LETTER TO JMG

Identification of a mutation in the Indian Hedgehog (*IHH*) gene causing brachydactyly type A1 and evidence for a third locus

T J Kirkpatrick, K-S Au, J M Mastrobattista, M E McCready, D E Bulman, H Northrup

J Med Genet 2003;**40**:42–44

Brachydactyly (BD) is a term used to describe inherited anomalies of the hands generally characterised by shortened phalanges or metacarpals. Initially, the brachydactylies were grouped into five different classes (A-E), with three subtypes of A.[1] Later work revised and extended the classification of BD.[2 3] In type A, shortening is primarily confined to the middle phalanges. Subtype A1 (BDA1, OMIM 112500) is distinguished by hypoplastic middle phalanges (especially the 2nd and 5th digits), with either distal or terminal symphalangism depending on the severity. In addition, shortening of the proximal phalanges of the thumb, the metacarpals, metatarsals, or the big toe are also observed in these patients. Short stature is often associated with BDA1 patients.

Despite being the first syndrome described with Mendelian autosomal dominant inheritance in 1903,[4], the genetic aetiology of BDA was not reported until 2001.[5] Suspecting that cell proliferation or differentiation factors could be the culprit causing BDA1, our group screened markers near several candidate genes in two families diagnosed with BDA1. No linkage was observed.[6] We also chose to look for mutations of the *PAX3* genes in these families by direct sequencing of all the exons, but no significant mutation was identified (unpublished data).

In 2000, a locus for BDA1 was mapped to 2q35-q36 in two unrelated Chinese families.[7] Refined mapping and mutation screening of candidate genes in the region by the same group identified missense mutations in the Indian Hedgehog gene (*IHH)* of the affected subjects in three unrelated families.[5] The missense mutations, located at the amino terminus of the IHH protein, are conserved among the Hedgehog family proteins. Local and long range cell proliferation signalling functions were suggested to reside within the amino-terminus domain. Interestingly, *ROR2* and *CDMP1* have been identified to cause brachydactyly types B and C, respectively.[8 9] The genes are important in chondrocyte proliferation and differentiation and joint morphogenesis.

Key points

- Brachydactyly type A1 (BDA1) is particularly interesting because it is the first syndrome characterised by Mendelian autosomal dominant inheritance.
- Three different missense mutations in the Indian Hedgehog gene (*IHH*) were recently identified in three BDA1 families. Another group showed linkage to an 11 cM region on chromosome 5p13.
- In one of our BDA1 families, we identified a missense mutation in *IHH* in all patients at a similar position to that previously reported. In another family, we excluded linkage to the 5p13 region and identified a polymorphism in *IHH* that segregated independent status.
- These data support the existence of a third locus for BDA1.

MATERIALS AND METHODS

The finding of *IHH* mutations in BDA1 patients led us to examine this gene in the two BDA1 families we previously studied.[6] We searched GenBank and extracted the genomic DNA sequence (NT_005289) using the cDNA sequence of *IHH*. *IHH* consists of three exons and spans approximately 5.5 kb of genomic DNA. The exon/intron boundaries of the three exons were identified and primers specifically targeting the splice sites and coding regions were designed using Oligo 4.1 Primer Analysis Software (©Rychlik 1992) for polymerase chain reaction (PCR) amplification and sequencing. PCR primers used are as follows 5′ to 3′: 1F (CGACGCGCCTGCCCATCA), 2F (TCCTCCGGCTGATTTCGCTC), 2R (TTCTCGGCACTACTCCTCCT), 3F (CAAGGGAGGGTCGTTGTGAC), 3R (GGACCCAGTACAGCAGTTCC). We also used primer 1R as described by Gao *et al*.[5] The three exons of *IHH* were then amplified by standard PCR and examined by electrophoresis on 3% MetaPhor® agarose gels. Shrimp alkaline phosphatase (1 U) and exonuclease I (10 U) were used to remove excess dNTPs and primers from our samples. We then sequenced the exons using BigDye Terminator (v.1) reagent following the manufacturer's protocol (Applied Biosystems, Foster City, CA). After purification through Sephadex® G-50 Fine columns, the sequenced products were run on an ABI Prism 3100 automated sequencer and analysed with the ABI Sequencing Analysis 3.7 software. Any changes noted in the patients' sequences were confirmed by sequencing in the reverse direction. The *IHH* gene was sequenced for all affected and unaffected people in the study.

RESULTS AND DISCUSSION

Our sequencing results identified a missense mutation in *IHH* in all affected members of family II, a family of Mexican descent (fig 1A). The missense mutation involved a 284A→G transition in exon 1 of these patients resulting in a Glu95→Gly mutation (fig 1B). Gao *et al*[5] reported a mutation of a different nucleotide (283G→A) in the same codon, resulting in a Glu95→Lys mutation. The recurrent mutation found within the same codon among unrelated patients of different ethnic backgrounds further supports the functional importance of Glu95 in the IHH peptide. The codon, Glu95, is conserved among hedgehog proteins and is suspected to play an important role in binding to the receptor for downstream cell cycle signalling. Gao *et al*[5] hypothesised that the novel mutations in *IHH* would cause BDA1 because of their position in the amino-terminal signalling domain. Amino acid changes in this domain of the closely related protein Sonic Hedgehog (SHH) have been shown to elicit a deleterious effect in SHH

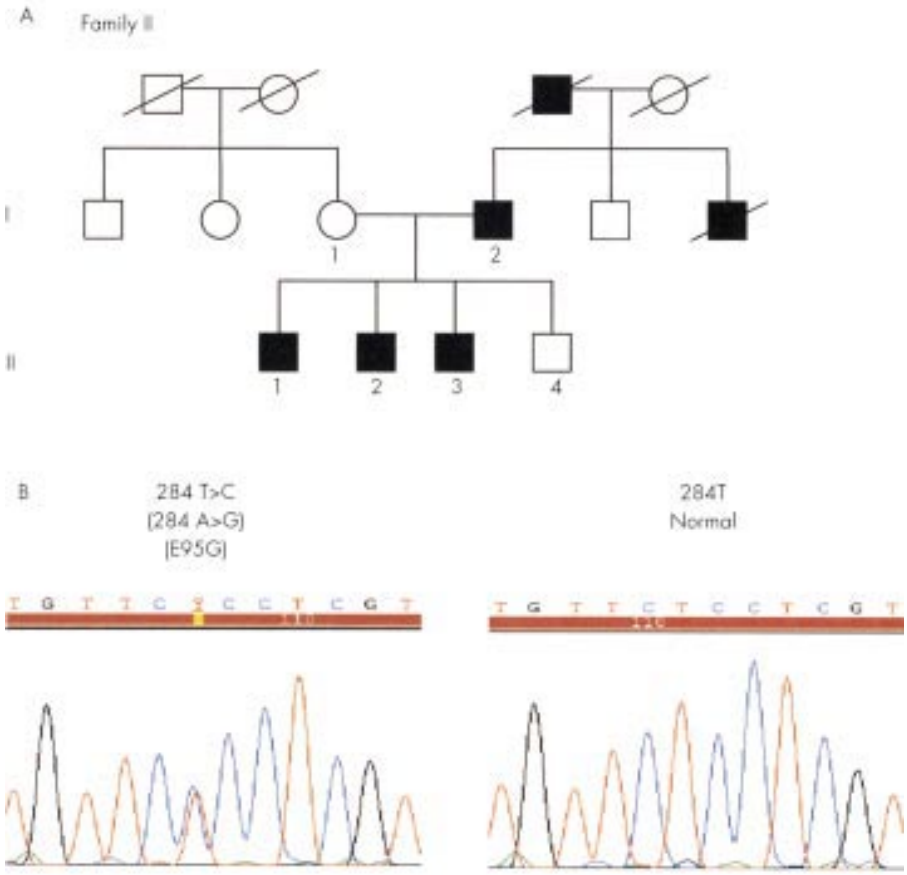

Figure 1 (A) Pedigree structure of family II. (B) Mutation analysis by direct sequencing on affected subject I.2 and unaffected subject II.4. Sequences shown are the result of reverse sequencing primers for exon 1 of IHH. Arrows indicate locations of changes where "Y" represents heterozygous T/C. Sequences of affected members II.1, II.2, and II.3 were the same as that of I.2. Similarly, the sequence of unaffected family member I.1 was the same as that of II.4.

binding with its receptor Patched (PTC), also a receptor for IHH.[10–12] Therefore, the three *IHH* mutations are also likely to disrupt binding of the IHH protein to PTC. If the missense mutations cause complete loss of binding, then BDA1 would be caused by haploinsufficiency of the wild type protein. The mutations may also result in partial loss or altered binding, the latter predicted to cause a gain of function effect. Our finding, together with that of Gao *et al*,[5] suggests the carboxyl side chain of Glu95 is vital to IHH protein function. Functional assays will elucidate the effect of these mutations.

Before discovery of *IHH* mutations, Fukushima *et al*[13] reported a case with an apparently balanced 5q11.2 and 17q23 translocation in a girl with BDA1 and Klippel-Feil syndrome. Armour *et al*[14] conducted a genome-wide linkage study on a four generation BDA1 family of Canadian descent, with special attention to chromosomes 5 and 17. Their study identified linkage of BDA1 to an 11 cM region on chromosome 5p13.3-13.2. The region contains two notable candidate genes, cadherin-6 (*CDH6*) and natriuretic peptide receptor C (*NPR3*), whose proteins are suggested to have roles in osteoclast differentiation and skeletal development.

Before the mutations in *IHH* were published, we examined markers (D5S819, D5S1986, D5S477, D5S1506, and D5S663) for our two BDA1 families to determine if there was linkage to the 5p13.3-p13.2 region. In family I, the same haplotype from I.1 is transmitted to affected and unaffected subjects in the second (II.1, II.2, II.4, and II.5) and third generations (III.2 and III.3) (fig 2A). However, affected subject III.1 did not inherit any of the chromosome 5 markers from the affected grandparent, I.1. Taken together, our family I does not show linkage to the 5p13.3-p13.2 candidate BDA1 region.

Direct sequencing analysis detected no significant changes in the exons and splice sites of *IHH* in affected members of family I. We did detect a 753T→C transition resulting in a silent variant (Pro251→Pro) and producing a novel restriction enzyme *Bsp*1286I site. Testing of all family members showed that the *Bsp*1286I$^-$ (T) allele segregated independently of BDA1 in the family (members II.5, II.6, III.2, and III.3), therefore excluding *IHH* as the disease causing gene (fig 2B). Our previous study using various genetic markers on several other chromosomes, and the chromosome 5 markers reported here, excluded non-paternity for II.6.[6] Since the variant is within exon 3 and *IHH* is small (5.5 kb), it is unlikely that other changes in the introns of *IHH* are causing BDA1 in family I.

Exclusion of linkage to both *IHH* and chromosome 5p13.3-13.2 in our BDA1 family I points to the existence of yet a third locus for BDA1. SNP linkage analysis of the receptors of *IHH*, its target genes, or the downstream targets for *CDH6* or *NPR3* are future studies that could uncover the disease gene in our family I.

ACKNOWLEDGEMENTS

We wish to acknowledge the support of the Shriners Hospital for Children in Houston. The work was supported in part by grants (Nos 8580 and 8520) from the Shriners Hospitals for Children National Organization to HN. The authors thank Phong X Tran and Mark R Kruzel for technical assistance and the participating family members for their participation. MEM is funded by a studentship from the Canadian Institute of Health Research (CIHR) and part of this work was funded by a research grant (DEB) from CIHR.

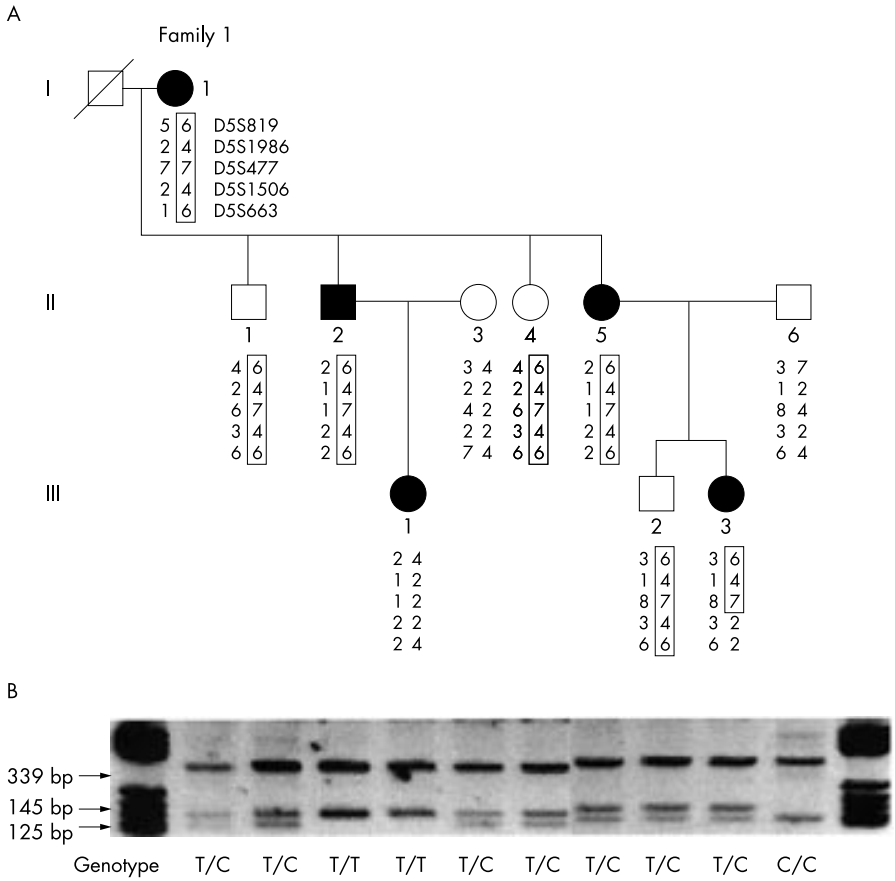

Figure 2 (A) Haplotype analysis of microsatellite markers on chromosome 5p13.3-13.2 in the three generations (I, II, and III) of family I with brachydactyly type A1 (BDA1). Affected subjects are denoted by filled symbols. Markers are listed in order from pter to centromere (D5S819, D5S1986, D5S477, D5S1506, D5S663) next to the genotypes of I.1. Genotypes of the transmitted chromosome from I.1 is boxed and shown to transmit to both affected and unaffected descendants in generations II and III. (B) Results of *Bsp*1286I polymorphism analysis (M = DNA molecular weight marker V, Boehringer Mannheim). Exon 3, 753 T→C silent variant creates a novel *Bsp*1286I site. The upper singlet appears in all samples and corresponds to 339 bp. The doublet (bottom two bands) is of interest and corresponds to 145 bp (753T) and 125 bp (753C), respectively. Horizontal arrows on the left point to the digested DNA fragments in the gel with corresponding sizes.

Authors' affiliations

T J Kirkpatrick, K-S Au, H Northrup, Department of Pediatrics, The University of Texas Medical School at Houston, Texas, USA
J M Mastrobattista, Department of Obstetrics, Gynecology and Reproductive Sciences, The University of Texas Medical School at Houston, Texas, USA
M E McCready, D E Bulman, Ottawa Health Research Institute, and the University of Ottawa Centre for Neuromuscular Disease, Ottawa, Ontario, Canada
M E McCready, D E Bulman, Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Ontario, Canada
D E Bulman, Department of Medicine, Division of Neurology, University of Ottawa, Ottawa, Ontario, Canada
H Northrup, Shriners Hospital for Children, Houston, Texas, USA

Correspondence to: Dr H Northrup, Department of Pediatrics, The University of Texas Medical School at Houston, 6431 Fannin, MSB 3.144, Houston, Texas 77030, USA; Hope.Northrup@uth.tmc.edu

REFERENCES

1 **Bell J**. In: Penrose LS, ed. *Treasury of human inheritance*. Vol 5. London: Cambridge University Press, 1951:1-31.
2 **Temtamy SA**, McKusick VA. Brachydactyly as an isolated malformation. In: Bergsma D, Mudge JR, Paul KW, eds. *The genetics of hand malformations*. New York: Alan R Liss, 1978:187-97.
3 **Fitch N**. Classification and identification of inherited brachydactylies. *J Med Genet* 1979;**16**:36-44.
4 **Farabee WC**. *Hereditary and sexual influence in meristic variation. A study of digital malformations in man*. Thesis, Harvard University, 1903.
5 **Gao B**, Guo J, She C, Shu A, Yang M, Tan Z, Yang X, Guo S, Feng G, He L. Mutations in *IHH*, encoding Indian Hedgehog, cause brachydactyly type A-1. *Nat Genet* 2001;**28**:386-8.
6 **Mastrobattista JM**, Dolle P, Blanton SH, Northrup H. Evaluation of candidate genes for familial brachydactyly. *J Med Genet* 1995;**32**:851-4.
7 **Yang X**, She C, Guo J, Yu AC, Lu Y, Shi X, Feng G, He L. A locus for brachydactyly type A-1 maps to chromosome 2q35-q36. *Am J Hum Genet* 2000;**66**:892-903.
8 **Oldridge M**, Fortuna AM, Maringa M, Propping P, Mansour S, Pollitt C, DeChiara TM, Kimble RB, Valenzuela DM, Yancopoulos GD, Wilkie AO. Dominant mutations in *ROR2*, encoding an orphan receptor tyrosine kinase, cause brachydactyly type B. *Nat Genet* 2000;**24**:275-8.
9 **Polinkovsky A**, Robin NH, Thomas JT, Irons M, Lynn A, Goodman FR, Reardon W, Kant SG, Brunner HG, van der Burgt I, Chitayat D, McGaughran J, Donnai D, Luyten FP, Warman ML. Mutations in CDMP1 cause autosomal dominant brachydactyly type C. *Nat Genet* 1997;**17**:18-19.
10 **Stone DM**, Hynes M, Armanini M, Swanson TA, Gu Q, Johnson RL, Scott MP, Pennica D, Goddard A, Phillips H, Noll M, Hooper JE, de Sauvage F, Rosenthal A. The tumour-suppressor gene patched encodes a candidate receptor for Sonic hedgehog. *Nature* 1996;**384**:129-34.
11 **Marigo V**, Davey RA, Zuo Y, Cunningham JM, Tabin CJ. Biochemical evidence that patched is the Hedgehog receptor. *Nature* 1996;**384**:176-9.
12 **Carpenter D**, Stone DM, Brush J, Ryan A, Armanini M, Frantz G, Rosenthal A, de Sauvage FJ. Characterization of two patched receptors for the vertebrate hedgehog protein family. *Proc Natl Acad Sci USA* 1998;**95**:13630-4.
13 **Fukushima Y**, Ohashi H, Wakui K, Nishimoto H, Sato M, Aihara T. De novo apparently balanced reciprocal translocation between 5q11.2 and 17q23 associated with Klippel-Feil anomaly and type A1 brachydactyly. *Am J Med Genet* 1995;**57**:447-9.
14 **Armour CM**, McCready ME, Baig A, Hunter AGW, Bulman DE. A novel locus for brachydactyly type A1 on chromosome 5p13.3-p13.2. *J Med Genet* 2002;**39**:186-9.