Multiplex Assay of Variant Effects for Kidney Disease. Exhaustive Contextual Measurement of *AGXT* Missense Effects and Development of *VHL* Functional Assays

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Abstract

Nearly every possible single nucleotide variant (SNV) compatible with life already exists in someone alive today. While a small fraction of SNVs is common, e.g. are present in greater than 1% of alleles in human populations, the vast majority have yet to be observed. For common (or even moderately rare) variants, evidence of pathogenicity can come from statistical association tests or single variant testing. However, single-variant testing must be more efficient to keep pace with variant discovery. This makes pathogenicity difficult to assess for most SNVs, and indeed, most missense variants are found to be “variants of uncertain significance” (VUSes). Therefore, as rare variants continue to be discovered, there is an urgent need to establish variant-phenotype relationships at the same speed as VUSes are being reported.

Recent improvements in synthetic biology and DNA sequencing have enabled high-throughput testing of all possible variants within genomic regions associated with genetic disease. A well-established multiplex assay of variant effect (MAVE) strategy can provide strong evidence for clinical variant interpretation.

To provide evidence for variant interpretation, I tested variants for the alanine glyoxylate aminotransferase (*AGXT)* and von Hippel Lindau (*VHL)* genes in large-scale genes causing different forms of renal disease. I used a deep mutational scanning (DMS) approach, making nearly all possible missense variants of these genes.

Because *AGXT* variants' effects can depend on context, I measured variant impact on the reference (major) allele and *in cis* with a common (minor) allele known to have a functional impact. For both major and minor alleles, I also systematically measured the impact of adding vitamin B6 (VB6), the precursor to pyridoxal 5'-phosphate (PLP), since PLP is a co-factor of the AGT enzyme, which can serve as a stabilizing molecular chaperone for some variants.

In parallel, I developed scalable variant impact assays based on the fitness of cell lines carrying different *VHL* variants and via two scalable assays for the ability of *VHL* variants to degrade two known *VHL* substrates (hypoxia-inducible factor or *HIF*) that are important for oxygen sensing in human cells.