ZPA Regulatory Sequence Variants in Chinese Patients With Preaxial Polydactyly: Genetic and Clinical Characteristics

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Preaxial polydactyly (PPD) is a common congenital abnormality with an incidence of 0.8–1.4% in Asians, characterized by the presence of extra digit(s) on the preaxial side of the hand or foot. PPD is genetically classified into four subtypes, PPD type I–IV. Variants in six genes/loci [including GLI family zinc finger 3 (GLI3), ZPA regulatory sequence (ZRS), and pre-ZRS region] have been identified in PPD cases. Among these loci, ZRS is, perhaps, the most special and well known, but most articles only reported one or a few cases. There is a lack of reports on the ZRS-variant frequency in patients with PPD. In this study, we recruited 167 sporadic or familial cases (including 154 sporadic patients and 13 families) with PPD from Central-South China and identified four ZRS variants in four patients (2.40%, 4/167), including two novel variants (ZRS131A>T/chr7:g.156584439A>T and ZRS474C>G/chr7:g.156584096C>G) and two known variants (ZRS428T>A/chr7:g.156584142T>A and ZRS619C>T/chr7:g.156583951C>T). ZRS131A>T and ZRS428T>A were detected in PPD I cases and ZRS474C>G and ZRS619C>T combinedly acted to cause PPD II. The detectable rate of ZRS variants in PPD I was 1.60% (2/125), while PPD II was significantly higher (9.52%, 2/21). Three bilateral PPD cases harbored ZRS variants (13.64%, 3/22), suggesting that bilateral PPD was more possibly caused by genetic etiologies. This study identified two novel ZRS variants, further confirmed the association between ZRS and PPD I and reported a rare PPD II case resulted from the compound heterozygote of ZRS. This investigation preliminarily evaluated a ZRS variants rate in patients with PPD and described the general picture of PPD in Central-South China.

Keywords: ZRS, preaxial polydactyly type I, preaxial polydactyly type II, enhancer, SHH

INTRODUCTION

Preaxial polydactyly (PPD) is a common congenital abnormality with an incidence of 0.8–1.4% in Asians, characterized by the presence of extra digit(s) on the preaxial side of the hand or foot (1). Severity varies from mere broadening of the distal phalanx with slight bifurcation at the tip to full duplication of the thumb, including the metacarpals (2). PPD is genetically classified into...
four subtypes, PPD type I–IV (Table 1) (3). PPD I (OMIM 174400) indicates the duplication of one or more of the skeletal components of biphalangeal thumbs, which is the most common subtype in many populations (2). PPD II (OMIM 174500) refers to isolated triphalangeal thumbs or the thumb duplication with triphalangeal components (4). PPD III (174600) is also known as index finger polydactyly. Thumbs of PPD III cases are replaced by one or two index fingers (5). PPD IV (174700) is polysyndactyly of the thumb (6).

Currently, only six genes/loci [GLI1, GLI3, serine/threonine kinase like domain containing 1 (STKLD1), ZPA regulatory sequence (ZRS), pre-ZRS region, and a deletion of 240 kb from the sonic hedgehog signaling molecule (SHH) promoter] have been identified in isolated PPD cases and ZRS is, perhaps, the most special and well known (7–12). ZRS, the zone of polarizing activity (ZPA) (located in the posterior region of the limb bud) regulatory sequence, is a limb-specific enhancer of SHH, which is located nearly 1 Mb from SHH and within intron 5 of Limb development membrane protein 1 (LMBR1) (4). ZRS can promote the expression of SHH in ZPA during the limb development. SHH diffuses from ZPA (posterior mesoderm) to anterior region of limb bud and there is no SHH in anterior region. The graded distribution of SHH determines the finger pattern. ZRS variants would alter the expression of SHH and cause limb deformities. ZRS variants and duplications had been shown to cause PPD I, PPD II, Werner mesomelic syndrome (WMS) (OMIM 188770), and other limb deformities (such as mirror-image polydactyly and radial ray deficiency) (12–16). The correlation between PPD and ZRS is definite, but most articles only reported one or a few cases, especially in PPD II cases. There is a lack of reports on the ZRS-variant frequency in patients with PPD.

In this study, we recruited 167 sporadic or familial cases with PPD from Central-South China. We identified four ZRS variants in four PPD cases (4/167, 2.40%), including two novel variants (ZRS131A>T and ZRS474C>G) and two known variants (ZRS428T>A and ZRS619C>T) (Table 1). This study preliminarily investigated the ZRS variant rate in patients with PPD living in Central-South China, expanded the spectrum of ZRS variants, furthered our understanding of PPD, and contributed to genetic diagnosis and counseling of patients with PPD.

**Materials and Methods**

**Patients and Subjects**

This study was approved by the Review Board of Xiangya Hospital of Central South University. A total of 167 sporadic or familial PPD cases admitted to the Department of Orthopaedics of Xiangya Hospital were recruited. They were all from Central-South China, especially Hunan province. Almost subjects were preschoolers and informed consent forms were obtained from the patients and their guardians. All the subjects and their guardians consented to participate in this study and to publication of the images. Blood was collected from patients and their family members to extract genomic DNA by the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, United States).

**Deoxyribonucleic Acid Extraction**

Peripheral blood samples were collected from patients and their family members to extract genomic DNA by the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, United States).

**Variant Screening**

The highly conserved 774-bp region of the ZRS (chr7: 156583796–156584569, hg19) was obtained from the National Center for Biotechnology Information (NCBI) database and primers were designed by Integrated DNA Technologies (IDT) (Table 2) (17). PCR was operated to amplify the target sequences by CFX384 Touch PCR Amplifier (Bio-Rad, Hercules, CA, United States). PCR product sequences were determined using the ABI 3100 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, United States) by Sanger sequencing performed by Boshang Biotechnology Co., Ltd. (Shanghai, China). For patients who were identified ZRS variants, further genetic screening (using PCR and Sanger sequencing) was used to detect whether they harbored pathogenic variants in GLI3 (NM_000168.6, NP_000159.3), GLI1 (NM_005269.3, NP_005260.1), STKLD1 (NM_153710.5, NP_714921.4), or pre-ZRS. Their primer pairs were also designed by IDT (Table 2).

**Prediction of Pathogenicity**

MutationTaster (http://www.mutationtaster.org/) was applied for predicting the pathogenicity of variants. GnomAD^3 was used to annotate the minimum allele frequency (MAF) of variants. ZRS sequences of species from Evgeny et al. (18) were used to compare the conservation of variant sites (18).

**Results**

We recruited 167 cases with PPD from Central-South China, including 154 sporadic patients and 13 families, named as PPD001–PPD167 depending on the order of recruitment. Among these cases, almost subjects (154/167, 92.22%) were Han Chinese and 148 patients had isolated PPD (Table 3). Based on PPD subtypes to divide subjects, 125 patients (74.85%) revealed PPD I, 21 patients (12.57%) had PPD II, and only four cases exhibited PPD III (1/167, 0.60%) or PPD IV (3/167, 1.80%). The rest of PPD subjects (17 cases, 10.18%) presented other organ malformations, such as congenital heart disease, radial ray deficiency, and anal atresia. There were 103 male patients (61.68%) and 64 female patients (38.32%). Most subjects were younger than 3 years old. Except 19 cases without clinical details, the overwhelming majority of PPD I/II was unilateral (109/127, 85.83%), in which PPD, on the right hand, accounted for almost two-thirds (72/109, 66.06%; Table 3).

In accordance with the flow diagram (Figure 1), we identified four ZRS variants in four patients (PPD003, PPD029, PPD116, and PPD154; Table 4). The detectable rate of ZRS variants in PPD

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1. https://www.ncbi.nlm.nih.gov/gene/103804841
2. http://sg.idtdna.com/Primerquest/Home/Index
3. http://www.mutationtaster.org/
4. http://gnomad-sg.org/
I was 1.60% (2/125), while PPD II was significantly higher (2/21, 9.52%). Three of these four patients were with bilateral thumbs involvement, occupying 13.64% of bilateral PPD (3/22). None ZRS variant was identified in patients with left PPD, although they were more than one-third total subjects (37.72%, 63/167).

**PPD029 Family**

The proband of PPD029 (III:1) was a 4-year-old girl, who presented bilateral triphalangeal thumbs (Figures 2A–C). She harbored compound heterozygous variants in ZRS (ZRS474C > G/chr7:g.15658496C > G and ZRS619C > T/chr7:g.156583951C > T) without GLI3, GLI1, STKLD1, or pre-ZRS variants (Figure 2D). ZRS474C > G was inherited from her father (II:2) and another variant was from her mother (II:3). Other family members without variants or with only one variant were unaffected.

**PPD154 Family**

The proband of PPD154 (II:1) was a boy with PPD I on the right hand (Figures 2E–G). He was admitted to our hospital for operative treatment at his age of 1.5 years. We identified a de novo variant in ZRS (ZRS131A > T/chr7:g.156584439A > T) in this patient and did not find suspicious variants in GLI3, GLI1, STKLD1, and pre-ZRS (Figure 2H). His parents were unaffected.

**PPD003 Family and PPD116 Family**

Known ZRS variant (ZRS428T > A/chr7:g.156584142T > A) was identified in two families with PPD II (PPD003) or PPD I (PPD116). Four variants identified in this study were highly evolutionarily conserved and were predicted to be disease-causing by MutationTaster (Figure 2I and Table 4).

**DISCUSSION**

Polydactyly is the most common limb malformation in China and PPD is over half (data from National Stocktaking Report on Birth Defect Prevention)\(^8\). PPD I is the most common subtype and PPD III is rarest (2, 19). In this study, 125 cases (74.85%, 125/167) had PPD I and only one patient (1.80%, 1/167) was diagnosed with PPD III. 17 patients with PPD (10.18%, 17/167) had other organ malformations, including congenital heart disease, radial ray deficiency, and anal atresia. These complications were relatively frequent in patients with PPD. Male patients with PPD are approximately twice as many as female (19). In this study, the proportion of male patients was 61.68% (103/167). This study showed that overwhelming majority of PPD I/II were unilateral (85.83%, 109/127), in which PPD, on the right hand, accounted for almost two-thirds (66.06%, 72/109), consistent with previous studies (19, 20).

In this study, we tested ZRS variants in 167 patients with PPD and identified unique variants in four cases (2.40%, 4/167). The detectable rate of ZRS variants in PPD I was 1.60% (2/125), while PPD II was significantly higher (9.52%, 2/21). Indeed, most known ZRS variants are identified in PPD II cases (data from the human gene mutation database (HGMD))\(^8\).

In this study, three ZRS variants were associated with bilateral PPD and 13.64% bilateral PPD cases (3/22) harbored ZRS variants, suggesting that bilateral PPD was more possibly caused by genetic etiologies. Compared with that no ZRS variant was detected by Xiang et al. (20) in 82 Chinese patients with PPD I/II or Rao et al. (21) in 72 Chinese patients with PPD, our identification was fortunate (20, 21). For the remaining 163 cases, we planned to applied chromosomal microarray analysis (CMA), whole-exon sequencing (WES), and genome-wide association study (GWAS) to detect their genetic etiologies. Furthermore, environmental factors, such as alcohol, are causes of limb deformities (22).

Of these four variants, ZRS131A > T and ZRS428T > A were novel and ZRS474C > G were reported in patients with PPD II (4, 15). ZRS428T > A was identified in both the patients with PPD I (PPD116) and PPD II (PPD003), suggesting the variability of ZRS428T > A-related clinical phenotypes. ZRS131A > T was identified in a sporadic case with PPD I (PPD154). Generally, ZRS variants are associated with PPD II and ZRS was first linked with PPD I by Xu et al. (12). Our report may be the second case worldwide, further demonstrating the correlation between ZRS and PPD I.

PPD029 was a rare case. We found that the proband harbored the compound heterozygote of ZRS (ZRS474C > G and ZRS619C > T). Given that Jacob et al. (23) reported ZRS variant carriers with minor anomalies and underlined the importance of accurate clinical examination in mild triphalangeal thumb families, we carefully checked the phenotypes of her family members with one ZRS variant and did not find any

8\[https://wenku.so.com/d/8c145f3c9e9b4892c02bf7c70aa83d01\]

8http://www.hgmd.cf.ac.uk/ac/search.php

### Table 1 | Classification of PPD and their clinical features and causative genes/loci.

| Subtype | OMIM | Clinical features | Heredity | Gene/Locus |
|---------|------|-------------------|----------|------------|
| PPD I   | 174400 | The duplication of one or more of the skeletal components of biphalangeal thumbs | AR | GLI1 |
| PPD II  | 174500 | Isolated triphalangeal thumb or thumb duplication with a triphalangeal component | AD | ZRS |
| PPD III | 174600 | Thumbs replaced by one or two index fingers | AD | pre-ZRS |
| PPD IV  | 174700 | Polydactyly of the thumb | AD | GLI family zinc finger 3 (GLI3) |

PPD, preaxial polydactyly; AD, autosomal dominant; AR, autosomal recessive.
### TABLE 2 | Primer pairs of ZPA regulatory sequence (ZRS), GLI3, GLI1, STKLD1, and pre-ZRS.

| Primer | Sequences (5′→3′) | Primer | Sequences (5′→3′) |
|--------|-------------------|--------|-------------------|
| ZRS 1f | GGAGGTATAACCTCTGGCCAGTG | ZRS 1r | CCGCTCCACCTCTGGTCAATCC |
| ZRS 2f | CCAGAGGCGTAGCAACAGGCTC | ZRS 2r | CAATTATGGATGATCAGTGC |
| ZRS 3f | TCAGGCCCTCCTAATCGAGAG | ZRS 3r | GAAATGGTATGTTGACAAAGT |
| GLI3 1f | GAAAGTTGATGGCTCTGTTGTTT | GLI3 1r | CAGGATCGAAAGACCTCAATCT |
| GLI3 2f | GCTCTCAAAGTTGCTGTGAATG | GLI3 2r | TGGGAAAGAAGTAGGCAAGATAG |
| GLI3 3f | CAGTTCGAGGGCCAGGGATAG | GLI3 3r | CAGTTCGAGGGCCCTGAAAGT |
| GLI3 4f | GCTCTGGTGATGATGAGAGGT | GLI3 4r | GGAGGTATGGCTCTGTTGTTT |
| GLI3 5f | TGTTGGTCTCTCCCTTTCTATTG | GLI3 5r | GCAATGCGGGTCAAGGTCTT |
| GLI3 6f | TCTCTCTCCCTCTTCTTCCCATG | GLI3 6r | GCAATGCGGGTCAAGGTCTT |
| GLI3 7f | TGTTGGTCTCTCCCTTTCTATTG | GLI3 7r | GCAATGCGGGTCAAGGTCTT |
| GLI3 8f | TGTTGGTCTCTCCCTTTCTATTG | GLI3 8r | GCAATGCGGGTCAAGGTCTT |
| GLI3 9f | TGTTGGTCTCTCCCTTTCTATTG | GLI3 9r | GCAATGCGGGTCAAGGTCTT |
| GLI3 10f | AGGAAGCATGCATACACAGTTA | GLI3 10r | CATCAGTTTGGCTCTCGAGGG |
| GLI3 11f | AACTTGGAGGGCGTGTTAG | GLI3 11r | CATCAGTTTGGCTCTCGAGGG |
| GLI3 12f | TACCTGCCTCCTGCTATTG | GLI3 12r | CATCAGTTTGGCTCTCGAGGG |
| GLI3 13f | ATTGGTCACTGGTGTATGTG | GLI3 13r | CATCAGTTTGGCTCTCGAGGG |
| GLI3 14-1f | TGGTCTCTCCCTTTCTATTG | GLI3 14-1r | CATCAGTTTGGCTCTCGAGGG |
| GLI3 14-2f | CAGCAGTACCGCCTCAAG | GLI3 14-2r | CATCAGTTTGGCTCTCGAGGG |
| GLI3 14-3f | CAGCACCCCGCAACTCACTC | GLI3 14-3r | CATCAGTTTGGCTCTCGAGGG |
| GLI3 14-4f | CACCCTAGTGAAGAAGGAT | GLI3 14-4r | CATCAGTTTGGCTCTCGAGGG |
| GLI3 14-5f | AGATGCGCATGGGACAGATG | GLI3 14-5r | CATCAGTTTGGCTCTCGAGGG |
| GLI3 14-6f | AGATGCGCATGGGACAGATG | GLI3 14-6r | CATCAGTTTGGCTCTCGAGGG |

**Continued**
TABLE 2 (Continued)

| Primer | Sequences (5'→3') | Primer | Sequences (5'→3') |
|--------|-------------------|--------|-------------------|
| STKLD1 17f | TTCTTGACATGTCCTGTTCA | STKLD1 17r | GCAGAATGAGGTGGAATTAA |
| STKLD1 18f | CCACACTAAACTCCACACTCA | STKLD1 18r | CAGGAAACTCCTTGAGAGC |
| pre-ZRS 1f | GGAAGTGCTGCTTAGTGTTAGT | pre-ZRS 1r | GTTCCACATAGGAGCACTAT |
| pre-ZRS 2f | GCTGTGACATGCTCCT | pre-ZRS 2r | GCCATATTTAGTGCCTCC |
| pre-ZRS 3f | AAATCTGGGCCTAGTGGAAC | pre-ZRS 3r | CCGTTGAGACAGTACGTAGTA |
| pre-ZRS 4f | TGATACCTAGAGGGAACACTAA | pre-ZRS 4r | CAGAGGCTGAGACTCATACAC |
| pre-ZRS 5f | ACATCAGGGACACTTGATTGG | pre-ZRS 5r | CACCCAAGGCTAGTAC |
| pre-ZRS 6f | ACTGGCCTGTAATACCTCAGT | pre-ZRS 6r | AACAATCTCCTGGCTTTGAT |

TABLE 3 | Characteristics and clinical phenotypes of all the subjects.

| Characteristics | PPD I | PPD II | PPD III | PPP IV | Others* | Total | PPD with ZRS variants |
|-----------------|-------|--------|---------|--------|---------|-------|-----------------------|
| Age (years)     | 3.326 ± 0.518 | 2.730 ± 0.695 | 0.9 | 3.400 ± 0.513 | 6.029 ± 1.904 | 3.529 ± 0.446 | 1.750 ± 0.777 |
| Gender          | 78 M; 47 F | 12 M; 9 F | 1 M; 0 F | 10 M; 7 F | 6 M; 6 F | 3 M; 2 F; 1 F | 10 M; 6 F |
| Ethnicity (Han) | 114 | 19 | 1 | 3 | 17 | 3 | 100.00% |
| Other ethnicities** | 11 | 2 | 0 | 0 | 0 | 13 | 0 |
| Number          | 125 | 21 | 1 | 3 | 17 | 167 | 4 |
| Proportion      | 74.85% | 12.57% | 0.60% | 1.80% | 10.18% | 100.00% | 2.40% |
| Unilateral      | 32 L; 63 R | 5 L; 9 R | 0 | 0 | – | 37 L; 72 R | 0 L (0.00%); 1 R (1.39%) |
| Bilateral       | 12 | 6 | 1 | 3 | – | 22 | 3 (13.64%) |
| Cases without details | 18 | 1 | 0 | 0 | – | 19 | 0 |
| Familial/sporadic | 8/117 | 3/18 | 0/1 | 1/2 | 1/16 | 13/154 | 1/3 |
| Isolated/syndromic | 125/0 | 21/0 | 1/0 | 1/2 | 0/17 | 148/19 | 4/0 |
| ZRS variants detection rate | 1.60% | 9.52% | 0.00% | 0.00% | 0.00% | 2.40% | 0.00% |

PPD, preaxial polydactyly; M, male; F, female; L, left thumb involved; R, right thumb involved. *PPD with multiple organ malformations, such as congenital heart disease, radial ray deficiency, anal atresia. **Other ethnicities include Tujia nationality, Miao nationality, and Hui nationality.

FIGURE 1 | The flow diagram of this study. PPD, preaxial polydactyly; CMA, chromosomal microarray analysis; WES, whole-exon sequencing; GWAS, genome-wide association study.
TABLE 4 | Phenotypes and genotypes of patients with PPD with ZRS variants.

| Patient | Age (years) | Gender | Phenotype | ZRS variant | Location (hg19) | MutationTaster | GnomAD |
|---------|-------------|--------|-----------|-------------|----------------|----------------|---------|
| PDD003  | 1           | M      | Bilateral PPD with triphalangeal thumb on the right hand | ZRS428T > A | Chr7:156584142 | D              | 0.00006 |
| PDD116  | 0.5         | M      | Bilateral PPD I | ZRS474C > G | Chr7:156584096 | D              | –       |
| PDD029  | 4           | F      | Bilateral triphalangeal thumbs | ZRS619C > T | Chr7:156583951 | D              | 0.00000 |
| PDD154  | 1.5         | M      | PPD I on the right hand | ZRS131A > T | Chr7:156584439 | D              | –       |

PPD, preaxial polydactyly; M, male; F, female; D, disease causing.

limb defects (cannot completely exclude the possibility of an extremely subtle anomaly) (23). We reasoned that PPD in this family was attributed to combinedly acting by these two variants. PPD II is an autosomal dominant disease and our description indicated that PPD II individuals can be affected with a pattern of autosomal recessive inheritance. A previous study indicated that compared with a heterozygous variant in ZRS (ZRS402C > T), the homozygote led to more severe phenotypes, WMS, manifesting the superimposed effect of ZRS variants and our detection demonstrated again this phenomenon (24). ZRS619C > T had been reported by Mohammad et al. (15) in a Saudi Arabian family presented with variable preaxial deformities of the upper limbs including isolated triphalangeal thumb, PPD, preaxial syndactyly, and absent thumb and radius (15). Some family members suffered from renal agenesis and congenital heart disease. The variant (ZRS619C > T) showed obvious phenotypic heterogeneity in the Saudi Arabian family, whereas the variant was unable to alone trigger PPD in PPD029 family. It suggested that the pathogenicity of ZRS variants may be affected by ethnic difference, individual variation, and/or environmental factor.
ZPA regulatory sequence is a limb-specific enhancer of SHH, which induces the expression of SHH within ZPA (25). SHH expands from posterior mesoderm to anterior region of limb buds and lacks within the anterior-proximal. The expression gradient of SHH is crucial in establishing the number and the identity of the digits during anteroposterior patterning of the limb (26). Duplications involved ZRS or gain-of-function variants in ZRS would promote the expression of SHH in ZPA and then trigger the ectopic expression within the anterior region, where proliferation of mesenchymal cells is increased to cause PPD I/II (27). For four ZRS variants identified by us, their biological functions were not clarified and further studies needed to be performed. But, we predicted the pathogenicity of these four ZRS variants and analyzed their conservation. GnomAD showed that these variants were absent from controls or extremely rare. Thus, we highly suspected that these ZRS variants were their genetic etiologies, which should be further investigated.

CONCLUSION

In summary, we recruited 167 sporadic or familial cases with PPD from Central-South China and identified four ZRS variants (ZRS131A>T/chr7:g.156584439A>T, ZRS428T>A/chr7:g.156584142T>A, ZRS474C>G/chr7:g.156584096C>G, and ZRS619C>T/chr7:g.156583951C>T) in four patients with PPD (2.39%). Our description about epidemiological investigation of PPD helped us to understand the general picture of PPD in Central-South China. Our detection of two novel ZRS variants not only enrich the genetic map of PPD, but also contributed to genetic diagnosis and counseling of patients with PPD. Furthermore, we reported two patients with PPD I harboring ZRS variants further supporting the link between ZRS and PPD I and a PPD II case caused by the compound heterozygote in ZRS contributing to our understanding of PPD II and its genetic mechanism.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

REFERENCES

1. Evanson BJ, Hosseinzadeh P, Riley SA, Burgess RC. Radial Polydactyly and the Incidence of Reoperation Using A New Classification System. J Pediatr Orthop. (2016) 36:158–60. doi: 10.1097/BPO.0000000000000395
2. Malik S. Polydactyly: phenotypes, genetics and classification. Clin Genet. (2014) 85:203–12. doi: 10.1111/cge.12276
3. Umair M, Ahmad F, Bilal M, Ahmad W, Alfadhel M. Clinical genetics of polydactyly: an updated review. Front Genet. (2018) 9:447. doi: 10.3389/fgene.2018.00447
4. Wu PF, Guo S, Fan XF, Fan LL, Jin JY, Tang JY, et al. A Novel ZRS Mutation in a Chinese Patient with Preaxial Polydactyly and Triphalangeal Thumb. Cytogenet Genome Res. (2016) 149:171–5. doi: 10.1159/000448820

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Review Board of Xiangya Hospital of Central South University. Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)’ legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

LZ performed the acquisition, analysis, and interpretation of the data. J-YJ contributed to conception and design, carried out the analysis, and interpretation of the data. F-ML and YS contributed to conception and design and wrote the original draft. RX revised the draft and finally approved the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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5. Atasu M. Hereditary index finger polydactyly: phenotypic, radiological, dermatoglyphic, and genetic findings in a large family. J Med Genet. (1976) 13:469–76. doi: 10.1136/jmg.13.6.469
6. Al-Qattan MM, Shamseldin HE, Salih MA, Alkuraya FS. GLI3-related polydactyly: a review. Clin Genet. (2017) 92:457–66. doi: 10.1111/cge.12952
7. Fujioka H, Ariga T, Horiuchi K, Otsu M, Igawa H, Kawashima K, et al. Molecular analysis of non-syndromic preaxial polydactyly: preaxial polydactyly type-IV and preaxial polydactyly type-I. Clin Genet. (2005) 67:429–33. doi: 10.1111/j.1399-0004.2005.00431.x
8. Petit F, Jourdain AS, Holder-Espinasse M, Keren B, Andreix J, Duterque-Coquillaud M, et al. The disruption of a novel limb cis-regulatory element of SHH is associated with autosomal dominant preaxial polydactyly-hypertrichosis. Eur J Hum Genet. (2016) 24:37–43. doi: 10.1038/ejhg.2015.53
Potuijt JWP, Baas M, Sukenik-Haley R, Douhen B, Nguyen P Venter DJ, et al. A point mutation in the pre-ZRS disrupts sonic hedgehog expression in the limb bud and results in triphalangeal thumb-polydactyly syndrome. *Clin Genet.* (2018) 95:134–9. doi: 10.1111/cge.13495

Umair M, Bilal M, Alhaddad B, Ahmad F, Abdullah, et al. Whole-exome sequencing revealed a nonsense mutation in STKLD1 causing non-syndromic pre-axial polydactyly type A affecting only upper limb. *Clin Genet.* (2019) 96:134–9. doi: 10.1111/cge.13547

Xu C, Yang X, Zhou H, Li Y, Xing C, Zhou T, et al. A novel ZRS variant causes preaxial polydactyly type I by increased sonic hedgehog expression in the developing limb bud. *Clin Genet.* (2020) 100:2189–98. doi: 10.1111/cge.14136-019-0626-7

Lettice LA, Heaney SJ, Purdie LA, Li L, de Beer P, Oostra BA, et al. A long-range Shh enhancer regulates expression in the developing limb and fin and is associated with preaxial polydactyly. *Hum Mol Genet.* (2003) 12:1725–35. doi: 10.1093/hmg/ddg180

Wieczorek D, Pawlik B, Li Y, Akarsu NA, Caliebe A, May KJ, et al. A specific mutation in the distant sonic hedgehog (SHH) cis-regulator (ZRS) causes Werner mesomelic syndrome (WMS) while complete ZRS duplications underlie Haas type polysyndactyly and preaxial polydactyly (PPD) with or without triphalangeal thumb. *Hum Mutat.* (2010) 31:81–9. doi: 10.1002/humu.21142

Al-Qattan MM, Al Abdulkareem I, Al Haibin Y, Al Balwi M. A novel mutation in the SHH long-range regulator (ZRS) is associated with preaxial polydactyly, triphalangeal thumb, and severe radial ray deficiency. *Am J Med Genet A.* (2012) 158A:2610–5. doi: 10.1002/ajmg.a.35584

Vanlerberge C, Fauve L, Petit F, Fruchart O, Jourdain AS, Clavier F, et al. Intrafamilial variability of ZRS-associated syndrome: characterization of a mosaic ZRS mutation by pyrosequencing. *Clin Genet.* (2015) 88:479–83. doi: 10.1111/cge.12534

Gurnett CA, Bowcock AM, Dietz FR, Morcuende JA, Murray JC, Dobbs MB. Two novel point mutations in the long-range SHH enhancer in three families with triphalangeal thumb and preaxial polydactyly. *Am J Med Genet A.* (2007) 143A:27–32. doi: 10.1002/ajmg.a.31563

Kvon EZ, Kamneva OK, Melo US, Barozzi I, Osterwalder M, Mannion BJ, et al. Progressive Loss of Function in a Limb Enhancer during Snake Evolution. *Cell.* (2016) 167:633–642e611. doi: 10.1016/j.cell.2016.09.028

Xiang Y, Bian J, Wang Z, Xu Y, Fu Q. Clinical study of 459 polydactyly cases in China, 2010 to 2014. *Congenit Anom.* (2016) 56:226–32. doi: 10.1111/cga.12163

Xiang Y, Jiang L, Wang B, Xu Y, Cai H, Fu Q. Mutational screening of GLI3, SHH, preZRS, and ZRS in 102 Chinese children with nonsyndromic polydactyly. *Dev Dyn.* (2017) 246:392–402. doi: 10.1002/dvdy.24488

Rao C, Chen J, Peng Q, Mo Q, Xia X, Lu X. Mutational Screening of GLI3, SHH, and SHH ZRS in 78 Chinese Children with Nonsyndromic Polydactyly. *Genet Test Mol Biomark.* (2018) 22:577–81. doi: 10.1089/gtmb.2018.0096

Pauli RM, Feldman PF. Major limb malformations following intrauterine exposure to ethanol: two additional cases and literature review. *Teratology.* (1986) 33:273–80. doi: 10.1002/tera.1400330304

Potuijt JWP, Hoogeboom J, de Graaff E, van Nieuwenhoven CA, Galjaard RH. Variable expression of subclinical phenotypes instead of reduced penetrance in families with mild triphalangeal thumb phenotypes. *J Med Genet.* (2020) 57:660–3. doi: 10.1136/jmedgenet-2019-106685

VanderMeer JE, Lozano R, Sun M, Xue Y, Daentl D, Jabs EW, et al. A novel ZRS mutation leads to preaxial polydactyly type 2 in a heterozygous form and Werner mesomelic syndrome in a homozygous form. *Hum Mutat.* (2014) 35:945–8. doi: 10.1002/humu.22581

Al-Qattan MM. Zone of polarizing activity regulatory sequence mutations/duplications with preaxial polydactyly and longitudinal preaxial ray deficiency in the phenotype: a review of human cases, animal models, and insights regarding the pathogenesis. *Biomed Res Int.* (2018) 2018:1573871. doi: 10.1155/2018/1573871

Tickle C, Towers M. Sonic Hedgehog Signaling in Limb Development. *Front Cell Dev Biol.* (2017) 5:3. doi: 10.3389/fcell.2017.00014

Johnson EJ, Neely DM, Dunn IC, Davie MG. Direct functional consequences of ZRS enhancer mutation combine with secondary long range SHH signalling effects to cause preaxial polydactyly. *Dev Biol.* (2014) 392:209–20. doi: 10.1016/j.ydbio.2014.05.025

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