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ORIGINAL ARTICLE

Variant in the zinc finger domain of *GLI1* underlie postaxial polydactyly type B

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ABSTRACT

Background: Polydactyly is a hereditary condition in humans resulting from abnormalities in genes related to the development of autopods. This disorder can be inherited in an autosomal dominant or autosomal recessive pattern. GLI1 functions as a moderator in the hedgehog (Hh) signaling pathway. Upon binding the Hh to its receptor, GLI proteins are activated, leading to the expression of genes responsible for bone patterning and establishment.

Methods and Result: Here, we describe the clinical and molecular findings of Pakistani-origin family with postaxial polydactyly type B. Whole exome sequencing followed by Sanger sequencing identified a homozygous variant [c.1133C > T, p.(Ser378Leu)] residing in the zinc finger (ZF) domain of the Glioma-Associated Oncogene Family ZF (GLI1).

Conclusion: This study will facilitate genetic counseling and proper diagnosis of families with related and same disorders in the Pakistani population.

Keywords: Polydactyly, *GLI1*, zinc finger domain, novel variant, phenotypic variability.

Introduction

Polydactyly is an inherited condition clinically illustrated by an extra supernumerary digit or toe, which may or may not have a bony element. Polydactyly is categorized into three different types: central polydactyly (axial), preaxial polydactyly (radial), and postaxial polydactyly (PAP) (ulnar) (1-3). In humans, there are 13 known genes associated with nonsyndromic polydactyly, namely, *GLI3* (OMIM 165240), *EFCAB7* (OMIM 617632), *STKLD1* (OMIM 618530), *GLI1* (OMIM 165220), *SMO* (OMIM 601500), *ZNF141* (OMIM 194648), *DACH1* (OMIM 603803), *IQCE* (OMIM 617631), *MIPOL1* (OMIM 606850), *LRP4* (OMIM 604270), *PITX1* (OMIM 602149), *KIAA0825* (OMIM 617266), *LMBR1/ZRS* (OMIM 605522), and *FAM92A1* (OMIM 617273) (4-17).

The GLI and hedgehog pathway is an extremely maintained signaling mechanism crucial for managing cell determination, cell-to-cell interactions, and modeling of tissue during embryonic growth. Sonic hedgehog plays a pivotal role in regulating digit numbering throughout embryonic development by modulating the functions of transcription factors belonging to the GLI family, such as GLI3, GLI1, and GLI2 (18,19). GLI proteins bind DNA using a consecutive set of five C2H2 zinc finger (ZF) motifs and feature a carboxy-terminal transactivation domain (20). In addition, GLI3 and GLI2 possess an N-terminal repressor region, enabling them to serve as dual transcription factors, In contrast, *GLI1* functions exclusively as a transcriptional activator. *GLI1* transcript levels increase in response to hedgehog (Hh) ligands, indicating that *GLI1* is a target gene and amplifier within the Hh pathway (21). Canonical stimulation of the GLI–Hh signaling pathway begins when the Hh molecule

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binds to Patched 1 (PTCH1; 12 TM receptor), leading to the activation of the G protein-coupled receptor Smoothened (SMO; seven-pass transmembrane), which functions as a repressor. Functional SMO originates a multiplex intracellular cascade that ultimately results in the triggering of the three GLI transcription factors (GLI1, GLI2, and GLI3), serving as the ultimate effecters of the GLI–Hh pathway (Figure 1 (22)).

In the current study, we recruited a Pakistani family exhibiting polydactyly inherited in an autosomal recessive pattern. Our analysis revealed a homozygous missense variant, [c.1133C > T, p.(Ser378Leu)], in the *GLI1* gene. This variant has been previously reported and is associated with phenotypic variability in affected individuals. The findings suggest that while the variant in *GLI1* is known, its phenotypic expression may vary, underscoring the complexity of genotype–phenotype correlations in polydactyly.

Methods

Photographs were taken of all participating family members or their guardians and provided written informed consent for both publication and genetic analysis. Pedigrees were constructed based on knowledge provided by well-informed family members. Venous blood samples (3–5 ml) were collected from both healthy and affected individuals in the family using EDTA vacutainer tubes. Genomic DNA from the blood samples was isolated using a commercially available DNA extraction and purification kit. Quantification of the purified DNA was conducted using the Thermo Scientific NanoDrop.

Whole Exome Sequencing (WES)

The WES on DNA from IV-3 in the family was performed using Illumina HiSeq-5200 using standard protocols. The average sequencing depth achieved was approximately 100×, with at least 95% of the target regions covered at a minimum depth of 20×. After exome enrichment, reads were obtained and aligned against human genome assembly hg19 (GRCh37) using Burrows-Wheeler Aligner (BWA v.0.7.5) (23). Duplicate exclusion, quality recalibration, indel rearrangement, variant calling, and identification were executed utilizing Picard and the genome analysis toolkit (24). The variants underwent annotation via ANNOVAR (25). The criteria for selecting variants included a minor allele frequency of <0.001 in normal human databases (26), a CADD-phred score exceeding 13, and variants located within splice sites (±12 bp) and exonic regions (27).

Primer Designing and Sanger Sequencing Validation

The selected variant sequence was acquired from the UCSC genome browser. Primers were generated using the online tool Primer3, and their specificity was verified using Primer Stats. To confirm the results from WES and assess the co-segregation of the identified variant, DNA from both affected and healthy individuals was Sanger sequenced. The resulting Sanger sequencing chromatograms were analyzed using the BioEdit sequence alignment editor (BioEdit v.0.7.2). The disease-causing potential of the variant was confirmed using

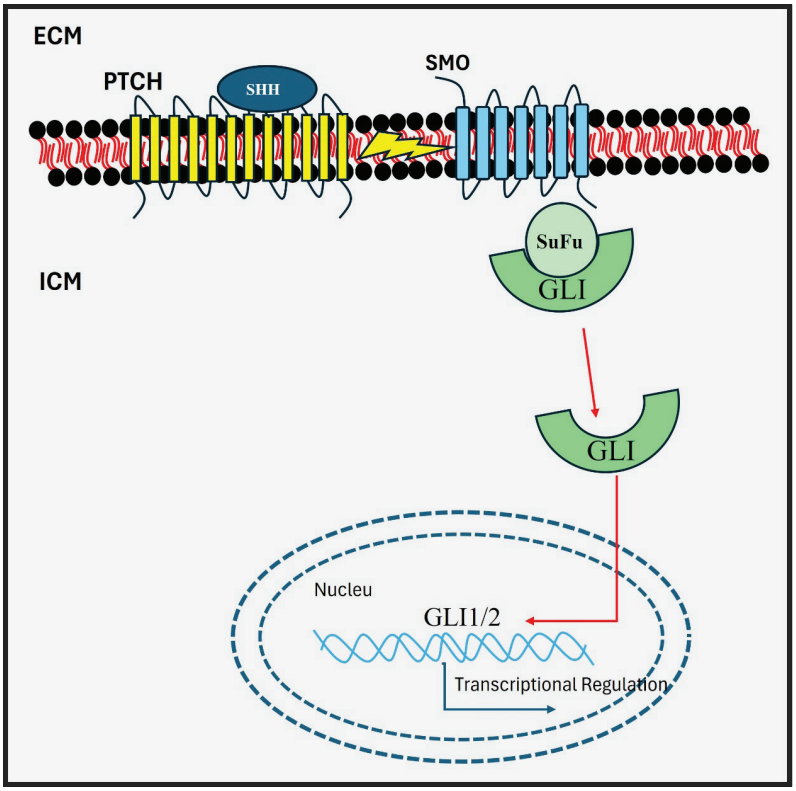

Figure 1. *Activation of GLI through hedgehog signaling pathway.*

available online tools such as Polyphen-2, Mutation Taster, and SIFT.

Results

Clinical assessments

In family A, three normal members (III-2, III3, and IV-1) and two affected individuals (IV-1 and IV-2) contributed to the study (Figure 2b). The affected member (IV-2) exhibited bilateral PAP type B in hands only. The affected individual (IV-3) manifested PAP type B in the right hand only. No other abnormalities were seen in all affected individuals (Figure 2b, c).

Genetic investigation

Affected member (IV-3) in family A underwent exome sequencing. WES analysis revealed a homozygous variant [c.1133C > T, p.(Ser378Leu)] in family A (Figure 2d) in the *GLI1*. Variant segregated within the respective family, indicating potential inheritance patterns. The [c.1133C > T, p.(Ser378Leu)] variant was not present in gnomAD v2.1.1 in homozygous form. The homozygous variant [c.1133C > T, p.(Ser378Leu)] has a GERP++ score of 4.53 and a CADD and Phred score of 27.1. The variant was evaluated using various online tools, including Mutation Taster, CADD Phred, and GERP++ scores, and predicted to be disease-causing. Variant is classified as "likely pathogenic" according to the ACMG classification.

Discussion

A Pakistani-origin family, demonstrating isolated PAP type B, was clinically and molecularly characterized in the study presented here. WES followed by Sangar sequencing revealed a homozygous variant p.(Ser378Leu))] in the *GLI1* gene.

The *GLI1* gene, residing on the 12q13.3 chromosome, encodes the GLI1 protein comprising 1106 amino acids (28). This protein functions as a moderator in the Hh signaling pathway. Upon the interaction of the Hh molecule with its receptor, GLI proteins become activated, leading to the target gene transcription involved in bone development and modeling (29). The GLI1 protein harbors specific regions, comprising a ZF domain spanning amino acids 235 to 387, degron degradation signals at amino acids 77 to 116 and amino acids 464 to 469, SUFU binding domains at amino acids 111 to 125, and the C-terminus, a nuclear localization signal from amino acids 380 to 420, and the transactivation domain between amino acids 1020 and 1091.

Variant [p. (Ser378Leu)] identified in the current study resides in the ZF domain of *GLI1*. The variant is supposed to prevent protein and DNA binding, leading to the disturbance of the Hh pathway, which regulates the growth of limbs and the formation of digits. During the Shh-dependent phase of limb development, Shh induction triggers *GLI1* expression in the posterior limb region and increases levels of full-length GLI activators (*GLI1*, GLI2, and GLI3FL), crucial for anterior–posterior patterning and digit formation. These activators are essential for the proliferation and sustenance of both posterior and anterior progenitor cells. *GLI1* and Gli2 protein levels increase until E10.75 (Embryonic Day 10.75) and then stabilize through E12.5 (Embryonic Day 10.75). This observation aligns with earlier research demonstrating a significant expansion of cells expressing Shh and responding to it, which concurrently express *GLI1* during this developmental period (30,31).

In previous studies, seven variants within the *GLI1* gene have been identified, each associated with various forms of polydactyly, with PAP type A being the most common

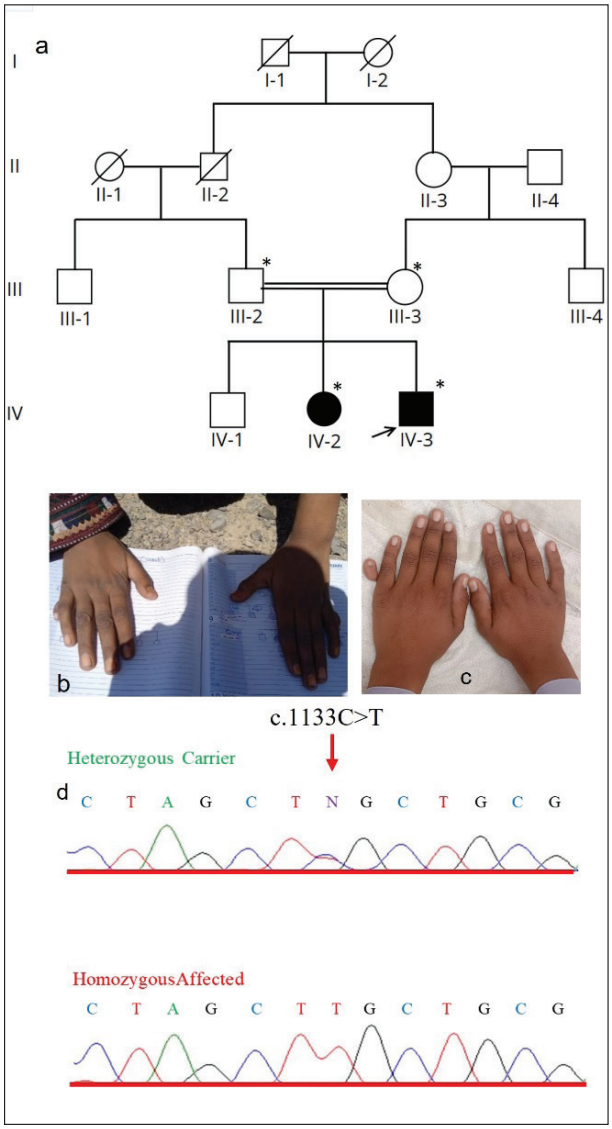

***Figure 2. a**: Pedigree of family segregating postaxial polydactyly in an autosomal recessive manner. Squares represent male family members and circles represent female family members. Filled symbols designate affected individuals. An asterisk indicates from whom the DNA sample was obtained. All affected individuals manifest bilateral postaxial polydactyl in their hands. **b,c:** Hands of individual IV-2 and IV-3 of family exhibiting PAP type B. **d:** Electropherograms obtained from Sanger sequencing showing variant in the GLI1 gene. The upper panel shows the nucleotide sequence in the heterozygous carrier, while the homozygous affected individual [c.1133C > T, p.(Ser378Leu)] is in the lower panel.*

Table 1. *Previously reported variants, phenotypes, and origin of families in GLI1.*

Mutation type and nature	Nucleotide change	Protein	Reported phenotypes	Origin
Missense homozygous	c.1517T > A	p.L506Q	Preaxial polydactyly	Pakistani
Nonsense homozygous	c.337C > T	p.R113X	Postaxial polydactyly	Pakistani
Missense homozygous (current study)	c.1133C > T	p.S378L	Postaxial polydactyly A/B	Pakistani
Missense heterozygous	c.1064C > A	p.T355N	Postaxial polydactyly	Pakistani
Nonsense homozygous	c.2340G > A	p.W780X	Ranging from simple postaxial polydactyly to EVC syndrome	Turkish
Nonsense homozygous	c.1930C > T	p.Q644X	Ranging from simple postaxial polydactyly to EVC syndrome	Turkish
Missense homozygous	c.1010C > T	Ser337Leu	Postaxial polydactyly	Pakistani

type observed. Among these, the distinct subtype known as PAP type B—characterized by an extra digit that is often underdeveloped and lacks normal functionality—had been linked to only a single variant, as shown in Table 1. In our current study, however, we observed the specific phenotype of PAP type B in both individuals carrying this variant, further corroborating its association with this distinct subtype. Our identification of a variant in *GLI1* correlated with this subtype adds valuable insights to the genetic complexity of polydactyly disorders.

Prenatal genetic screening and newborn screening have experienced significant advancements in recent years, boosted by the accessibility and speed of next-generation sequencing technologies (32). Sequencing cell-free DNA with NGS technologies from maternal plasma has resulted in the creation of remarkably sensitive screening tests for fetal aneuploidies. Likewise, prenatal diagnosis for monogenic disorders (PGT-M) can be achieved during early gestation stages (33). As NGS-based sequencing technology continues to improve, with reduced costs and faster data analysis, cell-free nucleic acid sequencing is expected to take on a progressively more vital part in prenatal diagnosis, monitoring, screening, and risk assessment for both maternal and fetal conditions (34).

In conclusion, we studied a Pakistani family exhibiting PAP type B in autosomal recessive manners. WES uncovered a variant in the *GLI1* gene. The study not only broadened the range of variants identified in *GLI1* but also underscored the clinical variability present within the families. This research will aid in the diagnosis and genetic counseling of patients with limb disorders within the Pakistani population.

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Declaration of conflicting interests
The authors have no affiliations with or involvement in any organization or entity with any financial interest or nonfinancial interest in the subject matter or materials discussed in this manuscript.

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Consent to participate
Informed consent was obtained from all individual participants included in the study.

Consent for publication
The authors affirm that human research participants provided informed consent for the publication of the images in Figure 2.

Ethics approval
This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of COMSATS University Islamabad, Pakistan (IRB-CUI-20087).

Authors' contributions
Zaheer Ahmed performed experimental work and prepared the manuscript. Syed Nasir Abbas Shah, Abdul Jabbar, and Adeel Shahid visited the family, drew pedigrees, and collected blood samples. Rimsha Zahid and Nizam Uddin Baloch analyzed the data. Muhammad Jawad Khan and Muhammad Umair designed the study, provided funds, and finalized the manuscript. All authors read and approved the final manuscript.

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