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ORIGINAL ARTICLE

A novel missense variant located within the zinc finger domain of the *GLI3* gene was identified in a Vietnamese pedigree with index finger polydactyly

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Abstract

Background: Polydactyly, particularly of the index finger, remains an intriguing anomaly for which no specific gene or locus has been definitively linked to this phenotype. In this study, we conducted an investigation of a three-generation family displaying index finger polydactyly.

Methods: Exome sequencing was conducted on the patient, with a filtration to identify potential causal variation. Validation of the obtained variant was conducted by Sanger sequencing, encompassing all family members.

Results: Exome analysis uncovered a novel heterozygous missense variant (c.1482A>T; p.Gln494His) at the zinc finger DNA-binding domain of the GLI3 protein within the proband and all affected family members. Remarkably, the variant was absent in unaffected individuals within the pedigree, underscoring its association with the polydactyly phenotype. Computational analyses revealed that GLI3 p.Gln494His impacts a residue that is highly conserved across species.

Conclusion: The GLI3 zinc finger DNA-binding region is an essential part of the Sonic hedgehog signaling pathway, orchestrating crucial aspects of embryonic development through the regulation of target gene expression. This novel finding not only contributes valuable insights into the molecular pathways governing polydactyly during embryonic development but also has the potential to enhance diagnostic and screening capabilities for this condition in clinical settings.

KEYWORDS
exome sequencing, GLI3, polydactyly, sonic hedgehog pathway, zinc-finger domain

1 | INTRODUCTION

Polydactyly is a congenital disorder characterized by the presence of extra fingers or toes on the hands or feet of a patient. The extra digits can range in size and shape, ranging from small, nonfunctional rudimentary appendages to fully formed and functional fingers or toes. Polydactyly is a relatively common condition, occurring in approximately 0.3–3.6 out of every 1000 live births, with the incidence rate in males being reported to be twice as high as in females (Umair et al., 2018). In terms of phenotype, polydactyly is a very heterogeneous deformity, with a significant propensity for the involvement of the right hand over the left, upper limbs over the lower and left foot over the right (Malik et al., 2014).

Polydactyly can occur as an isolated abnormality (non-syndromic polydactyly) or be associated with other genetic syndromes or birth defects (syndromic polydactyly). The extra digits can be variable in size and shape, ranging from small, nonfunctional rudimentary appendages to fully formed and functional fingers or toes. There are different types of polydactyly based on the location and characteristics of the extra digit. The most common types are postaxial polydactyly with the extra digit, which is located on the outside (little finger or toe side) of the normal hand or foot structure, and preaxial polydactyly with the extra digit, which is located on the thumb or big toe side of the hand or foot (Bubshait, 2022).

Although the phenotype of polydactyly is well described, it is still unclear which genetic factors contribute to this disorder. Autosomal dominant inheritance accounts for the majority of polydactyly cases, while X-linked and autosomal recessive inheritance patterns are more likely to occur in a few cases. Genetic research into polydactyly is more complex than that of single-gene diseases since it involves a large number of genes and intricate molecular network interactions (Ahmed et al., 2017). Several signaling pathways were demonstrated to be involved in the formation of limbs; digits interact on multiple levels and play distinct roles depending on the stage of embryonic development (Hu & He, 2008), such as the sonic hedgehog pathway (Klein et al., 2019) or the PI3K/AKT/mTOR signaling pathway (Vahidnezhad et al., 2016). Patients with Short-Rib Polydactyly exhibit genetic abnormalities that impact primary cilia, structures present on the majority of mammalian cells and considered essential for canonical hedgehog signal transduction (Loo et al., 2021). Different genes have been linked to polydactyly, both syndromic and nonsyndromic, including *GLI1*, *GLI3*, *MIPOL1*, *SHH*, *ZNF141*, and other genes, thanks to the development of molecular tools (Biesecker, 2011; Wang et al., 2014).

In this study, we report a three-generation Vietnamese pedigree with polydactyly of index fingers in every generation. The primary aim of this study was to sequence the exome of a patient with polydactyly, followed by filtration to identify potential causative variants and validation through Sanger sequencing, involving both the patient and his family members.

2 | MATERIALS AND METHODS

2.1 | Study subjects

A three-generation pedigree was enrolled in the study. All members belonged to the Kinh ethnic, located in Thai Nguyen, a province in northeastern Vietnam. All of the blood samples were collected at the Vietnam National Children's Hospital's Department of Orthopedics and kept in tubes with EDTA at −20°C. After being fully informed about the research project, each participant signed a consent form granting permission to use their samples.

2.2 | Exome sequencing

Genomic DNA was isolated from 200 μL of peripheral whole blood samples using the QIAamp DNA Blood Mini Kit (QIAGEN) in accordance with the manufacturer's instructions. Genomic DNA samples were then sheared, ligated to adapter, and hybridized with exome bait. A DNA library of exome fragments was created with 151-bp paired-end reads using Exome capture Sure select V7 and high-throughput sequenced on the NovaSeq 6000 system (Illumina, USA). Obtained sequence reads were pretreated to remove low-quality reads, duplication, and adapters using Trimomatic v 0.40, aligned, and mapped on the human reference genome (GRCh38) using BWA-0.7.17. Variant calling was conducted using the Genome Analysis Toolkit v4.1.2.0 (GATK) guidelines. The ANNOVAR tool was used to annotate sequencing variants, including single-nucleotide variants (SNVs) and short insertions or deletions (INDELs).

2.3 | Sanger sequencing confirmation and in-silico analyses

The inheritance pattern of the interest variant obtained from exome sequencing analysis was validated using Sanger sequencing. The polymerase chain reaction (PCR) products were amplified in all family members using the following pair of primers: Forward primer (5′-GTTACCGGGTCTTGGAAGGG-3′), Reverse primer (5′-CAGGGCAGCAAAGTTTGACC-3′), and then purified using the QIAquick PCR Purification Kit from Qiagen. The sequence of the DNA fragment (765 bp) was identified using the BigDye™ Terminator v3.1 cycle sequencing kit (Thermo) on the Sanger ABI PRISM 3500 Genetic Analyzer sequencer.

The potential pathogenic impact of the missense genetic variant was assessed in silico using Mutation Taster, PredictSNP, Sorting Intolerant From Tolerant (SIFT), and PolyPhen2. Amino acid sequence conservation across various species was investigated utilizing the UCSC Genome Database version human GrCh38/hg38.

3 | RESULTS

3.1 | Case presentation

The proband, a 6-year-old boy, presented with bilateral polydactyly affecting both hands, featuring an additional index finger, while his feet displayed a normal configuration. The digit present as a sixth finger exhibited complete functionality, enabling it to perform tasks and movements akin to those of the other fingers within the hand structure. X-ray radiographic analysis of the hand indicated that the extra digit had a fully developed structure of skeletal, muscular, and neural tissues and exhibits normal structure and function comparable to the other fingers. Based on the distinctive phenotypic traits observed, the patient received a diagnosis of nonsyndromic central polydactyly (also known as Preaxial Polydactyly type 3, MIM 174600; Umair et al., 2018). Within the family pedigree, both the mother (32-year-old) and the grandfather (70-year-old) exhibited bilateral polydactyly affecting their hands, similar to the proband. This manifestation includes an additional index finger on both hands. The extra digit present in both the mother and the grandfather may also exhibit complete functionality, allowing normal movements similar to those of other fingers. The grandfather exhibited an abnormal foot morphology, characterized by the divergence of the hallux from the alignment of the remaining toes, while the mother has a normal foot structure (Figure 1). The proband's sister, father, and other family members exhibited normal hands and feet (Figure 2a).

3.2 | Variant calling

The exome sequencing data yielded a total of 4,462,444,714 read bases, comprising 29,552,614 paired-end reads. The quality of the reads proved favorable for subsequent analysis, with 97.7% of bases exhibiting a Phred quality score exceeding 20, and 93.94% with a score surpassing 30. A comprehensive dataset of more than 50,000 single nucleotide polymorphisms (SNPs) and 18,000 indels was obtained. Following the exclusion of polymorphisms in intronic regions and synonymous variants, the dataset was refined to 4260 SNPs and 641 indels for further investigation. Our attention was directed toward 100 genes implicated in polydactyly and previously anticipated to play a role in limb formation during embryonic development. Additionally, variants with a minor allele frequency exceeding 0.01 in either the 1000 Genome Project or ExAC were excluded from consideration. Consequently, we identified a novel heterozygous substitution variant in exon 10/15 of the *GLI3* gene (OMIM ID: 165240), specifically NM_000168.6:c.1482A>T (p.Gln494His).

FIGURE 1 Phenotypic characteristics of polydactyly in the hands and feet among affected individuals in the family.

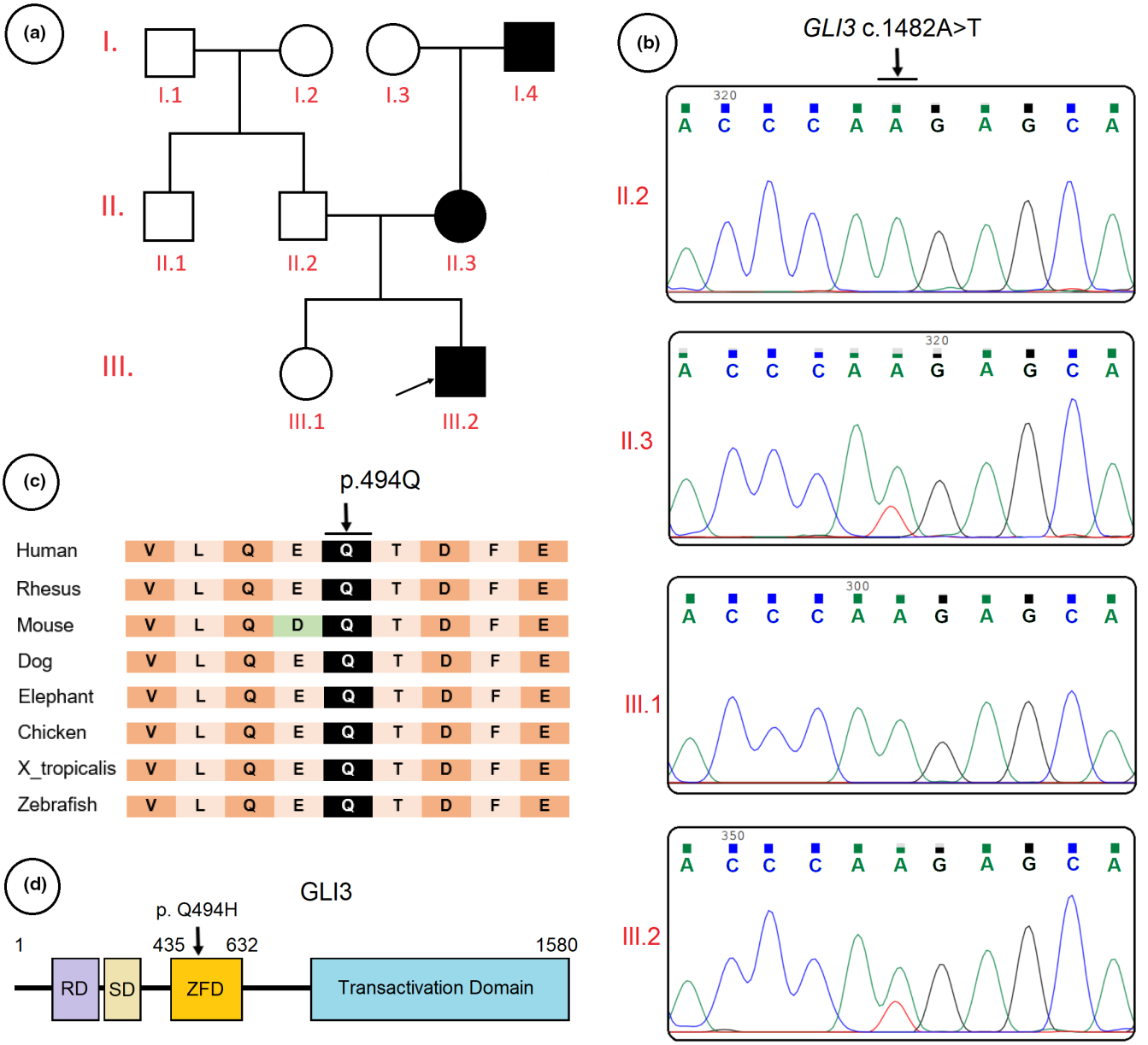

FIGURE 2 (a) Pedigree analysis of the studied family. (b) Verification of the mutation through Sanger sequencing. (c) Analysis of the conservation of the GLI3 p.494Gln position across various vertebrates. (d) Position of p.Gln494His in the biochemical domains of human GLI3-FL: RD (repressor domain), SD (SUFU-binding domain), and ZFD (Zinc finger binding domain).

3.3 | Sanger sequencing and in-silico analyses

The *GLI3* c.1482A>T variant was confirmed to be present in the proband, the proband's mother, and the proband's grandfather in a heterozygous form by Sanger sequencing. Notably, this variant was not detected in the genomes of other family members (Figure 2b). In-depth in silico analyses employing several tools such as Mutation Taster, PredictSNP, SIFT, and Polyphen2 suggested a potential detrimental impact on the protein structure since this variant causes a substitution of a polar uncharged side chain amino acid (Glutamine) by one with a positive charged side chain (Histidine). Additionally, multiple sequence alignments demonstrated that the amino acid position p.494Gln was relatively conserved across different vertebrates (Figure 2c).

4 | DISCUSSION

Polydactyly, a frequently encountered congenital limb deformity, arises from anomalies in the intricate genetic and developmental mechanisms orchestrating limb formation during embryogenesis. The majority of sporadic polydactyly cases typically present unilaterally, while

most of the family cases are bilateral (Malik et al., 2014). Nonsyndromic polydactyly can be categorized into three subtypes: pre-axial polydactyly, central polydactyly, or postaxial polydactyly, depending on which finger is duplicated (Ahmad et al., 2023).

GLI3 is a member of the *GLI* gene family implicated in the orchestration of tissue and organ patterning during embryonic development. Through the binding of proteins encoded by *GLI* family genes to specific DNA regions, regulation of gene expression, that is, activation or repression, is facilitated. The classification of GLI proteins as transcription factors stems from their regulatory role in this process. GLI3 is a transcription factor and repressor, plays an important role in the Hedgehog (Hh) signaling pathway. Variants in the Hedgehog (Hh)/GLI signaling pathway result in perturbations in patterning, consequently impacting normal developmental processes in different animal models (Sigafoos et al., 2021). The Hedgehog (Hh) signaling pathway is stimulated by three ligands: Sonic Hedgehog (Shh), Indian Hedgehog (Ihh), and Desert Hedgehog (Dhh). Of these, Shh exhibits the highest expression level and consequently serves as the principal inducer of a wide array of Hh-related functions, including the development of the brain, limbs, and spinal cord (Fattahi et al., 2018). The inactivation of the *GLI3* gene in medaka fish leads to the development of enlarged dorsal and paired fins with features resembling polydactyly observed in Gli3-deficient mice, revealing a regulatory mechanism mediated by Shh-GLI signaling governing fin size control, likely predating the evolutionary emergence of paired fins and indicating an ancestral resemblance between distal fins and digits in tetrapods (Letelier et al., 2021).

The GLI3 protein exists in two different forms: the full-length (GLI3-FL) and repressor (GLI3-R) forms. In the absence of the Shh signaling pathway, GLI3 undergoes phosphorylation and partial degradation or conversion to GLI3-R, resulting in the repression of Shh signaling. Conversely, when SHH signaling is active, GLI3-FL selectively binds to the promoter region of GLI1 to modulate the expression of genes associated with the SHH signaling pathway. The novel variant detected in this study, *GLI3* c.1482A>T, induces an alteration at the 494th amino acid position within the DNA-binding domain of the protein (from residue 435 to 632), which contains 5 zinc finger repeats (Figure 2d). This domain is shared by both the full-length (GLI3-FL) and repressor (GLI3-R) forms (Matissek & Elsawa, 2020).

Variants within the *GLI3* gene have been detected in individuals presenting with various congenital limb anomalies, including syndactyly (Ngoc et al., 2020), polydactyly, both nonsyndromic (Ahmad et al., 2023; Shen et al., 2022), and syndromic (Speksnijder et al., 2013) types. A frameshift variant of *GLI3* (c.3567_3568insG; p.Ala1190Glyfs*57) located in the transcriptional activator domain was observed in all affected individuals of a large pedigree associated with nonsyndromic postaxial polydactyly in Pakistan (Umair et al., 2019). *GLI3* c.1802A>G (p.His601Arg), a variant also situated within the zinc finger domain of GLI3, has been detected in 14 individuals with variable polydactyly-syndactyly complex in a large Jewish Moroccan family with 4 generations (Volodarsky et al., 2014). Other research revealed *GLI3* c.1826G>A; p.Cys609Tyr, another variant in the zinc finger domain, was associated with digital and skeleton anomalies by exhibiting diminished transcriptional activation capacity and inhibited expression of Hedgehog target genes regulated by additional GLI proteins (Crapster et al., 2017). Substantial evidence suggests that the gene sequence within the zinc finger protein region of the *GLI3* gene plays an indispensable role in embryonic development, shaping the hands and feet.

The result of this study not only expands our understanding of the genetic underpinnings of polydactyly but also holds significant implications for ongoing research into the molecular pathways governing embryonic development. Furthermore, the identified variant presents a promising avenue for the improvement of diagnostic and screening approaches for polydactyly in clinical settings. Our result contributes valuable information that may guide future investigations into the intricate genetic mechanisms involved in limb development and provide valuable insights for clinical applications in polydactyly diagnosis and management.

5 | CONCLUSION

In this study, we investigated a three-generation family exhibiting polydactyly of the index finger, a type for which no responsible gene or locus has been identified to date. Analysis of exome sequencing revealed a novel missense variant located in the DNA-binding domain of the *GLI3* gene (c.1482A>T; p.Gln494His) in the proband, suggesting its potential causative role in the disorder. Notably, this variant was consistently present in all affected family members but not in unaffected individuals within the pedigree. This finding has the potential to significantly contribute to ongoing research on the molecular pathways involved in polydactyly during embryonic development. Moreover, it holds promise for enhancing diagnostic and screening capabilities for polydactyly in clinical settings.

AUTHOR CONTRIBUTIONS

T.N Nguyen designed the study and received the grant for the study. H.D Hoang carried out the sampling. T.N Nguyen conducted the laboratory work. T.N Nguyen, L.G Nguyen and G.S Tran analyzed the data. T.N Nguyen wrote the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest regarding the research presented in this manuscript.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available upon request.

ETHICS STATEMENT

This study was conducted following ethical standards and guidelines. This research was approved by the Council for Ethics in Biomedical Research of the Vietnam National Children's Hospital (No. 564/BVNTW-VNCSKTE). Written informed consent was obtained from all family members involved in this study. Consent was specifically secured for genetic testing and the publication of anonymized data.

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