BMC
Medical Genetics

RESEARCH ARTICLE

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Discontinuous microduplications at chromosome 10q24.31 identified in a Chinese family with split hand and foot malformation

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Abstract

Background: Split hand/foot malformation (SHFM) is a congenital disorder characterized by a cleft of the hands and/or feet due to dificiency of central rays. Genomic rearrangement at 10q24 has been found to cause nonsyndromic SHFM (SHFM3).

Methods: Four patients and fourteen unaffected individuals from a four-generation Chinese pedigree with typical SHFM3 phenotypes were recruited for this study. After informed consent was obtained, genome-wide copy number analysis was performed on all patients and two normal family members using the Affymetrix Cytogenetics Whole-Genome 2.7M Array. The results were then confirmed by real-time quantitative polymerase chain reaction in all available individuals of this pedigree. Candidate genes were further screened for mutation through sequence analyses.

Results: Copy number analysis showed a microduplication at chromosome 10q24.31-q24.32 co-segregating with the SHFM phenotype. Compared to other known genomic duplications for SHFM3, the duplication described here contains two discontinuous DNA fragments. The minimal centromeric duplicated segment of 259 kb involves *LBX1*, *POLL* and a disrupted *BTRC*. The minimal telomeric duplication of 114 kb encompasses *DPCD* and one part of *FBXW4*. No coding and splice-site mutations of candidate genes in the region were found.

Conclusions: Genomic duplications at chromosome 10q24.3, which were identified in the current study, provide further evidence for limb-specific cis-regulatory sequences in this region, highlighting the importance of chromosome 10q24.31-q24.32 in limb development and SHFM pathogenesis.

Keywords: SHFM, Microduplication, Han Chinese

Background

Split hand/foot malformation (SHFM) is a congenital disorder characterized by variable degrees of median clefts of hands and/or feet due to absence of the central ray digits. It also presents syndactyly, and aplasia/hypoplasia of the phalanges, metacarpals and metatarsals. SHFM shows highly variability in phenotypes between and/or within families, even between limbs of a single patient, ranging from mild syndactyly to severe central clefting of the autopods, oligodactyly or monodactyly [1]. The condition can occur as an isolated entity or a part of other anomalies, with an estimated incidence of 1/8,500 ~1/25,000 [2].

Currently, six loci for nonsyndromic SHFM phenotype have been identified: SHFM1 on 7q21 (OMIM 183600) [3], SHFM2 on Xq26 (OMIM 313350) [4-6], SHFM3 on 10q24 (OMIM 246560) [7-9], SHFM4 on 3q27 (OMIM 605289) [10], SHFM5 on 2q31 (OMIM 606708) [11,12] and SHFM6 on 12q13 (OMIM 225300) [13]. The autosomal dominant inherited SHFM1, 3 and 4 have been associated with deletions and translocations on 7q, genomic duplications on 10q24 and mutations in *TP63*, respectively. The only autosomal recessive SHFM6 have been found to be caused by mutations in *WNT10B* [13-15]. However, genetic causes for SHFM2 and SHFM5 are still unknown.

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The SHFM3 locus was initially mapped to chromosome 10q24 by linkage analysis [7-9,16], then narrowed to a 0.5-Mb region through the identified genomic duplications involving *LBX1*, *BTRC* and *FBXW4* [17]. No intragenic mutations to date have been detected in candidate genes within or flanking duplicated regions. The relationship has been well established between SHFM3 and genomic duplications in 10q24 [17-20], a few were documented in Chinese patients [21] though a relative high birth prevalence rate of 0.64/10000 for nonsyndromic SHFM was reported [22]. In this study, we conducted an array-based genome-wide copy number variation (CNV) analysis on a Chinese family with SHFM and identified a discontinuous microduplication on 10q24.31-24.32 as the likely cause.

Methods

Subjects

A SHFM family with four-generation-span was recruited after informed consent (Figure 1). Four affected (II:5, III:9, III:10 and IV:3) and 14 unaffected family members received full clinical evaluation and blood samples were collected for further studies. Clinical records and radiographic images were published under the patients' written permission. This study was approved by the Research Ethics Committee of Sichuan University.

Mutation screening

Genomic DNA was isolated from blood samples using a commercially available kit (Bio Teke, Beijing, China). As *TP63* is the only disease-causing gene for autosomal dominant SHFM, we first searched for possible mutations in the gene in family members as outlined previously [23]. We also screened candidate genes within the regions defined by array-based copy number variation analysis. Exons and their flanking intronic sequences of the *LBX1*, *BTRC*, *DPCD* and *FBXW4* were amplified by polymerase chain reaction (PCR). All the resulting products were purified, and sequenced bi-directionally using an ABI 3730xl sequencer. All primer sequences are available on request.

Microarray analysis

Using the Affymetrix Cytogenetics Whole-Genome 2.7M array (Affymetrix Inc, USA), four affected and two unaffected individuals (II:6, III:8) in this family were selected to undertake CNV analysis. Experiments were carried out by Peking CapitalBio Company (Beijing, China) according to the manufacturer's protocol. The Affymetrix Chromosome Analysis Suite Software (ChAS) was used for genotype calling, quality control and CNV identification.
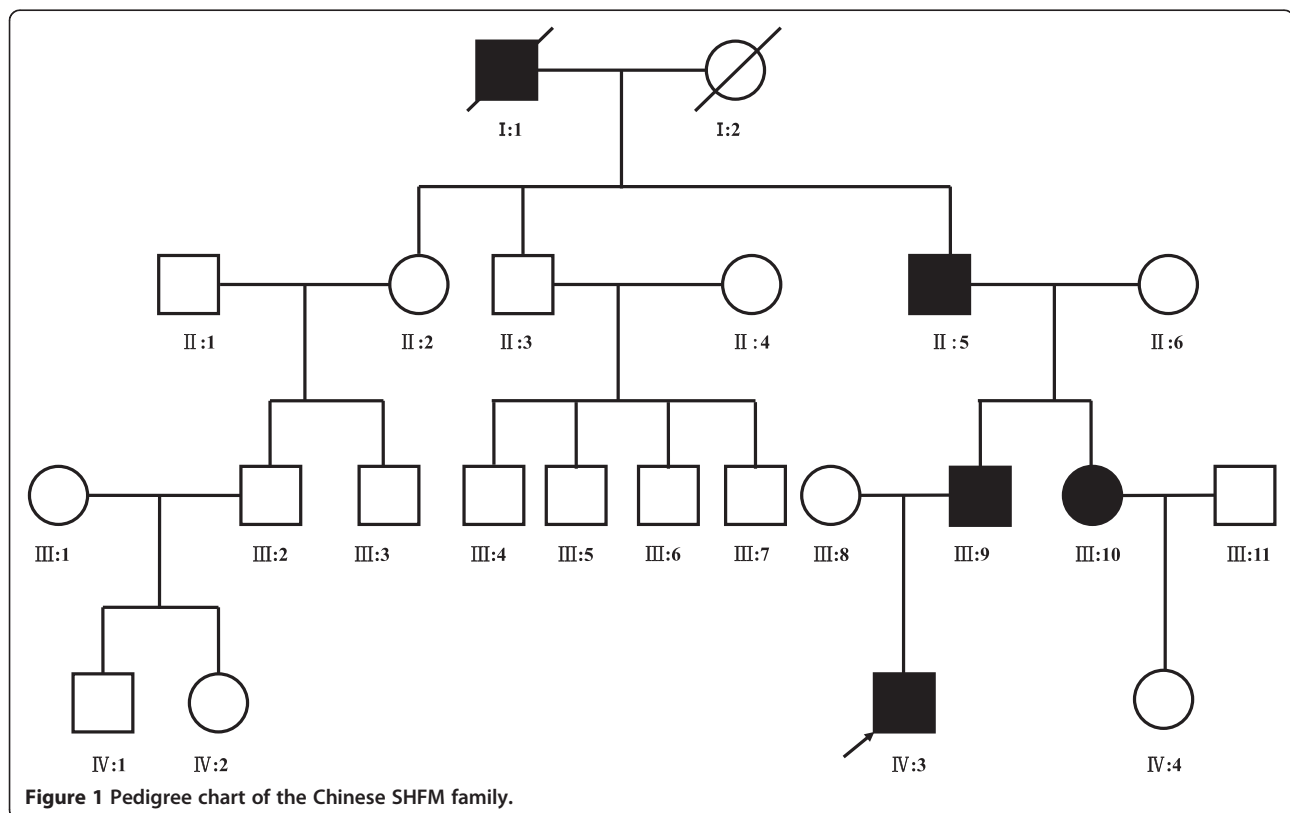

Figure 1 Pedigree chart of the Chinese SHFM family.

Identification of the duplication by quantitative PCR

To confirm duplications at 10q24.31-q24.32 mapped by CNV analysis, six EvaGreen assays were designed to determine the relative copy number (RCN) for all family members. Primers for amplifying selected fragments in duplicated regions were described previously [20]. PCR conditions and locations of assays are presented in Additional file 1: Table S1. All reactions used 500nM of each primer, 10 ng of genomic DNA, and SsoFast EvaGreen Supermix quantitative PCR (qPCR) buffer (Bio-Rad, California, USA). PCRs were performed by using a Bio-Rad C1000™ Thermal Cycler (Bio-Rad, USA) in a 10-µl volume in 96-well plates with four replicates per sample. Reactions were run in a Bio-Rad CFX96™ Real-Time System with the following conditions: 98°C for 2 min, 98°C for 1 sec, annealing for 5 sec, and 40 cycles of 98°C 1 sec. The quantification of the target sequences was normalized to one assay from chromosome 11 [18], the RCN was determined on the basis of the comparative $2^{-\Delta\Delta Ct}$ method with a normal control DNA as the calibrator [24]. The experiments were repeated three times. A ~1.5-fold RCN was used for duplication. Relative DNA copy number was obtained by pair-wise comparisons of test and control DNAs.

Results

Clinical report

Five individuals in this family were affected by SHFM with autosomal dominant transmission, but only four of them were available for this study (Figure 1). The proband (IV:3), one three-year-old boy, was affected by severe distal deficiency affecting all 4 limbs. Other three patients exhibited similar phenotypes including ectrodactyly of central digits, bilateral median cleft in feet, 3/4 finger or toe syndactly, hypoplasia of metacarpals and metatarsals. Representative clinical observations from photographs and X-ray data are summarized in Figure 2 and Additional file 2: Table S2. Notably, phenotypic variations were observed between affected family members. Triphalangeal thumb was only identified in patient II:5, and complete 1/2 toe syndactyly in individual IV:3. Similar hypoplasia/agenesis of 1st ray existed in III:9, III:10 and IV:3. No non-limb malformations were identified in any patient.

Mutation screening in candidate genes

Before genome-wide genotyping, we did not identify any mutation in exons of *TP63* gene using PCR and direct sequencing analysis. Also we sequenced four known genes in the critical regions at 10q24, and found no pathogenic mutation in the coding region of these genes.

Copy number variation analysis

Using array-based genome-wide CNV, we identified similar duplications at 10q24.31-q24.32 in the four patients,
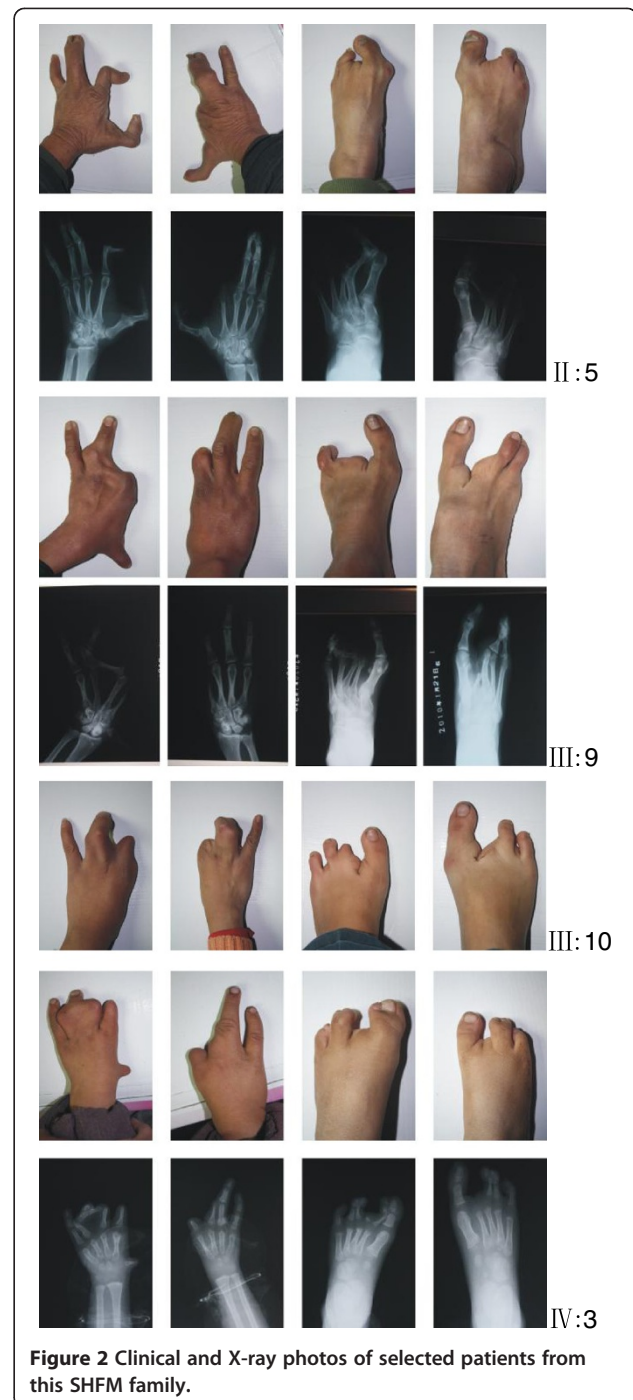

Figure 2 Clinical and X-ray photos of selected patients from this SHFM family.

but not in the two unaffected family members. The duplicated genomic contents contain two segments. The centromeric duplications, ranging from 247 kb to 260 kb, share a common overlapping segment containing *LBX1*, *POLL* and a disrupted *BTRC*. The telomeric duplications of 114–125 kb comprise *DPCD* and one part of *FBXW4* gene (Additional file 3: Table S3, Additional file 4: Figure S1). With use of qPCR assay, we confirmed the duplication co-segregating with SHFM phenotypes in

the family. As predicted by ENCODE (http://www.genome.gov/10005107), this genomic region contains a variety of regulatory elements including enhancers, promoters and multiple transcription factor-binding sites. Particularly, the centromeric duplications involve two negative enhancers, hs324 and hs939, while the region between two duplications harbors a human limb-development-related positive enhancer, hs326 (chr10:103,266,649-103,267,972, http://enhancer.lbl.gov).

Discussion

SHFM is a complicated limb malformation that shows highly phenotypic variability and genetic heterogeneity. Even though six SHFM loci have been mapped to date, causal genes for a large number of cases are still unknown. At the gene level, mutations in *TP63* and *WNT10B* can lead to autosomal dominant SHFM4 and autosomal recessive SHFM6, respectively. Recently a point mutation of *DLX5* gene was reported to be responsible for autosomal recessive SHFM [25]. Genomic rearrangements at 10q23-q24 have been identified in many familial or sporadic SHFM cases [17-20], the mechanisms remain to be elucidated. In current study, we identified a previously unreported copy number variation at 10q24.31-q24.32, segregating with SHFM in a four-generation Chinese family. The minimal duplicated genomic contents include a 247-kb fragment comprising *LBX1* and part of *BTRC*, and another fragment of 114 Kb containing *POLL*, *DPCD*, and disrupted *FBXW4*. Our findings expand the knowledge of genomic rearrangements leading to SHFM, and highlight the important role of chromosomal 10q24 region in regulating limb development.

A genotype-phenotype study showed that preaxial involvement of the upper limbs can be locus discriminators for SHFM3 [26]. In the current study, various preaxial upper limb anomalies were found in the affected individuals including proximal placed thumb, triphalangeal thumb, preaxial polydactyly, and absence of paraxial rays. In addition, more severe affected feet than hands, and worsening phenotype in patients of younger generations, were observed in the family. As noted in literature [9,19], the observed phenotypes strongly suggested that current disorder should be mapped to SHFM3 locus, chromosomal 10q24.

SHFM is considered a failure to maintain medial apical ectodermal ridge (AER) [1], so genes expressed in AER are reasonable candidates for SHFM3, such as the three known SHFM genes *TP63*, *WNT10B* and *DLX5*. Microduplications involving *LBX1*, *BTRC*, *POLL*, *DPCD* and one part of *FBXW4* (human *dactylin* gene) were shown for SHFM3 in two studies [17,19]. Another study reported that a 325-kb duplication containing *BTRC* and *POLL* leads to SHFM3, which is the smallest one identified to date [20]. A small number of genes at 10q24 like

BTRC, *FBXW4*, *SUFU* and *FGF8* are also expressed in the developing limbs [1]. Among them, *FBXW4* has been considered as the best candidate gene due to its role in the SHFM3 'like' *Dactylaplasia* mouse model [27,28]. *BTRC* is another promising candidate, because its product gets involved in essential pathways for limb development, such as the canonical WNT/β-catenin and NKFα signaling pathways. Despite the frequent observations of genomic rearrangements at 10q24 in SHFM patients, no exon mutations were found in these candidate genes.

In this study, the duplicated segments contain three entire genes (*LBX1*, *DPCD* and *POLL*) and one part of two genes (*BTRC* and *FBXW4*). The duplicated portion of *BTRC* gene contains its promoter and exon 1, while the duplicated segment of *FBXW4* includes 3′ end of the gene. Since *BTRC* and *FBXW4* are only partially duplicated in our SHFM cases, it is less likely that overexpression of these two gene causes SHFM. Comparing our findings with those of previous studies, *BTRC* is the most frequently identified gene involved in the genomic rearrangements. A duplication affecting *BTRC* and *POLL* only has been reported to be responsible for SHFM3 [20]. We speculate that limb-specific regulatory elements around or in *BTRC* gene may play an important role in SHFM pathogenesis, by controlling the expression of genes at 10q24 or the related downstream genes.

It is unclear how genomic rearrangement at this critical region causes the SHFM phenotype. The elevated expression of *BTRC* and *SUFU* in lymphoblastoid cells detected in SHFM3 patients suggested that the gene dosage (*BTRC*) or long-range control mechanisms (*SUFU*) may underlie overexpression [20]. In the *Dactylaplasia* mouse model, changes in the expression levels of *Lbx1*, *Btrc*, *Poll*, and *Dpcd* were not observed except for reduced levels of normal *Fbxw4* transcript [19], which implies duplications may disrupt normal expression of related genes. An increasing number of studies have shown that copy number variations are a common cause of human genetic disorders [29]. Duplications of regulatory elements have been described for human limb malformations. Brachydactyly type A2 is caused by a tandem duplication of a 5.5 kb region 3′ of *BMP2* [30]. Triphalangeal thumb-polysyndactyly syndrome is associated with duplications of ZPA regulatory sequence, a long range cis-regulator for *SSH* gene [31]. Based on current understanding of the molecular mechanisms for limb anomalies associated with duplications, the duplications described here may alter the dosage of a regulatory element involved in limb development or disrupt the co-expression domains, consequently leading to SHFM phenotypes.

Conclusion

In summary, this report describes a discontinuous duplication at 10q24.3 responsible for a typical familial

SHFM3. Our data suggest that chromosome 10q24.3 may contain key elements for regulating the coordinate expression of many genes for limb development. Future studies on the developmental effects of genomic rearrangements at this region in animal models would thus complement the human data presented here. Our findings could be of value to molecular diagnosis and deeper understanding of pathogenesis of this disorder.

Additional file

Additional file 1: Table S1. Quantitatibe PCR Primers used in the study.

Additional file 2: Table S2. Clinical features of four patients in the SHFM family.

Additional file 3: Table S3. Genomic duplications at 10q21.31-q21.32 identified in SHFM patients.

Additional file 4: Figure S1. Schematic overview of microduplications at chromosome 10q24.3 associated with SHFM. This graph was generated by the Affymetrix ChAS software.

Abbreviations
SHFM: Split hand/foot malformation; *BTRC*: Beta-transducin repeat containing E3 ubiquitin protein ligase; *LBX1*: Ladybird homeobox 1; *DPCD*: Deleted in primary ciliary dyskinesia homolog (mouse); *POLL*: Polymerase (DNA directed), lambda; *FBXW4*: F-box and WD repeat domain containing 4; *SUFU*: Suppressor of fused homolog (Drosophila); *BMP2*: Bone morphogenetic protein 2.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
LD studied the family, designed research plan and prepared manuscript. YD, NL and LX performed the molecular genetic studies. MM participated in clinical evaluation. JZ participated in collecting specimens. All authors reviewed the final manuscript and approved the publication of the clinical images. All authors read and approved the final manuscript.

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