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

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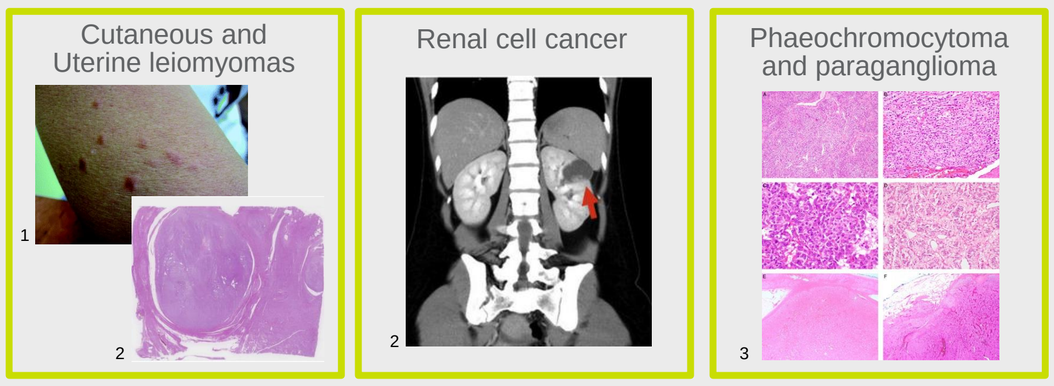
Introduction

Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC) is a rare cancer susceptibility syndrome exclusively attributable to **pathogenic variants in *FH*.**



In the 2015 ACMG/AMP variant interpretation framework published by Richards et al[4], a deliberately conservative **default weighting of 'supporting'** was assigned for code PP4.

In current practice, the degree to which PP4 (or variably PS4) is scored for phenotypic observations varies widely by disease, but such scoring has **not yet been quantified for *FH*.**

We have previously explored this for ***SDHB* and *SDHD*** in phaeochromocytoma and paraganglioma in Garrett et al., 2023[5].

Methods

FH germline diagnostic genetic testing data was collated from three UK diagnostic laboratories (Birmingham, Sheffield, and Leeds), and one US-based central testing laboratory (Ambry):

Total Case Dataset
Suspected HLRCC Patients: **387**
Renal cancer patients: **1,780**

Birmingham	Sheffield	Leeds	Ambry
• HLRCC: 158 • Renal: 952	• HLRCC: 229 • Renal: 131	• Renal: 359	• Renal: 338

Total Population Controls: 562,295

1000 Genomes Project	gnomAD v2.1.1	UK Biobank
• N = 2,504	• N = 118,474	• N = 441,317

To calculate a population frequency threshold below which variants may be described as "very rare", a Maximum Tolerated Allele Frequency (MTAFpred) was calculated using the methods described by Whiffin et al[6]:

- **Prevalence of renal cancer:** 1 in 50
- **Renal cancer penetrance:** 15%
- **Genetic heterogeneity**: 0.22%
- **Allelic Heterogeneity**: 0.1

→ **MTAF: 0.0015%**

Positive likelihood ratios were calculated for **very rare missense** and very **rare truncating variants** in FH, 'very rare' variant having an allele frequency < MTAF.

Likelihood ratio were converted to evidence points using a log base 2.08 conversion as prescribed in the Bayesian update of the ACMG/AMP variant classification framework described by Tavtigian et al., 2020.

Regional enrichment of very rare missense variants in HLRCC cases was examined using the clustering algorithm and windowing method as described by Walsh et al.[7]

Results

		Cases with variant	Controls with variant (/562,290 controls)	Likelihood Ratio (95% Confidence Intervals)	ACMG Evidence (exponent) points
HLRCC (/387 cases)	Rare Truncating Variants	79	43	2669.39 (1843.44-3881.25)	+10.77
	Rare Missense Variants	146	988	214.71 (185.01-246.87)	+7.33
	Rare Missense Variants, known pathogenic removed*	30	754	57.81 (39.79-82.71)	+5.54
Renal (/1780 cases)	Rare Truncating Variants	13	43	95.50 (48.93-183.0)	+6.23
	Rare Missense Variants	18	988	5.76 (3.51-9.30)	+2.39
	Rare Missense Variants, known pathogenic removed*	4	754	1.68 (0.54-4.61)	+0.71

Table 1: Pan-gene likelihood ratios for very rare missense and truncating variants observed in HLRCC cases and unselected Renal cancer cases.

*Known pathogenic variants defined as variants which are Pathogenic or Likely Pathogenic in ClinVar, with submission of at least one star.

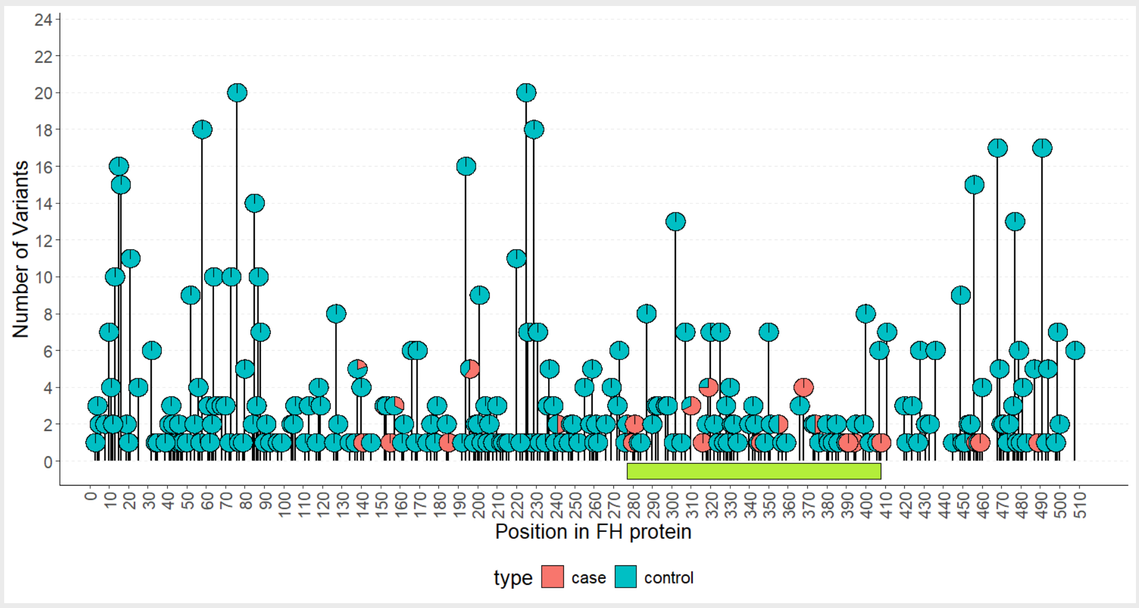


Figure 1: Enrichment of very rare missense variants in HLRCC across the *FH* gene. Variants shown exclude variants which are known pathogenic (at least one star in ClinVar). Enriched region is from p.277-p.408.

- Likelihood ratio within the enriched region = 166.37 **(+6.98 EPs)**
- Likelihood ratio outside the enriched region = 34.98 **(+4.95 EPs)**

Conclusion

- **Phenotypic specificity (PP4)** for rare missense and truncating variants in *FH* **has been quantified for different phenotypic contexts**
- Phenotypic evidence for rare variants in **HLRCC should be weighted more strongly** than in a general renal cancer context.
- Enriched clusters of rare missense variants in HLRCC cases **confirm the presence of enriched 'hotspots' in FH** (PM1).

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