

Whole-exome sequencing identifies a novel *IHH* insertion in an Ontario family with brachydactyly type A1

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Abstract

Isolated brachydactyly is an umbrella term describing disproportionately shortened fingers and toes, often following an autosomal dominant mode of inheritance. Various forms of brachydactyly have been characterized and several causative genes have been found, but many types remain genetically undefined. We describe an Ontario family with mild brachydactyly in which whole-exome sequencing identified a novel variant for brachydactyly type A1 (exon 1, c.285_287dupGAA, p.Glu95_Asn96insLys) in the Indian hedgehog (*IHH*) gene. This rare variant co-segregated with affected status in the pedigree and was associated with (1) shortened middle phalange length by 21.1% ($p < 0.001$); (2) shortened palm length by 13.8% ($p < 0.01$); (3) reduced digit-palm ratio by 6.8% ($p < 0.03$); and (4) reduced stature by 9.5% ($p < 0.001$). We report the first *IHH* in-frame insertion causing brachydactyly type A1.

Keywords

Brachydactyly type A1, Indian hedgehog, signalling molecule, autosomal dominant disorder, skeletal disorder, whole-exome sequencing

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Introduction

In the early 1900s, Farabee¹ and Drinkwater^{2,3} published the first human trait with an autosomal dominant inheritance pattern, namely brachydactyly. The brachydactylies are a group of malformations characterized by shortened phalanges and/or metacarpals. Over the past century, several forms of isolated brachydactyly have been identified.⁴ Brachydactyly type A features the shortening or absence of the middle phalanges, with shortened proximal phalanges of the thumb and big toe. Type A is further subdivided into four subtypes, of which type A1 is the most severe with involvement of all fingers, while the other subtypes A2, A3, and A4 display milder malformations involving index fingers, fifth fingers, and both index and fifth fingers, respectively. In contrast, brachydactyly types B–E feature shortened or absent terminal phalanges, the involvement of the middle phalanges and metacarpals, shortened thumbs and big toes, and shortened metacarpals and metatarsals, respectively.⁴

Brachydactyly type A1 (BDA1) is an autosomal dominant disorder in which affected individuals present with short broad hands and proportionately shortened digits. Drinkwater subdivided BDA1 into severe and minor brachydactyly.^{2,3} In severely affected individuals, the fingers are shortened to half

the normal length, with complete absence of middle phalanges and occasional fusion of the middle to the terminal phalanges. This shortening can extend across the entire skeleton, resulting in short stature due to shortened bones. In addition, BDA1 can occur as part of a complex syndrome, accompanied by nystagmus, musculoskeletal abnormalities, developmental delay, or scoliosis.⁵ Here, we describe a family presenting with isolated brachydactyly (Figure 1). Whole-exome sequencing performed on the proband's DNA identified a rare heterozygous, novel in-frame insertion variant in *IHH*.

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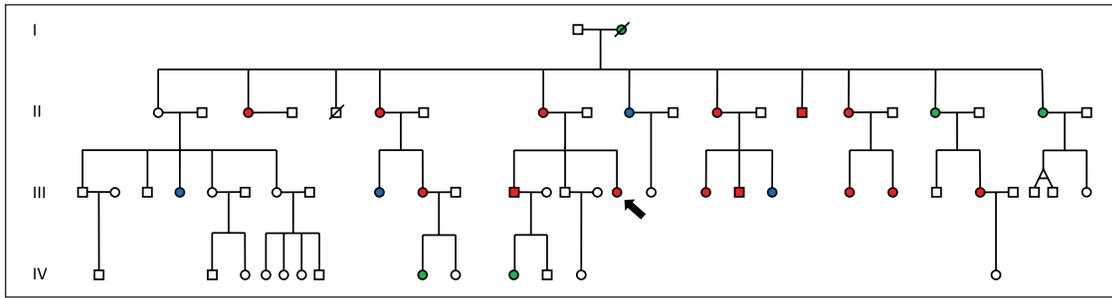


Figure 1. Pedigree of a Canadian autosomal dominant brachydactyly type A1-affected family. The proband, as indicated by the black arrow, was the first patient ascertained. All red and blue shaded individuals represent those whose DNA samples were Sanger sequenced for the *IHH* variant (exon 1, c.285_287dupGAA, p.Glu95_Asn96insLys). The reddened squares (males) and circles (females) represent affected individuals with brachydactyly type A1 who carry the *IHH* variant. The blue coloured squares and circles indicate those who are unaffected by brachydactyly and do not carry the *IHH* variant. The green coloured squares and circles represent the individuals clinically diagnosed with brachydactyly whose DNA was not obtained or sequenced. Diagonal lines indicate deceased individuals.

Case report

In 2003, a 31-year-old female from Ontario was referred for medical consultation for a lifelong history of short stature and shortening of all digits of the upper and lower extremities. There was no history of other medical conditions, specifically no nystagmus, musculoskeletal abnormalities, developmental delay, or scoliosis. Upon examination, all digits of upper and lower extremities were noted to be short, with missing middle phalanges and splayed fingers. The proband's parents were both of short stature, but only her mother shared identical characteristics including very short fingers and toes. Seven maternal aunts and one uncle reported abnormally shortened fingers and stature of variable severity. The proband's brother and his daughter had similarly shortened digits. Seventeen additional family members were subsequently assessed, of whom 13 were clinically affected (Figure 1). The radiological presentation was similar to that reported by Yang et al.⁶ Sanger sequencing was performed for all known brachydactyly genes; however, the case was left unsolved from standard sequencing. Informed consent was obtained in compliance with the Ethics Review Board at Western University (Certificate Number 07920E). DNA was extracted from whole blood using the Puregene[®] Blood Extraction Kit (Gentra Systems, Qiagen Inc., Mississauga, ON). The proband's DNA was indexed and pooled using the Illumina TruSeq Rapid Capture Exome Library Prep Kit (Illumina, San Diego, CA) and subjected to whole-exome sequencing on the Illumina NextSeq 500 (Illumina, San Diego, CA) at the London Regional Genomics Centre (<http://www.lrgc.ca>). Sequence data in the form of two FASTQ files were aligned (CLC Bio Genomics Workbench v10, CLC Bio, Aarhus, Denmark) with the human reference genome (build hg19) to generate coverage statistics and a variant call format file, which was input into the Golden Helix VarSeq[®] software (Golden Helix, Inc., Bozeman, MT), where variants were fully annotated.

A heterozygous *IHH* c.285_287dupGAA (p.Glu95_Asp96insLys) variant was detected (Figure 2) and validated using Sanger sequencing. This variant was absent from gnomAD, 1000 Genomes, Exome Variant Server, and ExAC databases. Seventeen family members were genotyped for the mutation using direct Sanger sequencing (primers and conditions available upon request). In family-based association analyses using the Wilcoxon–Mann–Whitney test, the *IHH* variant was associated with shortened length of middle phalanges by 21.1% ($p < 0.001$) and palms by 13.8% ($p < 0.01$), of middle digit to palm ratio by 6.8% ($p < 0.03$) and of stature by 9.5% ($p < 0.001$), consistent with the relative mild clinical phenotype.

Discussion

We report an Ontario family with autosomal dominant BDA1, characterized by variably short stature and shortened digits. Ascertained 15 years ago, this case was left unsolved following Sanger sequencing of all known brachydactyly genes. With the recent use of whole-exome sequencing, it was found that affected family members carry a heterozygous in-frame insertion in *IHH*, designated c.285_287dupGAA, p.Glu95_Asn96insLys, explaining their brachydactyly phenotype (Figures 1 and 2). This *IHH* variant is predicted to exert a damaging effect on protein function from multiple *in silico* prediction tools, co-segregates with disease status in the family, is considered novel in multiple control population databases, and has not been previously reported in the literature.

BDA1 results from causative mutations within the Indian hedgehog gene (*IHH*)^{6–8} on chromosome 2q35–36. *IHH* encodes the IHH protein, a member of the hedgehog family of signalling proteins. Along with sonic and desert hedgehog, IHH regulates patterning processes in both vertebrate and invertebrate development.⁹ The hedgehog family is involved in limb polarity and chondrogenesis, with IHH playing a critical role in human skeletal development. IHH mutations impair chondrocyte maturation and proliferation, with failure

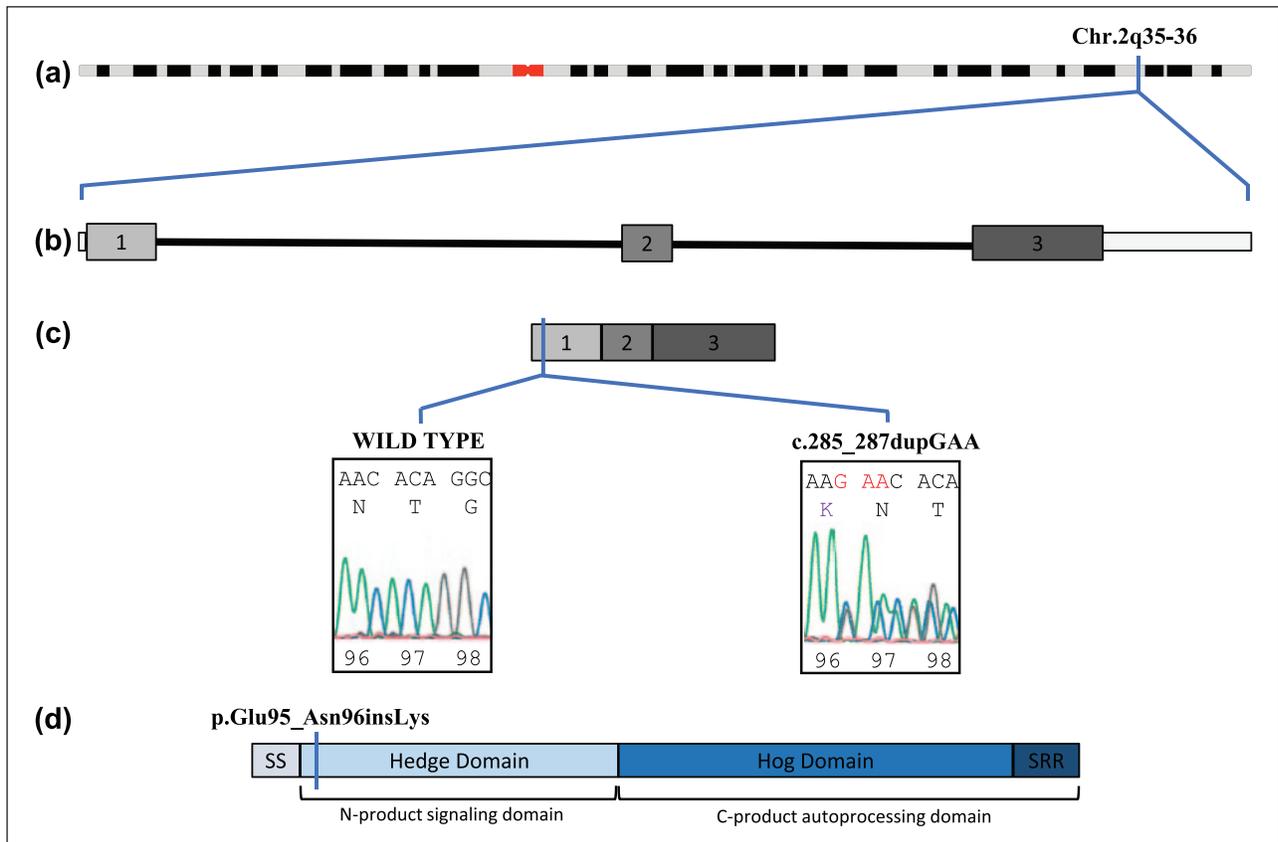


Figure 2. Chromosome location, gene structure, sequence analysis and protein structure of Indian Hedgehog. (a) Chromosome 2 and the location of the *IHH* gene drawn to scale. (b) The genomic DNA map for the *IHH* gene. (c) The mRNA for *IHH* with three numbered exons and the position of the mutation indicated. Electrophoretic tracings for the *IHH* variant for both unaffected and affected family members are also shown with the three-nucleotide codons, single-letter amino acid codes and corresponding numbered amino acid residues. (d) The IHH protein structure with key domains, regions, and the mutation indicated. SS: signal sequence; SRR: sterol recognition region.

of osteoblast development in endochondral bones.¹⁰ In *Ihh*^{-/-} mice, the loss of IHH signalling leads to reduction defects in the forelimbs and digits.¹¹ Dominant mutations in *IHH* are causative of BDA1, while recessive mutations have been linked to acrocapitofemoral dysplasia, which features short stature, short limbs, and cone-shaped epiphyses.¹²

Several other mutations in the *IHH* gene have been previously reported. All mutations responsible for BDA1 have been limited to the N-terminal active fragment of *IHH*.⁵ These mutations have predominantly affected codon positions 95, 100, 131, and 154, with ours as no exception.^{7,13,14} This solidifies the importance of the N-terminal region in bone development and differentiation, especially since mutations causing acrocapitofemoral dysplasia differ and are exclusively located at the distal N- and C- terminal regions.¹²

The IHH protein operates through a feedback control mechanism. IHH binds to the receptor Patched (PTC), which, in turn, functions with Smoothened (SMO) to activate the GLI complex of transcription factors.¹⁵ From there, these transcription factors continue to signal and regulate downstream genes affecting patterning. Because IHH acts later in

endochondral bone formation, mutations in *IHH* lead to the delay in bone growth and subsequent digit abnormality. Studies in *Ihh*^{-/-} mice uncovered the association between *Ihh* mutations and BDA1, as loss of IHH signalling leads to shortened forelimbs and abnormal digits.⁹ We propose our novel variant is acting in the same manner. More specifically, although our variant is the first insertion identified in BDA1, we suggest that our variant acts similar to the p.Glu95Lys missense mutation identified by Ma et al.,¹⁵ where the mutation results in the conversion of a negatively charged area to a positively charged area in a critical calcium-binding groove.

Conclusion

We used whole-exome sequencing and a bioinformatics pipeline to detect a new *IHH* variant in a family with classic BDA1, previously unsolved using Sanger sequencing. These data confirm the power of next-generation sequencing to rapidly solve or clarify the genetic basis of patients with known genetic syndromes. Expanding the genetic spectrum of BDA1 will allow for further understanding of disease mechanisms

and will aid other health-care providers caring for families with the same condition caused by the same gene.

Declaration of conflicting interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: R.A.H. has received honoraria from serving on advisory boards and speakers' bureaus for Aegerion Pharmaceuticals, Akcea and Ionis, Amgen, Gemphire Therapeutics, Regeneron Pharmaceuticals, Sanofi, and Valeant, all unrelated to the topic of this article. The other authors declare no other conflicts of interest.

Ethical approval

The study was approved by the Ethics Review Board at Western University (Certificate Number 07920E).

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Informed consent

Signed informed consent was obtained from all participants for anonymized information to be published in this article. Written informed consent was obtained from the patient(s) for their anonymized information to be published in this article.

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