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ORIGINAL ARTICLE

Novel variant in CBP domain of GLI3 underlying postaxial polydactyly

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ABSTRACT

Background: Polydactyly is a common congenital limb anomaly characterized by having extra fingers or toes and can be either syndromic or non-syndromic. GLI3 plays an important role in limb development via the Sonic Hedgehog signaling system.

Methods and Results: In this study, we examined a Pakistani family with postaxial polydactyly in an autosomal dominant manner. Clinical assessment confirmed a non-syndromic presentation without further systemic abnormalities. Whole exome sequencing and Sanger validation identified a novel heterozygous missense variant in *GLI3* (Glioma-associated oncogene homolog 3) [NM_000168.6: c.3199C>T; p. (Pro1067Ser)].

Conclusion: This novel variant expands the GLI3 mutation spectrum and highlights the importance of comprehensive genetic screening for accurate diagnosis and counseling of families with isolated polydactyly.

Keywords: GLI3, missense variant, polydactyly, whole exome sequencing.

Introduction

Polydactyly is a prevalent congenital disorder defined by having extra finger or toe. It is among the most common limb abnormalities, including both isolated and syndromic types. The expression of the disorder varies greatly, ranging from a little soft-tissue digit to a completely developed, functional finger and toe. Its prevalence can be affected by family history, population type, as well as mode of inheritance. This makes it a subject of clinical and genetic interest (1). Recent research studies have discovered several genes related to polydactyly (2).

GLI3, a gene related to glioma oncogenesis, which exists in two isoforms: active full-length GLI3 and repressor truncated GLI3 (GLI3R). SHH signaling promotes the GLI3R production (3). Functional investigations in mice have shown that SHH is important for proper limb development, as SHH null mutants show significant digit loss, whereas GLI3 mutant mice show polydactyly. SHH and GLI3 mutations in humans cause a variety of limb malformations, including preaxial or postaxial polydactyly, as well as more severe diseases such as Greig cephalopolysyndactyly syndrome (GCPS) and Pallister-Hall Syndrome (4).

Herein, we report a Pakistani family with isolated postaxial polydactyly and identified a novel missense variant by whole-exome sequencing and Sanger sequencing in the binding protein (CBP) domain of GLI3. To our knowledge, no prior reports have described variants in this domain causing isolated polydactyly, underscoring the novelty and significance of our findings.

Materials and Methods

Ethical approvals and family recruitment

Ethical approvals were obtained from the Institutional Review Board of the University of Balochistan, Quetta and the National Institute of Health Sciences, Islamabad, Pakistan. A family from the region of Balochistan was recruited and each participant gave written informed consent. After being informed in their native language.

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Consent granted authority to publish images and clinical data.

Genomic DNA extraction and blood collection

Genomic DNA was isolated through a commercially available kit, according to the manufacturer's standard protocol. DNA concentration as well as purity were assessed using a spectrophotometer by measuring absorbance at 260 nm and 280 nm. The A260/A280 ratio was utilized as an indication of DNA purity with values of 1.8-2.0 indicator of DNA purity, with values between 1.8 and 2.0 considered suitable for downstream genetic analysis.

Whole exome sequencing

The whole exome sequencing (WES) of IV-1 in the family was done with an Illumina Hiseq-5200 following standard protocols. After the exome enrichment, the sequencing reads were collected and aligned to human genome assemblies hg19 (GRChg37; 5). Duplicate removal, quality recalibration, indel realignment, calling, and variant detection were performed using the Genome Analysis Toolkit and Picard tools (6). Variants were annotated with ANNOVAR **(7)**. The average on-target sequencing depth was ~100×, with 98% of targeted bases at ≥20×. At the c.3199C>T site in *GLI3* (CBP-binding domain), the alternate allele fraction at variant position was 82× raw read depth and 48%, as expected for a heterozygous alteration. The variant selection criteria included a minor allele frequency of >0.01 in gnomAD CADD-Phred scores >13, exonic variation, and splice sites (±12 bp) (8).

Sanger sequencing

The identified variant was validated using Sanger sequencing. PCR primers were designed using the *GLI3* reference sequence from the Esembl Genome Browser. Primer3 was used to generate variant-specific primers, which were then confirmed with Primer Stats. Sanger Sequencing was executed on DNA samples from two affected [III-2, IV-1] and one unaffected participant [IV-2] using the BigDye Terminator V3.1 according to the manufacturer's protocols (9).

Results

Clinical features

The pedigree of the family manifested autosomal dominant inheritance patterns (Figure 1A). In individual IV-1, bilateral postaxial polydactyly type B was observed, along with postaxial polydactyly type A in the right foot. Radial deviation of the distal phalanx of the fifth toe in the left foot was noted in IV-1 (Figure 1C). Individual III-1 exhibited bilateral postaxial polydactyly type A in the feet. No other anomalies were detected in any of the affected individuals, signifying an isolated disease pattern (Table 1).

Genetic analysis

Whole-exome sequencing revealed a heterozygous missense variant in the *GLI3* gene [NM_000168.6: c.3199C>T; p. (Pro1067Ser): Figure 1B]. This nucleotide change was not observed in normal human population databases, including gnomAD v2.1.1. In silico prediction programs SIFT, PolyPhen-2, and Mutation Taster all predict a potentially deleterious impact on GLI3 protein function. Segregation of the variant with phenotype was observed within the family. Based on current ACMG guidelines, it is classified as a likely pathogenic variant. The variant had a CADD score of 25.4, signifying deleteriousness, and a GERP++ score of 5.47, supporting strong evolutionary conservation.

Discussion

GfLI3 is an essential regulator of tissue development and patterning. It is one of the three transcriptional factors in GLI (GLI1, GLI2, GLI3) that play a vital role in the canonical Hedgehog signaling pathway (10). The gene comprises 15 exons that encode a 1,580 amino acid protein distributed into multiple functional domains, such as N-terminus transcriptional repressor, a proteolytic cleavage site, zinc finger DNA binding motifs, CB-binding regions (TA/CB), and two transcriptional activation domains at the C-terminal region. The missense variant identified in the present study is located within the conserved CREB-binding protein domain, which is essential for transcriptional activation capacity.

This study identified a rare heterozygous missense variation in the *GLI3* gene [NM_000168.6: c.3199C>T; p. (Pro1067Ser)] through WES, underlying isolated polydactyly. The variant found in exon 15 affects a highly conserved amino acid residue in the C-terminal transcriptional activation domain, which plays an important role in the *GLI3* gene function as a transcriptional activator. The CBP-binding domain of GLI3 is essential for its transcriptional regulation, since it facilitates interaction with CREB-binding protein (CBP), a chromatin remodeling- and gene expression-associated

Table 1. *Clinical features of affected individuals with isolated postaxial polydactyly.*

Individuals	Affected limbs	Type of polydactyly	Additional features
Upper limbs			
IV-1	Both hands	Postaxial, Type B	None
Lower limbs			
IV-1	Right foot	Postaxial, Type A	Radial deviation of distal phalanx of left fifth toe
III-1	Boot feet	Postaxial, Type A	None

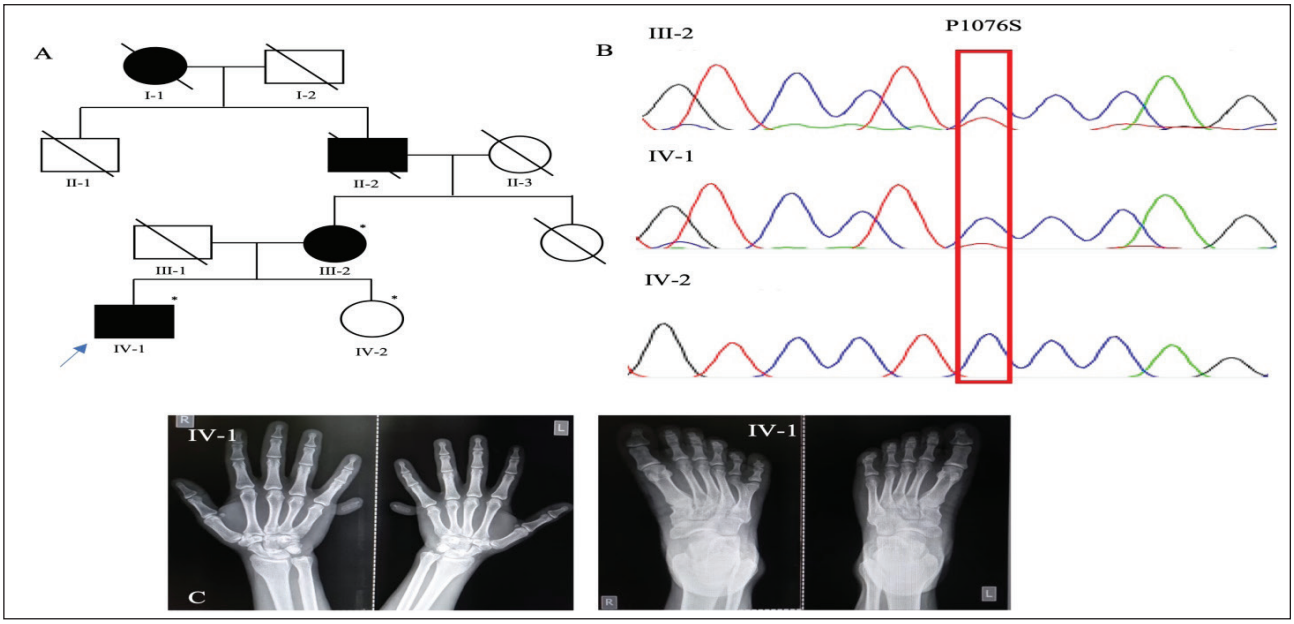

Figure 1.*A: Family pedigree with the pattern of autosomal dominant inheritance. Squares indicate males, circles indicate females, asterisks indicate family members who were enrolled for genetic and clinical evaluation, and the arrow indicates the person who was subjected to whole exome sequencing. B: Sanger sequencing chromatograms of the individuals with the nucleotide change. C: X-ray photographs of individual IV-1 showing polydactyly in hands and feet. D: Schematic representation of GLI3 protein domains and associated disorders.*

co-activator. GLI3-FL binds to CBP in phosphorylation-independent fashion at residues 827 and 1132 in its C-terminal domain. This binding is required for GLI3-FL–mediated activation of the GLI1 promoter and is seen only when SHH is present, implying that mutations within the CBP-binding domain would interfere with this binding and SHH-dependent transcriptional activity (11).

The importance of phenotype–genotype correlations in GLI3-associated disorders has been highlighted in a number of studies, with a focus on the domain-specific location of mutations (12). GCPS is more commonly linked to variants found in the N-terminal and C-terminal sections of the GLI3 protein, while Pallister-Hall syndrome (PHS) is more commonly associated with mutations found in the central region of the protein (13,14; Figure 1D). The phenotypic boundaries for isolated (non-syndromic) polydactyly are still unclear in spite of these well-established patterns. Interestingly, GLI3 mutations that cause non-syndromic polydactyly have been identified in several of the protein's functional domains, excluding the TA/CBP-binding domain (15). The current variant is the first report of polydactyly in the CBP binding domain.

In conclusion, this study reports a novel heterozygous missense variant in the *GLI3* gene in a Pakistani family having isolated polydactyly. The variant is located within the CBP-binding domain. This is the first report of a GLI3 missense variant in the CBP-binding domain causing isolated postaxial polydactyly. These findings expand the phenotypic spectrum and underscore the importance of domain-specific variant interpretation in *GLI3*.

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List of Abbreviations

ACMG	American College of Medical and Genetics
Bp	Base pairs
CADD	Combined Annotation-Dependent Depletion
CBP	CREB-binding protein
CB	CREB-binding
DNA	Deoxyribonucleic acid
GATK	Genome Analysis Toolkit
GCPS	Greig Cephalopolysanydactyly syndrome
GERP++	Genomic evolutionary rate profiling
GLI1	GLI1 Family zinc finger transcription factor 1
GLI2	GLI2 Family zinc finger transcription factor 2
GLI3	Glioma-associated oncogene homolog 3
GLI3F	Full length GLI3
GLI3R	Repressor truncated GLI3
gnomAD	Gene Aggregation Database
IRB	Institutional Review Board
nm	Nanometer
PCR	Polymerase chain reaction
PHS	Pallister-Hall syndrome
PolyPhen-2	Polymorphism phenotyping v2
SHH	Sonic hedgehog
SIFT	Sorting intolerant from tolerant
TA	Transactivation domain
WES	Whole exome sequencing
ZRS	Zone of polarizing activating regulatory sequence

Conflict of interest

The authors of this article have no affiliations with or involvement in any organization or entity with any financial

interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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Author contributions
Aneela carried out the experimental work in the laboratory and wrote the manuscript. Bibi Ayesha Zehri and Nadeem Hameed sampled the families and analyzed the data. Muhammad Sharif Hasni and Mehraj Gull designed the study, provided funds, and finalized the manuscript.

Data availability Statement
Available upon request.

Ethical approval
Written informed consent was provided by all individuals who were over the age of 18. For individuals who were minors, informed consent was obtained from their parents. IRB-NIH-0812, Dated 2-January-2024.

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