Sub-Exome Target Sequencing in a Family With Syndactyly Type IV Due to a Novel Partial Duplication of the LMBR1 Gene: First Case Report in Fujian Province of China

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Syndactyly is one of the most frequent hereditary limb malformations with clinical and genetic complexity. Autosomal dominant syndactyly type IV (SD4) is a rare form of syndactyly, caused by heterozygous mutations in a sonic hedgehog (SHH) regulatory element (ZRS) which resides in intron 5 of the LMBR1 gene on chromosome 7q36.3. SD4 is characterized by complete cutaneous syndactyly of the fingers, accompanied by cup-shaped hands due to flexion of the fingers and polydactyly. Here, for the first time, we reported a large Chinese family from Fujian province, manifesting cup-shaped hands consistent with SD4 and intrafamilial heterogeneity in clinical phenotype of tibial and fibular shortening, triphalangeal thumb-polysyndactyly syndrome (TPTPS). We identified a novel duplication of ∼222 kb covering exons 2–17 of the LMBR1 gene in this family by sub-exome target sequencing. This case expands our new clinical understanding of SD4 phenotype and again confirms the feasibility to detect copy number variation by sub-exome target sequencing.

Keywords: syndactyly type IV, LMBR1, triphalangeal thumb-polysyndactyly syndrome, tibial and fibular shortening, sub-exome target sequencing

BACKGROUND

Syndactyly is one of the most frequent hereditary limb malformations (Tonkin, 2009). The occurrence rate of syndactyly is variable due to geographical and registry differences, ranging from 1.1/10,000 in the northern Netherlands (Vasluian et al., 2013) to 1.3/10,000 in New York State (Goldfarb et al., 2017). The incidence of syndactyly in China is 7.4/10,000 in 1998–2009 (Sun et al., 2011). Currently, five pathogenic genes for syndactyly have been reported, which are associated with nonsyndromic syndactyly types I-c, II-a, II-b, III, IV, V, VII, VIII (Ahmed et al., 2017). Syndactyly
type IV (SD4; OMIM 186200), also known as Haas polysyndactyly, is an autosomal dominant condition occurring as a result of heterozygous mutations of a sonic hedgehog (SHH) regulatory element, designated ZRS, within intron 5 of the LMBR1 gene (limb development membrane protein 1 gene) on chromosome 7q36.3 (Lettice et al., 2003). SD4 is characterized by complete cutaneous syndactyly of all fingers, cup-shaped hands due to flexion of the fingers, accompanied by polydactyly. The phenotypic and the genotypic variability in syndactyly may pose a diagnostic challenge for clinicians. Point mutations within the ZRS region have been described associated with preaxial polydactyly or triphalangeal thumb with preaxial polydactyly (Farooq et al., 2010; Albuisson et al., 2011). A large study of Chinese families showed the occurrence of small duplications that affect ZRS locus both in SD4 and the more severe triphalangeal thumb-polydactyly syndrome (TPTPS) (Klopacki et al., 2008; Wieczorek et al., 2010). Some small duplications (<80 kb) are associated with a more severe phenotype, named Laurin-Sandrow syndrome (Lohan et al., 2014). The ZRS which maps approximately 1 Mb away from SHH (Lettice et al., 2003) controls the expression of SHH in the developing limb and is conserved among mammals and fish (Lettice et al., 2003). The LMBR1 mutant mouse displayed syndactyly involving digits II to V (Al-Qattan et al., 2013), which is in line with the phenotypes of SD4.

Until now, 14 cases, mainly diagnosed as TPTPS or SD4 or TPTPS accompanying SD4, were reported to have duplication covering ZRS locus by different technologies (Dai et al., 2013; Lohan et al., 2014; Liu et al., 2017) (Supplementary Table 1). No lower limb dysplasia was reported in these patients, only two SD4 cases were with fibula dysplasia, but the evidences were insufficient: either lack of X-ray image of fibula dysplasia (Wu et al., 2009) or without precise description of the genomic duplication (Sato et al., 2007). Here, we report a large Chinese family with a novel duplication of ~222 kb covering exons 2–17 of the LMBR1 gene, underlying that cup-shaped hands were correlated with SD4 and intrafamilial heterogeneity in clinical phenotype by sub-exome target sequencing and imaging evidence.

CASE PRESENTATION

The proband (II-6) (Figure 1A) was a 26-year-old woman with twin pregnancy, presented malformed hands and fingers. Both hands have complete syndactyly. The left foot had six toes and a preaxial polydactyly; on the right foot, syndactyly was between the fourth and fifth toe. She had a first pregnancy with a fetus interrupted due to hand malformations identified at ultrasound screening in Quanzhou Woman’s and Children’s Hospital. In a second pregnancy, prenatal ultrasound at 22 weeks revealed two twins both with fetal hands in a fist-shaped posture and the absence of finger-to-finger interval, hands and feet deformity could not be ascertained. Investigating her family history, we found she belongs to a large Chinese family segregating autosomal dominant non-syndromic syndactyly. Eight members from three generations were affected, including three males and five females (Figure 1A). Two of these cases (III-1, III-4) had syndactyly confirmed by prenatal ultrasonography and were interrupted (Figure 1A). None of the affected family members exhibited intellectual anomalies.

By physical examination, we found all patients had the typical clinical phenotype of SD4: cup-shaped hands caused by the fingers together with cutaneous syndactyly, accompanied by limited flexion of the fingers, but no fusion of phalanges and metacarpus. The clinical phenotypic diversity of our family members is characterized by varying degrees of syndactyly and polydactyly in different fingers, skin fusion, preaxial polydactyly and fused fingernails. In most of the affected patients, the presentation was bilateral and symmetrical, accompanied by syndactyly and polydactyly (Figure 1B). Phenotypic spectrum in all affected subjects can be seen in Supplementary Table 2. Some affected individuals have other abnormalities in their fingers and lower limb. In appearance, I-1 presented with triphalangeal thumb on her right hand, and the phenotype of II-2 is the most variable. His hands had a complete syndactyly (surgically treated) and triphalangeal thumb, with seven-toes deformity and the asymmetrical syndactyly on the left and right toes and accompanied by 1–2 small toes of both feet, the third big toe shaped like a thumb, appeared limply walking. In addition, III-6 had a congenital cleft lip and palate, which has been treated surgically. Meanwhile, special attention should be paid to differences in the malformations of the twins. The right toe of II-7 is normal and the left toe of II-7 is an extra postaxial toe. The right fourth and fifth toes of II-6 are syndactyly, and the left toe of II-6 are two extra postaxial toes.

Clinical X-ray examination of the proband (II-6) (Figure 1C-c, e) and II-2 (Figure 1C-a, b, d) revealed the characteristics of the deformity of both hands and feet and the changes of the bilateral tibial and fibular. II-2 is represented by unilateral shortened tibia and fibula (Figure 1C-d), triphalangeal thumb on his right hand (Figure 1C-c), but it is not sure on his left hand because of all bent and overlapping fingers by X-ray. X-rays from other family members were not available.

LABORATORY INVESTIGATIONS AND TEST RESULTS

Our research was approved by the institutional review board of Quanzhou Woman’s and Children’s Hospital. Written consent for reporting clinical results was obtained from all the participants. Targeted gene testing of peripheral blood samples from six patients (II-6, III-6, II-7, III-7, II-4, II-2) were performed to make molecular pathogenic diagnosis, genomic DNA were sequenced using a BGISEQ-500 sequencer, which average coverage depth is nearly 400×. The targeted sequences were captured using the Genetic Disease Chip from internal laboratory (named sub-exome target sequencing), which contained 3,299 genes and covered seven genes [HOXD13, FBLNI, ZRS(LMBR1), LRPL4, FGFI6, BHLHA9, GJA1] related to syndactyly according to OMIM. The point mutations and
Copy number variations of genetic diseases-related genes can be detected more comprehensively by the sub-exome target sequencing. The library was prepared by shearing 1 μg of genomic DNA into a small fragment of 200–300 bps of DNA. The methods used for target capture, enrichments, and elution followed previously described protocols with slight modifications. Sequencing was performed using the BGISEQ-500 platform. Bioinformatic analysis included low-quality read removal with SOAPnuke, alignment to UCSC hg19 with BWA, and variant detection with GATK, and variant annotation was conducted as described previously (Liu et al., 2015; Patch et al., 2018; Zheng et al., 2018). This research uses CNVkit software to detect copy number variation (Talevich et al., 2016). This software uses both the on-target reads and the nonspecifically captured off-target reads to calculate log2 copy ratios across the genome for each sample based on controls (Supplementary Table 3). We found a heterozygous duplication spanning exons 2–17 of the LMBR1 gene (NM_022458.3) in four patients of our family (II-6, III-6, II-7, II-2); the duplication was absent from two unaffected members (II-4, III-7). Demonstration of duplication mutation covering exons 2–17 of the LMBR1 gene analyzed by Integrative Genomics Viewer (IGV) can be seen in Supplementary Figure 1. In order to further clarify the breakpoints of duplication and check the probability of larger fragment duplication, proband (II-6) was tested by low-coverage pair-end whole-genome sequencing as previously reported (Dan et al., 2012) (Supplementary Figure 2), and the breakpoint mapping and size of duplication mutation (chr17:156,460,343–156,682,575; hg19) were confirmed in II-6. This duplicated region covered only exons 2–17 of one OMIM pathogenic gene LMBR1. The identified variant was confirmed by quantitative PCR (qPCR) (Supplementary Table 4). A part of the protein-coding region (CDS5, CDS8, CDS15) of the LMBR1 gene was representatively selected for qPCR verification. qPCR for pedigree analysis confirmed the mutation is cosegregation in this family (Supplementary Figure 3). According to the
In this study, we found a novel microduplication encompassing exons 2–17 in LMBR1 gene (covering the SHH limb enhancer ZRS) in a large family. The patients showed clinical heterogeneity: most of them manifested SD4 and TPTPS, one of them had severe phenotype with shortened tibia and fibula. Considering previous reports together, it seems that there is no positive correlation between severity of disease and size of duplicating regions (Figure 2). For example, TPTPS was observed for the patient with known largest duplication covering ZRS (up to 589 kb) while severer Laurin-Sandrow syndrome was found in patient harboring ZRS duplication with size less than 80 kb (Supplementary Table 1). Thus, we suggest that other genetic or nongenetic determinants might influence the phenotype beyond the duplication. The mechanism how point variants within ZRS influence the expression pattern of SHH has been well investigated, but the mechanism of duplication variants of the ZRS related with phenotype is unclear. There are several speculations for the pathogenesis of duplicated variant. For example, tandem duplications of the ZRS might simultaneously increase the copy number of cis-regulatory elements changing the SHH expression profile. Meanwhile, duplications might affect 3D structure of the DNA and therefore influence SHH expression or affect the interacting between ZRS and SHH (Spielmann and Mundlos, 2013). As reported previously, limb-specific SHH expression was regulated by the ZRS enhancer, demonstrated by a topologically associating domain (TAD) over the SHH/ZRS genomic region SHH identified by chromosome conformation capture (5C). It was suggested that close SHH and ZRS proximity instigate the expression of SHH (Williamson et al., 2016). Therefore, the duplications in this locus likely alter the structure of TAD influencing SHH expression profile and finally associated with disease, yet more experimental studies are needed for this hypothesis.

One more point to consider is that the reported duplication variations (including our case) were identified by Array CGH, qPCR assays, and targeted sequencing. Although duplicates were found, it was impossible to determine the location of the duplicated regions, or whether they have been inserted into or translocated to other chromosomes, thereby influencing the spatial expression pattern or dosage of SHH during limb bud development. Future applications of transcriptome sequencing or whole-genome sequencing may help answer this question.

**Treatment and Management of Syndactyly**

The treatment and management of syndactyly is still by surgical correction. Because of varying degrees of deformity and functional requirements by different patients, it must take into account effects on psychological, economic, and social aspects of the patients, and a higher incidence of complications was observed in patients undergoing complex syndactyly repair (McQuillan et al., 2017); one reported patient with SD4 had 18 surgical corrections (Wieczorek et al., 2010). Therefore, the best prevention for family members with syndactyly is popular science education of genetic diseases and genetic counseling from professionals. Prenatal tests may be offered to at-risk couples.

**A Potential Application in Diagnosis of Genetic Diseases**

Reported testing methods for duplication variation were array CGH, qPCR assays, or linkage and haplotype analysis (Sun et al., 2008; Wu et al., 2009), we first used sub-exome target sequencing (average coverage depth nearly 400×) to test both copy number variant (CNV) and single nucleotide variant (SNV) mutations. The
duplication segments of exons are given relatively accurately, although discontinuous exon duplication was occasionally found. Undeniably, that is the disadvantage of target capture sequencing due to capture efficiency issues. But the reliability of the results was again confirmed by low-coverage pair-end whole-genome sequencing and qPCR. This work demonstrates that sub-exome target sequencing could be an alternative application in the diagnosis of genetic diseases. Meanwhile, this method also needs mutual verification by other methods in order to be used as a candidate for clinical applications.

In summary, we first described an affected family with heterozygous duplication of exons 2–17 in LMBR1 gene by sub-exome capture sequencing, who presented with SD4 accompanied with a diverse phenotype of triphalangeal thumb, and tibia and fibula shortening in Quanzhou City, Fujian Province. It again confirmed the genetic homogeneity of TPTPS and SD4 and demonstrated intrafamilial phenotypic heterogeneity.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding authors on reasonable request. The data reported in this study are available in the CNGB Nucleotide Sequence Archive (CNSA: https://db.cngb.org/cnsa; accession number CNP 000157).

ETHICS STATEMENT

Our research was approved by the institutional review board of Quanzhou Woman’s and Children’s Hospital. Written consent for reporting clinical results was obtained from all the participants.

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AUTHOR CONTRIBUTIONS

LM, HC, and XW conducted the next generation sequencing, NGS procedure and subsequent analysis/interpretation. QJ and WF conducted the genetic counseling process and the follow-up. RH and WF contributed to physical examination and clinical X-ray examination. KL performed image modification. LC and YY conducted the qPCR analysis. YW and HH instructed and supervised this study. The manuscript was drafted by LS and edited by JW. All authors have read and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2020.00130/full#supplementary-material

SUPPLEMENTARY FIGURE 1 | Demonstration of duplication covering exons 2-17 of LMBR1 gene tested by low-coverage pair-end whole-genome sequencing and sub-exome target sequencing.

SUPPLEMENTARY FIGURE 2 | The breakpoint mapping of duplication mutation on chromosome 7q36.3.

SUPPLEMENTARY FIGURE 3 | Quantitative real-time PCR validation of partial LMBR1 duplication.
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Conflit of Interest: Author JW and HH were employed by company BGI Genomics, BGI-Shenzhen.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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