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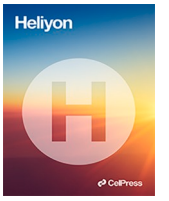

Case report

A novel *GLI3* frameshift mutation in a Chinese pedigree with polydactyly: A case report

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ABSTRACT

Background: *GLI3* gene mutations can result in various forms of polysyndactyly, such as Greig cephalopolysyndactyly syndrome (GCPS, MIM: #175700), Pallister–Hall syndrome (PHS, MIM: #146510), and isolated polydactyly (IPD, MIM: #174200, #174700). Reports on IPD-associated *GLI3* mutations are rare. In this study, a novel *GLI3* mutation was identified in a Chinese family with IPD.

Results: We report a family with six members affected by IPD. The family members demonstrated several special phenotypes, including sex differences, abnormal finger joint development, and different polydactyly types. We identified a novel frameshift variant in the *GLI3* gene (NM_000168.6: c.1820_1821del, NP_000159.3: p.Tyr607Cysfs*9) by whole-exome sequencing. Further analysis suggested that this mutation was the cause of polydactyly in this family.

Conclusions: The discovery of this novel frameshift variant in our study further solidifies the relationship between IPD and *GLI3* and expands the previously established spectrum of *GLI3* mutations and associated phenotypes.

1. Introduction

Polydactyly is a common limb malformation in humans characterised by the presence of extra fingers or toes. It is estimated to occur in 0.3–3.6 per 1000 live births and 1.6–10.7 per 1000 individuals in the general population [1–4]. Extensive research has identified over 100 genes associated with human polydactyly, including *GLI3*, *GLI2*, *HOXD13*, *SHH*, *FGF8*, and *WNT7A*. Mutations in these genes have been associated with limb malformation [5–9]. Notably, *GLI3* gene mutations cause various polydactyly phenotypes [10,11].

Greig cephalopolysyndactyly syndrome (GCPS, MIM: #175700) is a *GLI3*-related disorder characterised by craniofacial dysmorphology, polydactyly and syndactyly, and central nervous system development anomalies [12]. The *GLI3*–related Pallister–Hall syndrome (PHS, MIM: #146510) affects the development of multiple organs and tissues and is characterised by polydactyly, hypothalamic hamartoma, anal and respiratory system development anomalies, and other potential abnormalities, such as kidney and heart defects [13].

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In addition to syndromic polydactyly, *GLI3* mutations are associated with isolated polysyndactyly (IPD), including polydactyly preaxial type IV (PPD–IV, MIM: #174700) and postaxial types A and B (PAP–A and –B, MIM: #174200) [9,11]. PAP–A and PAP–B involve extra digits on the hand or foot's outer side (little finger or little toe), respectively. Those with PAP–A exhibit a fully formed extra digit, whereas those with PAP–B have a smaller, partially formed digit that may resemble a skin tag. PPD–IV involves the presence of extra digits on the inner side (thumb or big toe) of the hand or foot. The degree of thumb duplication is usually mild, whereas fingers 3 and 4 exhibit varying syndactyly degrees. Duplication of part or all of the first toe is commonly observed, accompanied by syndactyly affecting all of the toes, particularly the second and third [1,14]. However, due to the scarcity of reports on *GLI3* mutations, how different mutations induce symptoms of different severity needs further study.

In this study, we investigated a family with six individuals affected by IPD. A novel variant within the *GLI3* gene was identified by whole-exome sequencing (WES) in four affected individuals in the family, further expanding the spectrum of *GLI3* mutations and phenotypes.

2. Case presentation

2.1. Clinical presentation

The proband of this study was a 2-year-old male admitted to Jinhua Municipal Central Hospital in November 2016 for polydactyly. The family history examination revealed 6 patients (1 female and 5 male) with polydactyly deformities in the four-generation pedigree (Fig. 1). All the family members were native southern Chinese individuals born in Jinhua, Zhejiang Province, China. This study was approved by the Medical Ethics Committee of Jinhua Central Hospital (Approval No. 2016LS169) and written informed consent was obtained from all participating individuals.

The proband (IV2) presented with great toe duplication in both feet (preaxial polysyndactyly type IV, PPD–IV). He demonstrated fusion of the first and second toes in the right foot and fusion of the first, second, and third toes in the left foot (Fig. 2A). X-ray imaging revealed fully developed bones in the toes (Fig. 2B). The extra toes in both feet were removed surgically with good recovery (post-operative figures not provided).

The father (III2) also had bilateral great toe duplication involving fusion of the first and second toes (PPD–IV) (Fig. 2C). An important phenotypic difference between the proband and his father is that the proband exhibited fusion of the first, second, and third toes in his left foot, whereas his father demonstrated fusion of the first and second toes in both feet. In addition, the father's extra toes did not have full bone structure (Fig. 2D).

The grandmother (II2) of the proband, in contrast to her son and grandson, presented a more severe manifestation of bilateral PPD–IV (Fig. 2E). Specifically, there was a duplicated toe segment on her right foot consisting of only one phalanx (Fig. 2F). Moreover, fusion occurred between the first and second toes and the third, fourth, and fifth toes on both feet.

Individual II4 displayed unique symptoms, presenting with a soft tissue protrusion on the ulnar side of the right hand (PAP–B)

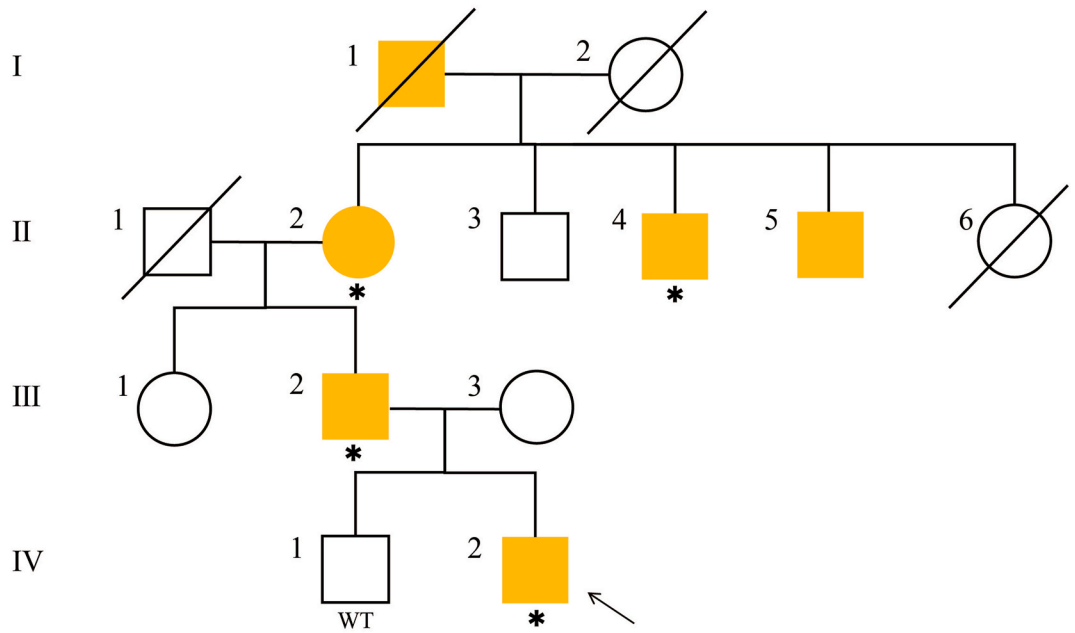

Fig. 1. Genetic pedigree map of the *GLI3* gene mutation in the family. The arrow indicates the proband. The * symbol represents individuals carrying the NM_000168.6:c.1820_1821del heterozygous mutation, while WT represents individuals with the wild–type allele. I1 was also affected by polydactyly, but the cause of his symptoms remains unknown.

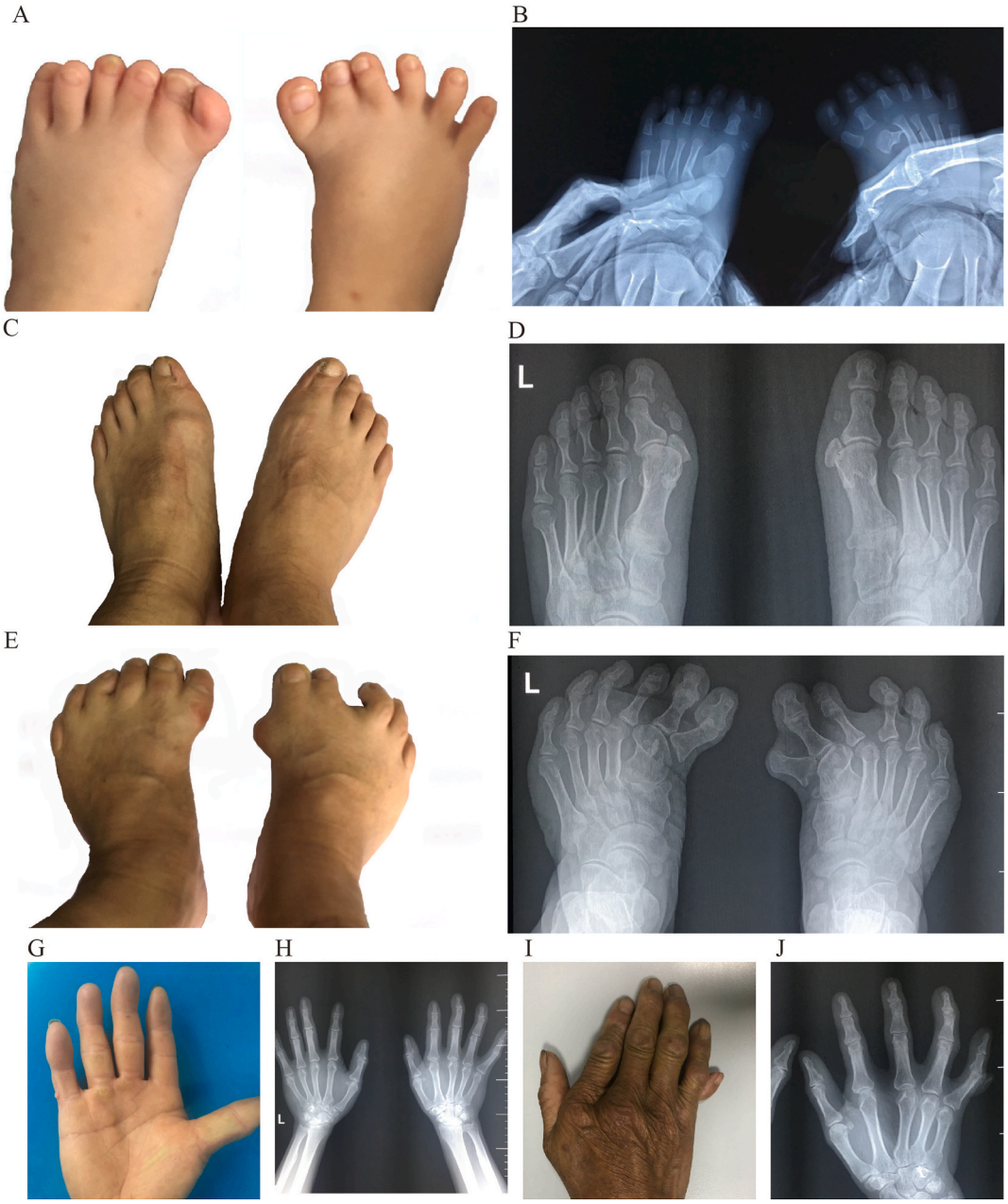

Fig. 2. Phenotypic manifestations of the *GLI3* gene mutation in the family. (A), (B) Proband (IV2): Bilateral PPD–IV, fusion of the first and second toes in the right foot, and fusion of the first, second, and third toes in the left foot. (C), (D) Father (III2): Bilateral PPD–IV with a fusion of the first and second toes on both feet. Additional toes were surgically removed. (E), (F) Grandmother (II2): Severe bilateral PPD–IV. Fusion of the first, second, third, fourth, and fifth toes on both feet. The duplicated toe segment in the right foot consisted of one phalanx. (G), (H) Individual II4: Soft tissue protrusion on the ulnar side of the right hand, absence of bone structure and nail. The little finger of the right hand appeared to have only one joint; however, no abnormalities were observed on X–ray. (I), (K) Individual II5: PAP–B. Underdeveloped accessory digit with a soft tissue protrusion on the ulnar side with a nonosseous connection to the normal portion.

lacking bone structure and nails (Fig. 2G). The little finger of the right hand had only one joint, but an X–ray examination showed no abnormalities (Fig. 2H).

Individual II5 also had PAP–B, one of the most common types of polydactyly (Fig. 2I). The accessory digit was underdeveloped, with a soft tissue protrusion on the ulnar side, forming a nonosseous connection to the normal portion (Fig. 2J).

All members of this family exhibited normal intelligence, language, and cognitive abilities.

2.2. Identification of a novel candidate mutation in the family

To determine the cause of polydactyly in this family, we performed WES on peripheral blood samples collected from the proband (IV2), unaffected brother (IV1), and affected father (II2). The WES results revealed a novel frameshift mutation, NC_000007.13: g.42012220_42012221del (GRCh37), NM_000168.6:c.1820_1821del, NP_000159.3:p.Tyr607Cysfs*9, within the 13th exon of the *GLI3*

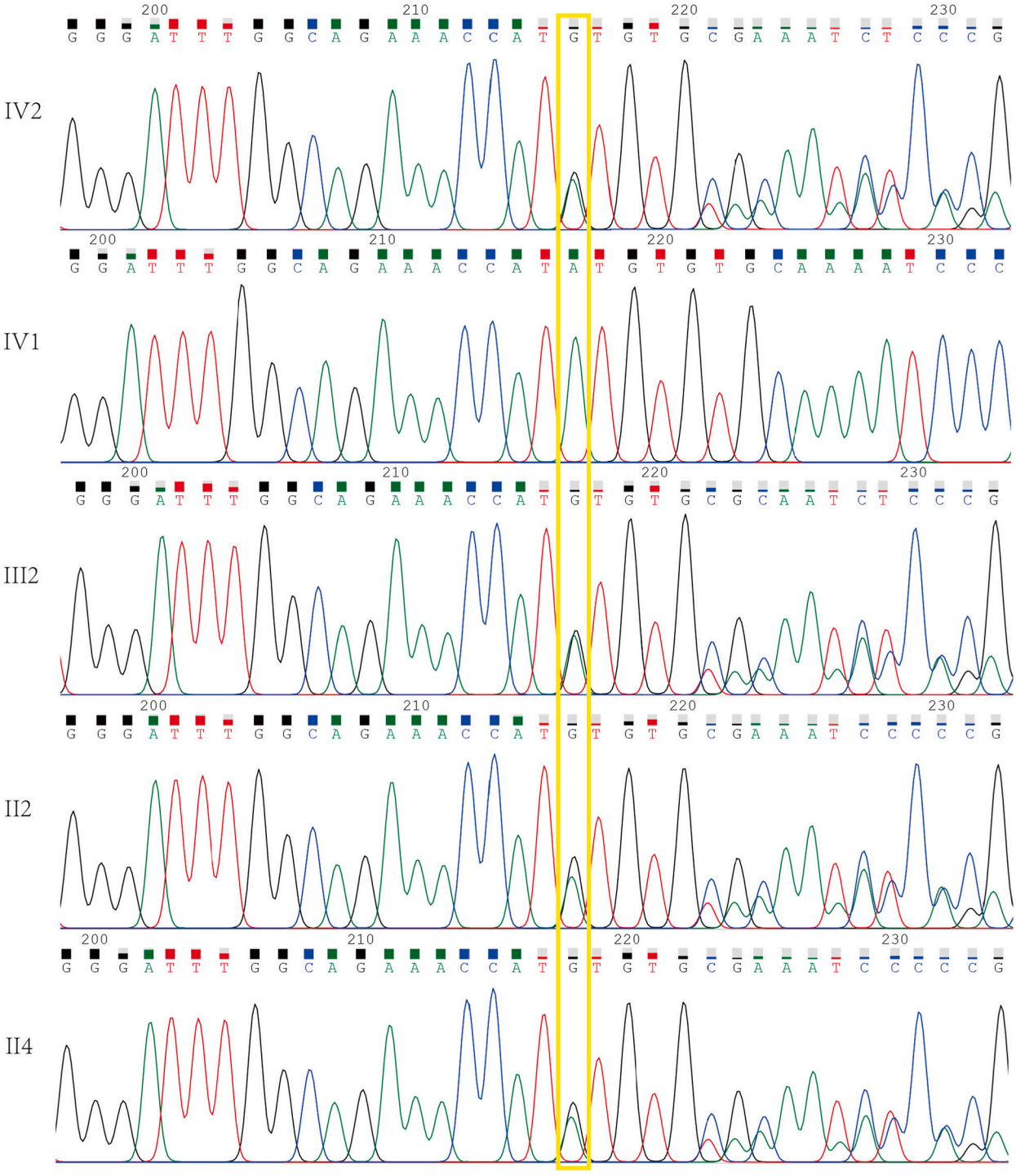

Fig. 3. Sanger sequencing of the *GLI3* gene. The results revealed heterozygous NM_000168.6:c.1820_1821del mutations in IV2, III2, II2, and II4. IV1, who remains unaffected, does not carry this mutation. The yellow box indicates the location of the mutation.

gene. No other rare pathogenic variants were detected in any other genes associated with skeletal malformation.

We conducted Sanger sequencing to verify the presence of the identified mutations within the proband's family and confirmed that four patients (IV2, III2, II2, II4) were heterozygous for *GLI3* c.1820_1821del (Fig. 3). The proband's unaffected brother (IV1) did not carry this mutation, indicating that this mutation co-segregated with polydactyly in this family. These observations are consistent with an autosomal dominant inheritance pattern.

2.3. Pathogenicity evaluation of candidate mutations according to ACMG guidelines

We followed the standards and guidelines set by the American College of Medical Genetics and Genomics (ACMG) to evaluate the pathogenicity of *GLI3* c.1820_1821del.

According to gnomAD, *GLI3* is intolerant of protein-truncating variations at pLI = 1 and o/e = 0.09, indicating that loss of function is the causative mechanism. This frameshift mutation was predicted to result in a premature termination codon (PTC) in the ninth codon after the mutation, leading to PTC-triggered nonsense-mediated mRNA decay (NMD) (PVS1). In addition, we compared the variant with publicly available databases and found no records of it in the gnomAD, 1000 Genomes, dbSNP, Mastermind, ClinVar, or HGMD databases (PM2_Supporting). This mutation also co-segregated with five individuals in the family, further supporting its potential pathogenicity (PP1_Moderate). These criteria provide compelling evidence supporting *GLI3* c.1820_1821del as a pathogenic mutation, with one category of very strong evidence and two categories of moderate evidence (PVS1+PM2_Supporting + PP1_Moderate). These findings suggest that the identified variant is the cause of polydactyly in this family.

3. Discussion

Genetic testing on a family with digit anomalies revealed a novel frameshift mutation in the *GLI3* gene, named NM_000168.6: c.1820_1821del. According to the standards set by the ACMG, this mutation can be classified as pathogenic. During genetic counselling, the doctor recommended that the affected family members undergo a third-generation in vitro fertilisation technique before pregnancy or prenatal diagnosis during pregnancy to prevent the inheritance of polydactyly.

It is suggested that there is substantial clinical heterogeneity in *GLI3* mutation-associated IPD when comparing the deformities of this family with previously reported cases [10,15–17]. In this family, three members exhibited bilateral PPD–IV with varying degrees of syndactyly in their feet, while two members displayed unilateral PAP–B in their right hands. This kind of heterogeneity within the family, including the types of polydactyly (PPD and/or PAP) and affected organs (hands and/or feet, unilateral and/or bilateral), has also been described in other reported pedigrees (Supplementary Table 1, family ID F1, F2, F5, F6, F7, F8, F12, F14). Another interesting observation in our study is that the only female patient (II2) exhibited more severe symptoms than her son (III2) and grandson (IV2). Similar differences in sexual symptoms between females and males have also been reported previously (see Supplementary Table 1, family ID F1). However, this is not absolute, as some females only exhibit syndactyly or have fewer affected organs than their male relatives in the family (Supplementary Table 1, family ID F5, F6, F14). Thus, further investigation is needed to determine if there are sex differences in *GLI3*–associated polydactyly.

In addition to the common symptoms of PAP–B and PDP–IV, we also identified a condition affecting the little finger in family member II4. The little finger's external appearance indicated only two phalanges, resembling a thumb; however, an X–ray revealed normal anatomy. Other sporadic deformities, such as clino–symphalangism and central polydactyly (Supplementary Table 1, family ID F1, F8), have also been reported in *GLI3*–associated IPD, aligning with our findings. In July 2013, Carsten G. Bönnemann et al. identified two children with unique characteristics, namely unusually short and rigid little fingers with small nails, multiple tongue frenulum syndrome, hypothalamic hamartoma, and mild to moderate neurological impairments [18]. These symptoms appear similar to those of hereditary *GLI3*–associated PHS but demonstrate certain distinctions. Despite extensive analyses, no pathogenic or likely pathogenic variants were detected in the *GLI3* gene of these two children. Thus, a detailed and comprehensive phenotype analysis is required for all individuals carrying *GLI3* mutations to identify mild symptoms and make a diagnosis.

The novel variant identified in our study, p.Tyr607Cysfs*9, results from a frameshift *GLI3* mutation that shifts the reading frame after tyrosine 607 in the zinc finger domain (ZFD) region. A new stop codon is generated at the ninth codon downstream of the mutation, potentially triggering NMD. NMD is an RNA surveillance mechanism that recognises and degrades aberrant mRNA molecules containing PTCs [19]. NMD can lead to *GLI3* gene haploinsufficiency, resulting in the manifestation of limb abnormalities commonly observed in GCPS in the proband and other affected family members. Some studies have suggested that IPD represents a milder form of GCPS rather than a separate entity, and milder forms of GCPS with subtle phenotypes, such as mild craniofacial dysmorphisms, might have been missed [10,20].

The *GLI3* gene encodes a polypeptide chain consisting of 1580 amino acids. The protein is divided into three domains: the ZFD, the cyclic AMP–binding protein–binding domain (CBPD), and two transactivation domains (TA1 and TA2). Then ZFD comprises five highly conserved tandem zinc finger structures that possess specific DNA-binding abilities [11,21,22]. *GLI3* is a key zinc finger transcription factor critical to the Sonic Hedgehog (Shh) signalling pathway and plays a vital role in limb development [10,23]. *GLI3* has dual functions as a transcriptional activator and a repressor within this pathway. After phosphorylation and nuclear translocation, the full-length *GLI3* form (Gli3FL), referred to as Gli3A, functions as an activator. Conversely, its C–terminal truncated form, Gli3R, acts as a repressor [24]. Maintaining a proper balance between the Gli3A activator and the repressor Gli3R is important for specifying the digit number and identity during limb development [1,11]. However, the pathogenesis of disorders caused by *GLI3* mutations, especially why different *GLI3* mutations lead to various disorders, has not been determined. It is important to elucidate the specific pathogenic mechanisms that may contribute to developing precise treatments for GLI3–associated disorders.

Previous studies have indicated that the phenotype of *GLI3*–related disorders is correlated with the mutation location [11,22]. GCPS is associated with variants affecting either the N–terminal (upstream and ZFD region) or the C–terminal third of the protein. Most PHS pathogenic variants affect the middle third of the *GLI3* protein, while IPD occurs when variants affect the C–terminal third of the *GLI3* protein [11]. The type of *GLI3* mutation may also affect disease severity. PTC–triggered NMD might induce milder symptoms in some diseases caused by genes with dominant negative effects, including *CFTR*–associated cystic fibrosis and *HTT* mutation-induced Huntington's disease [25,26]. In fact, recent research has also suggested that nonsense variants causing IPD involve almost the entire coding region of *GLI3* [10]. Thus, the symptoms of diseases caused by *GLI3* mutation might be associated with both the location and type of mutation, and further investigation into the disease pathogenesis is required.

4. Conclusion

Our study successfully identified a novel frameshift mutation in the *GLI3* gene in a family with digit abnormalities. By adhering to the ACMG guidelines and providing strong evidence supporting the pathogenic nature of the identified variant, we have expanded our understanding of the phenotypic spectrum of developmental anomalies. The study suggests that polydactyly patients could benefit from family genetic testing for clinical diagnosis and management.

Ethics statement

Written informed consent was obtained from the individuals for publication of any potentially identifiable images or data included in this article.

Data availability statement

The data associated with our presented case has not been deposited into a publicly available repository for privacy concern. But the data will be made available on request.

CRediT authorship contribution statement

Chi Zhao: Resources, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization. **Chengcheng Gao:** Writing – original draft. **Yijun Zhu:** Methodology. **Qi Zhang:** Writing – original draft, Visualization, Software. **Ping Lin:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Chi Zhao reports financial support was provided by Jinhua Municipal Central Hospital Young and Middle-aged Scientific Research Start-up Fund. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28638.

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