Complete functional map of *FGFR3* variants reveals a broad spectrum of effects and underdiagnosed skeletal dysplasia

#P107

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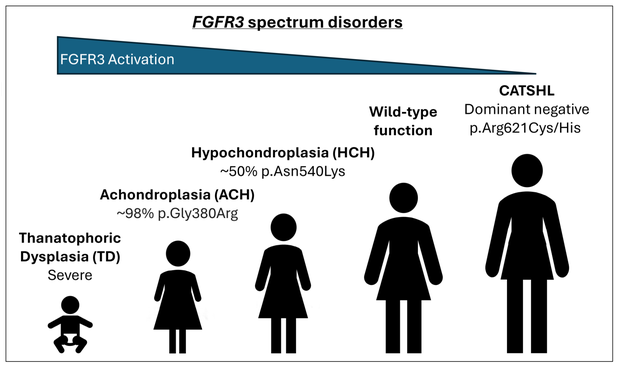
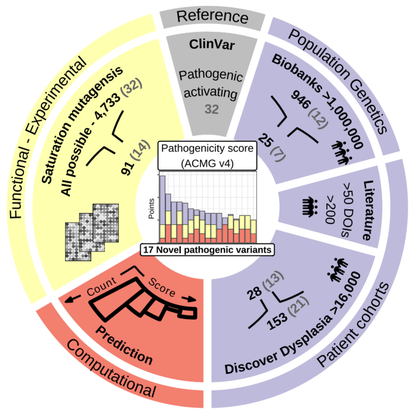
Abstract

Hypochondroplasia is among the most common *FGFR3*-related skeletal dysplasias, but it is challenging to diagnose due to phenotypic and allelic heterogeneity. We conducted a comprehensive functional screen utilizing saturation mutagenesis, testing every possible single nucleotide variant in FGFR3. We integrated these findings with population level data from Biobanks, a skeletal dysplasia clinical testing program and the literature. Following current ACMG v4 guidelines for data integration, **we identified twenty novel putatively pathogenic *FGFR3* variants at the nucleotide level, encompassing seventeen amino acid substitutions**. Here, we present evidence for the pathogenicity of variants with currently conflicting or uncertain significance status, namely p.Ser217Cys, p.Ser249Phe, p.Leu324His, p.Asn328Ile, p.Leu377Arg/His, p.Tyr379Asp, p.Val381Glu, p.Gly382Asp, p.Met528Ile, p.Ile538Val, p.Val555Leu/Met, p.Cys613Tyr/Trp/Phe, and p.Arg669Gly. Based on gnomAD allele frequencies and biobank penetrance estimates for short stature, the estimated prevalence of hypochondroplasia was 2.2 per 100,000 and 2.4 per 100,000 for previously known and novel pathogenic variants respectively. Our study provides a comprehensive overview of the mutational spectrum of *FGFR3* and will **improve the diagnostic yield of genetic testing for skeletal dysplasia**. Furthermore, our analysis provides a **framework for analyzing and integrating diverse data types and sources that is generally applicable for clinically relevant genes.**



In all figures: Novel - novel pathogenic variants according to current work; TD - Thanatophoric Dysplasia; ACH - Achondroplasia; HCH - Hypochondroplasia; CS - Craniosynostosis; CATSHL - Camptodactyly, Tall Stature, and Hearing Loss syndrome; Benign - reference benign missense variants.

Methods



Variant reference: Pathogenicity classification of germline *FGFR3* variants were annotated using ClinVar[1]. GnomAD[2] was used as an allele frequency reference (general population).

Population biobanks: Genetic and phenotypic data from UK Biobank[3] and All of Us[4] were used to estimate the effect of *FGFR3* variants on height and sitting/standing height ratio using REGENIE[5]. We have received an exception to the Data and Statistics Dissemination Policy from the *All of Us* Resource Access Board.

Discover Dysplasias cohort: De-identified clinical data associated with *FGFR3* variants was collected via the US Discover Dysplasias[6] no-cost testing program from December 2019 to January 2025 (LabCorp Genetics, formerly Invitae).

Functional assay of all missense variants in *FGFR3*: We performed a high-throughput screen measuring the effect of all possible single nucleotide variants in the *FGFR3* gene to identify gain-of-function activating variants. Plasmid libraries were transduced into MCF10A cells in assay media without growth factors[7]. Fold-changes and P-values are based on differential analysis utilizing negative binomial generalized linear models (edgeR[8]).

Pathogenicity scoring
Pathogenicity scores were based on draft ACMG v4 guidelines[9], with a gnomAD frequency cutoff of $<4.5\times10^{-5}$. Observational support from biobanks, Discover Dysplasias cohort and the literature. Functional support designation of +3 points for significant activating variants was based on calculation of an odds of pathogenicity[10] of 30. Computational support was based on the variant effect predictor REVEL[11].

Functional saturation mutational scanning of all *FGFR3* single nucleotide variants correlate with population-scale analysis of *FGFR3* variants affecting height

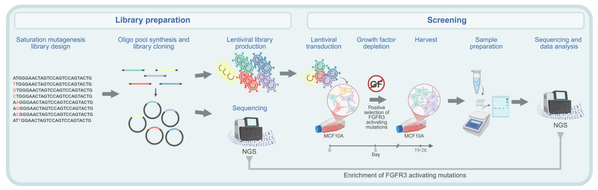


Figure 1 (right): Overview of functional screen experimental workflow.

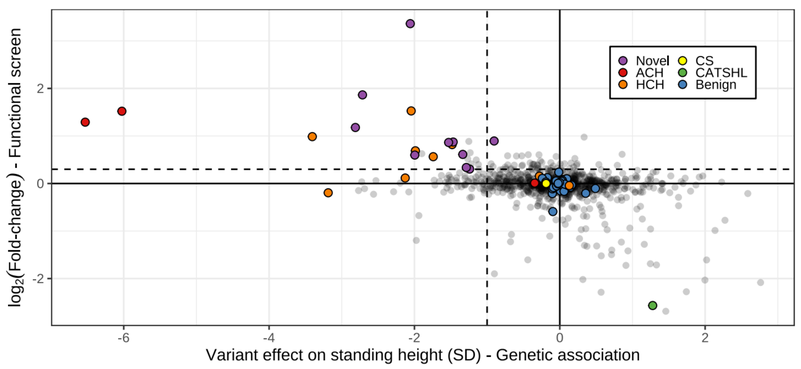


Figure 3 (left): Variant effects on height from the biobank analysis compared to functional impact according to the screen. To emphasize magnitude, the dotted lines correspond to variant effects of –1 SD on height and a log2 fold-change functional impact of 0.3 and do not necessarily reflect statistical significance.

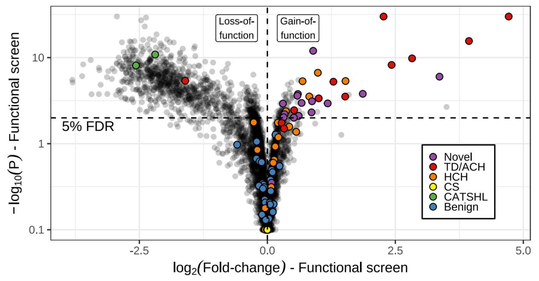


Figure 2 (top): Fold-changes and P-values from negative binomial generalized models. Positive fold-changes correspond to activating GoF variants; negative fold-changes correspond to LoF or dominant negative variants. The dotted line corresponds to a 5% false discovery rate. Y-axis is on a logarithmic scale.

Novel variants affect disproportionality and are enriched in a skeletal dysplasia clinical testing program

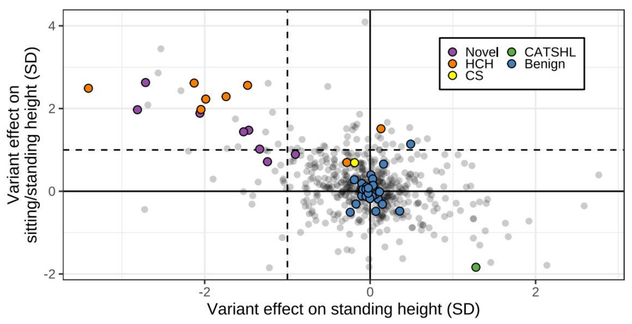


Figure 4 (top): Variant effects from single variant meta-analysis of UKBB and All of us participants genetic associations for height and single variant meta-analysis of UKBB participants genetic associations for sitting/standing height ratio. The average sitting/standing ratio in this population was 0.53, with an effect of +2 SD corresponding to 0.56. ACH variants were removed for clarity. To emphasize magnitude, the dotted lines correspond to variant effects of –1 SD on height and +1 SD on sitting/standing height ratio and do not necessarily reflect statistical significance.

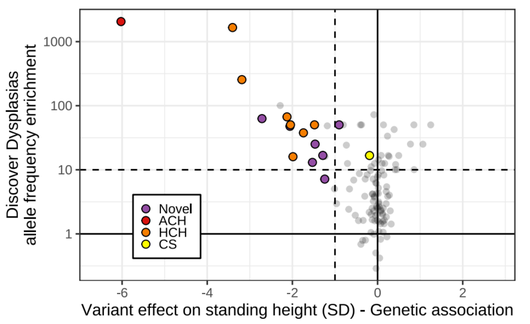


Figure 5 (top): Variant effects on height from the biobank analysis compared to variant enrichment in a skeletal dysplasia clinical testing patient cohort over the population background. Enrichment is calculated based on gnomAD allele frequency. To emphasize magnitude, the dotted lines correspond to a variant effect of –1 SD on height and 10-fold variant enrichment and do not necessarily reflect statistical significance. Y-axis is on a logarithmic scale.

Application of draft v4 ACMG guidelines for reclassification of pathogenic variants in *FGFR3*

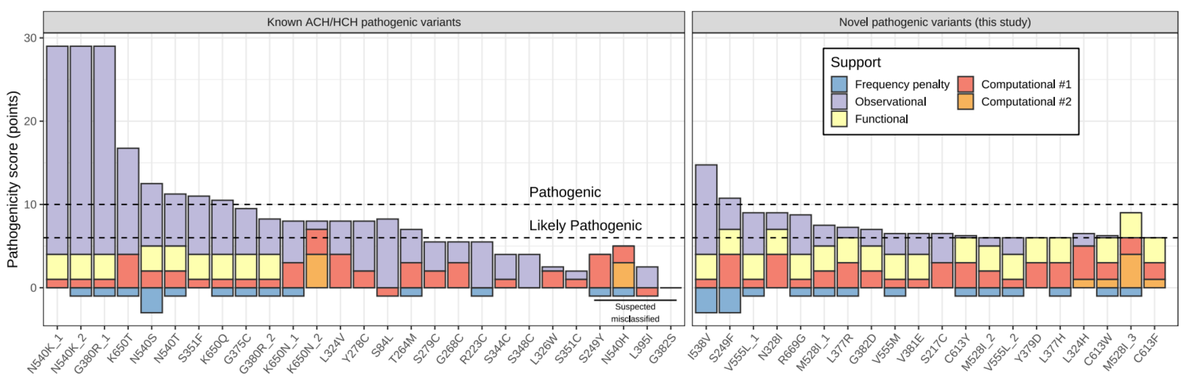


Figure 6 (left): Analysis of DNA-level variant support for pathogenicity based on draft ACMG v4 guidelines. Point contributions were grouped and colored into observational, functional, or computational support. Computational #1 represents predictive support and the first round of comparison variants; computational #2 represents additional support from the second round of comparison variants. All reference variants currently defined as pathogenic or likely pathogenic for ACH/HCH are shown in the left panel. All variants not currently defined as pathogenic with >=6 points (not exclusively computational support) are shown in the right panel. Dotted lines correspond to thresholds for a "likely pathogenic" or a "pathogenic" classification. Frequency penalties are shown but were not considered.

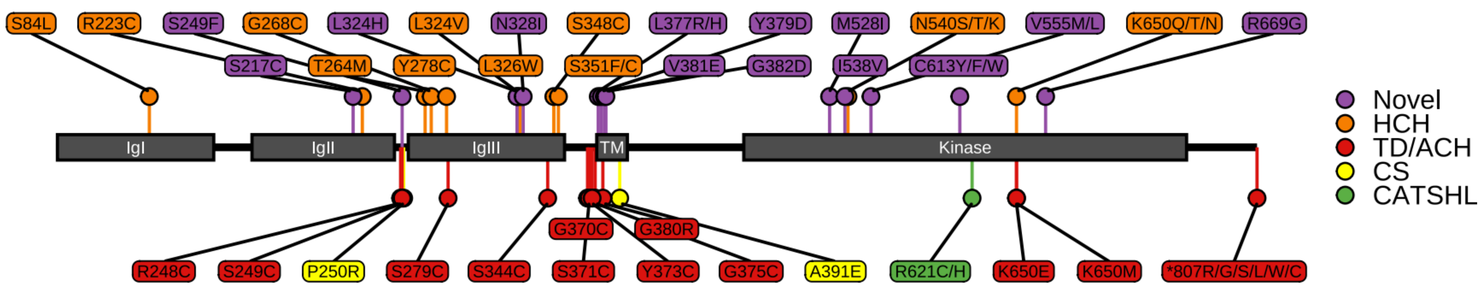


Figure 7 (left): Position of known and novel FGFR3 pathogenic missense variants. Annotation excludes reference variants suspected to be misclassified. Protein domains are annotated. IgI/II/III - immunoglobin like domains I / II / III; TM – transmembrane domain.

Conclusions & Future directions

- An experimental cellular FGFR3 saturation mutagenesis assay identified known pathogenic variants and supported novel gain-of-function activating variants, while it is significantly correlated with effects on stature.
- Biobanks and the Discover Dysplasias cohort enabled identification of novel *FGFR3* variants associated with disproportionate short stature. Clinician interviews provided additional information, including family segregation.
- S217C, S249F, N328I, L377R, V381E, G382D, M528I, V555L/M, I538V, C613Y and R669G are strong candidate pathogenic activating variants.
- Diagnosis of Hypochondroplasia can be challenging and clinicians rely upon genetic testing for confirmation. We continue to follow ACMG guidelines to reclassify variants and coordinate with genetic testing companies to improve diagnostic yield.

Acknowledgments & References

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1. Landrum M. et al. *Nucleic Acids Res*. 2014; **2.** Chen S. et al. *Nature*. 2024; **3.** UK Biobank: https://www.ukbiobank.ac.uk/ ; **4.** All of Us: https://allofus.nih.gov/ ; **5**. Mbatchou, J. et al. *Nature Genetics.* 2021; **6.** Discover Dysplasias: https://www.invitae.com/us/sponsored-testing/discover-dysplasias ; **7.** Tangermann C et al., *Nat Genet*. in press; **8**. Chen Y et al. *Nucleic Acids Res*. 2025; **9**. ACMG v4: tinyurl.com/ACMGv4 ; **10**. Brnich, SE. et al. *Genome Medicine.* 2020; **11**. Ioannidis NM et al. *American Journal of Human Genetics.* 201

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