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Considerations for Cardiovascular Family Studies

The primary goal of the cardiology family studies program is to obtain segregation data that will ultimately aid in the reclassification of variants of unknown significance (VUS) as either pathogenic or benign. As such, the cases approved for family studies are those that will be most informative in the variant classification process. Please note that the decision to approve or deny family studies occurs in the context of the particular variant and family.

Informative Family Members

The following family members are most likely to be informative for segregation analysis:

Affected individuals

Family members with the expected phenotype for the gene(s) requested, with clinical documentation of diagnosis (ideally, meeting established diagnostic criteria), and with few or no confounding comorbidities that may complicate diagnosis.

- Example, likely to be informative: In the case of an *MYH7* VUS, a family member has a diagnosis of hypertrophic cardiomyopathy and cardiovascular imaging demonstrating severe ventricular hypertrophy.
- Example, likely to be informative: In the case of a *KCNQ1* VUS, a family member with exercise-induced syncope and prolonged QTc interval on ECG.
- Example, unlikely to be informative: In the case of an *MYH7* VUS, a family member with long QT syndrome, or 'heart attack' at an older age without evidence of primary cardiomyopathy.
- Example, unlikely to be informative: In the case of a *COL3A1* VUS in a proband without clinical diagnosis of vascular EDS, a family member with only mild connective tissue features, such as easy bruising and some hypermobility of small joints, but no major criteria for vascular EDS.

Unaffected individuals

- Due to incomplete and age-related penetrance observed amongst cardiovascular disease genes, consideration of unaffected individuals may be disease, gene, and/or family specific. However, in most cases, family members older than 70 years with documentation of normal clinical evaluations for the disease(s) in question are more likely to be informative, and are more informative following demonstration of VUS co-segregation with disease in *affected* family members.
- For genes with significant rates of *de novo* occurrence and appropriate disease association (e.g., *RYR2* and CPVT, *FBN1* and Marfan syndrome), testing of parents may be approved provided that they are documented to be unaffected and there is no family history of the disease(s) in question.

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Documentation of Clinical History

To ensure accurate interpretation of results, additional clinical information will be requested prior to family studies approval. Some examples include:

- In the case of an *MYBPC3* VUS, we will request cardiovascular imaging records, such as echocardiogram, to confirm individuals are affected.
- In the case of an *LDLR* VUS, we will request serum lipid records to confirm that an unaffected relative does not have significantly elevated LDL cholesterol levels.
- In the case of a *COL5A1* VUS, if the proband has a relative with a clinical diagnosis of Marfan syndrome while the proband has mild aortic dilation, we may require documentation that *FBN1* testing was performed in the relative to avoid falsely attributing the clinical history to the *COL5A1* VUS.

Inheritance pattern

Testing of VUS will be considered in conjunction with appropriate inheritance pattern.

- Example: If an X-linked VUS is detected in a male proband, the proband's paternal relatives would not be considered for testing.

Exclusions & additional considerations

Clinically actionable alterations, such as mutations and likely pathogenic variants, are not eligible for family studies.

Single VUS detected in autosomal recessive genes are not eligible for family studies (unless co-occurring with a mutation or VLP in the same gene). If more than one VUS detected in an autosomal recessive gene, parental testing and/or testing of affected relatives to determine phase may be considered on a case-by-case basis.

VUS that are expected to result in haploinsufficiency (e.g., gross deletions/duplications, nonsense, frameshift, splicing alterations) in genes where loss of function is not established as a mechanism of disease are not automatically eligible for family studies, but may be considered on a case-by-case basis.

Segregation for VUS with certain characteristics may not be informative and are considered on a case-by-case basis. Some examples include the following:

- VUS occurring in regions of a gene that are not expressed in the appropriate tissue (e.g., brain-specific exon 38 of *ANK2*)
- Missense VUS occurring in a large gene with high VUS burden in which the mechanism of disease is loss of function (e.g., missense *TTN* VUS with limited evidence for/against pathogenicity)
- VUS with high general population frequency or frequency in unaffected cohorts
- Gene involved is not known to have primary phenotype consequences consistent with the proband's/family member's presentation

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