Brief report

Identity-by-descent refines mapping of candidate regions for preaxial polydactyly 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 *LMBR1* and *SHH* respectively. IBD approach is a suitable method for mapping cause 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, *LMBR1, 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 of polydactyly in Chinese ranks third in birth defects after heart defects, central nervous system diseases [4]. Polydactyly usually occurs in complicated hereditary patterns [5]. 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 chromosome 7q36 region [6]. Heus et al. further refined the candidate region to approximately 450 Kb including five genes: C7orf2 (i.e. LMBR1), C7orf3 (i.e. NOM1), C7orf4 (i.e. LINC00244), HLXB9 (i.e. MNX1) and RNF32 [7] by reconstructed a detailed physical map using a combination of exon trapping, cDNA selection, and EST mapping methods. Further evidence for PPD is caused by ectopic expression of SHH in mice, cats and humans [8]. The zone of polarizing activity regulatory sequence (ZRS), performs as the limb-specific cis-regulator, the expression of SHH. ZRS locates within intron 5 of the neighboring gene LMBR1, which is ~1 Mb upstream from SHH [9]. In a number of cases, mutations of ZRS disturb the expression of SHH at the anterior limb bud margin and consequently caused PPD [9-16]. Deletion of ZRS showed that it is both necessary and sufficient to drive expression of SHH in the limb bud [17].
Duplication of ZRS is unclear how this contributes to ectopic expression [9].

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 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 linkaged region spanning 1.7 Mb between D7S2465 and D7D2423 [18]. It includes the 450Kb candidate region previously identified by Henus [7]. Nevertheless, the other part 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 [18]. 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 [19]. PPD shows autosomal dominant inheritance in this pedigree.
SNP array

We genotyped 900015 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 [20] by using PLINK 1.9 [21]. 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 595534 autosomal SNPs for 43 individuals were utilized in subsequent analyses. The data have been deposited into Dryad (XXXXXX).

IBD detection

We used BEAGLE 4.0 [22] to phase and impute the genotype data referring to the pedigree information and the genetic map of HapMap II [23]. We detected the IBD segment with the refined IBD in BEAGLE 4.1 [24]. The IBD segment length shorter than 1cM and the logarithm of odds (LOD) score under 3 were excluded before permutation [25]. 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 (Fig. S1). The permutation result shows the significant segments distributing widely across genomes.
When considering the top 0.01% outliers of signals, we find the peak signals of 72 SNPs, of which 57 markers located at 7q36 chromosomal region (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 (Table S2). The IBD segments are more frequently in patient-patient (ratio; percentage) than normal-normal (ratio; percentage) (Table 2). We make annotation for the significant SNPs (Table S1). All the SNPs haven’t been reported to be associated with PPD before.

**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 (Table S2). The segment for LMBR1 includes three genes (i.e. LMBR1, NOM1, and RNF32) and lies within the 450Kb candidate region identified before [7]. Given that the intron 5 of LMBR1 performed as an enhancer for SHH playing an important role in the pathogenesis of PPD (Table 3). Li et al. sequenced the exons of the five candidate genes and the intron 5 of LMBR1. But no candidate mutations were found [18]. The candidate mutations for PPD may locate in the other introns of LMBR1.

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 [26]. The ectopic expression of SHH in the anterior limb margin can cause PPD in mouse [27]. This has been described as well in humans [27], Hemingway cats [8] and chicken [28]. The
candidate causal mutations are located in the intron 5 of LMBR1 [9]. The duplication of ZRS can cause polysyndactyly in the Triphalangeal thumb–polysyndactyly syndrome and syndactyly type IV but not PPD [29]. Its role in PPD is unknown. In the previous investigation of the same family, Li et al. ruled out the ZRS duplication by quantitative PCR and detected no pathogenic mutation in ZRS [18]. Consequently, the etiology of this PPD family may be another limb-specific regulatory element of SHH gene exists in the noncoding region. Recently, Petit et al. identified a 2 kb deletion occurring about 240 kb upstream from the SHH promoter in a large family with PPD-hypertrichosis. They found the 2 kb deletion repress the transcriptional activity of the SHH promoter in vitro [30]. 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 compared with previous results [7, 18]. Our study suggests that the IBD approach is a suitable method for mapping the cause genes of human diseases. Moreover, as disruptions of topological chromatin domains can result in limb malformations [31], more attention should be paid when studying PPD in the future on this aspect.

**Abbreviations**

IBD: Identity by descent; PPD: preaxial polydactyly; LMBR1: limb development membrane
protein 1; NOM1: nucleolar protein with MIF4G domain 1; LINC00244: long intergenic non-protein coding RNA 244; MNX1: motor neuron and pancreas homeobox 1; RNF32: ring finger protein 32

**Competing interests**

The authors declare no conflict of interest.

**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 (SMKX 2012013). 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 BeadChip previously as based data for our next study. The results of the analysis of clinical data has been described previously in Li et al. Our manuscript does not contain any individual person’s data.
Availability of data and supporting materials section

If the paper is accepted the data will be deposited into Dryad (XXXXXX).

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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.

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Legends of Figures and Tables

Fig. 1 Permutation analysis after filtering out regions with low IBD sharing.

The black line indicates genome-wide threshold and the red line is the 0.01 percentile of the permutation.
Table 1 Genetic variants in the two IBD segments.

Table 2 Pairwise statistics of LMBRI and SHH.

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Supplementary Figure and Table

**Fig. S1** Plot of the distribution of the IBD segments.

**Table S1** Top 0.01% peak signals.

**Table S2** IBD segments of *LMBR1* and *SHH*.

**Table S3** Mutations in intron 5 of *LMBR1*. 
Table 1 Genetic variants in the two IBD segments.

| Gene(7q36) | Position (GRCh37.p13) | SNP ID | REF | ALT | P-value | Note |
|------------|------------------------|--------|-----|-----|---------|------|
| LMBR1      | 156354434              | rs1860156 | T   | C   | 1.00E-06 | 116kb upstream of LMBR1 |
|            | 156401455              | kgp6282999 | C   | A   | 1.00E-06 | 69kb upstream of LMBR1 |
|            | 156477347              | kgp13575466 | C   | A   | 1.00E-06 |
|            | 156497668              | rs10228997 | A   | G   | 1.00E-06 |
|            | 156526645              | rs10224728 | T   | G   | 1.00E-06 | 199bp downstream of LMBR1 |
|            | 156686101              | kgp6457815 | C   | T   | 1.00E-06 | 492kb upstream of LMBR1 |
|            | 156687282              | kgp1716770 | C   | T   | 1.00E-06 | 1kb downstream of LMBR1 |
|            | 156716316              | kgp3747986 | T   | C   | 1.00E-06 | 30kb downstream of LMBR1 |
|            | 156730688              | kgp7566181 | T   | C   | 1.00E-06 | 45kb downstream of LMBR1 |
| SHH        | 155103781              | rs13223383 | G   | T   | 1.00E-06 | 492kb upstream of SHH |
|            | 155169143              | rs1990808  | C   | T   | 1.00E-06 | 426kb upstream of SHH |
|            | 155182442              | kgp9710825 | G   | A   | 1.00E-06 | 426kb upstream of SHH |
|            | 155716520              | rs4716928  | C   | T   | 1.00E-06 | 112kb downstream of SHH |
|            | 155718241              | rs4716930  | A   | C   | 1.00E-06 | 113kb downstream of SHH |
|            | 155721324              | rs11764820 | A   | G   | 1.00E-06 | 116kb downstream of SHH |
|            | 155721386              | rs11769663 | G   | T   | 1.00E-06 | 116kb downstream of SHH |
|            | 155722231              | rs6971588  | T   | G   | 1.00E-06 | 117kb downstream of SHH |
|            | 155723112              | kgp11597900 | C   | T   | 1.00E-06 | 118kb downstream of SHH |
Table 2 Pairwise statistics of *LMBR1* and *SHH*.

| Gene | patient-patient | normal-normal | patient-normal |
|------|-----------------|---------------|---------------|
|      | No. IBD in patient pairs | % IBD in patient pairs | No. IBD in normal pairs | % IBD in normal pairs | No. IBD in patient-normal pairs | % IBD in patient-normal pairs |
| *LMBR1* | 21 | 84 | 0.400 | 22 | 17 | 0.074 | 29 | 0.126 |
| *SHH* | 21 | 81 | 0.386 | 22 | 16 | 0.069 | 24 | 0.104 |

Note:

% IBD patient pairs = IBD patient pairs/case × (case-1)/2;
% IBD normal pairs = IBD normal pairs/normal × (normal-1)/2;
% IBD patient-normal pairs = IBD patient-normal pairs/case × normal/2.