A novel pathogenic variant of IHH for Brachydactyly type A1

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Abstract

Brachydactyly type A1 (BDA-1, MIM 112500) is a genetically heterogeneous autosomal-dominant disorder mainly characterized by shortening or missing of the middle phalanges. Brachydactyly type A1 (BDA-1) is caused by heterozygous pathogenic variants in a specific region of the N-terminal active fragment of Indian Hedgehog (IHH). In this study, we reported a Chinese family with 8 members affected with Brachydactyly type A1. After performing whole-exome sequencing in the proband, we identified a novel heterozygous missense variant c.299A>G (p.D100G) at the mutational hotspot of IHH gene. The variant co-segregated with BDA-1 in the pedigree, showed 100% penetrance for phalange phenotype with variable expressivity. This finding expanded the Brachydactyly type A1-related mutational spectrum of IHH gene.

Background

Brachydactyly (BD) is a group of inherited disorders of the hands, and is generally characterized by shortened and often malformed digits [1]. Heritable BDs have been classified into seven types, i.e: A1, A2, A3, B, C, D, and E on the basis of their patterns of skeletal involvement [2]. Brachydactyly A-1 (BDA1; MIM 112500) is inherited as an autosomal dominant disorder and is characterized by short stature and shortening of middle phalanges of all the digits. The middle phalanges are either rudimentary or fused with the terminal phalanges. About half of the BDA1 families are due to mutations in the IHH (Indian Hedgehog) gene [3]. To date, about 11 different IHH pathogenic variants had been identified in individuals with BDA1 (HGMD and ClinVar), and the pathogenic variants cluster in the central region of the N-terminal signaling fragment [4]. As a central signaling molecule in mediating skeletal development, IHH plays an important role in mediating skeletal condensation, growth and differentiation of chondrocyte, joint development and bone formation [5]. Here, we studied a five-generation Chinese family associated with a variation of BDA1 and identified a novel IHH pathogenic variant by whole-exome sequencing.

Materials And Methods

Patients
The BDA1-affected family and 200 unaffected subjects were recruited in our study. Diagnosis was based on physical examination, radiographic findings and family history. All the participants recruited in this study provided informed consent for the study approved by the ethics committee of the Maternal and Child Health Hospital of Guangxi Zhuang
Autonomous Region. The pedigree is shown in Figure 1.

Genetic analysis
Whole-exome sequencing and Sanger sequencing
Whole exome sequencing using the genomic DNA of proband IV-1(Figure 1) was performed. Agilent SureSelect Human All Exon V5 Kit (Agilent Technologies, Santa Clara, CA) was used for target capture. The library was sequenced on Hiseq2500 platform (Illumina, San Diego, CA, USA) according to the manufacturer’s instructions. A custom pipeline mainly built on the Genome Analysis Toolkit (GATK) was used for sequence data analysis and annotation. Identification of causal variant was aided by the TGex software (LifeMap Sciences,USA). The candidate \textit{IHH} variant was validated by Sanger sequencing and its pathogenicity classified following ACMG/AMP guidelines [6].

Results
Clinical phenotype
We ascertained a five-generation pedigree with BDA1. Twenty two family members, including 8 affected members and 14 unaffected family members, were participated in this study (Figure 1). The proband (IV-2) was a 30-year-old women, who presented with mild disproportionate short stature with a height SDS of -2.4SD. Her hand radiographs showed varying degrees of shortening of the middle phalanx of the second to fifth fingers, and the middle phalanges in digit five was fused to the terminal phalange as only one interdigital joint was visible. She also showed bilateral shortening of metacarpals bone 3-5. Her foot radiographs showed shortening of all digits, the middle phalanges of third to fifth toe were fused to the terminal phalange (Figure 2). She also had bilateral shortening of metatarsals bone 3-4. Her other affected family members all exhibited features consistent with BDA1. The toes of other affected individuals in the family were severely shortened (Figure 3B) and so were fingers (Figure 3A). Interestingly, short stature was not consistently presented affected individuals. The proband’s uncle (III-4) and cousin (IV-5) presented with mild disproportionate short stature with a height SDS of -2.4SD±0.3, but her father (III-2) and uncle (III-5)’s heights are within normal range. In addition, the proband (IV-5), her father (III-2) and cousin (III-5) showed radial deviation of the second finger (Figure 3A: III-2 and III-5). The uncle (III-5) showed radial deviation the 2nd and 3rd finger and flexion contracture of the 4nd finger (Figure 3A). No other abnormalities were observed in the family members.

Mutation analysis
Exome sequencing of the proband’s DNA identified a novel heterozygous A to G transversion in exon 1 at the position 299 of the \textit{IHH} gene. Validation by Sanger sequencing confirmed that the c.299A>G (D100G) mutation was shared in 6 affected individuals and not in the unaffected individual (Figure 4C).The variant is not present in the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/), HPSD (http://liweilab.genetics.ac.cn/HPSD/) and the dbSNP (http://www.ncbi.nlm.nih.gov/SNP/), nor it is present in DNA samples from 200 normal or in control databases (e.g. ExAC and gnomAD).

The variant is located in N-terminal signaling domain as the DD-peptidase zinc-binding domain of IHH protein. Functional predictions using SIFT, PolyPhen2.0 and Mutation Taster
revealed that the novel missense variant had deleterious effects. According to the ACMG standards and guidelines for the interpretation of sequence variants [6], the novel variant is pathogenic.

Discussion

Brachydactyly type A1 is characterized by hypoplasia/aplasia of the middle phalanges of digits 2–5. Indian hedgehog (IHH) was the first identified gene to be associated with BDA1 [7]. The IHH gene, which encodes a member of the Hedgehog family of signaling proteins, is known for its role in endochondral ossification: regulate the balance between growth and ossification of the developing bones [5]. The IHH protein operates through a feedback control mechanism. IHH binds to the patched (PTC) receptor, which functions in association with smoothened (SMO), to activate the GLI complex of transcription factors [8-10]. From there, these transcription factors continue to signal and regulate downstream genes affecting patterning.

IHH mutations affect Hh signaling at multiple levels, which impair chondrocyte maturation and proliferation, resulting in failure of osteoblast development in endochondral bones [7]. So far, about 14 IHH pathogenic variants have been reported to be association with BD [11-14][figure 4A], and the variants are restricted to the N-terminal active fragment, and exhibit a variable outcome [4]. Variants associated with brachydactyly type A1 are known to predominantly affect codon 95, 100, 131, and 154 [3, 15-16]. Based on the X-ray crystal structure of SHH, McLellan, et al showed that these residues are located within a calcium binding site, an important domain for mediating interactions with PTCH1, HIP1, CDO and GAS1 [17]. In particular, p.D100E had been shown to affect IHH interaction with PTCH1 and HIP1, reducing its capacity to induce cellular differentiation [16], whereas p.D100E was shown to change the Hh local tertiary structure and intracellular fate [5], causing abnormal bone development and abnormal digit formation. To date, six other BDA1-affected families of Italian, American, India, British and Chinese descent have been found to affect the same residue of IHH at the codon position of 100, demonstrating a mutational hot spot of IHH. The novel variant at the nucleotide position c.299 A>G of the IHH gene results in a novel amino acid substitution (p.D100G) at the hotspot. This novel variant co-segregated with the BDA1 phenotype in this Chinese family and demonstrated a high penetrance of this pathogenic variant in causing dactyl phenotypes. In addition, phenotypic variations were observed among affected family members in the extent/severity of affected phalanges and metacarpal/metatarsal bones, demonstrating considerable intra-familial variations in terms of expressivity.

IHH is expressed in the prehypertrophic chondrocytes of cartilage, and regulates growth of bones by coordinating chondrocyte proliferation and differentiation [18]. Thus in addition to affect phalange bone growth, disregulation of IHH signaling could also affect long bone and stature. Biallelic IHH pathogenic variants cause acrocapitofemoral dysplasia acrocapitofemoral dysplasia (MIM 607778), a disorder characterized by severe disproportionate short stature, BDA1 and cone-shaped epiphyses in hands and hips [19]. Short stature is often a component of the Brachydactyly, but it is less frequent among DBA1 individuals. So far, short stature had only been observed in DBA1 individuals with pathogenic variants affecting Asp100 residue of IHH gene [3, 20-21]. Not all DBA1 individuals with these pathogenic variants exhibited short stature, but they were shorter than non-carrier family members [3, 19-21], suggesting variants at this mutational hotspot
can affect the growth to various degree but with reduced penetrance for short stature. In this Chinese family with a novel variant at residue 100, short stature was not 100% penetrant: the proband (IV-2), proband’s uncle (III-4) and cousin (IV-5) had short stature, whereas her father (III-2) and uncle (III-5)’s height had normal stature. Other factors are believed to be involved in affecting the final stature of an individual with IHH pathogenic variants. Such pedigrees provided opportunities for identify co-determinants for human heights. Gabriela et al. observed that in Brazilian and Spanish populations heterozygous deleterious IHH variants are more frequent in short stature cohort (1.6%) than in general population (0.017% in gnomAD; P<0.001) [22], supporting the notion that reduced IHH signaling may be responsible for a reduced growth of the long bones and short stature [3, 7]. The role of IHH variants in non-syndromic short stature also needs further study.

In conclusion, a novel missense variant (c.299 A>G) affecting the mutational hotspot (residue 100) of IHH resulting in brachydactyly type A1 was identified in a Chinese family. Sufficient evidence support the pathogenicity of this novel variant. High penetrance for the phalange phenotype and variable expressivity were observed in this family. Short stature was only observed in a subset of affected family members. The findings of this report will further help our understanding the phenotype-genotype correlations of IHH pathogenic variants and related disorders including brachydactyly type A1.

Abbreviations

IHH: Indian Hedgehog;
BDA-1: Brachydactyly type A1.

Declarations

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Availability of data and materials
All data generated or analysed during this study are included in this published article.

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Author contributions
QY and YPS designed the study and drafted the manuscript; JW, QY, XXT, CF and JL extracted, analyzed, interpreted the data, and collected the clinical data QZ, XF, SY and MTL performed the targeted sequencing, analyzed and interpreted the data; YPS and YQ participated in the study coordination and revised the manuscript. All authors read and approved the final version of the manuscript.

Competing interests
The authors have no conflict of interest to declare.

Ethics approval and consent to participate
All procedures in this study were approved by the Institutional Review Boards and Ethics Committees of Guangxi Maternal and Child Health Hospital. Detailed written informed consent was obtained from all participants.

Consent for publication
Not applicable.

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Figures
Figure 1

Pedigree of a five generation family with brachydactyly type A1 (BDA1). Filled symbols represent affected individuals; open symbols unaffected individuals; squares depict males and circles females. Diagonal lines indicate deceased individuals. The proband is indicated by an arrow.
(A-D) The appearance and radiological findings of the proband with brachydactyly type A1(BDA1). (A) showing shortened fingers and absence of middle phalanges of the fifth finger and radial deviation of the second finger. (B) Radiographic images of proband's hand: shortening of the middle phalanges of digits II–V, fusion of middle and terminal phalanges of 5th finger, bilateral shortening of metacarpals bone 3-5. (C) showing short toes and absence of middle phalanges of the third to fifth toes. (D) Radiographic images of proband's foot: fusion of middle and terminal phalanges of third to fifth toe, bilateral shortening of metatarsals bone 3-4.
Figure 3

Features of other affected family members with brachydactyly type A1 (BDA1).

(A) showing shortened fingers and radial deviation of the second or/and third finger (III-2, III-5, IV-5) and flexion contracture of the 4nd finger (III-5). (B) showing abnormally shortened toes.
IHH pathogenic variants. (A) Boxes represent three different exons as indicated, and solid lines connecting these boxes represent the introns of IHH gene. The numbers above the boxes indicate the positions of the IHH complementary DNA at the start-stop sites and exon-intron boundaries. Vertical lines represent the locations of missense (above the boxes) or nonsense/frameshift/splicing (below the boxes) variants. (B) IHH protein structure with key domains, regions, and the mutation indicated. (C) Sanger sequencing chromatograms showing a missense variant c.299A>G(p.Asp100Gly) in the affected individuals in comparison to those of unaffected individuals.
