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

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Introduction: A variant observed in the general population at frequencies higher than expected for a given gene-disease relationship (GDR) is considered a strong (BS1) or stand-alone (BA1) line of evidence towards benign classification. Previous guidance recommended the use of generalized allele frequencies (e.g., BS1 at 1%, BA1 at 5%) for all characterized GDRs. This generalized one-size-fits-all approach, however, does not account for variation in the underlying disease-specific architectures and therefore may result in overly conservative classifications, resulting in a high burden of variants of unknown significance (VUS). To account for this biological variation, we posit that the application of BS1/BA1 evidence, when calculated as a function of disease-specific architecture, will result in decreased rates of VUS. *À la carte* population frequency thresholds are incorporated into several ClinGen Variant Curation Expert Panels (VCEPs); however, the clinical impact of this approach does not appear to be well documented, particularly in the setting of a large commercial testing laboratory, across a diverse set of clinically relevant genes.

Methods: The mode of inheritance, disease prevalence and penetrance, and heterogeneity (genetic and allelic) for independent GDRs were used to establish the maximum credible population allele frequency (BS1) thresholds using the statistical framework described by Whiffin et al. 2017; BA1 thresholds were set at one-order-of-magnitude higher. Calculated BS1 thresholds were stress tested against filtering allele frequency thresholds (gnomAD v2.1.1) for credible pLoF variants, published disease-causing variants and internally classified pathogenic/likely pathogenic variants. Single nucleotide variants (SNVs; exonic and flanking ±5bp intronic), with a current classification of VUS, likely benign or benign within a defined time window, were analyzed in a preliminary dataset of 7 genes, as a proof-of-concept, to determine the rate of VUS reduction using these gene-disease specific thresholds compared to a generalized approach. Variants were identified at a commercial laboratory as a part of routine genetic testing. To determine clinical impact, the reduction in VUS rates using gene-disease specific thresholds were compared to VUS rates utilizing a generalized approach across a 2-to-3-year period.

Results: A total of 3,134 eligible SNVs from 7 genes were assessed: *MLH1* (HGNC:7127), *MSH2* (HGNC:7325), *MSH6* (HGNC:7329), *PMS2* (HGNC:9

22) (causes of Lynch syndrome-2 year period from 1/2021 to 1/2024); *KCNQ1* (HGNC:6294), *KCNH2* (HGNC:6251) (causes of long QT syndrome or LQTS-3 year period from 2/2022 to 2/2024); and *SCN5A* (HGNC:10593) (also a cause of LQTS-3 year period from 5/2021 to 5/2024). The BS1 thresholds based on the underlying disease-specific architecture were 2-to-3 orders-of-magnitude lower than those utilized under a generalized approach (BS1 at 1%). Under a gene-disease specific threshold approach, an overall reduction of 4.34% (136/3134) in VUS rates was observed for BS1/BA1 eligible downgrades within the stated time window. This contrasts with a generalized approach, where no decrease in VUS rates was observed (0/3134) within the same time window. The reduction in VUS rates achieved using the 7 gene-disease specific thresholds impacted 12,073 individuals, either by a VUS being downgraded or a variant not being reported as it was classified as likely benign or benign.

Conclusion: Here we show that the application of BS1/BA1 evidence, when calculated as a function of the known disease-specific architecture, decreases the rate of VUS in clinically relevant genes compared to a generalized approach. This reduction in VUS impacts many individuals, reducing the burden associated with receiving an uncertain test result.