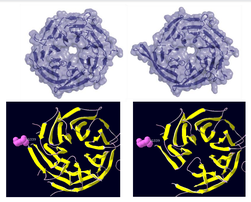
HGNC GENE	Description	Variant	In Silico Predictions & Domains Affected	On RDx List	Conserved Residue	ConDel Score	EVS MAF	Phenotype Association
PALLD	Palladin	E446Ter	Numerous domains affected which may disrupt interactions with VASP, SRC and ezrin at focal adhesions	No			0	Actin regulatory protein; No conclusive demonstration of disease association in humans
HABP2	Hyaluronan binding protein 2	R203Q	Within Kringle domain; Mutation taster predicts disease-causing	No	Highly conserved	0.865	0	Polymorphisms in this gene have been associated with carotid stenosis and venous thromboembolism
SOWAHB (ANKRD6)	Ankyrin repeat domain containing protein	R13H	Within an ankyrin repeat normally involved in protein interactions; Poly-phen2 predicts probably damaging	No	Highly conserved	0.932	0.0013	Affects Planar cell polarity in Drosophila and fusion of cardiac precursors in zebrafish
IQGAP1	IQ motif containing GTPase activating protein 1	I1071F	Within Ras-Gap domain; Mutation taster predicts disease-causing	Filamin-A STRING	Highly conserved	0.836	0	Regulates integrin-mediated cell migration and adhesion via interaction with filaminA
FAT3	FAT Tumor suppressor homolog 3	A3418S	Within cadherin domain; Poly-phen-2 predicts benign; Mut. Taster predicts disease-causing	No	Highly conserved	0.966	0.002	Paralog of *DCHS1*; Atypical cadherin; Mouse mutant has abnormal dendritic morphology; authors did not look at heart

NCG_00157

- In NCG_00157, we identified a heterozygous truncating variant in *NUP43* found at MAF .0001 in exome variant server.
- NUP43 is a component of the Nuclear Pore Complex which controls nucleo-cytoplasmic transport.
- **Hypothesis**: Impaired transport of TGFβ signaling molecules such as Smads may contribute to MVP/TAA



Sanger confirmation of WES-identified nonsense variant



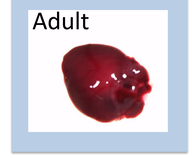
Two models showing the resulting truncated protein (right side in both panels) would be missing several β strands found at the 3' end of wild-type *NUP43* (left side in both panels), thereby affecting its surface structure.

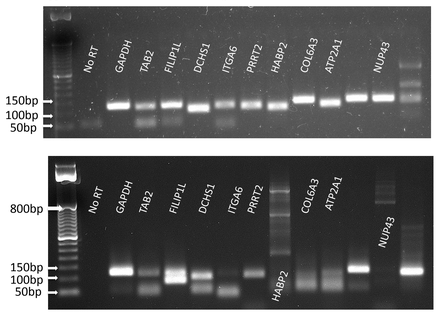
NCG_00354

Summary of candidate WES-identified variants

HGNC GENE	Description	Variant	In Silico Predictions & Domains Affected	On RDx List	Conserved Residue	ConDel Score	EVS MAF	Phenotype Association
TAB2	TGF-beta activated kinase 1/MAP3K7 binding protein 2	R347Ter	Missing NZF domain which binds Ubiquitin	TGF-beta Signaling genes			0	Outflow tract defects; aortic stenosis and cardiac arrhythmias; residual aortic regurgitation; HF; bicuspid aortic valve; aortic dilation
FILIP1L	filamin A interacting protein 1-like	R156Ter	Coiled-coil region; low complexity	No			0	FLNA is assoc. with X-linked cardiac valvular dysplasia; When overexpressed in endothelial cells, FILIP1L leads to inhibition of cell proliferation and migration and an increase in apoptosis.
ATP2A1	SERCA1; cardiac muscle; fast-twitch	R989L	Very near 3' end of protein; Cytoplasmic domain per Uniprot; Not in MSV3d; Mut. Taster says DP	16p12.1-11.2 MMVP1 locus	Highly Conserved	0.99	0	May be associated with AR Brody Myopathy; SERCA defects assoc. with a number of conditions: HF, sperm motility defects, cataracts, carcinogenesis, diabetes, cardiac hypertension and hypertrophy
COL6A3	Collagen type 6, alpha 3	D253H	VWA -MIDAS (metal ion mediated cell adhesion); Not in MSV3d; Mutation Taster says Disease Predicting	ECM	Highly Conserved	0.718	0	Bethlem myopathy; Ulrich congenital myopathy, neither of which have MVP, but do have joint problems; Collagen abnormalities are often present in both MVP and Joint Hypermobility
DCHS1	cadherin superfamily of cell-cell adhesion molecules	S667C	Cadherin-6 domain; MSV3d= VUS; Sift (D); Mutation taster says disease predicting;	11p15.4 MMVP2 locus	Highly conserved	0.896	.00035	Per S. Slaughenhaupt at HMS, MVP is part of mutant phenotype; atrial septal defect in knockout mouse
ITGA6	Integrin alpha 6	K1082N	"GFFKR" motif, involved in α and β subunit stabilization; Not in MSV3d; MT says DP	FBN1 String	Conserved but not in Chicken	0.764	0	Integrins are known to participate in cell adhesion as well as cell-surface mediated signaling; OMIM=Junctional Epidermolysis Bullosa with Pyloric Atresia
PRRT2	Proline-rich Transmembrane protein 2	D143E	Low complexity	16p12.1-11.2 MMVP1 locus	Not particularly conserved	0.653	0	OMIM=Seizures, dyskinesia; interacts with Snap-25
HABP2	Hyaluronan Binding Protein 2	D87Y	EG-like domain	No	Not Conserved	0.666	0	OMIM=one variant assoc. with reduced activation of pro-urokinase and strong risk for carotid stenosis; PLAU has been linked to MVP; some literature exists re:hyaluronan and valve formation, MVP

Candidate MVP genes in embryonic and adult mouse heart





Total RNA was isolated from embryonic or adult mouse hearts and 1µg was reverse transcribed into cDNA. 75ng of cDNA was used as template in PCR to screen for expression of a subset of candidate genes in relation to the housekeeping gene, *GAPDH*

CONCLUSIONS & 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 pathological characteristics associated with specific genotypes.

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