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SHORT COMMUNICATION

A novel heterozygous mutation in the Indian hedgehog gene (*IHH*) is associated with brachydactyly type A1 in a Chinese family

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Abstract Brachydactyly type A1 (BDA1) is caused by mutations in the Indian hedgehog gene, *IHH*, on chromosome 2q35-36. In this study, a large five-generation Chinese family with BDA1 was identified and characterized. All affected family members demonstrated significant homogeneous phenotype and some unique clinical features different from those associated with the reported BDA1 mutations in *IHH*. Linkage analysis showed that the BDA1 gene in the family was linked to marker *D2S126* close to *IHH* with a LOD score of 4.74 at a recombination fraction of 0. DNA sequence analysis revealed a heterozygous C to T transition at nucleotide 461 of *IHH*, resulting in a novel T154I substitution. The T154I mutation co-segregated with all affected individuals in the family, and was not present in normal family members or 200 normal controls. These results expand the spectrum of clinical phenotype associated with IHH mutations.

Keywords *IHH* · Brachydactyly type A1 · Limb malformation · Linkage analysis · Mutation · Genetics

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Introduction

Brachydactyly (BD) is an inherited anomaly of the hands, and is generally characterized by shortened phalanges or metacarpals. As an autosomal dominant trait, Type A1 brachydactyly (BDA1; MIM 112500) was the first genetic disorder to be described (Farabee 1903). It is characterized by rudimentary middle phalanges of all the digits or fusion of the middle phalanges with the terminal phalanges, and shortness of the proximal phalanges of the thumbs and big toes.

The gene for BDA1 was mapped to 2q35–q36 by linkage analysis in two Chinese families (Yang et al. 2000). Subsequently, three distinct mutations were identified within the Indian hedgehog gene (*IHH*) (Gao et al. 2001). Later studies established that heterozygous missense mutations in *IHH* resulted in BDA1, whereas homozygous transition resulted in acrocapitofemoral dysplasia (ACFD; MIM 607778), which is characterized by short stature of variable severity with postnatal onset (Hellemans et al. 2003).

In this study, we studied a large five-generation Chinese family associated with a variation of BDA1 and identified a novel *IHH* mutation.

Materials and methods

Patients

One Chinese family with a clinical diagnosis of BDA1 was identified and characterized. The study enrolled 22 individuals from the family, including 10 affected individuals. The stature of the affected members, either children or adults, was indistinguishable from the unaffected members. Radiographic analysis of the proband's hands showed bilateral shortening of the middle phalanges of digits 2–4, and fusion of the middle and terminal phalanges of digit 5, but the proximal phalange of digit 1 was unaffected (Fig. 1). Radiographic analysis of the proband's feet revealed no apparent abnormalities (data not shown). The clinical features of all patients in the family are very similar.

Linkage analysis

Genomic DNA was prepared from venous blood. This study was performed in accordance with the study protocols approved by the Ethics Committee of Huazhong University of Science and Technology.

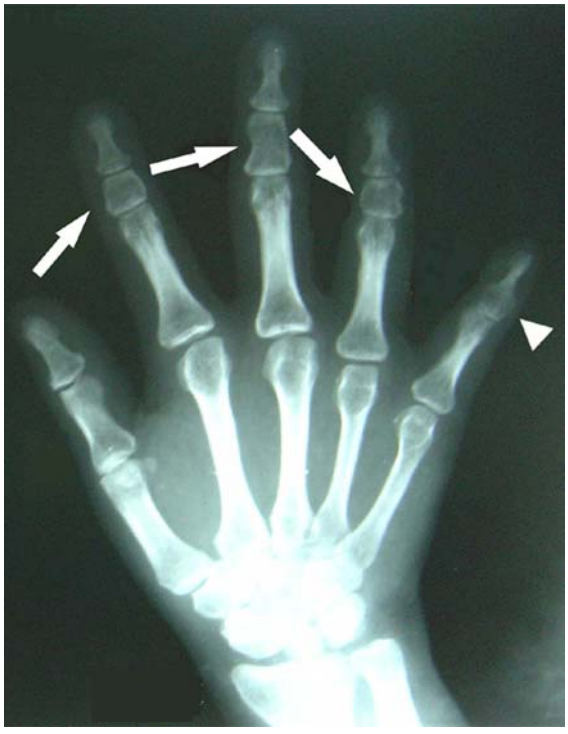

Fig. 1 Radiography of the right hand of the proband. *Arrows* Shortening of the middle phalanges of digits 2–4, *triangle* fusion of the middle and terminal phalanges of digit 5. Note the unaffected proximal phalange of digit 1, which contrasts to classical Type A1 brachydactyly (BDA1) phenotype

Linkage analysis was carried out as described previously (Wang et al. 2005). Markers were genotyped using an ABI 3100 Genetic Analyzer and genotypes were analyzed using the GeneMapper 2 Software program (Applied Biosystems, Foster City, CA). The markers used for linkage analysis include *D2S2382* and *D2S126* for *IHH* (OMIM *600726), *D20S195* and *D20S107* for *GDF5* (OMIM *601146), *D4S414* and *D4S1572* for *BMPR1B* (OMIM *603248), *D9S283* and *D9S287* for *ROR2* (OMIM *602337), *D2S335* and *D2S364* for *HOXD13* (OMIM *142989), and *D17S1868* and *D17S787* for *NOG* (OMIM *602991) as well as *D5S630*, *D5S416*, *D5S419*, *D5S426*, *D5S418* and *D5S407* for the chromosome 5p13.3–13.2 BDA1B locus (OMIM %607004).

Mutation detection

All three exons and intron-exon boundaries of the *IHH* gene were amplified by PCR (sequences for PCR primers are available upon request). The PCR products were purified using the QIA Quick Gel Extraction Kit (Qiagen, Valencia, CA), and sequenced using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) as described previously (Wang et al. 2005).

PCR-SSCP analysis

SSCP (single strand conformation polymorphism) analysis was performed using the standard method to confirm the mutation and to test whether the mutation co-segregates with the BDA1 disease in the Chinese family, and is present in 200 normal Chinese controls (Chen et al. 1998). The 425 bp fragment containing exon 2 of *IHH*, where the c.461C > T mutation is located, was amplified by PCR using the forward primer 5′-GCG CCT ACA CCT GCA CCT C-3′ and the reverse primer 5′-GGC GGG CTC TTC ACC TTC TC-3′.

Results

To identify the chromosomal location of the BDA1 gene in the Chinese family, we performed linkage analysis with markers that span the known genes and one genetic locus for malformations of digits, including *IHH*, *GDF5*, *BMPR1B*, *ROR2*, *HOXD13* and *NOG*, and the chromosome 5p13.3–13.2 locus. Only marker *D2S126*, which is 2.2 Mb from *IHH*, showed complete linkage with the disease in the family (Fig. 2a). A LOD score of 4.74 was obtained

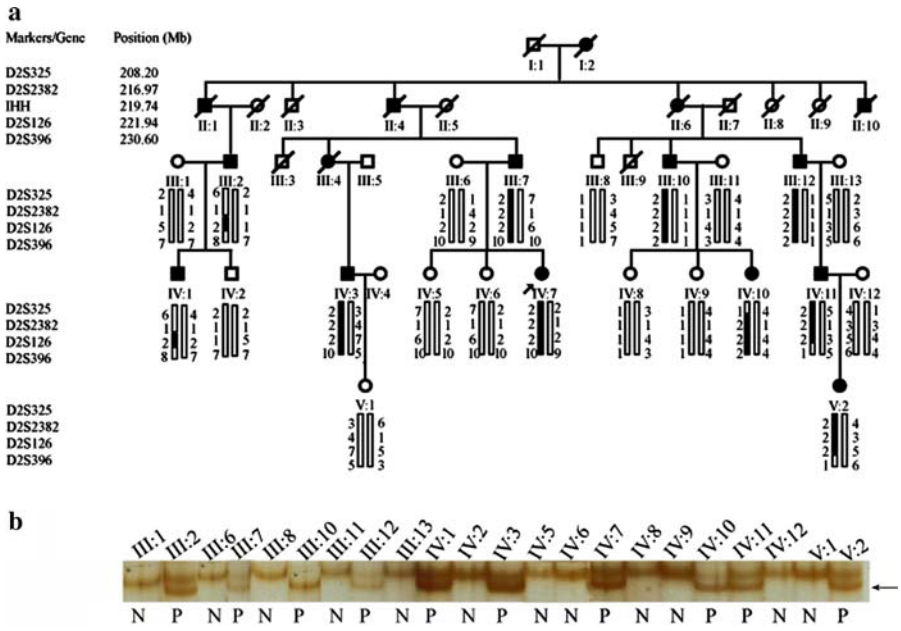

Fig. 2 Fine mapping of the BDA1 gene in the Chinese family and co-segregation of the Indian hedgehog gene (*IHH*) mutation T154I with disease. **a** Pedigree structure. *Squares* Males, *circles* females, *solid symbols* affected individuals, *open symbols* unaffected individuals, *diagonal lines* deceased individuals, *arrow* proband. The results of haplotype analysis are shown below each symbol: *black bar* disease allele, *open bar* normal allele. **b** SSCP analysis of mutation T154I in the Chinese BDA1 family. *Arrow* Abnormal SSCP band, *N* unaffected family member, *P* affected family member

at the recombination fraction of 0. DNA sequence analysis identified a mutation in the *IHH* gene: c.461C > T, in exon 2. The c.461C > T transition results in the substitution of a hydrophilic threonine residue by a hydrophobic isoleucine residue (T154I). SSCP analysis showed that the T154I mutation co-segregated with all affected individuals in the family (Fig. 2b), which was confirmed by DNA sequence analysis. Alignment of the IHH sequences from different species showed that the T154I mutation altered the evolutionarily conserved amino acid residue T154 of IHH (Fig. 3). Further SSCP analysis revealed that the *IHH* mutation T154I was not present in 200 normal Chinese controls.

Discussion

In this study, we identified a novel mutation, a heterozygous C-to-T transition that resulted in a T154I missense mutation in the *IHH* gene in a five-generation Chinese family with BDA1. To date, five IHH mutations have been previously associated with BDA1 (Gao et al. 2001; McCready et al. 2002, 2005; Giordano et al. 2003; Kirkpatrick et al. 2003; Table 1). The unique clinical feature that distinguishes mutation T154I from other mutations is the unaffected proximal phalanges of digit one (Fig. 1). It is interesting to note that this distinct feature is different from the definition of the typical BDA1 type (BDA1; MIM 112500), which

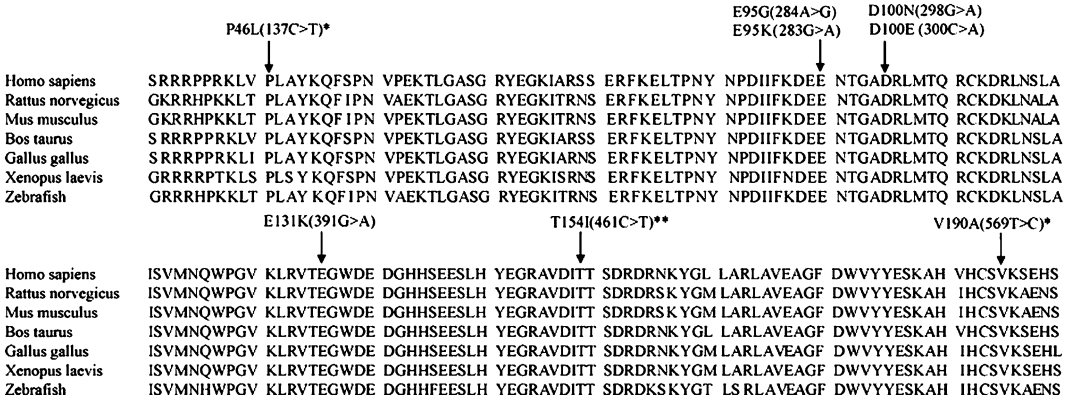

Fig. 3 The T154 residue of IHH is evolutionally conserved among different species. *Double asterisks* T154I mutation for BDA1 in this study, *single asterisk* homozygous mutations associated with acrocapitofemoral dysplasia (ACFD). Other IHH mutations associated with BDA1 are also indicated

Table 1 Summary of clinical features of Type A1 brachydactyly (BDA1) associated with mutations in the Indian hedgehog gene (*IHH*)

Mutation	Ethnic origin	Clinical features	Reference
c.283G > A (p. E95K)	Chinese	Middle phalanges missing or fused to the distal phalanges; short proximal phalanges of digits 1; one to three fingers or toes missing, or fused phalanges	Gao et al. 2001
c.284A > G (p. E95G)	Mexican	No detailed clinical features described	Kirkpatrick et al. 2003
c.298G > A (p. D100N)	British	Bilateral dysplasia of the carpals, metacarpals, and phalanges; short and broad phalanges; middle phalanx missing in digits 2, 3, and 5; spurs on the radial side of the trapezium; variety of musculoskeletal problems; pain experienced in knees and hips	McCready et al. 2002
c.298G > A (p. D100N)	Italian	Proportionate shortening of all digits; most severely shortened middle phalanges	Giordano et al. 2003
c.298G > A (p. D100N)	British	Bilateral shortening of the middle phalanges of digits 1–4; fusion of the middle and terminal phalanges of digit 5	McCready et al. 2005
c.300C > A (p. D100E)	Chinese	Similar to clinical features associated with mutation E95K; short stature	Gao et al. 2001
c.391G > A (p. E131K)	Chinese	Similar to clinical features associated with mutation E95K	Gao et al. 2001
c.461C > T (p. T154I)	Chinese	Bilateral shortness of the middle phalanges of digits 2–4; fusion of the middle and terminal phalanges of digit 5; unaffected proximal phalanges of digit 1 of both hands; unaffected feet; homogeneous phenotype	This study

has short proximal phalanges of the thumbs and big toes.

IHH is synthesized as a 45 kDa precursor that undergoes autocatalytic internal cleavage. This results in an amino-terminal domain of approximately 20 kDa that has all the known signaling activity and a 25 kDa C-terminal domain responsible for autoproteolytic processing (Porter et al. 1996). The IHH mutations identified to date for BDA1 and ACFD are all located in the amino-terminal signaling domain. The three-dimensional structure of the mouse IHH protein, Shh, has been determined [1VHH (Molecular Modeling DataBase accession number 3860); Hall et al. 1995]. Due to high homology of the mouse Shh protein to human IHH protein, we used the Shh structure to infer the spatial localization of the different mutations in IHH. The autosomal dominant BDA1 mutations E95G, E95K, D100N, D100E, and E131K are clustered together at one surface region of Shh, whereas the autosomal recessive ACFD mutations P46L and V190A are localized on the opposite side of the tertiary structure. All these mutations occur in α-helices, but mutation T154I identified in this study is in a β-sheet. Mutation T154I is located in the central section of the protein, between the BDA1 mutations and the amino terminal mutation P46L. It is interesting to note that T154I appears to be located on the same surface as other BDA1 mutations; however, it remains to be determined whether it acts by a mechanism similar to that of other BDA1 mutations.

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