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, 57markers 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

(Fig. 1). 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 unknwon. 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 *LMBR1* and *SHH.*

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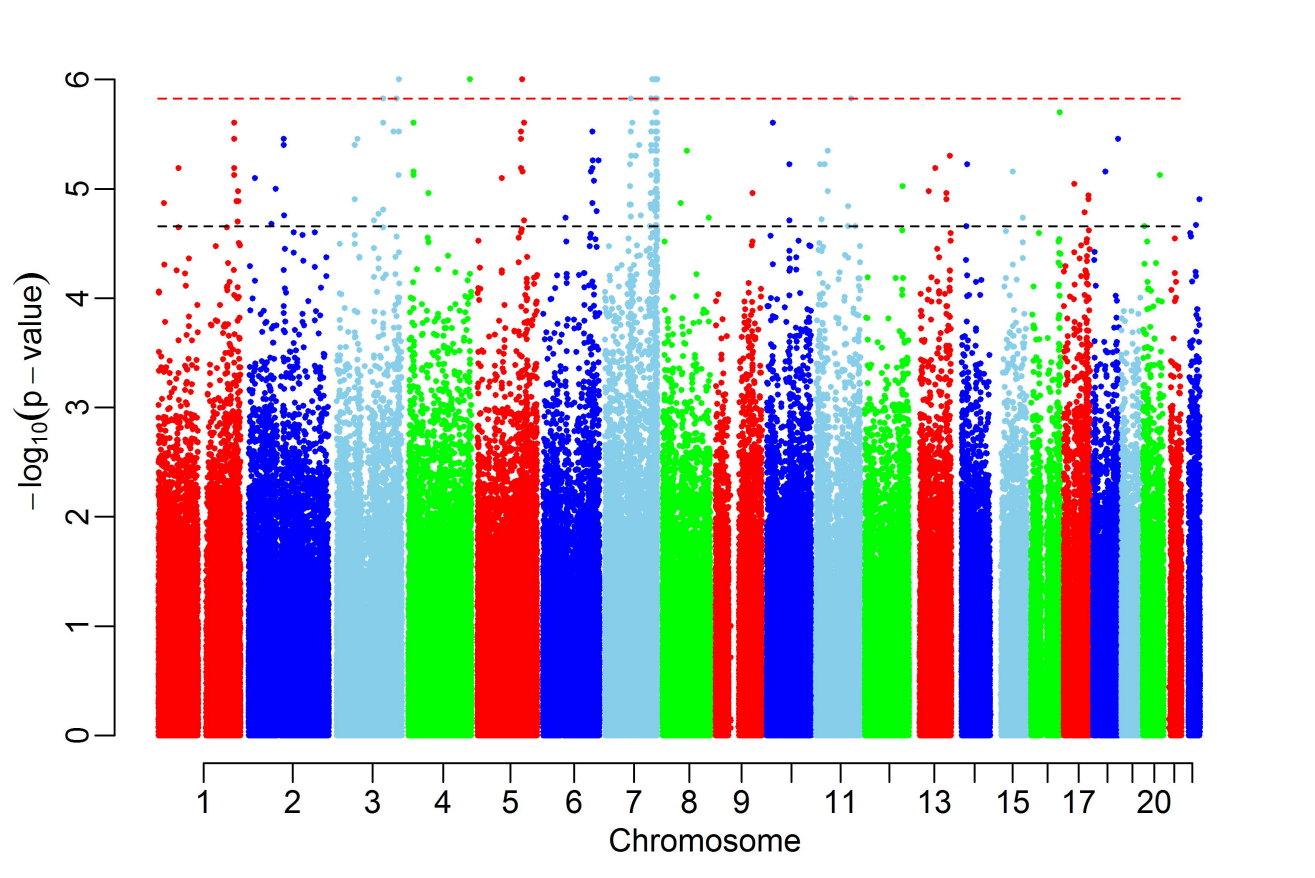
Supplementary Figure and Table

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*.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Chromosome

Table 1 Genetic variants in the two IBD segments.

Gene(7q36))	Position (GRCH37.p13)	SNP ID	REF	ALT	P-valve	Note
	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	
LMBR1	156526645	rs10224728	T	G	1.00E-06	
156470537...156685902	156686101	kgp6457815	C	T	1.00E-06	199bp downstream 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*
	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*
SHH	155718241	rs4716930	A	C	1.00E-06	113kb downstream of *SHH*
155595558...155604967	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. patient	No.IBD in patient pairs	% IBD in patient pairs	No. normal	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 x (case-1)/2;
% IBD normal pairs =IBD normal pairs/normal x (normal-1)/2;
% IBD patient-normal pairs =IBD patient-normal pairs/case x normal/2.