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ORIGINAL ARTICLE

IHH gene variants in North Indian individuals with brachydactyly A1

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ABSTRACT

Background: Brachydactyly A1 (BDA1) is an autosomal dominant disorder. It manifests as shortness/absence of the middle phalanges in the hands and feet. It is caused by a variation in the Indian hedgehog (*IHH*) gene. The *IHH* gene plays an important role in the development of limbs. IHH is expressed in the pre-hypertrophic chondrocytes of cartilage elements, where it regulates the rate of hypertrophic differentiation. A lack of IHH prevents proliferating chondrocytes from initiating the hypertrophic differentiation process. This study aimed to identify *IHH* gene variants in BDA1 individuals.

Methods: The present study used Sanger sequencing to analyze *IHH* variants in three exons and *in silico* tools for detailed variant analysis.

Results: A total of 44 individuals were sequenced - 14 BDA1 individuals and 30 healthy controls. Sanger sequencing revealed a novel variant in the *IHH* gene in exon 1 and exon 2. A variant was found to segregate affected individuals in a family.

Conclusion: The present study findings further expand the mutation spectrum of the *IHH* gene, and provide detailed mutant protein changes by *in silico* methods. The study also provides additional evidence that *IHH* plays an important role in limb development and short stature.

Keywords: Brachydactyly, missense, Indian hedgehog, phalanges, metacarpals, metatarsals, Sanger sequencing, short hands.

Introduction

Brachydactyly (BD) is characterized by shortening or missing phalanges and toes and abnormal development of the phalanges, metacarpals, and metatarsals [1-4], BD was the first disorder described in terms of autosomal dominant Mendelian inheritance [5,6]. In 2001, BD was added to the international nosology and classification of genetic skeletal disorders [7]. The International Nosology Classification of Skeletal Disorders has divided different skeletal disorders into 42 groups, one of which is BD with extra-skeletal manifestations, and another is BD without extra-skeletal manifestations [8]. It was initially classified by Julia Bell in 1951 into five types, namely, BD types A, B, C, D, and E. Type A is further divided into types A1, A2, and A3 based on the shortening of phalanges. It can be present in isolated forms and is involved in syndromes such as Temtamy preaxial syndrome, Feingold syndrome, Robinow syndrome, and achondroplasia. Types A3 and D were approximately 2% prevalent among all types of BD. The most common types of BD found are A and E. The mechanism of BD has not been extensively explored. It can be associated with other hand and foot anomalies, such as polydactyly, syndactyly, and clinodactyly. The hands and feet of the affected individual appear short, occurring as an isolated form or as a part of the syndrome [9-11].

Brachydactyly A1 (*BDA1*) (OMIM: 112,500) is characterized by hypoplasia/aplasia of the middle phalanges of digits 2-5. *BDA1* can be found in an isolated form and can also present with additional malformations, such as short stature, scoliosis, craniofacial dysmorphism, developmental delay, or intellectual disability. It exists in an autosomal dominant manner. The genetic basis

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of *BDA1* on chromosome number 2q35 in the Indian Hedgehog (*IHH*) gene was first described by Gao et al. [11]. It is caused by heterozygous pathogenic variants in a specific region of the N-terminal active fragment of the *IHH* gene [12-15] *IHH* plays important roles in meditating skeletal condensation, growth, patterning, morphogenesis, pre-hypertrophic chondrocyte formation, chondrocyte differentiation, joint development, and bone formation. IHH was the first gene associated with BDA1 [1,3,11,16,17,18]. To date, only a few pathogenic variants causing BDA have been identified. The *IHH* gene encodes a member of the hedgehog family located on chromosome number 2. IHH is a peptide hormone that cleaves proteins to form N-terminal functional signals and C-termini, which further regulate the protein [11,18,19]. Secreted IHH proteins play a role in regulating embryonic development, including growth, patterning, and morphogenesis [18,20,21]. The protein encoded by this gene specifically plays a role in skeletal development and differentiation. It acts with factors including proteins involved in bone morphogenesis and other growth factors to induce differentiation of osteoblasts [22,23,24]. In this study, we investigated the clinical and genetic characteristics of individuals with BDA1. We present the clinical and radiographic findings and novel *IHH* variations in related and unrelated individuals with BDA1 and short stature.

Subjects and Methods

This is a case cohort study in which 44 individuals, including 14 patients, and 30 controls were enrolled from an outpatient tertiary care center. Patients belonged to Himachal Pradesh, Punjab, Haryana Chandigarh, Uttar Pradesh, and Bihar. Individuals included in this study were phenotyped by clinical geneticists and senior residents undergoing advanced training. Peripheral blood samples (2-3 ml) were obtained from all individuals of related and unrelated families. Sanger sequencing was performed to analyze *IHH* gene variants of the patients. The patients or family members/caretakers provided written informed consent for the publication of the clinical details. This study was ethically approved by the Ethics Committee of the Institute (Ref No: NK17963/PhD/638).

Genomic DNA was extracted from the peripheral blood of the patients. The three exons of the *IHH* gene (forward and reverse) in the NM_002181.4 transcript were amplified with primers from the NCBI via primer blast. All three exons and intron-exon boundaries of *IHH* were amplified via PCR and subjected to Sanger sequencing (Table 1). Blood samples were collected in an EDTA vial, and then DNA was extracted using the Qiagen DNA Extraction Kit. The PCR program was set to a denaturation temperature of 95°C for 5 minutes, an annealing temperature of 580°C for 1 minute, and an extension temperature of 72°C for 7 minutes. This PCR cycle was run 35 times. We used a kit (Promega Corporation) to purify the PCR products. The polymerase chain reaction products were bidirectionally sequenced using PCR primers and an ABI Prism Big Dye Terminator Cycle Sequencing Kit and analyzed on an ABI 3100/3700XL sequencer. After capillary electrophoresis, the Sanger sequencing results were analyzed by Finch TV, and *in silico* tools, including Mutation Taster, Franklin, Mutalyzer, and Varsome, and the pathogenicity of the variants was classified following the American College of Medical Genetics (ACMG)/ Association for Molecular Pathology (AMP) guidelines [25].

Table 1. *IHH gene forward, reverse primers of three exons used for Sanger sequencing.*

Exon	Primer	Sequence 5' to 3'
IHH Exon1	Forward primer	CGGCCTATTTATTGG-CGG
	Reverse primer	TGCCAGCCAGTC-GAGAAAAT
IHH Exon2	Forward Primer	TTCCAGCTCCCTTGG-GTGT
	Reverse Primer	ATGTCCTCTTCCCCCG-GAT
IHH Exon3	Forward Primer	ATATGGTGACGGG-GGCTCT
	Reverse Primer	GGTATCCGGGATG-GTCCCT

Results

Clinical phenotypes

A total of 44 individuals were examined and 14 affected BDA1 individuals belonged to families from nearby geographical regions. A total of 30 healthy unrelated controls of age group 7-35 years were enrolled in this study, these individuals did not have any skeletal, neurodevelopmental, other genetic disorders or BD. All the affected individuals had abnormalities, including short hands, short feet, growth retardation, and short stature. Three affected individuals belonged to a single family, whereas the others were unrelated. An anthropometric examination was performed for all individuals in the growth laboratory. The height and weight of the bodies of the affected individuals were measured. X-rays were evaluated for the identification of short, missing phalanges in the hands and feet. All 14 BDA1 individuals were found to have short stature. The interpretation of short stature, underweight status, and overweight status is given in Table 1. The hand phenotypes and X-ray images are shown in Figure 1.

Familial case

A family with an AD inheritance pattern was examined in the outpatient clinic and 3 BDA1 individuals in the family underwent genetic evaluation and genetic counseling was provided. The clinical details of the affected members of the family included short stature, short limbs, and abnormalities according to their hands and feet radiographs. There were 7 individuals in the BDA1-affected family, 3 of whom exhibited BD. We clinically examined 3 of the subjects via physical examination and imaging studies, including radiographs of the hands and feet and family history. The younger

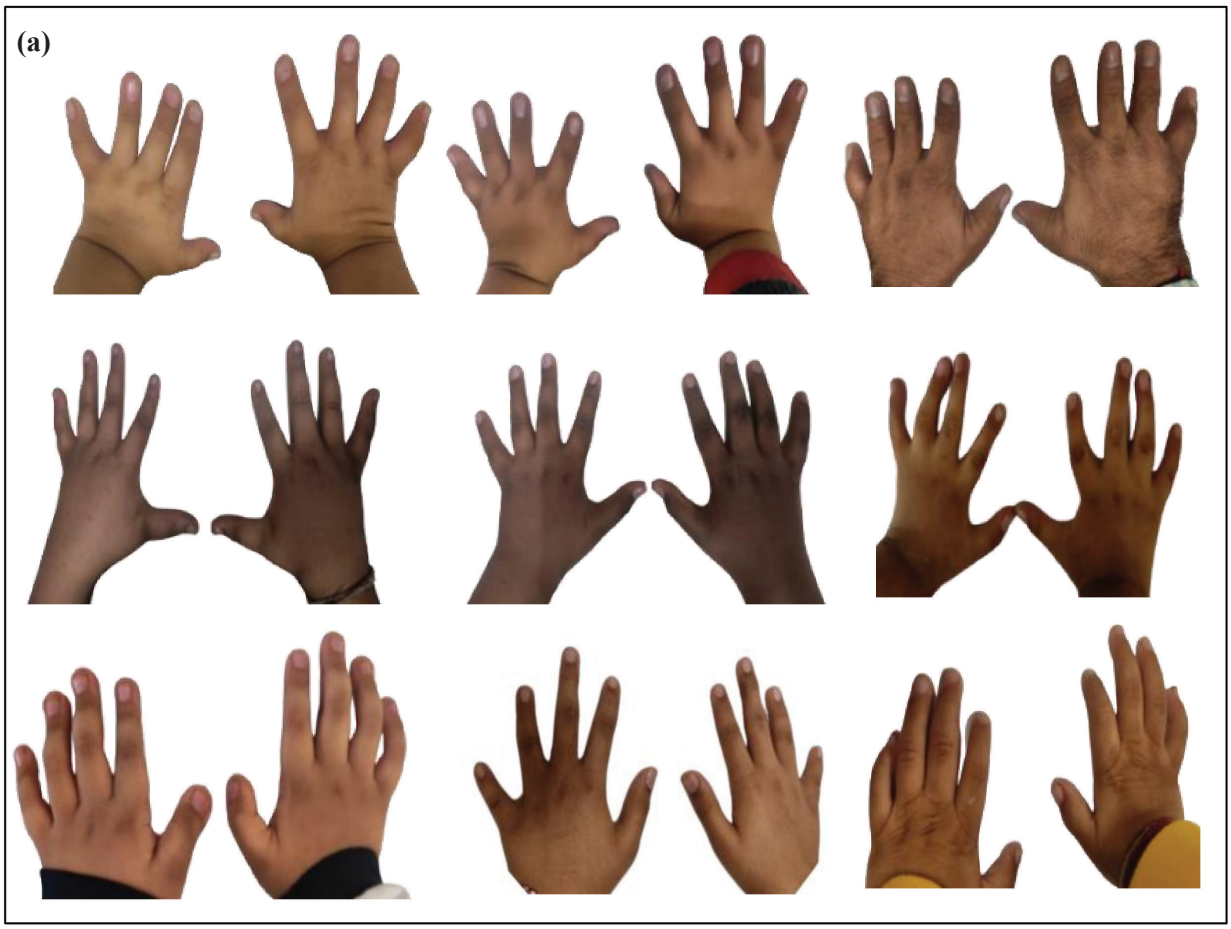

Figure 1. *(a) Phenotype of short hands and X-ray (b) of adult showing missing middle phalanges and short proximal phalanx of thumb in BDA1.*

girl was 7 years old and presented with short hands and feet with a height of 103 cm and a weight of 18 kg. On clinical evaluation, it was found that the other sibling of the girl also had similar short hands and feet, and the father also had similar short hands and feet (Figure 2). On X-ray examination, the middle phalanges of the right and left hands of the three affected individuals were missing (HP:0010239). X-ray of the father's feet showed shortened 4th and 5th metatarsals. Another member of the family was found to be affected with down syndrome.

Genetic analysis

Genetic analysis of 44 individuals was done and 14 affected individuals were identified with three different

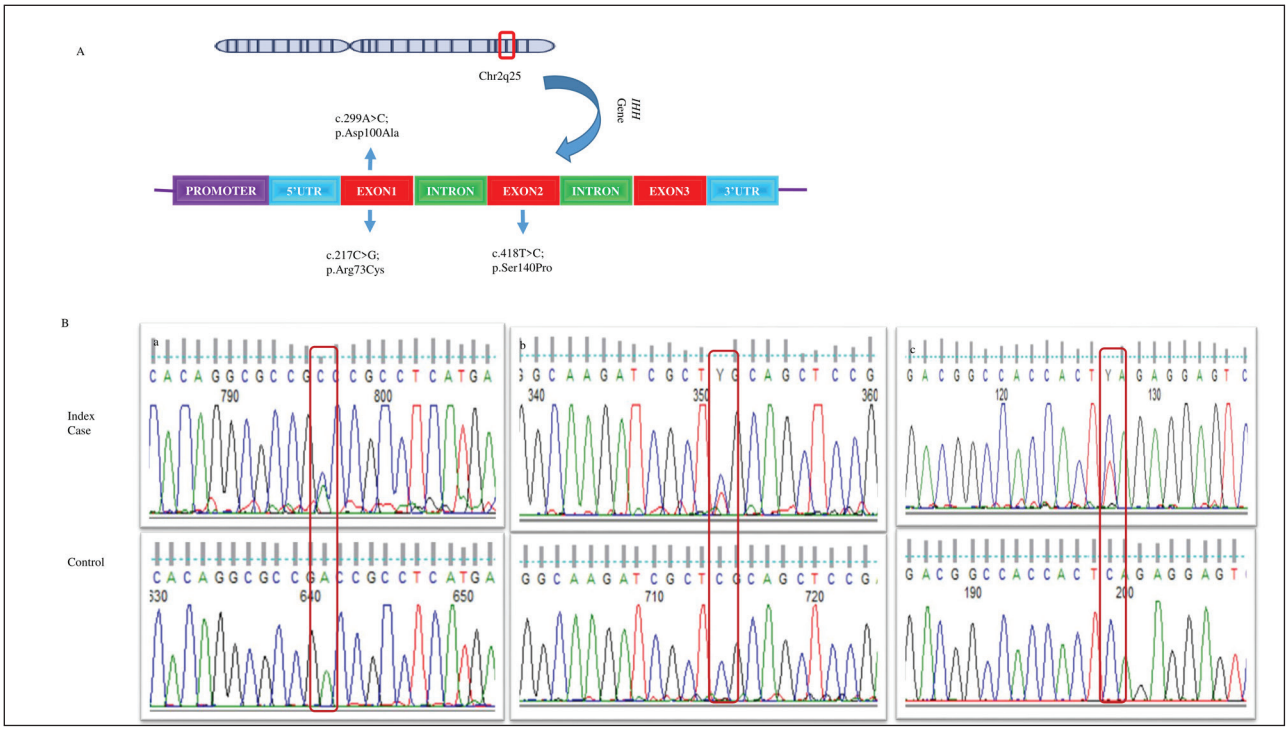

Figure 2. *A. diagrammatic representation of IHH gene on chromosome number 2 at 2q25 with three exonic regions. B. Sanger chromatograms for heterozygous variation a. IHH: c.299A>C, b. IHH:c.217C>T, c. IHH: c.418T>C in index case and normal in control.*

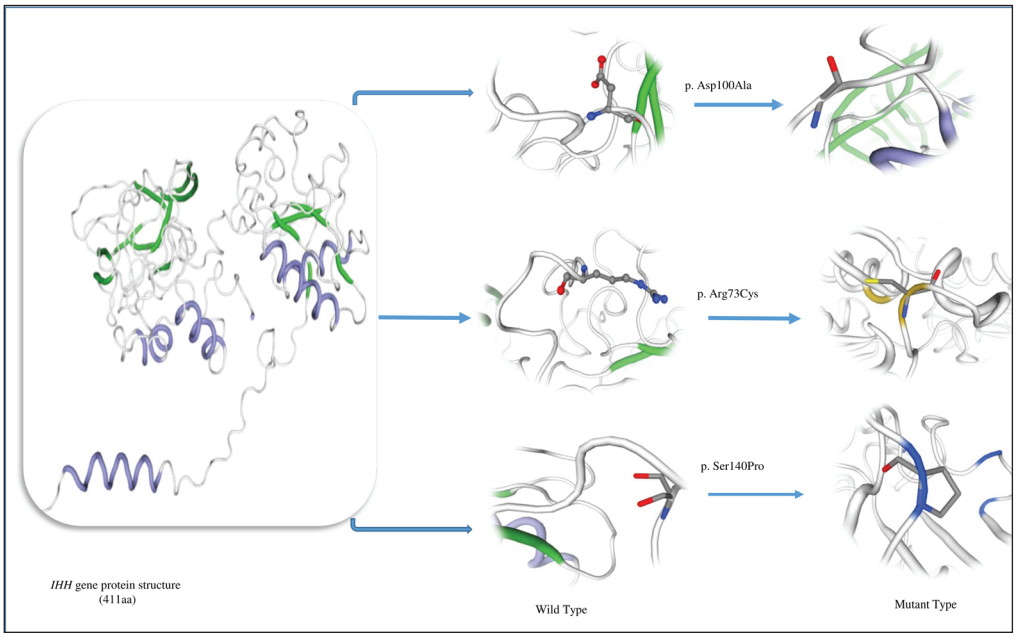

Figure 3. *IHH gene variant and protein change in wild type to mutant type.*

heterozygous *IHH* variants. The genetic testing by Sanger sequencing revealed a missense variant in exon 1, NM_002181.4 *IHH*: c.299A>C; p. Asp100Ala, in 3 affected individuals of the family with BDA1. Another variant was detected in exon 1 in the 4 individuals with NM_002181.4 *IHH:* c.217C>T; p.Arg73Cys; in addition, 7 individuals had a variant in exon 2 namely, NM_002181.4 *IHH:* c.418T>C; p.Ser140Pro. They had short hands, short feet, and short stature. The Sanger sequencing chromatograms are shown in Figure 2. The affected individuals did not present any other specific signs of other skeletal dysplasia on limb and spine radiographs. The unaffected family members of the patients were not found with a variance of unknown significance (VUS). The list of variants identified in 14 individuals is provided in Table 2. We also performed the sequencing of all *IHH* exons in the control group, but no significant variants were identified.

Table 2. *Detailed characteristics of BDA1 patients with identified IHH variants.*

Lab Enrollment ID	Patients-ID's	Age/ Gender	Upper limb	Lower limb	BMI	Height for age (HAZ)	Height interpretation	Weight for Age (WAZ)	Weight interpretation	Variant Identified by Sanger sequencing	Zygosity	ACMG classification
En4	BDA-1	7YM	Short hands	Short feet	17.36	-2.81	Short stature	0.48	Healthy weight	*IHH*:c.217C>T; p.Arg73Cys	Heterozygous	Uncertain significance
En9	BDA-2	10YF	Short hands	-	13.9	-2.72	Short stature	-1.54	Healthy weight	*IHH*:c.217C>T; p.Arg73Cys	Heterozygous	Uncertain significance
En11	BDA-3	18YF	Short hands	Short feet	14.6	-2.87	Short stature	-2.54	Underweight	*IHH:*c.217C>T; p.Arg73Cys	Heterozygous	Uncertain significance
En12	BDA-4	8YF	Short hands	Short feet	16.83	-3.65	Short stature	-1.56	Healthy weight	*IHH:* c.418T>C ;p.Ser140Pro	Heterozygous	Uncertain significance
En 15	BDA-5	10YF	Short hands	Short feet	12.7	-3.08	Short stature	1.62	Healthy weight	*IHH:* c.418T>C; p.Ser140Pro	Heterozygous	Uncertain significance
En 16	BDA-6	10YM	Short hands	Short feet	13.6	-2.72	Short stature	-1.11	Healthy weight	*IHH:* c.418T>C; p.Ser140Pro	Heterozygous	Uncertain significance
En 17	BDA-7	11YM	Short hands	Short feet	15.7	-3.04	Short stature	-2.66	Underweight	*IHH:* c.418T>C; p.Ser140Pro	Heterozygous	Uncertain significance
En18	BDA-8	34YF	Short hands	Short toes	22.3	-2.87	Short stature	-2.56	Underweight	*IHH:* c.418T>C; p.Ser140Pro	Heterozygous	Uncertain significance
En 19	BDA-9	8YM	Short hands	Short feet	15.1	-3.45	Short stature	-1.99	Healthy weight	*IHH*:c.217C>T, p.Arg73Cys	Heterozygous	Uncertain significance
En 21	BDA-10	10YM	Short hands	Short feet	12.7	-2.58	Short stature	-9.2	Underweight	*IHH:* c.418T>C; p.Ser140Pro	Heterozygous	Uncertain significance
En 23	BDA-11	7YF	Missing middle phalange of hands	-	13.16	-3.65	Short stature	-2.62	Underweight	*IHH*:c.299A>C,p. Asp100Ala	Heterozygous	Likely pathogenic
En 27	BDA-12	10.3YF	Missing middle phalange of hands	-	14.8	-3.08	Short stature	-1.99	Healthy weight	*IHH:*c.299A>C,p. Asp100Ala	Heterozygous	Likey pathogenic
En28	BDA-13	37YM	Missing middle phalange of hands	Short 3rd, 4th metatarsals	25.2	-2.01	Short stature	2.22	Overweight	*IHH*:c.299A>C,p. Asp100Ala	Heterozygous	Likely Pathogenic
En29	BDA-14	10YM	Short hands	Short feet	12.7	-2.58	Short stature	-9.2	Underweight	*IHH:* c.418T>C; p.Ser140Pro	Heterozygous	Uncertain significance

In silico analysis of the ACMG/AMP classification of identified variants

The ACMG and the AMP guidelines were followed to interpret the pathogenicity of the identified variants. The allele frequencies for rare and pathogenic alleles in our patients were compared with those in public databases. To provide additional genetic evidence for the association of IHH with the short-stature phenotype, we performed variant analyses by comparing allele frequencies between our cohorts of patients and public databases. The variant was not found to be homozygous in public databases, such as ExAC, gnomAD (Exome), gnomAD (Genome), ESP 6,500, 1,000 Genomes, Iranome, GenomeAsia, GME Variome, Turkish Variome, Mexican DB, India DB, 4.7KJPN and TOPMed Bravo. The rare nonsynonymous missense variants were predicted to be pathogenic and to be of uncertain significance by the *in-silico* tools Revel, Alpha Missense, Varity, SIFT, Meta-LR, Primate AI, Meta-RNN, Mutation Assessor, Mutation Taster, PROVEAN, and Aggregated Prediction. The details of the prediction scores of the identified variants are given in Table 3.

In-silico analysis of the protein parameters of the identified variants

Variant c.299A>C, p. Asp100Ala showed a negatively charged amino acid change to a nonpolar, aliphatic amino acid. Despite the variance in the uncertainty of the significance of the protein change, c.217C>T and p.Arg>Cys altered the positively charged amino acid to a highly reactive amino acid. Another variant, c.418 T>C, p.Ser>Pro changes the polar, neutral amino acid to a nonpolar, neutral, and aliphatic imino group amino acid. An overall change in the *in-silico* structure of the protein was observed. The change in the structure is shown in Figure 3.

To analyze the changes in the proteins of the identified variants, we performed an extensive analysis of the protein parameters by using the tool ExPASy https://web.expasy.org/protparam/. This provides us with the various physical and chemical parameters required for a change in a mutant protein. Here, we compared the changes in wild-type protein parameters with those of the identified likely pathogenic and variants of uncertain significance. The protein parameters included the amino acid number, which was the same for the mutant and wild-type strains. The molecular weight, isoelectric point, instability index, and grand average of hydropathicity (GRAVY) slightly changed in all the mutant strains compared to those in the wild type. All the parameters are given in Table 4.

Individuals with mutant type *IHH*:c.299A>C; p.Asp100Ala were found with missing middle phalanges in the hands and short middle phalanges and metacarpals in the feet. This patient's protein parameter isoelectric point is increased by 9.09, molecular weight decreased with respect to wild-type IHH. Patients with p.Asp100Ala have 40 negatively charged amino acids in the *IHH* gene. Aliphatic index and instability index are also increased in the patients with p.Asp100Ala. These changes in parameters significantly correlate and segregate with the phenotypic changes in the patients and thus p.Asp100Ala is likely to be pathogenic.

In mutant c.217C>T; p.Arg73Cys, patients were found with short hands and feet, short 3rd, 4th, and 5th metacarpals, and short stature. Protein parameters in the patients included decreased isoelectric point 8. 78, and decreased molecular weight with respect to wild-type protein parameters. +vly charged amino acid is also decreased 46 with respect to wild type 47 in IHH. Grand average hydropathicity decreased in the patients

Table 3. *In silico analysis prediction score and ACMG/AMP classification of IHH-Identified variants.*

	***IHH* gene variant**	**c.299A>C;p. Asp100Ala**	**c.217C>T; p.Arg73Cys**	**c.418T>C; p.Ser140Pro**
In Silico Tools	**Domain**	**N-terminal signaling domain**	**N-terminal signaling domain**	**N-terminal signaling domain**
	Revel	0.88	0.92	0.82
	Alpha missense	0.99	0.995	0.86
	Varity	0.96	0.95	0.94
	SIFT	0	0.001	0.154
	Meta LR	0.99	0.99	0.96
	Primate AI	0.83	0.69	0.74
	Meta RNN	0.99	0.98	0.71
	Mutation assessor	2.73	2.99	1.24
	Mutation taster	0.99	1	1
	PROVEAN	-6.32	-6.09	-3.17
	Aggregated prediction	0.87	0.88	0.85
	ACMG/ AMP classification	Likely pathogenic PM2 PM5 PM1 PP3 PP2	Uncertain significance PP3 PM2 PP2	Uncertain significance PM2 PP3 PM1 PP2

from the actual value of −0.200. Aliphatic and instability index remain the same as the wild-type parameters of proteins. These changed parameters may play a role in the phenotypic changes in the patients and interpreted as uncertain significant variants. With variant c.418T>C: p.Ser140Pro; the Molecular weight of IHH amino acids increased. Protein parameters like aliphatic and instability index decreased, but other parameters like isoelectric point, +vly, -vly charged remained the same as wild-type protein parameters. Thus, it was interpreted as an uncertain significance variant.

Discussion

BD is mostly seen in isolated form but can manifest as a complex syndrome. The overall prevalence rate in the population is not known because many individuals do not present to clinicians. Our study involved the clinical and molecular analysis of BDA1 in 14 individuals from related and unrelated families. A missense heterozygous novel variation was identified in the family of the three affected individuals with heterozygous novel variation in exon 1 of the *IHH* gene This finding demonstrated the complete penetrance of the gene in the affected individual's phenotype. The affected individuals presented with short hands, feet and short stature. The middle phalanges of the hands of the affected individuals were missing on X-ray examination. In this study, we found the novel pathogenic variant, NM_002181.4 (*IHH*): c.299A>C; p. Asp100Ala, which segregates with BDA1 and the persons also had short stature. A pathogenic variant in the same region with different amino acid changes has been described earlier in a Chinese family [12]. Additionally, since multiple affected members of the family harbored the variant and because *in silico* tools supported the pathogenicity of the variant, we assumed that the variant was likely the cause of the phenotype in the family with short stature and BD.

Two other variants were analyzed by the *in-silico* tools. The ACMG guidelines were predicted to be VUS. The allele frequencies of the identified variants in the patients in different population databases are not presented. These two variants NM_002181.4 (*IHH*): c.217C>T; p.Arg73Cys and NM_002181.4 (*IHH*): c.418T>C; and p.Ser140Pro were not reported in the ClinVar database. Our study demonstrated that *IHH* gene screening by sequencing should be done for individuals with BDA1 or other hand-foot malformations, especially if associated with short stature, in related and unrelated individuals. Further description of the BDA1 multiplex families and also a long-term follow-up of these individuals, especially children can give further insights into the possible correlation of genotype (variant location) with phenotype. Additionally, functional studies will be important for validating VUSs. To date, a few variants in the N-terminal and C-terminal domains of *the IHH* gene have been characterized [26,27]. First, it is important to phenotype patients by detailed clinical examination, including anthropometric assessments and radiological assessments. Furthermore, parental segregation based on phenotypic appearance is a necessary part of clinical examination. This approach helps reveal the familial nature of the disorder. Targeted gene sequencing of the *IHH* gene is the gold standard method for exploring monogenic cases of BD and short stature. The best way to detect the genetics of minor skeletal abnormalities is currently available at various centers. This study is a pilot study, and a greater number of patients could not be enrolled because usually syndromic patients are seen in our clinic and isolated BD patients do not visit the hospital. A few unaffected family members did not agree to give samples for testing. Funding issues are also a reason for a limited number of enrollment of patients and controls.

Table 4. *In silico analysis, protein parameters of identified variants in the present study.*

		Wild type *IHH* Gene	Mutant type *IHH*: c.299A>C;p. Asp100Ala	Mutant type *IHH*: c.217C>T; p.Arg-73Cys	Mutant type *IHH*: c.418T>C; p. Ser-140Pro
Protein Parameters ENSG00000163501	Amino acid	411	411	411	411
	Molecular weight	45,250.67	4,5206.66	4,5197.62	45260.71
	Isoelectric point	8.98	9.09	8.78	8.98
	+vly charged amino acid	47	47	46	47
	-vly charged amino acid	41	40	41	41
	Formula	$C_{2028}H_{3161}N_{589}O_{569}S_{11}$	$C_{2027}H_{3161}N_{589}O_{567}S_{11}$	$C_{2025}H_{3154}N_{586}O_{569}S_{12}$	$C_{2030}H_{3163}N_{589}O_{568}S_{11}$
	Total number of atoms:	6,358	6,355	6,346	6361
	Aliphatic index:	86.18	86.42	86.18	86.18
	The instability index	37.62	38.01	37.62	37.50
	Grand average of hydropathicity (GRAVY)	-0.200	-0.187	-0.183	-0.202

Conclusion

This study is the first to report a familial case of a novel variant in the northern region of India. Additionally, two variants of uncertain significance were also identified in patients with BDA1 and short stature. *In silico* tools are helpful for interpretation according to ACMG guidelines. However, additional research is essential to determine the exact pathogenic processes in the development of short stature and BD with the variants, and further *ex vivo* studies may be needed for a better understanding of this phenomenon.

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List of Abbreviations

ACMG/AMP	American College of Medical Genetics/ American Molecular Pathology
BD	Brachydactyly
BDA	Brachydactyly type A
DB	Database
DNA	Deoxyribonucleic acid
GRAVY	Grand average of hydropathicity
IHH	Indian Hedgehog
PCR	polymerase chain reaction
PROTEAN	Protein Variation Effect Analyzer
SIFT	Sorting Intolerant from Tolerant
VUS	variant of unknown significance

Declaration of conflicting interests

The authors of this article have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Consent to participate

Informed consent was obtained from the patients.

Funding

None.

Ethical approval

The present study was approved by the Ethics Committee of the Institute (Ref No: NK17963/PhD/638) at the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India.

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References

1. Yang Q, Wang J, Tian X, Shen F, Lan J, Zhang Q, et al. A novel variant of IHH in a Chinese family with brachydactyly type 1. BMC Med Genet. 2020 Dec;21:1–7 https://doi.org/10.1186/s12881-020-01000-6
2. Zhu T, Guan L, Luo Y, Zhu M, Sun R, Shi J, et al. A Novel heterozygous variant in IHH may contribute to fetal Brachydactyly type A1. Available from: SSRN 4356060.
3. Bechtold TE, Kurio N, Nah HD, Saunders C, Billings PC, Koyama E. The roles of Indian hedgehog signaling in TMJ formation. Int J Mol Sci. 2019 Dec 13;20(24):6300. https://doi.org/10.3390/ijms20246300
4. Vasques GA, Funari MF, Ferreira FM, Aza-Carmona M, Sentchordi-Montané L, Barraza-García J, et al. IHH gene mutations causing short stature with nonspecific skeletal abnormalities and response to growth hormone therapy. J Clin Endocrinol Metabol. 2018 Feb;103(2):604–14. https://doi.org/10.1210/jc.2017-02026
5. Temtamy SA, Aglan MS. Brachydactyly. Orphanet J Rare Dis. 2008 Dec;3(1):1–6. https://doi.org/10.1186/1750-1172-3-15
6. Byrnes AM, Racacho L, Grimsey A, Hudgins L, Kwan AC, Sangalli M, et al. Brachydactyly A-1 mutations restricted to the central region of the N-terminal active fragment of Indian Hedgehog. Eur J Hum Genet. 2009 Sep;17(9):1112–20. https://doi.org/10.1038/ejhg.2009.18
7. Hall CM. International nosology and classification of constitutional disorders of bone (2001). Am J Med Genet. 2002 Nov 15;113(1):65–77. https://doi.org/10.1002/ajmg.10828
8. Mortier GR, Cohn DH, Cormier-Daire V, Hall C, Krakow D, Mundlos S, et al. Nosology and classification of genetic skeletal disorders: 2019 revision. Am J Med Genet Part A. 2019 Dec;179(12):2393–419. https://doi.org/10.1002/ajmg.a.61366
9. Fitch N. Classification and identification of inherited brachydactylies. J Med Genet. 1979 Feb 1;16(1):36–44. https://doi.org/10.1136/jmg.16.1.36
10. Giordano N, Gennari L, Bruttini M, Mari F, Meloni I, Baldi C, et al. Mild brachydactyly type A1 maps to chromosome 2q35-q36 and is caused by a novel IHH mutation in a three generation family. J Med Genet. 2003 Feb 1;40(2):132–5. https://doi.org/10.1136/jmg.40.2.132
11. Gao B, Guo J, She C, Shu A, Yang M, Tan Z, et al.. Mutations in IHH, encoding Indian hedgehog, cause brachydactyly type A-1. Nature Genet. 2001 Aug 1;28(4):386–8. https://doi.org/10.1038/ng577
12. Liu M, Wang X, Cai Z, Tang Z, Cao K, Liang B, et al. A novel heterozygous mutation in the Indian hedgehog gene (IHH) is associated with brachydactyly type A1 in a Chinese family. J Hum Genet. 2006 Aug;51(8):727–31. https://doi.org/10.1007/s10038-006-0012-6
13. Lodder EM, Hoogeboom AJ, Coert JH, de Graaff E. Deletion of 1 amino acid in Indian hedgehog leads to brachydactyly A1. Am J Med Genet Part A. 2008 Aug 15;146(16):2152–4. https://doi.org/10.1002/ajmg.a.32441
14. McCready EM, Sweeney E, Fryer AE, Donnai D, Baig A, Racacho L, et al. A novel mutation in the IHH gene causes brachydactyly type A1: a 95-year-old mystery resolved. Hum Genet. 2002 Oct;111:368–75. https://doi.org/10.1007/s00439-002-0815-2
15. Salian S, Shukla A, Nishimura G, Girisha KM. Severe form of brachydactyly type A1 in a child with a c. 298A>G mutation in IHH gene. J Pediatr Genet. 2017 Sep;6(03):177–80 https://doi.org/10.1055/s-0037-1599201

16. Chen Y, Struhl G. Dual roles for patched in sequestering and transducing Hedgehog. Cell. 1996 Nov 1;87(3):553–63. https://doi.org/10.1016/S0092-8674(00)81374-4

17. Daoussis D, Filippopoulou A, Liossis SN, Sirinian C, Klavdianou K, Bouris P, et al. Anti-TNFα treatment decreases the previously increased serum Indian Hedgehog levels in patients with ankylosing spondylitis and affects the expression of functional Hedgehog pathway target genes. Semin Arthritis Rheum. 2015 Jun 1;446:646–51. https://doi.org/10.1016/j.semarthrit.2015.01.004

18. Gao B, Hu J, Stricker S, Cheung M, Ma G, Law KF, et al. A mutation in Ihh that causes digit abnormalities alters its signaling capacity and range. Nature. 2009 Apr 30;458(7242):1196–200. https://doi.org/10.1038/nature07862

19. Kirkpatrick TJ, Au KS, Mastrobattista JM, McCready ME, Bulman DE, Northrup H. Identification of a mutation in the Indian Hedgehog (IHH) gene causing brachydactyly type A1 and evidence for a third locus. Jo Med Genet. 2003 Jan 1;40(1):42–4. https://doi.org/10.1136/jmg.40.1.42

20. Lee J, Platt KA, Censullo P, Altaba AR. Gli1 is a target of Sonic Hedgehog that induces ventral neural tube development. Development. 1997 Jul 1;124(13):2537–52. https://doi.org/10.1242/dev.124.13.2537

21. Marigo V, Johnson RL, Vortkamp A, Tabin CJ. Sonic Hedgehog differentially regulates expression of GLI and GLI3 during limb development. Dev Biol. 1996 Nov 25;180(1):273–83 https://doi.org/10.1006/dbio.1996.0300

22. McLellan JS, Zheng X, Hauk G, Ghirlando R, Beachy PA, Leahy DJ. The mode of Hedgehog binding to Ihog homologs is not conserved across different phyla. Nature. 2008 Oct 16;455(7215):979–83. https://doi.org/10.1038/nature07358

23. Paula MA, Carvalho A, Aragão L, Sousa M, Sales M, Granjeiro C, et al. Case report: small stature associated with uncertain significance variant of The IHH Gene. IMPE Abstracts. 2023 Feb 14;96.

24. Puvabanditsin S, Gorbonosov M, Blackledge K, Manzano J, Federici M, Mehta R. Spondylocostal dysplasia and brachydactyly associated with TBX6 and IHH variants: a case report. Clinical Case Rep. 2022 Jul;10(7):e6000. https://doi.org/10.1002/ccr3.6000

25. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015 May;17(5):405–23. https://doi.org/10.1038/gim.2015.30

26. Ho R, McIntyre AD, Kennedy BA, Hegele RA. Whole-exome sequencing identifies a novel IHH insertion in an Ontario family with brachydactyly type A1. SAGE Open Med Case Rep. 2018 Dec;6:2050313X18818711.

27. Ma G, Yu J, Xiao Y, Chan D, Gao B, Hu J, et al. Indian hedgehog mutations causing brachydactyly type A1 impair Hedgehog signal transduction at multiple levels. Cell Res. 2011 Sep;21(9):1343–57.