Identity-by-descent refines mapping of candidate regions for preaxial polydactyly II/III in a large Chinese pedigree

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Abstract
Preaxial polydactyly (PPD) is congenital hand malformation characterized by the duplication of digit. Herein, we scan the genome-wide SNPs for a large Chinese family with PPD-II/III. We employ the refined IBD algorithm to identify the identity-by-decent (IBD) segments and compare the frequency among the patients and normal relatives. A total of 72 markers of 0.01 percentile of the permutation are identified as the peak signals. Among of them, 57 markers locate on chromosome 7q36 which is associated with PPD. Further analyses refine the mapping of candidate region in chromosome 7q36 into two 380 Kb fragments within \textit{LMBR1} and \textit{SHH} respectively. IBD approach is a suitable method for mapping causal gene of human disease. Target-enrichment sequencing as well as functional experiments are required to illustrate the pathogenic mechanisms for PPD in the future.

Keywords: PPD, IBD, 7q36, \textit{LMBR1}, \textit{SHH}

Main text

Background
Preaxial polydactyly (PPD; OMIM#188740) is characterized as complete or partial duplication of the thumb [1]. It is one of the most common congenital deformities [2]. The worldwide incidence of PPD is 1 in 3000 births [3]. The prevalence rate of polydactyly in Chinese ranks third in birth defects after congenital heart diseases and central nervous system diseases [4]. Polydactyly has genetic and clinical heterogeneity [2]. The mainstream treatment is resection for excess digits.

A series of efforts have been performed to investigate the genetic basis for PPD. Zguricas et al. conducted linkage analysis for Dutch, British, Turkish, Cuban pedigrees and mapped the candidate region to a 1.9 cM interval between D7S550 and D7S2423 of 7q36 region [5]. Heus et al. further refined the candidate region to approximately 450 Kb including five genes: \textit{C7orf2} (i.e. \textit{LMBR1}), \textit{C7orf3} (i.e. \textit{NOM1}), \textit{C7orf4} (i.e. \textit{LINC00244}), \textit{HLXB9} (i.e. \textit{MNX1}) and \textit{RNF32} [6] by reconstructed a detailed physical map using a combination of exon trapping, cDNA selection, and EST mapping methods. Further evidence shows that PPD is caused by ectopic expression of \textit{SHH} in mice, cats and humans [7]. The zone of polarizing activity regulatory sequence (ZRS), performs as the limb-specific cis-regulator, in controlling the expression of \textit{SHH}. ZRS locates within intron 5 of the neighboring gene \textit{LMBR1}, which is ~1 Mb upstream from \textit{SHH} [8]. In a number of cases, mutations of ZRS disturb the expression of \textit{SHH} at the anterior limb bud margin and consequently caused PPD [8–15]. Homozygous deletion of ZRS can cause limb-specific absence of \textit{SHH} expression in the acheropodia [16]. It actually exists in the snake species and a limbless newt [17]. Duplication of ZRS results in Triphalangeal thumb–polysyndactyly syndrome (TPTPS; OMIM#174500), that is a subtype of PPD. It also can lead to syndactyly type IV (SD4; OMIM#186200) [18].

The common PPD only involves in hands/feet. In extreme and rare cases, PPD occur both in hands and feet. To investigate the genetic basis, Li et al. adopted a candidate gene approach to genotype nine

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microsatellite markers of 7q36 chromosomal region in a Chinese family with PPD both in hands and feet. By linkage analysis and haplotype construction, they located the linked region spanning 1.7 Mb between D7S2465 and D7D2423 [19]. It includes the 450 kb candidate region previously identified by Henus [6]. Nevertheless, the other parts of genome is not investigated yet. Herein, we genotyped genome-wide SNPs and employed the identity-by-descent (IBD) to refine the mapping of potential candidate loci for PPD in the same family.

**Methods**

**Patients**

This study has been approved by the internal review board of Kunming Institute of Zoology, Chinese Academy of Sciences (SMKX 2012013). The six-generation pedigree (including 21 patients and 24 normal relatives) involved in this study has been described previously in Li et al. [19]. All patients show hexadactyly of hands and feet. They have been diagnosed by physical examination & X-ray and assigned as isolated PPD-II on hand and isolated PPD-III on feet according to Temtamy and McKusick’s classification [20]. PPD shows autosomal dominant inheritance in this pedigree.

**SNP array**

We genotyped 900,015 markers in 45 individual with HumanOmniZhongHua-8 BeadChip v1.0 (Illumina). We exported the chip data in accordance with the reference sequence GRCh37 into PLINK format via GenomeStudio (Illumina). The markers on mitochondrial DNA and sex chromosomes were disregarded. We adopted a series of quality control strategies [21] by using PLINK 1.9 [22]. Two individuals with call rate < 90% were removed. The SNPs with call rate < 90%, minor allele frequency < 1%, and deviation of Hardy–Weinberg equilibrium \( P < 1e^{-6} \) were excluded. After filtering, a total of 595,534 autosomal SNPs for 43 individuals were utilized in subsequent

| Gene(7q36) | Position (GRCh37.p13) | SNP ID   | REF | ALT | P-value | Note                          |
|------------|-----------------------|----------|-----|-----|---------|-------------------------------|
| LMBR1 156470537..156685902 | 156354434              | rs1860156 | T   | C   | 1.00E-06 | 116 kb upstream of LMBR1      |
|            | 156401455              | kgp2828999 | C   | A   | 1.00E-06 | 69 kb upstream of LMBR1      |
|            | 156477347              | kgp13575466 | C   | A   | 1.00E-06 | 450 kb upstream of LMBR1     |
|            | 156497668              | rs10228997 | A   | G   | 1.00E-06 | 1 kb downstream of LMBR1     |
|            | 156526645              | rs10224728 | T   | G   | 1.00E-06 | 1 kb downstream of LMBR1     |
|            | 156686101              | kgp6457815 | C   | T   | 1.00E-06 | 199 bp downstream of LMBR1   |
|            | 156687282              | kgp1716770 | C   | T   | 1.00E-06 | 1 kb downstream of LMBR1     |
|            | 156716316              | kgp3747986 | T   | C   | 1.00E-06 | 30 kb downstream of LMBR1    |
|            | 156730688              | kgp7566181 | T   | C   | 1.00E-06 | 45 kb downstream of LMBR1    |
|            | 155103781              | rs13223383 | G   | T   | 1.00E-06 | 492 kb upstream of SHH       |
|            | 155169143              | rs1990808  | C   | T   | 1.00E-06 | 426 kb upstream of SHH       |
|            | 155182442              | kgp9710825 | G   | A   | 1.00E-06 | 426 kb upstream of SHH       |
|            | 155716520              | rs4716928  | C   | T   | 1.00E-06 | 112 kb downstream of SHH     |
|            | 155718241              | rs4716930  | A   | C   | 1.00E-06 | 113 kb downstream of SHH     |
|            | 155721324              | rs11764820 | A   | G   | 1.00E-06 | 116 kb downstream of SHH     |
|            | 155721386              | rs11769663 | G   | T   | 1.00E-06 | 116 kb downstream of SHH     |
|            | 155722231              | rs6971588  | T   | G   | 1.00E-06 | 117 kb downstream of SHH     |
|            | 155723112              | kgp11597900 | C   | T   | 1.00E-06 | 118 kb downstream of SHH     |
analyses. The data have been deposited into the Genome Variation Map [23] (GVM0000001).

**IBD detection**

We used BEAGLE 4.0 [24] to phase and impute the genotype data referring to the pedigree information and the genetic map of HapMapII [25]. We detected the IBD segment with the refined IBD in BEAGLE 4.1 [26]. The IBD segment length shorter than 1 cM and the logarithm of odds (LOD) score under 3 were excluded before permutation [27]. The threshold of the genome-wide significance was set to the 0.05 percentile of the distribution of the permutation $p$-value.

**Results**

The length distribution of detected IBD segments approximates a Pareto distribution (Additional file 1: Figure S1). The permutation result shows the significant segments distributing widely across genomes (Fig. 1). When considering the top 0.01% outliers of signals, we find the peak signals of 72 SNPs, of which 57 markers locate at 7q36 chromosomal region (Additional file 2: Table S1). We map the markers into the IBD fragments including LMBR1 and SHH (Table 1). The minimal IBD segments within LMBR1 and SHH are around 380 Kb, respectively (Additional file 3: Table S2). The IBD segments are more frequently in patient-patient (ratio; percentage) than normal-normal (Table 2). We make annotation for the significant SNPs (Additional file 2: Table S1). All the SNPs are not haven’t been reported to be associated with PPD before.

| Gene  | No. patient-patient | % IBD in patient-patient pairs | No. normal-normal | % IBD in normal-normal pairs | No. patient-normal | % IBD in patient-normal pairs |
|-------|----------------------|---------------------------------|-------------------|-------------------------------|-------------------|-------------------------------|
| LMBR1 | 21                   | 0.400                           | 22                | 0.074                         | 29                | 0.126                         |
| SHH   | 21                   | 0.386                           | 22                | 0.069                         | 24                | 0.104                         |

% IBD patient-normal pairs = IBD patient-normal pairs/case x (case-1)/2
% IBD normal-normal pairs = IBD normal-normal pairs/normal x (normal-1)/2
% IBD patient-normal pairs = IBD patient-normal pairs/case x normal/2

In addition to the segment of LMBR1, we also identified a segment of SHH. The SHH gene encodes sonic hedgehog, a secreted protein, which plays a key role in the limb development [28]. The ectopic expression of SHH in the anterior limb margin can cause PPD in human, in mouse [29], Hemingway cat [7] and chicken [30]. Recently, Petit et al. identified a 2 kb deletion occurring about 240 kb upstream from the SHH promoter in a large family with PDD-hypertrichosis. They found the 2 kb deletion repress the transcriptional activity of the SHH promoter in vitro [31]. It raises a possibility that long range regulation may be an explanation for the PPD. Further target-enrichment sequencing and further functional experiments for LMBR1 and SHH are required to identify the pathogenic mutation(s).

In summary, we refine the mapping of the candidate regions for PPD based on high-density genomic SNPs. The potential candidate mutations are most likely to locate in LMBR1 and/or SHH gene. It is much improved compred with previous results [6, 19]. Our study suggests that the IBD approach is a suitable method for mapping the causal genes of human diseases. Moreover, as disruptions of topological chromatin domains can result in limb malformations [32], more attention should be paid when studying PPD in the future on this aspect.

**Discussion**

Our IBD analyses refine the mapping of the candidate regions for PPD into two ~380 Kb segments in 7q36 referring to LMBR1 and SHH genes, respectively (Additional file 3: Table S2). The segment for LMBR1 includes three genes (i.e. LMBR1, NOM1, and RNF32) and lies within the 450 kb candidate region identified before [6]. Mutations in the ZRS is playing an important role in the pathogenesis of PPD (Additional file 4: Table S3). The duplication of ZRS can cause TPTPS and SD4 [18]. Its role in PPD-II /PPD-III is unclear. In the previous investigation of the same family, Li et al. detected no pathogenic mutation in ZRS as well as no duplication of ZRS [19]. Consequently, the etiology of this PPD family may be another limb-specific regulatory element of SHH gene exists in the noncoding region.

**Additional files**

Additional file 1: Figure S1. Plot of the distribution of the IBD segments.
Additional file 2: Table S1. Top 0.01% peak signals.
Additional file 3: Table S2. IBD segments of LMBR1 and SHH.
Additional file 4: Table S3. Mutations in intron 5 of LMBR1.

**Abbreviations**

IBD: Identity by descent; LINC00244: Long intergenic non-protein coding RNA 244; LMBR1: Limb development membrane protein 1; MNX1: Motor neuron and pancreas homeobox 1; NOM1: Nucleolar protein with MIF4G domain 1; PPD: Preaxial polydactyly; RNF32: Ring finger protein 32
The authors declare no conflict of interest.

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The research protocol of the study entitled “Identity-by-descent Refines Mapping of Candidate Regions for Preaxial Polydactyly II/III in a Large Chinese Pedigree”, has been reviewed and approved by the internal review board of Kunming Institute of Zoology, Chinese Academy of Sciences. This study was supported by grants from Bureau of Science and Technology of Yunnan Province, China.

Availability of data and materials
The variation data reported in this paper have been deposited in the Genome Variation Map (GVM) in Big Data Center, Beijing Institute of Genomics (BIG), Chinese Academy of Science, (GVM; http://bigd.big.ac.cn/gvm/; accession numbers GVM0000001).

Authors’ contributions
XY analyzed the SNP array data and wrote the manuscript. IBD was carried out by XY and assisted by QS. XS revised the manuscript. HL performed experiments and provided patients data. MP participated in its design and revised the manuscript. All processes were guided by Dr. MP and Pro. YZ. All authors read and approved the final manuscript.

Ethics approval and consent to participate
This study has been approved by the internal review board of Kunming Institute of Zoology, Chinese Academy of Sciences (SMXK 2013). The patients consent to participate in this study by signing a Consent Form allowing the use of biological samples and clinical data.

Consent for publication
A six-generation family consisting of 45 individuals including 21 affected members and 24 normal relatives was located in a rural area of Zhejiang Province, China. All patients show hexadactyly of hands and feet, diagnosed by physical examination & X-ray. According to Temtamy and McKusick’s classification it is classified as isolated PPD-II on hand and isolated PPD-III on feet. We used raw data have genotyped by Illumina HumanOmniZhongHua-8 Beadby physical examination & X-ray. According to Temtamy and McKusick’s members and 24 normal relatives was located in a rural area of Zhejiang Province.

Consent to participate
This study has been approved by the internal review board of Kunming Institute of Zoology, Chinese Academy of Sciences (SMXK 2013). The patients consent to participate in this study by signing a Consent Form allowing the use of biological samples and clinical data.

Competing interests
The authors declare no conflict of interest.

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