A199 · Leveraging Mutational Coldspots to Build an Atlas of Variant Effects

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Approximately 90% of missense variants in ClinVar remain classified as Variants of Uncertain Significance (VUS), limiting their clinical utility for diagnosis and risk assessment. Although most VUS are predicted to be benign, the current clinical variant classification framework provides limited opportunities to accumulate sufficient benign evidence for reclassification. While mutational hotspots are well-characterized, mutational coldspots—regions enriched for benign missense variation—remain underutilized across disease-associated genes. Here, we present a multivariate Hidden Markov Model (mvHMM) trained on the established BRCA1 exon 11 coldspot to identify similar mutationally tolerant regions across genes by integrating four unsupervised predictive and evolutionary tracks: AlphaMissense, ESM1b, EVE, and phyloP. Applied across 2,748 disease-associated genes with at least one pathogenic missense variant in ClinVar, the mvHMM identified 2,729 coldspots across 1,336 genes, capturing 17% of ClinVar VUS. In ClinVar, coldspots contained 26% of benign variants but only 0.58% of pathogenic variants, corresponding to an odds ratio (OR) of 60.3. Independent validation using experimentally derived functional data showed that 43% of functionally normal variants fell within predicted coldspots, compared with 1.4% of functionally abnormal variants (OR = 50.08). Coldspot membership yielded a negative likelihood ratio (LR–) of 0.022, corresponding to very strong benign evidence under the Bayesian adaptation of the current clinical variant classification framework. Incorporating coldspot membership as strong benign evidence reduced VUS by 95% among ClinGen-curated variants and could provide additional evidence for reclassification of 85,929 VUS across these disease-associated genes.