Segregation analysis offers a mechanism for variant reclassification in a small subset of cases but is especially powerful in classifying deleterious mutations

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Introduction

Accurate classification of variants in regards to their clinical significance is a critical challenge associated with gene sequencing tests, with this challenge expected to increase dramatically as next generation sequencing technologies are more widely used. Thus, it is important to evaluate the effectiveness of various strategies for variant reclassification.

At Myriad Genetic Laboratories Inc., we utilize multiple lines of evidence to evaluate and reclassify variants of uncertain significance (VUS), and new classification methods are continually being assessed by Myriad scientists. Here we describe the use of segregation analysis for the reclassification of variants in the genes associated with Hereditary Breast and Ovarian Cancer syndrome (HBOC). We report on the participation rate of Myriad's family testing for segregation analysis through its Variant Classification Program.

We also show that for the discovery of benign variants, segregation analysis is a comparatively weak method compared to other methodologies developed at Myriad. However, for the identification of truly deleterious mutations, we have demonstrated that segregation analysis is a powerful method and compliments the other variant reclassification methods employed at Myriad.

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Reclassification Methods in Myriad's Variant Classification Program

MYRIAD

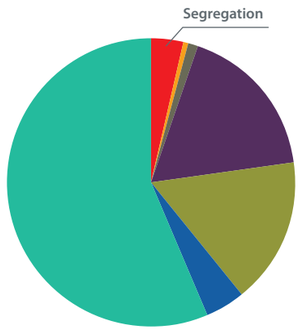
- **History Weighting Algorithm** - Steps 1 & 2 needed
- **Mutation Co-Occurrence** - Steps 1 & 2 needed
- ***in trans*** - Steps 1, 2 & occasionally 9 needed
- **Segregation** - Steps 1 - 10 needed

PUBLIC DATA

- **Literature Review**
- **Population Frequency**
- **Evolutionary Conservation**

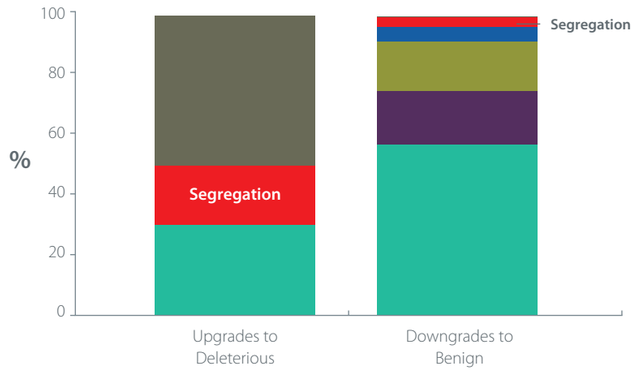
Frequency of use by Reclassification Method

Oct 2011 – Nov 2012



Percentage of Reclassification Upgrades and Downgrades Achieved by Each of Myriad's Reclassification Methods

Oct 2011 – Nov 2012



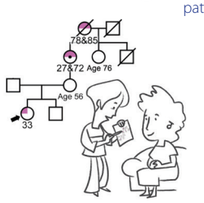
Relative Effectiveness of Reclassification Methods by Proband Count

Oct 2011 – Nov 2012

Reclassification Method	Average Number of Probands per Downgrade & (minimum)**	Average Number of Probands per Upgrade & (minimum)**
in trans	17.5 (1 min)	NA
Mutation Co-Occurence	31.2 (2 min)	NA
Segregation	41.8 (9 min)	32 (13 min)
History Weighting Algorithm	19.4 (6 min)	33.7 (28 min)
Evolutionary Conservation	15.4 (NA)	NA
Literature Review	7 (NA)	12.6 (NA)
Population Frequency	8.5 (NA)	NA

** These represent the real life minimum data requirements necessary for reclassification in the Oct 2011 – Nov 2012 time period. They do not necessarily represent theoretical minimums.

1. Clinician identifies patient for genetic testing.

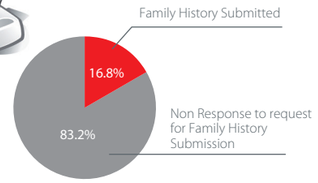


2. Sample with clinical history sent to Myriad and a VUS is detected[1,2].

3. VUS report sent to clinician with invitation to submit a detailed pedigree.

4. Clinician discloses VUS result and collects detailed pedigree.

5. 16.8% of clinicians receiving VUS or Favor Polymorphism results submit a detailed pedigree to Myriad for further evaluation.



Oct 2011 – Aug 2012

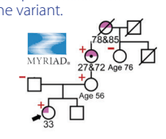
6. Myriad's scientists evaluate each pedigree and select additional family members for variant testing. On average, 2.3 relatives per proband are offered testing.

7. Myriad mails the clinician the no-cost family testing offers.

8. The clinician provides the patient with the family testing offers.

9. 24% of targeted family members accept the no-cost variant testing offers for segregation analysis.

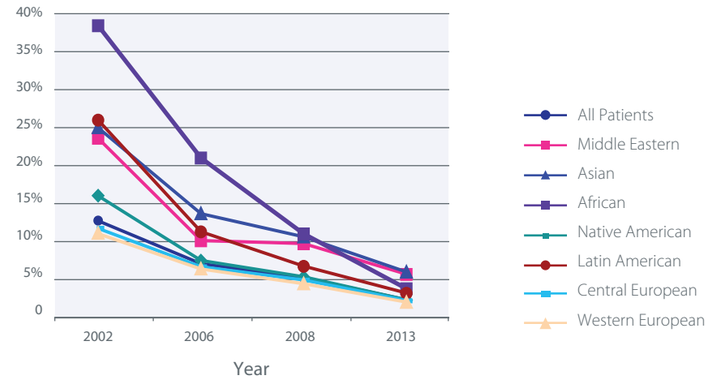
10. Segregation data is gathered from multiple families with the same variant.



11.

A variant is reclassified from "VUS"to "Suspected Deleterious" when one of the reclassification methods achieves significance. The variant is further upgraded to "Deleterious" when a different reclassification method achieves significance.

Decline in Rate of *BRCA1/2* Variants of Uncertain Significance found in USA Patients



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Conclusions:

The majority of *BRCA1/2* VUSs are discovered to be benign through a variety of methods, with History Weighting Algorithm being the most robust method. Segregation analysis shows particular power in identifying deleterious variants rather than benign variants. Considering laboratories and community research centers have finite resources, these data therefore suggest that the tailoring of family analysis to specific families with higher likelihoods of having a deleterious mutation may be the most productive use of resources.

References

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2. Frank TS et al. J Clin Oncol. 2002. 20(6):1480-1490.
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4. Easton DF et al. Am J Hum Genet. 2007. 81(5):873-883.

Concurrent ESHG 2013 Poster number P11.047: "A Clinical History Weighting Algorithm Accurately Classifies *BRCA1* and *BRCA2* Variants, Bowles et al".

*All authors are employees of Myriad Genetics, Inc., and Myriad Genetic Laboratories, Inc. and receive salary and stock options as compensation.

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