A novel variant of IHH in a Chinese family with brachydactyly type 1

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Abstract

Brachydactyly type A1 (BDA-1) is an autosomal dominant disorder which is caused by heterozygous pathogenic variants in a specific region of the N-terminal active fragment of Indian Hedgehog (IHH). The disorder is mainly characterized by shortening or missing of the middle phalanges. The following study revealed a novel heterozygous missense variant c.299A>G (p.D100G) at the mutational hotspot of IHH gene after performing whole-exome sequencing in the proband of a Chinese family with BDA-1. The variant co-segregated with BDA-1 in the pedigree, showed 100% penetrance for phalange phenotype with variable expressivity. This finding expanded the variants on IHH gene which contribute to the cause of BDA-1.

Background

Brachydactyly (BD) is generally characterized by shortened and often malformed digits of the hands [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 14 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

Subjects

The BDA-1 affected family was referred to Guangxi Maternal and Child Health Hospital for shortened and malformed digits and requested genetic testing for all 22 family members. Diagnosis was based on physical examination, radiographic findings and family history. There were 20 individuals tested in the BDA1-affected family including 6 individuals who were diagnosed with BDA1. In order to rule out the possibility that the variant is unique to the region, we recruited 200 local residents for alternative allele frequency testing in 2018. The control group consisted of 100 females and 100 males which aged between 20 to 40. The height of each individual in the control group is taller than 160cm. The fingers and toes of the control group appears normal. All 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

Genomic DNA was extracted from peripheral blood using standard protocols of Lab-Aid DNA kit (Zeesan Biotech Co., Ltd., Xiamen, China). Agilent SureSelect Human All Exon V5 Kit (Agilent Technologies, Santa Clara, CA) was used for target capture. The library was sequenced on the Hiseq 2500 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). We performed genotyping on 200 subjects by Sanger sequencing. The candidate IHH variant was
validated by Sanger sequencing and its pathogenicity classified following ACMG/AMP guidelines [6].

Results

Clinical phenotype

We constructed a pedigree of the five-generation family that participated in this study, which includes 6 family members affected by BDA-1, 2 affected members have been deceased, and 14 family members who are unaffected (Figure 1, Table 1). We identified a mutation c.299A>G / p.Asp100Gly of the IHH gene with co-segregation of all 6 affected individuals in this family. The genotype-phenotype co-segregation of LOD score is 1.5 indicate the novel variant co-segregated with the BDA1 phenotype in this Chinese family. Statistical test for the mean digit length cannot be performed because of the lack of standard measurement for fingers and toes among the Chinese population. Finger 2 and 3 revealed that the affected individuals have relatively shortened fingers and toes to varied degree. We compared the height of each family members to the height growth chart for Chinese population. Non-affected individuals have normal stature. Some of the affected individuals have normal stature and some have short stature. The proband (IV-2) was a 30-year-old female, who presented with mild disproportionate short stature with a Height Standard Deviation Score (HSDS) of -2.4SD. The radiograph of her hands 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. The radiograph of her feet 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. All of the other affected family members 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 among the affected individuals. The proband’s uncle (III-4) and cousin (IV-5) presented with mild disproportionate short stature with a HSDS 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 affected family members.

**Mutation analysis of whole exome sequencing**

Whole exome sequencing using the genomic DNA of proband IV-1(Figure 1) was performed. In total, 850 million uniquely mapped reads with MAPQ ≥30 were generated which covering 96% of exome target regions at least 20x. Exome sequencing called a total of 120,764 variants which excluded variants in non-functional variants such as intronic changes. A total of 16040 variants were found in exonic and splice site regions. Among these variants, 1009 variants had the MAF less than 0.01 then neutral and benign variants were also omitted according to ClinVar. Based on the TGex software (LifeMap Sciences, USA), we found 17 variants existed in genes whose functions matched with known phenotypes. Variants from 6 genes associated with IHH, NOTCH1, HMN, ATRX, BBS1 and L1CAM were extracted, leading to the identification of a novel variant in IHH c.299A>G / p.Asp100Gly that co-segregated with the disease phenotype in the examined family (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 the DNA samples from 200 normal or in the 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 CADD (https://cadd.gs.washington.edu/snv), SIFT (http://sift.jcvi.org/), PolyPhen2 (http://genetics.bwh.harvard.edu/pper2/), Mutation Taster (www.mutationtaster.org) It is 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 (PM1, PM2, PM5, PP1, PP3, PP4).

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 signaling protein of the Hedgehog family, is known for its role in endochondral ossification: regulate the balance between growth and ossification of developing bones [5]. The IHH protein operates through a feedback control mechanism where IHH binds to the patched (PTC) receptor which functions in association with smoothened (SMO), in order to activate the GLI complex of transcription factors [8-10]. From there, these transcription factors continue to signal and regulate down-stream genes affecting patterning.

IHH mutations affect Hh signaling at multiple levels. It impairs 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 associated with BD [11-14][figure 4A]. All of the pathogenic variants are restricted to the N-terminal active fragment yet exhibit a variable outcome [4]. Variants associated with brachydactyly type A1 are known to affect codon 95, 100, 131, and 154 predominantly [3, 15-16]. Based on the X-ray crystal structure of IHH, 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]. Previous study showed that p.D100E affects IHH interaction with
PTCH1 and HIP1 which resulted reduced capacity to induce cellular differentiation [16], and p.D100N changes the Hh local tertiary structure and intracellular fate [5], causing abnormal bone development and abnormal digit formation. We suggest that our variant acts similar to the p.D100E or p.D100N missense mutation, the mutation affect Hh signaling at multiple levels, 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 high penetrance of this pathogenic variant in causing dactyl phenotypes. In addition, phenotypic variations were observed among affected family members in terms of the severity of affected phalanges and metacarpal/metatarsal bones, demonstrating considerable intra-familial variations for expressivity.

IHH is expressed in the prehypertrophic chondrocytes of cartilage, and regulates growth of bones by coordinating chondrocyte proliferation and differentiation [18]. Not only it affects phalange bone growth, dysregulation of IHH signaling could also affect long bone and stature.

Although short stature is often a component of the Brachydactyly, it has been reported less frequently in type A1. Short stature only present in brachydactyly type A1 patients with the mutation on Asp100 in IHH gene [3, 19-20]. Interestingly, in the study of Gao et al, all of the affected subjects were shorter than unaffected subjects in the same family. However, short stature was only presented for some of the same family BDA-1 subjects of other studies [3, 19-20].
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) had normal stature. Pedigree studies provided the opportunity to identify co-determinants for human heights. Gabriela et al. observed that heterozygous deleterious IHH variants are more frequent in short stature cohort (1.6%) in Brazilian and Spanish populations compared to the general population (0.017% in gnomAD; P<0.001) [20], supporting the notion that reduced IHH signaling may be responsible for a reduced growth of the long bones and short stature [3, 7]. We suggest that the mutation on Asp100 reduced IHH signaling capacity which consequently reduced the growth of the long bones, thus resulted associated short stature. Certainly, there are other factors involved in affecting the final stature of individuals with IHH pathogenic variants. 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 a mutational hotspot (residue 100) of IHH was identified in a Chinese family affected by BDA-1. Sufficient evidence have been provided to 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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Table

Table 1 Clinical features of the patients with the IHH variant

| Patient | Gender | Age at last Examination (years) | Height | Shared features | Additional features |
|---------|--------|---------------------------------|--------|-----------------|---------------------|
| III-2   | Male   | 50y                             | 168cm (normal) | Shortened fingers and toes | -                   |
| III-4   | Male   | 49y                             | 157cm (<-2.4SD) | Shortened fingers and toes | Radial deviation of the third finger bilaterally |
| III-5   | Male   | 47y                             | 169cm (normal) | Shortened fingers and toes | Flexion contracture of the fourth finger |
| IV-2    | Female | 30y                             | 154cm (<-2.4SD) | Shortened fingers and toes, absence of middle phalanges of the fifth finger, absence of middle phalanges of the third to fifth toes, fusion of middle and terminal phalanges of third to fifth toe, | Radial deviation of the second finger bilaterally, shortening of metacarpals bone 3-5, bilateral shortening of metatarsals bone 3-4 |
| IV-5    | Male   | 19y                             | 155cm (<-2.5SD) | Shortened fingers and toes, absence of middle phalanges of the fourth toes. | Radial deviation of the second finger bilaterally |
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.
Figure 2

(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.
