The Road Not Taken: Exome "Results" That Were Not Returned

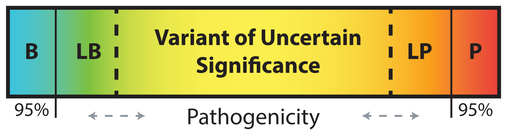
N.T. Strande[1,2], G.T. Haskell[1], J.K. Booker[1,2], A.K.M. Foreman[1], K. Lee[1], J.M. O'Daniel[1], B.C. Powell[1], B.A. Seifert[1], J.P. Evans[1], K.E. Weck[1,2], J.S. Berg[1]

[1]Department of Genetics, UNC-Chapel Hill, [2]Department of Pathology & Laboratory Medicine, UNC-Chapel Hill

Introduction

Massively parallel sequencing (MPS) is now increasingly employed in the clinic for multi-gene diagnostic purposes and research settings, unveiling an unprecedented number of unique sequence variants. A significant challenge is therefore interpreting the results at both the variant level and the case level.

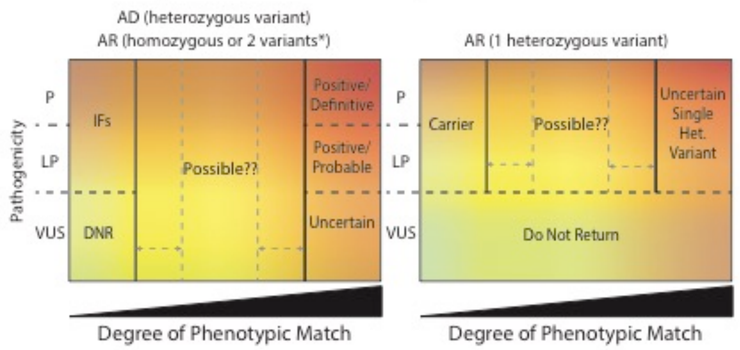
Variant Level Interpretation



Variant pathogenicity is described using the above five categories in the ACMG sequence variant interpretation guidelines.

- Multiple criteria required to determine pathogenicity
- Lots of variants are considered to be of "uncertain clinical significance" (VUS)
- Variability in the threshold for VUS between laboratories

Case Level Interpretation



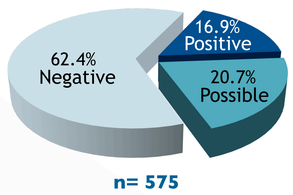
The above figure illustrates the complexity associated with interpreting results at the case level and the subjectivity that goes into determining each threshold. The following factors influence the overall outcome of a particular case:

- Variant pathogenicity
- Whether the suspected inheritance pattern in the family is consistent with the disease-gene in consideration
- Phenotypic match between the patient and phenotype associated with the gene-disease pair

NCGENES

The North Carolina Clinical Genomic Evaluation by Next-generation Exome Sequencing (NCGENES) project examines the application of whole exome sequencing (WES) in a diagnostic context among a diverse population of adult and pediatric patients with heterogeneous clinical indications.

Overall Diagnostic Results



n= 575

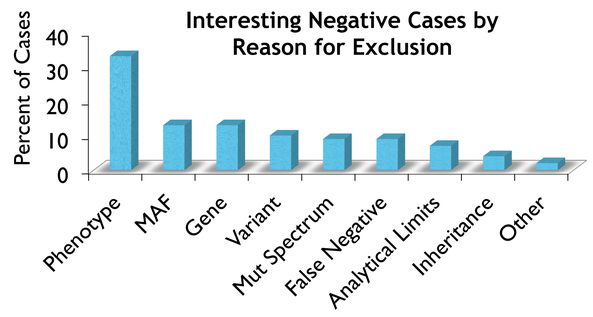
Methods

Potential candidate variants were discussed by a committee of diverse genetics professionals prior to making a final decision for the overall case-level result. Notes regarding these discussions were recorded and used to evaluate our negative cases. The following are typical reasons a result may not have been reported:

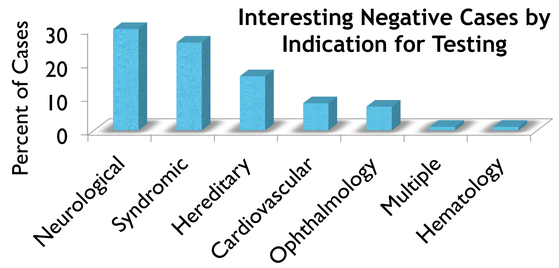
1. Limited evidence supporting variant pathogenicity
2. Limited evidence supporting a causal role for the gene in a Mendelian disorder
3. Variant outside the predicted mutation spectrum for the gene-disease pair
4. Minor allele frequency higher than expected for the Mendelian disorder
5. Suspected inheritance pattern was not consistent with gene associated with an disease
6. Patient's phenotype was not consistent with the phenotype associated with the gene-disease pair

Results

Approximately 24.5% of our 363 negative cases had one or more variants that were heavily discussed but ultimately not returned (89/580 or 15.4% of total cases).



Sub-threshold "results" by indication for testing varied to some extent, possibly reflecting the different number and nature of genes comprising the diagnostic gene lists used to filter WES variants.



Examples

Cardiovascular Disorders:

- Case 1: Poor gene-disease association
  - 51yr old AA male with non-ischemic DCM @ 39
  - Identified a VUS in *ABCC9*
  - *ABCC9* - ATP-binding cassette, subfamily C, member 9 or sulfonylurea receptor 2
    - Forms part of a potassium ion channel
    - Associated with hypertrichotic osteochondrodysplasia
    - A single report associates *ABCC9* with DCM and atrial fibrillation

Neurological Disorders:

- Case 2: Poor phenotypic Match
  - 68 yr old female with Charcot-Marie Tooth @ 50
  - Identified a heterozygous *POLG* missense variant that had been previously reported for progressive external ophthalmoplegia
  - *POLG* – DNA polymerase gamma

Conclusions

One of the many challenges with interpreting genomic data is setting a consistent threshold for calling result positive or negative. These internal thresholds can impact a laboratory's overall diagnostic yield, therefore we recommend that laboratories provide the following information when reporting results:

- The laboratory's analytical limitations
- The potential types of "results" that are not returned to the ordering clinician
- The thresholds used to inform return of results
- The criteria used to define these thresholds

It is also important that the testing lab have access to the patient's phenotypic information or a working relationship with the clinician.

References

Richards, S. *et al.* Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* **17**, 405-24 (2015).

Acknowledgements

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