Interdisciplinary Variant Re-Classification: *FGFR3* as an example of genotypic investigation in suspected skeletal dysplasia population

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P213

Background

With limited clinical awareness/identification of most rare genetic conditions, various streams of evidence are required to better classify variants as pathogenic or benign.

- The fibroblast growth factor receptor 3 (*FGFR3*) gene is linked to skeletal disorders including achondroplasia (ACH) and hypochondroplasia (HCH), both inherited in an autosomal dominant pattern
- Most cases of HCH (50–70%) are due to *de novo* mutations[1-3]
- Inconclusive genetic test results remain a significant challenge to HCH diagnosis, e.g., a 1:1 ratio of HCH positive to inconclusive results from a skeletal disorder gene panel program (Discover Dysplasias)[4,5]
- Interpreting VUSs can involve biobank, registry, clinical review data and is limited in part by the lack of high-throughput functional assays.[6,7] However, new assay methods are emerging[8]
- Effort to improve diagnosis requires evidence-based criteria and coordinated, impartial involvement from healthcare professionals, researchers, statisticians, and laboratories

Methods

To demonstrate the impact of a collaborative approach to reclassifying *FGFR3* variants of uncertain significance (VUSs) inconclusive for HCH, we initiated a project with the following steps:

Data collection: De-identified clinical data associated with *FGFR3* VUSs were collected via the US Discover Dysplasias[9] no-cost testing program from December 2019 to April 2024 (LabCorp Genetics, formerly Invitae). *VUSs found in patients with a positive molecular diagnosis in another gene were excluded.*

Evidence collection:

Assessment of existing databases: Data from external sources, including the UK Biobank (UKBB), gnomAD, and other relevant databases, were assessed to identify frequency, phenotypic correlations, and potential pathogenicity of the variants.

Ordering Clinician Interview: Genetic Counselor with expertise in skeletal dysplasias and genetic testing provided clinical context for the variants under review by interviewing clinicians for patient level phenotypic information.

Analysis & review: An established process was used to review all available evidence objectively. This included using pre-defined criteria for variant classification to ensure consistency and impartiality in re-evaluation decisions.

Results

Evidence collection

as of February 2025

- A total of 168 inconclusive *FGFR3* molecular results were reviewed, involving 119 unique nucleotide variants (113 unique predicted protein variants; from 137 ordering clinicians).
- Data from ClinVar, UK Biobank (UKBB), and gnomAD was collected and analyzed. 83 variants were tested for frequency enrichment and effects on disproportionate short stature.
- 60 clinicians were approached, and 28 clinician interviews were conducted including patient chart review and family pedigree discussion.

Figure 1. Population Overview

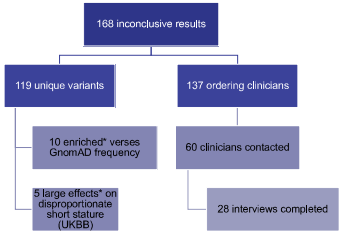


Figure 2. Example Pedigree Created via Ordering Clinician Interview (p.Val555Leu)

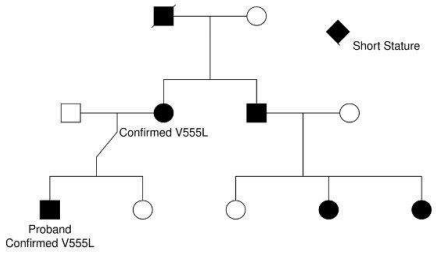
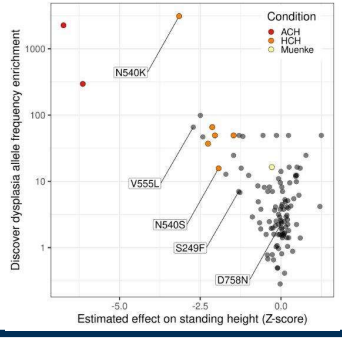


Figure 3. *FGFR3* Variant Effect Size on Standing Height



Analysis & Review

as of February 2025

- Clinician interviews provided a better understanding of clinical presentation and diagnostic accuracy in some patients with a VUS within *FGFR3*
- For many of the variants remaining "VUS" after data/literature & clinical data, functional assay data is likely to provide pivotal data to determine classification

Table 1. Summary of Variant Evidence & Classification

Variant	# of Probands	Data & literature highlights	Clinical data highlights	Functional Data	Resulting Classification
p.Val555Leu	5 (3.0%)	Alphamissense:	Co-segregation with phenotype in 2 families (3 probands)	Top hit activating, (p=0.0002*)	Likely pathogenic
p.Ser249Phe	2 (1.2%)	Same AA position as pathogenic variant Pathogenic analogous variant in FGFR1 Alphamissense:	Phenotype consistent with HCH, co-segregation confirmed in one family	TBD	Likely pathogenic
p.Asp758Asn	9 (5.4%)	Alphamissense:	Alternative MDx, suspected MDx, and/or inconsistent phenotype in at least 75% of interviewed	No effect, (p=0.89*)	Likely benign

*variant-wise exact tests testing for differences in means between two groups of negative-binomially distributed counts, enrichment of variant counts in the assay samples (n=6) compared to plasmid library (n=3) indicates gain of function and FGFR3 activation

Conclusions

- Concerted collection and analysis of clinical and laboratory data are required to facilitate variant classification (in addition to publicly available sources).
- Functional assays along with the elucidation of phenotypic presentation in patients can provide crucial missing evidence to support variant classification in rare genetic conditions.
- With emerging technologies and therapeutics, variant classification is an essential component to improving diagnosis and patient care.

References

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Acknowledgements

Interviewed clinicians that provided deidentified phenotypic information.

Disclosures

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