Quantifying phenotypic specificity (PP4) of rare germline *FH* variants from diagnostic laboratory testing for HLRCC and renal cancer

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Background:

Under the current ACMG/AMP variant classification guidelines for PP4, there is often uncertainty and inconsistency in evidence weighting for very rare variant (VRV) observations. This is particularly the case where variants can be identified in both highly specific and less specific phenotypes, for example identification of very rare *FH* variants in HLRCC (Hereditary Leiomyomatosis and Renal Cell Cancer) versus unselected renal cancer.

Methods:

We collated germline testing data from four diagnostic laboratories (3 UK, 1 USA (Ambry)) for 387 affected probands who would meet the eligibility criteria for R365 (Fumarate hydratase-related tumour syndromes), and for 1,780 patients with renal cancer who otherwise underwent FH testing as part of a renal panel. We defined a VRV as a variant with an allele frequency of <0.000015 (calculated MTAF), i.e. plausibly pathogenic. We compared the frequency of VRVs in each phenotypic cohort against a total of 562,295 controls to calculate pan-gene likelihood ratios (PG-VRV-LR), and performed spatial clustering analysis to identify enriched regions of very rare missense variants within the gene.

Results:

For HLRCC, the PG-VRV-LR was estimated to be 2,669.4 (95% CI: 1,843.4-3,881.2, LLR 10.77) for truncating variants and 214.7 (185.0-246.9, LLR 7.33) for missense variants. For renal cancer, the PG-VRV-LR was 95.5 (48.9-183.0, LLR 6.23) for truncating variants and 5.8 (3.5-9.3, LLR 2.39) for missense variants. Clustering analysis of very rare missense variant enrichment in HLRCC cases revealed three ‘hotspot’ regions wherein the domain-specific LR increased to 1226.9.

Conclusions:

These analyses illustrate the highly enriched association between putatively pathogenic variants and a highly specific phenotype versus that for a less specific phenotype. These data provide quantitative measures which may be applicable in clinical variant classification for very rare variants in FH. This methodology is potentially applicable in other diseases to quantify the specificity of the relationship between phenotype, gene, and variant type.