TITLE: Development of population frequency thresholds specific for rare neurodevelopmental disorders

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Variant classification at scale requires accurate population frequency thresholds for application of benign population evidence codes and determination of a variant's rarity. Current approaches for calculating population frequency thresholds require at least determination of disease prevalence and/or characterization of the population frequency of pathogenic variants. However, these approaches have limited utility for rare and newly characterized diseases, particularly neurodevelopmental disorders, which tend to be characterized by variable expressivity, high genetic heterogeneity, and relatively non-specific clinical features. (For such disorders, prevalence is often unknown, and there may be a paucity of characterized pathogenic variants.) To address this, we developed a set of binned population frequency thresholds for neurodevelopmental disorders based on mode of inheritance, penetrance, severity, and *de novo* rate or estimated prevalence. We subsequently refined these criteria with the intention of applying them on a per-gene basis, using the frequencies and/or allele counts of disease-associated variants found in gnomAD v4.1.0. A proof-of-concept study based on publicly available data in gnomAD and ClinVar demonstrates that these population frequency thresholds can discriminate well between disease-associated variants and benign variants, and that their application can result in a decreased rate of variants being classified as Variants of Uncertain Significance (VUSs). For example, for *TBL1XR1*, which is associated with two moderately severe autosomal dominant neurodevelopmental disorders in which pathogenic variants are commonly found *de novo*, no variants (0 out of 105) classified as Pathogenic or Likely Pathogenic in ClinVar meet the threshold for assigning benign population weight based on data in gnomAD v4.1.0, whereas 36% of ClinVar Likely Benign/Benign variants and 10% of ClinVar VUSs meet this threshold. For *ZNF142*, which is associated with a rare, moderately severe autosomal recessive neurodevelopmental disorder, no variants (0 out of 25) classified as Pathogenic or Likely Pathogenic in ClinVar meet the threshold for assigning benign population weight based on data in gnomAD v4.1.0, whereas 39% of ClinVar Likely Benign/Benign variants and 9% of ClinVar VUSs meet this threshold. In conclusion, the application of binned population frequency thresholds for neurodevelopmental disorders, refined with gene-specific criteria, can effectively discriminate between disease-associated and benign variants, and can thereby reduce the number of variants classified as VUS.