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BS1/BA1 thresholds derived from disease-specific genetic architectures result in decreased rates of VUS in clinically relevant genes

Matthew P. Johnson[1], Ashley P. L. Marsh[1], Laura I. Hudish[1], Marcy E. Richardson[1], Steven M. Harrison[1]
[1] Ambry Genetics

BACKGROUND

- Variants observed at frequencies higher than expected for a given disease relationship (GDR) are considered for strong (BS1) or stand-alone (BA1) lines of evidence towards benign classification.
- Previous guidance recommended the use of a generalized allele frequency approach for characterized diseases. This is particularly true for BA1 (a variant with a frequency > 5%).[refs 1, 2] For BS1, a generalized approach may be based on the definition of a "polymorphism" (i.e., a variant with a frequency > 1%).
- However, generalized approaches do not account for variation in the underlying disease-specific architectures (DSA; i.e., mode of inheritance, disease prevalence and penetrance, genetic and allelic heterogeneity) and may result in conservative classifications and a high rate of variants of uncertain significance (VUS).
- Hypothesis: The application of BA1/BS1 evidence, when calculated as a function of DSA, will result in decreased rates of VUS in clinically relevant genes relative to a generalized approach.

METHODS

Using the statistical framework described by Whiffin et al. 2017[ref 3] and DSAs, we established GDR-specific BS1/BA1 thresholds.

Calculated BS1 thresholds were tested against filtering allele frequencies (FAFs) (gnomAD v2.1.1) for published and internally classified pathogenic variants.

Single nucleotide variants (SNVs; exonic and flanking ±5bp intronic), with a current classification of VUS, likely benign (LB) or benign (B) were analyzed in a dataset of 12 genes.

To determine clinical impact, the reduction in VUS rates using GDR-specific BS1/BA1 thresholds were compared to VUS rates utilizing a generalized approach across a 2-to-3-year period.

Variants were identified at a commercial laboratory as part of routine multigene panel testing.

REFERENCES

1. Richards S, et al. (2015) *Genetics in Medicine* 17(5), 405-424.
2. Ghosh R, et al. (2018) *Human Mutation* 39(11), 1525-1530.
3. Whiffin N, et al. (2017) *Genetics in Medicine* 19(10), 1151-1158.

RESULTS

- A total of 6,114 eligible SNVs (VUS, LB, B) from 12 genes were assessed:

Gene	HGNC	Phenotype Category	Disease	Start Date	End Date	Eligible Variants (n)
MLH1	7127	Oncology	Lynch syndrome	JAN 2021	JAN 2024	380
MSH2	7325	Oncology	Lynch syndrome	JAN 2021	JAN 2024	973
MSH6	7329	Oncology	Lynch syndrome	JAN 2021	JAN 2024	907
PMS2	9122	Oncology	Lynch syndrome	JAN 2021	JAN 2024	581
BRCA1	1100	Oncology	HBOC*	FEB 2021	FEB 2024	730
BRCA2	1101	Oncology	HBOC*	FEB 2021	FEB 2024	1,432
KCNQ1	6294	Cardiovascular	Long QT syndrome	FEB 2022	FEB 2024	46
KCNH2	6251	Cardiovascular	Long QT syndrome	FEB 2022	FEB 2024	79
SCN5A	10593	Cardiovascular	Long QT syndrome	MAY 2021	MAY 2024	168
LDLR	6547	Cardiovascular	FH#	MAY 2021	MAY 2024	65
APOB	603	Cardiovascular	FH#	MAY 2021	MAY 2024	635
PCSK9	20001	Cardiovascular	FH#	MAY 2021	MAY 2024	118

* HBOC: Hereditary Breast and Ovarian Cancer; # FH: Familial Hypercholesterolemia

- An overall reduction of 4.43% in VUS was observed for the DSA approach, which contrasts with 0% for the generalized approach:

Gene	Eligible Variants# (n)	BS1 Threshold^ (%)	Gene-Disease Approach (FAF > BS1 or BA1)		Generalized Approach (FAF > BS1 [1%] or BA1 [5%])	
			VUS to LB/B (n)	VUS Rate Reduction (%)	VUS to LB/B (n)	VUS Rate Reduction (%)
ALL GENES	6,114		271*	4.43	0	0
MLH1	380	0.01	17	4.47	0	0
MSH2	973	0.01	24	2.47	0	0
MSH6	907	0.022	22	2.43	0	0
PMS2	581	0.028	9	1.55	0	0
BRCA1	730	0.01	16	2.19	0	0
BRCA2	1,432	0.01	41*	2.86	0	0
KCNQ1	46	0.006	6	13.04	0	0
KCNH2	79	0.003	22	27.85	0	0
SCN5A	168	0.0125	36	21.43	0	0
LDLR	65	0.02	9*	13.85	0	0
APOB	635	0.02	51	8.03	0	0
PCSK9	118	0.02	18	15.25	0	0

SNVs with a current classification of VUS, LB or B
^ BA1 thresholds were set at one-order-of-magnitude higher than BS1.
* 5 variants (3 *BRCA2*, 2 *LDLR*) with FAF > BS1 or BA1 remained VUS due to conflicting evidence in the pathogenic direction.

- The reduction in VUS rates achieved using the gene-disease specific thresholds impacted 20,489 individuals, either by a VUS being downgraded or a variant not being reported as it was classified as LB/B.

TAKE HOME POINTS

- VUS rates were reduced by 4.43% when BS1/BA1 thresholds were calculated as a function of the disease-specific architecture.
- Improved accuracy of genetic risk reduced the clinical and psychological burden associated with an uncertain test result in 20,489 individuals across a 2-to-3-year period.