

Mild brachydactyly type A1 maps to chromosome 2q35-q36 and is caused by a novel *IHH* mutation in a three generation family

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The brachydactylies are a heterogeneous group of inherited digital abnormalities originally classified into five types, on the basis of malformation of the digits.¹ Among them, brachydactyly type A1 (BDA-1, MIM 112500), also referred to as Farrabee or Fitch type 9, is mainly characterised by short middle phalanges, which may be fused to the terminal ones.^{1,2} All the small tubular bones tend to be reduced in size, but the middle phalanges are the most severely shortened. Several phenotypes have been described for this disease. BDA-1 may occur as an isolated condition or maybe associated with other manifestations. Patients often have short stature and additional features, such as radial and/or ulnar clinodactyly, malformed or absent epiphyses, scoliosis, abnormal menisci, and club feet.^{3,4} Some patients also showed mental retardation, nystagmus, squint, and sixth nerve palsy.^{3,6}

Despite the fact that BDA-1 has been identified as a Mendelian disorder since 1903,⁷ until recently few molecular genetic studies on this malformation have been performed. Several candidate genes including *MSX1*, *MSX2*, *FGF1*, *FGF2*, and the *HOXD* gene cluster have been excluded for this autosomal dominant disease.⁸ In 2000, a linkage study performed in two large Chinese families mapped the locus for BDA-1 to chromosome 2q35-q36⁹ and subsequent analysis in the same families identified mutations in the Indian hedgehog gene (*IHH*) in affected subjects.¹⁰ The *IHH* gene lies within the critical region on chromosome 2q35-q36, and codes for a signalling molecule that is known to mediate condensation, growth, and differentiation of cartilage. To date, there have been no other reports linking *IHH* mutations to BDA-1. Moreover, the affected subjects who showed a mutation in the *IHH* gene had physical manifestations beyond those described in BDA-1 by Fitch,² and a recent study in a family with typical features of BDA-1 identified the presence of a novel locus on chromosome 5p13.3-p13.2.¹¹ Thus, the different BDA-1 phenotypes might be the result of locus heterogeneity.

In this study we report a family affected by a mild form of BDA-1 with a novel mutation in the *IHH* gene.

SUBJECTS AND METHODS

Patients

We examined an Italian BDA-1 family from Tuscany with three affected members in three different generations. A pedigree of the family is shown in fig 1.

Case III.1, 33 year old male (proband)

The proband was admitted to our clinic in October 1999, because of a four month history of increasing low back pain. He was 1.60 m tall (below the 3rd centile) and 58 kg in weight. Physical examination showed an upper/lower segment ratio of 1.4 and brachydactyly. In particular, the middle finger was 9 cm and the palm was 5.2 cm in length (fig 2A). The remainder of the clinical examination was within normal limits. Clinical,

Key points

- The brachydactylies are a group of inherited disorders in which different subtypes have been defined on the basis of the specific pattern of digital bones involved. Recently, mutations in the Indian hedgehog (*IHH*) gene on chromosome 2q2q35-q36 have been shown to cause BDA-1 in three large families from China. However, several phenotypes and locus heterogeneity have been described for BDA-1, with an additional locus mapped on chromosome 5p13.3-p13.2.
- We describe a three generation family of Italian descent affected by mild BDA-1, without any additional clinical abnormality except for short stature. Linkage analysis showed segregation compatible with a locus at 2q35-q36 and mutation screening of the *IHH* gene showed the presence of a heterozygous c.298G>A transition in exon 1, resulting in the substitution of asparagine for aspartic acid at residue 100.
- This paper confirms *IHH* as a major gene responsible for BDA-1.

laboratory, and biochemical findings excluded endocrine diseases or other heritable disorders of connective tissue, such as the chondrodysplasias. Skeletal x rays showed signs of brachydactyly of the hands (fig 2D) and feet, in accordance with the clinical diagnosis of BDA-1.

Case II.6, 65 year old mother

The proband's mother was born in southern Italy. She was 1.42 m tall (below the 3rd centile) and 50 kg in weight. She showed marked brachydactyly, with the palm 7.5 cm and the middle finger 4 cm in length (fig 2B). X rays of the hands (fig 2E) and feet confirmed the clinical evidence of BDA-1.

Case IV.2, 10 year old daughter

The proband's daughter was born in 1991. At birth, she was 48 cm long (25th centile) and 3820 g in weight (50th centile). She has been previously seen in a paediatric department on several occasions and she always showed normal psychophysical development. We first observed the child in November 1999 when she was 8 years old, 122 cm tall (25th centile), and 25 kg in weight (50th centile). The head circumference was 51 cm (50th centile). Her physical examination was normal, except for signs of brachydactyly, with the palm 5 cm and the middle finger 4.3 cm in length (fig 2C). X rays of the hands (fig 2F) and feet showed a less severe degree of disease than her grandmother and her father. The evaluation of the skeletal age¹² corresponded to the age of 7 years 7 months.

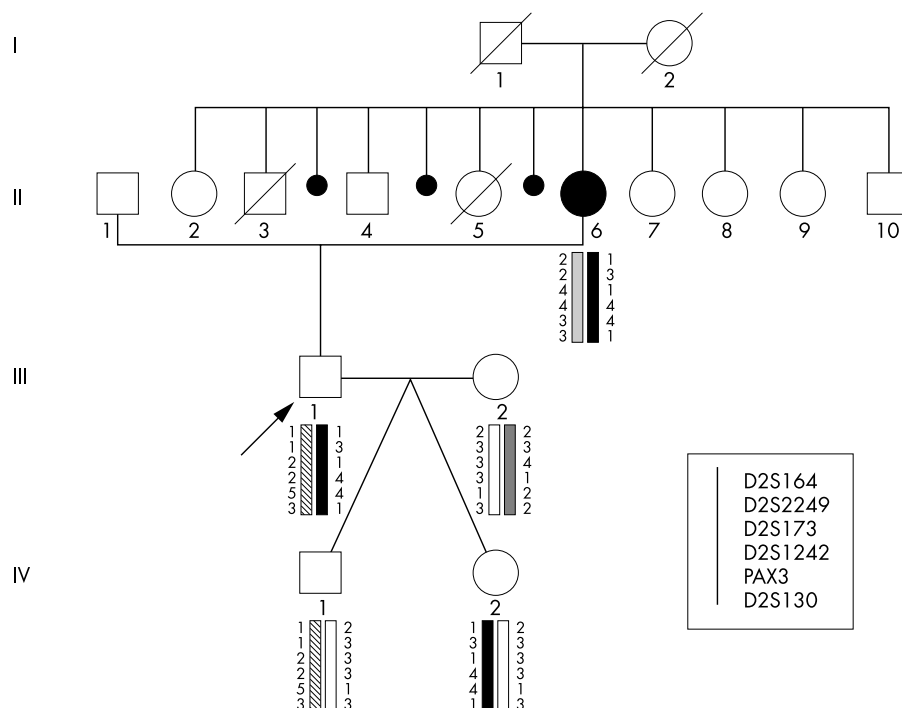


Figure 1 Pedigree and haplotype analysis of the BDA-1 family. Black symbols indicate affected subjects and an arrow indicates the proband. The haplotype shared by affected subjects is indicated by a black bar.

Linkage and mutation analysis

Peripheral blood samples were taken from available family members and standard procedures were used to isolate genomic DNA.

Linkage analysis was performed by using six markers on chromosome 2q35-q36 (D2S164, D2S2249, D2S173, D2S1242, PAX3, D2S130). The primer sequences and allele sizes of these markers were obtained from Genome Database. PCR products were run in vertical polyacrylamide gel electrophoresis (6%) and silver stained.

PCR amplification of part of the coding region of the *IHH* gene (part of exon 1, exon 2, and exon 3) was performed by using the published primers.¹⁰ Genomic DNA (100 ng) was denatured at 97°C for five minutes, mixed with 10× buffer, 0.5 μmol/l primers, 200 mmol/l dNTP, 1 U *Taq* polymerase in a final volume of 25 μl, and cycled 35 × at 94°C for one minute, 62°C for one minute, 72°C for one minute, and finally incubated at 72°C for five minutes. DNA sequencing was performed with Big dye terminator cycle sequencing kit (Applied Biosystems) on an ABI 310 Automated Sequencer and analysed with the Genescan package software. In order to sequence the entire exon 3, we designed an internal primer (5′ gcagctgtctcacacagtgg 3′).

RESULTS

Linkage analysis suggested segregation of the disease with a locus on chromosome 2q35-q36, since all affected subjects shared the same 131441 haplotype (fig 1). Sequence analysis of the three exons of the *IHH* gene showed the presence of a novel mutation in the three affected patients. The mutation was a heterozygous transition, c.298G>A, in exon 1, resulting in the substitution of asparagine for aspartic acid at residue 100 (fig 3). The presence of the mutated amino acid eliminates a *BseI* restriction site. By restriction endonuclease digestion we confirmed the mutation in the affected subjects and excluded its presence in the two unaffected analysed members of the family (III.2 and IV.1), as well as in 100 unrelated Italian subjects.

DISCUSSION

In this paper, we describe a family with clinical and radiological findings of a mild form of BDA-1. Affected subjects showed broad hands, with proportionate shortening of all the digits and the most severely shortened bones represented by the middle phalanges. Moreover, some of the middle phalanges were absent in patients III.1 and II.6. Persistent epiphyseal nucleus of the third metacarpus and absence or hypoplasia of the styloid ulnaris were also seen, as in previous reports.²⁻¹³ By contrast, the involvement of the first metacarpal in patients III.1 and IV.2 and of the third metacarpal in patient II.6 appears to be uncommon.¹⁴⁻¹⁵ The feet showed a similar pattern of abnormalities, with an absent and/or rudimentary middle phalanx and proximal phalanx of the big toe. In contrast with other cases,¹³ the bone age of patient IV.2 was not delayed.

A previous report by Yang *et al*⁹ mapped the locus for BDA-1 to a 8.1 cM interval on chromosome 2q35-q36 in two large unrelated families from China.⁹ Consistent with this observation, the same authors recently reported that BDA-1 is the result of mutations in the *IHH* gene on chromosome 2q35-q36.¹⁰ *IHH* is a secreted signalling protein member of the mammalian hedgehog family, which is implicated in cell-cell interaction.¹⁶⁻¹⁷ Its mouse orthologue, *Ihh*, is expressed in gut and cartilage and coordinates proliferation and differentiation of chondrocytes during endochondral bone development.¹⁷⁻¹⁹ To date, there have been no other reports confirming the association of BDA-1 with mutations in the *IHH* gene. By contrast, a recent study in a Canadian kindred linked BDA-1 to a novel locus on chromosome 5p13.2-13.3,¹¹ indicating that the disease may be genetically heterogeneous. Importantly, some phenotypic differences existed between the Canadian and the Chinese BDA-1 families. While the former showed a mild form of the disease, the latter had some features that were apparently beyond Fitch's description,² such as severely shortened distal phalanges, shortened third metacarpals, and shortened proximal phalanges of the fifth digit.⁹ These differences were not surprising given that different loci have been implicated. Thus, different disease causing genes may be

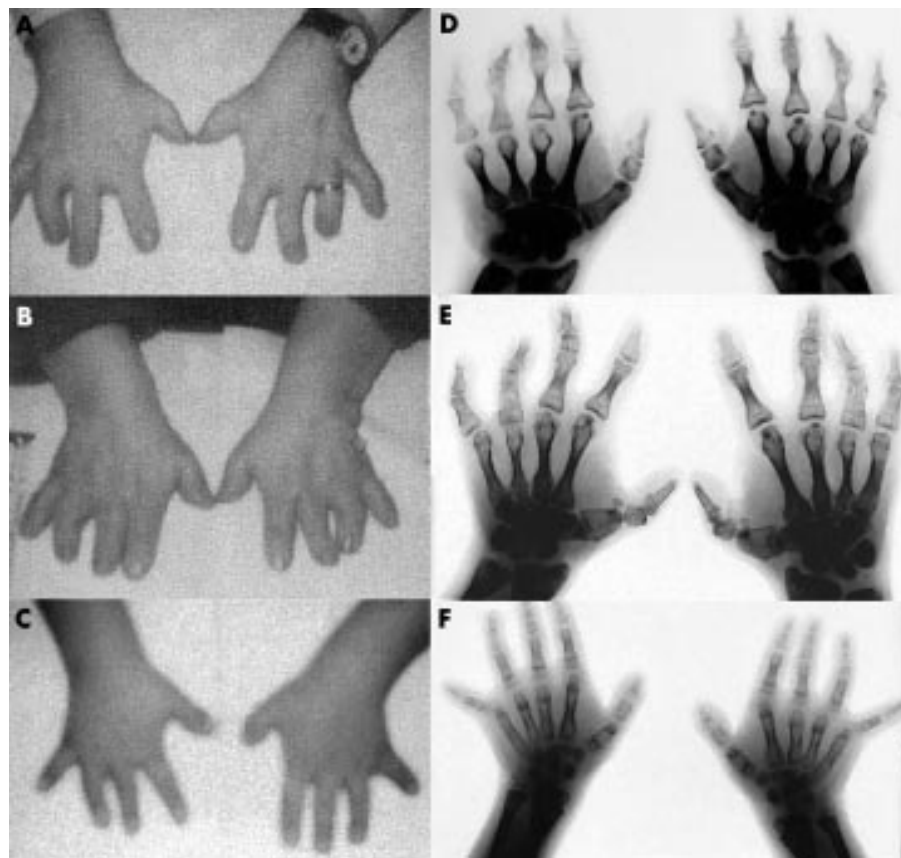


Figure 2 Phenotypic features of the BDA-1 family. On the left is shown the dorsal view of the hands of patient III.1 (proband, A), patient II.9 (mother, B), and patient IV.2 (daughter, C). On the right are shown the x ray films of patients III.1 (D), II.9 (E), and IV.2 (F), respectively.

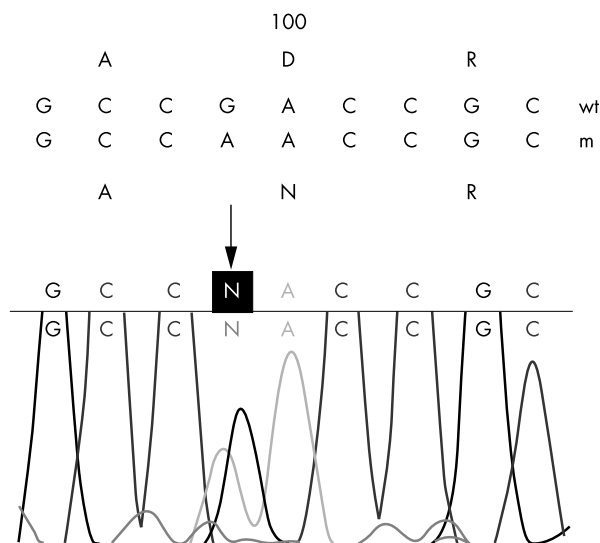


Figure 3 Chromatogram of the mutated sequence. Both the nucleotide and the amino acid change are shown above the chromatogram. An arrow indicates the site of mutation. wt = wild type sequence, m = mutated sequence.

responsible for the variable phenotypes often described for BDA-1. A similar feature has been also proposed for other subtypes of brachydactyly, such as brachydactyly type B.²⁰ In this paper, we confirm the association of BDA-1 with mutations in the *IHH* gene in a three generation family showing variable clinical features. Our kindred had clinical and radiological characteristics different from both the Canadian

and the Chinese families, indicating a relatively moderate form of BDA-1. In particular the middle phalanges and the proximal first phalanges were mainly involved, while the distal phalanges were of normal size. The *IHH* mutation we found in our family was different from those previously described.¹⁰ However, it falls in the same residue (aspartic acid 100) that is substituted in the D100E missense mutation found in one of the three pedigrees analysed by Gao *et al.*¹⁰ This amino acid is highly conserved across all vertebrates and intervertebrates studied so far and it is predicted to lie on the surface of the protein and to interact with the Patched receptor.¹⁰ Therefore, D100N mutation may cause aberrant signalling by interfering with *IHH* binding to its receptor or promoting its binding to other receptors.

Short stature is often a component of the brachydactylies even if less frequent in type A1 and more frequent in type C.^{2 15} Interestingly, all the affected subjects showing an Asp100 mutation in the study of Gao *et al.*¹⁰ were shorter than unaffected subjects in the same family, while BDA-1 subjects with different *IHH* mutations were of normal height. In the reported BDA-1 family showing a similar mutation on Asp100, patients III.1 and II.6 were short in stature, while patient IV.2 had normal growth. Thus, it is unlikely that short stature is phenotypically linked to a particular *IHH* mutation, such as that involving Asp100.

Finally, a decrease in the severity of phenotypes from the first to the third generation was observed in our BDA-1 family. However, since BDA-1 is known to exhibit considerable variation in severity both between and within affected families, the phenotypic differences observed in this Italian family may also be the result of variable expressivity occurring by chance in different generations. A detailed phenotypic description of the large families described by Gao *et al.*¹⁰ would have been useful to define this issue.

In summary, results from the present study confirm that BDA-1 is a phenotypically heterogeneous disease resulting from mutation in the *IHH* gene and that the same *IHH* mutation may exhibit considerable variation in severity both between and within affected families.

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Electronic database information: Accession numbers and URLs for data in this article are as follows: Genome Database, The, <http://gdbwww.gdb.org/> (for primer sequences). Online Mendelian Inheritance in Men (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for BDA-1 (MIM 112500)).

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