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A novel homozygous variant in *BMPR1B* underlies acromesomelic dysplasia Hunter–Thompson type

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Abstract

Acromesomelic dysplasia is genetically heterogeneous group of skeletal disorders characterized by short stature and acromelia and mesomelia of limbs. Acromesomelic dysplasia segregates in an autosomal recessive pattern and is caused by biallelic sequence variants in three genes (*NPR2, GDF5*, and *BMPR1B*).

A consanguineous family of Pakistani origin segregating a subtype of acromesomelic dysplasia called Hunter–Thompson was clinically and genetically evaluated. Geno-typing of microsatellite markers and linkage analysis revealed a 7.78 Mb homozygous region on chromosome 4q22.3, which harbors *BMPR1B*. Sequence analysis of the gene revealed a novel homozygous missense variant (c.1190T > G, p.Met397Arg) that segregates with the disease phenotype within the family and produced a Logarithm of odds (LOD) score of 3.9 with the disease phenotype. This study reports on the first familial case of acromesomelic dysplasia Hunter–Thompson type. It is also the first report of *BMPR1B* underlying the etiology of acromesomelic dysplasia Hunter–Thompson type.

Keywords

acromesomelic dysplasia Hunter–Thompson type; BMPR1B; missense variant

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AUTHOR'S CONTRIBUTIONS

A. U. and W. A. designed the study.
A. U., M. U. and D. M Collected data.
A. U. and M. B. Performed experiment and analyzed data.
A. U., W. A and S. M. L. Prepared manuscript.
W. A., and S. M. L. Supervised the study, provided funds and critically reviewed the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

1| INTRODUCTION

Acromesomelic dysplasia is a group of hereditary skeletal disorders characterized by abnormal growth of bones, cartilages, and connective tissues. It is further classified into five subgroups, namely acromesomelic dysplasia Maroteaux type (AMDM, MIM 602875), acromesomelic dysplasia Grebe type (AMDG, MIM 200700), acromesomelic dysplasia Hunter–Thompson type (AMDH, MIM 201250), acromesomelic dysplasia DuPan type (AMDDu, MIM 228900), and acromesomelic dysplasia Demirhan type (AMDD, MIM 609441) (Bonafe et al., 2015; Warman, Cormier-Daire, & Hall, 2011).

Acromesomelic dysplasia Maroteaux type is characterized by short stature and shortening of the middle and distal segments of the appendicular and axial skeleton. This is caused by biallelic sequence variants in the natriuretic peptide receptor-2 (*NPR2*) (Bartels et al., 2004; Irfanullah, Umair, Khan, & Ahmad, 2015; Khan et al., 2012).

Acromesomelic dysplasia Grebe type is characterized by short stature and abnormalities confined to the limbs, namely toe-like fingers, fusion or absence of carpals and tarsals, and missing joints. Biallelic sequence variants in the growth and differentiation factor 5 (*GDF5*) or its receptor bone morphogenetic protein receptor-1b (*BMPR1B*) cause AMDG (Basit et al., 2008; Graul-Neumann et al., 2014; Khan, Basit, Khan, Muhammad, & Ahmad, 2016; Thomas et al., 1996; Umair et al., 2016).

Acromesomelic dysplasia Hunter–Thompson type is characterized by shortening of the limbs. Lower limbs are more affected than the upper limbs. Lower limb anomalies include short femurs, tibias, and fibulas as well as dislocated ankles, and those of the upper limbs include hands with short phalanges and metacarpals, short humerus, and a curved radius and ulna. The middle and distal limbs are most affected. AMDH is caused by biallelic variants in the *GDF5* (Thomas et al., 1996).

Acromesomelic dysplasia DuPan type, also known as fibular hypoplasia and complex brachydactyly, is characterized by mild shortening of the limbs. Affected individuals have normal radius, ulna, femur, carpals, metacarpals, and phalanges. Tarsals, metatarsals, and phalanges of toes are mildly affected with impalpable fibulae. This type of acromesomelic dysplasia is caused by biallelic variants in the *GDF5* and *BMPR1B* genes (Stange et al., 2015).

In AMDD, limb shortening is associated with genital anomalies, namely hypoplasia of the uterus and premature ovarian failure leading to hypogonadotropic hypogonadism. Demirhan et al. (2005) reported on an 8 bp deletion (c.361_368del8) in *BMPR1B* causing AMDD in a Turkish family. AMDD differs from AMDG because of the presence of genital anomalies and the distinct limb phenotype.

Here, we present a consanguineous Pakistani family from the Sindh province with four members affected with AMDH caused by a homozygous sequence variant in the *BMPR1B* gene.

2| MATERIALS AND METHODS

The present study was approved by the institutional review board of Quaid-i-Azam University Islamabad, Pakistan. Informed consent was obtained from all family members who participated in the study. Blood samples from four affected (IV-8, V-1, V-3, and V-4) and three unaffected members (IV-2, IV-4, and IV-6) of the family were collected in ethylenediaminetetraacetic acid tubes (BD, Franklin Lakes, NJ, USA). Genomic DNA was extracted using the Sigma-Aldrich GenElute Blood Genomic DNA Kit (St. Louis, MO, USA).

2.1| Genotyping and analysis

Genotyping was performed using microsatellite markers flanking *NPR2* on chromosome 9p12–21 and *BMPR1B* on chromosome 4q22.3. PCR-based genotyping was carried out as previously described (Ullah et al., 2015). PCR products were resolved on 8% nondenaturing polyacrylamide gel and analyzed after staining with ethidium bromide. The haplotype was constructed using 10 microsatellite markers (D4S2929, D4S2460, D4S2461, D4S3245, D4S3006, D4S423, D4S2364, D4S2433, D4S2909, and D4S1560) linked to *BMPR1B* (Fig. 1a). Two-point and multipoint linkage analyses was performed using Superlink (Silberstein et al., 2006) assuming an autosomal-recessive mode of inheritance with no reduced penetrance or phenocopies. Because of the rarity of AMDH, a disease frequency of 0.00001 was used in the analysis. Equal allele frequencies were used for each marker. Two-point linkage analysis was also performed for the identified variant, assuming an allele frequency of 0.0000001 for the alternative allele since the variant was not observed in gnomAD.

2.2| Sequencing *GDF5* and *BMPR1B*

Sequences of *GDF5* and *BMPR1B* were downloaded from Ensembl genome browser (https://asia.ensembl.org/index.html) and primers for the coding exons and splice sites were designed for the intronic regions using Primer3 software (https://bioinfo.ut.ee/primer3-0.4.0/primer3/) (Untergasser et al., 2012). The primer sequences are available on request. PCR products were analyzed on 2% agarose gel and purified using a commercially available kit (Axygen Inc., CA, USA). DNA sequencing was performed using DTCS Quick Start sequencing kit (Beckman Coulter, Fullerton, CA, USA). Sequencing results were aligned through BIOEDIT sequence alignment editor version 6.0.7 (Ibis Biosciences, Carlsbad, CA, USA). Pathogenitcity and the impact of the variant on the protein function of the identified variant was analyzed using MutationTaster (https://www.mutationtaster.org/index.html) and Polymorphism Phenotyping v2 (PolyPhen-2) (https://genetics.bwh.harvard.edu/pph2/), respectively.

3| RESULTS

All four affected members of the family presented clinical features of AMDH. At the time of this study, the ages of the affected pedigree members were between 24 and 30 years, and their height ranged from 140 to 141 cm. They displayed a severe form of short stature with abnormalities limited to an appendicular skeleton. Anomalies observed included short radii/ulnae, shorts and bowed tibias/fibulas, and short fingers and toes. The axial and craniofacial

skeleton were not affected. Detailed clinical and radiological features, recorded in two affected members (V-1 and V-4) compared to an unaffected population of same ethnicity group, are presented in Figure 2b–i (Table 1). Endocrinological studies in two affected members (V-1 and V-4) revealed normal levels of gonadotropins, particularly luteinizing hormone and follicle-stimulating hormone. The two affected females (IV-8 and V-3) declined to provide photographs for presentation in publications. Affected members were able to work in an adequate manner despite having limb anomalies.

3.1| Genotyping and sequence analysis

Biallelic variants in the *GDF5* gene have been reported to cause AMDH; therefore, coding regions of the gene were Sanger sequenced in all four affected members of the family. However, analysis of the sequences failed to detect any variants in the *GDF5* gene. Genotyping using microsatellite markers linked to *BMPR1B* (D4S2929, D4S2460, D4S2451, D4S3245, D4S3006, D4S423, D4S2364, D4S2433, D4S2909, and D4S1560) and *NPR2* (D9S1118,D9S1788, D9S1817, D9S761, D9S1794, D9S50, D9S772, and D9S55) was then performed using DNA samples from all available affected and unaffected family members. Haplotype analysis revealed a 7.78 Mb homozygous region among affected members on chromosome 4q22.3, which harbors *BMPR1B* (Fig. 1a). Microsatellite markers linked to *NPR2* were heterozygous in both affected and unaffected individuals, suggesting an absence of linkage to chromosome 9p12–21. No recombination events were observed with markers D4S2461, D4S3245, D4S3006, D4S423, D4S2364,D4S2433, and D4S2909, and they each produced a logarithm of odds (LOD) score of 1.5 at theta = 0. Multipoint linkage analysis produced a maximum LOD score of 2.7.Sequence analysis revealed a homozygous missense variant (c.1190T > G, p.Met397Arg) in exon 9 of the *BMPR1B* gene in affected family members (Fig. 2a). Further sequencing using DNA samples from unaffected members validated segregation of the variant with disease phenotype in the family (Fig. 2c–e). Two-point linkage analysis performed between the disease phenotype and c.1190T > G variant produced an LOD score of 3.9 at theta = 0.

The mutation effect prediction tool MutationTaster predicted the substitution of methionine at amino acid position 397 with arginine as disease causing. PolyPhen-2 predicted that the substitution (p.Met397Arg), present in the kinase domain of the *BMPR1B* gene, has a damaging effect on protein structure and function. Clustal Omega analysis showed that the methionine at position 397 of the *BMPR1B* gene is completely conserved among all species known to have a *BMPR1B* ortholog (Fig. 2f). The identified variant was not found in available databases of genetic variations (i.e. dbSNP, EVS, and gnomAD) or in the 200 ethnically matched control individuals of Pakistani origin sequenced in the present study.

4| DISCUSSION

Three different types of acromesomelic dysplasia, namely Grebe, DuPan and Hunter-Thompson, are characterized by shortening of the appendicular skeleton, which segregate in an autosomal-recessive pattern. Short stature with abnormalities limited to limbs, with lower limbs more affected than the upper limbs, are the key features of AMDH. Feet are very short, often bowed with short great toes forcing affected individuals to walk on knees. Some

toes are ball-shaped and functionless. The proximal and middle phalanges are either short or absent; however, distal phalanges are normal in both hands (Faivre & Cormier-Daire, 2005; Thomas et al., 1996). Toe-like fingers and proportionate involvement of the upper and lower limbs differentiate AMDG from AMDDu and AMDH. Similarly, mild short stature and milder limb defects in AMDDu, namely fibular hypoplasia and complex brachydactyly, differentiate it from AMDH (Stange et al., 2015).

Affected individuals in the family, presented here, showed phenotypes of AMDH. Patients have short but functional toes with the ability to walk on their feet. Shortening of the limbs was limited to the medial and distal parts. Shortening of hands, metacarpals and distal phalanges, forearms with bowed radius, and feet and forelegs with bowed tibias/fibulas were observed in all of the patients. The observed phenotypes were consistent for all affected family members. Normal levels of luteinizing hormone and follicle-stimulating hormone, short fingers, and a normal axial skeleton support the diagnosis of AMDH in the family.

Acromesomelic dysplasia types Grebe and DuPan are caused by biallelic sequence variants in the *GDF5* gene and its receptor coding gene *BMPR1B*. Biallelic sequence variants in the *GDF5* gene also cause AMDH. However, variants in the *BMPR1B* gene causing AMDH have not been reported previously. To the best of our knowledge, our study reported the first familial case of AMDH caused by a homozygous sequence variant in the *BMPR1B* gene. Genotyping using microsatellite markers followed by Sanger sequencing of *BMPR1B* revealed a homozygous missense variant (p.Met397Arg) in all the affected members of the family.

GDF5 belongs to bone morphogenetic protein (BMP) family and the transforming growth factor-β (TGF-β) superfamily. BMPs are multifunctional proteins regulating cell growth, differentiation and apoptosis. GDF5 is a ligand for BMP receptors type 1 and 2. Binding of GDF5 to its receptor activates BMPR1B by trans-phosphorylation through BMPR2. Activated BMPR1B phosphorylates receptor-regulated SMADs, namely Smad1, Smad5, and Smad8. These then interact with Smad4 (common-mediator Smad) and translocate into the nucleus to regulate transcription of the target genes (Derynck, Zhang, & Feng, 1998; Massague & Wotton et al., 2000). BMP receptors also activate p38 MAPK and JNK pathways (Heldin, Miyazono, & ten Dijke, 1997). These signal cascades regulate transcription of specific target genes, which are involved in bone formation (Gilboa et al., 2000; von Bubnoff & Cho, 2011). Biallelic sequence variants in either *GDF5* or *BMPR1B* can cause considerable phenotypic variations of acromesomelic dysplasia, and the latter seem to represent a severity continuum with mutation in either of these two genes encoding a ligand-receptor pair.

To date, four sequence variants in the *BMPR1B* gene causing acromesomelic dysplasia have been reported (Table 2). The sequence variant (p.Met397Arg), identified in our family, is located in serine-threonine kinase domain of BMPR1B (Fig. 2a–b). The kinase domain of BMPR1B (at amino acid positions 204–494) phosphorylates Smads (Smad1, Smad5 and Smad8) in TGF-β/Smad Signaling. The missense variant (p.Met397Arg) is located in the kinase domain, which most likely leads to impairment of BMPR1B's ability to phosphorylate Smads. A homozygous nonsense variant (p.Trp219*) in the same domain, in a

family of Pakistani origin reported by Graul-Neumann et al. (2014), caused more severe form of acromesomelic dysplasia, AMDG. It is highly likely that the nonsense variant in the kinase domain results in complete loss of function of protein through nonsense-mediated mRNA decay and/or protein truncation.

In the present study, we believe that we have presented evidence of the first familial case of AMDH caused by a sequence variant in the *BMPR1B* gene.

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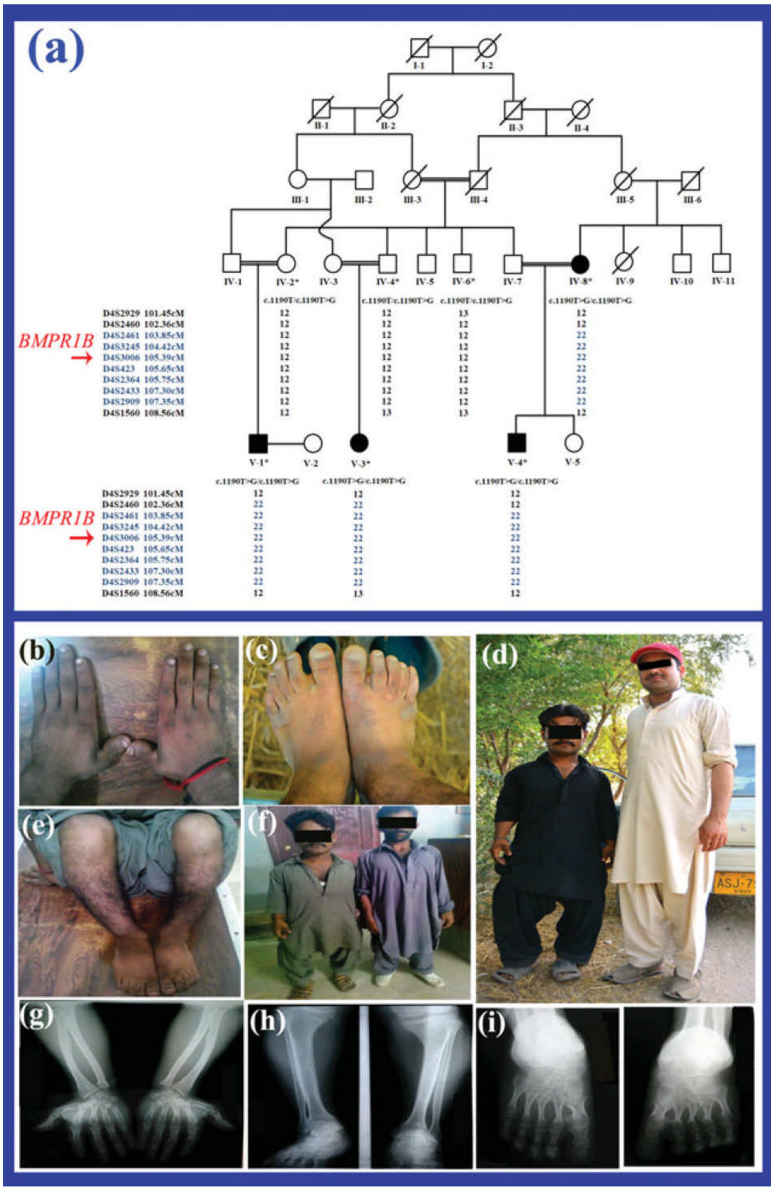

FIGURE 1.

Pedigree of Pakistani family segregating acromesomelic dysplasia Hunter–Thompson type in an autosomal-recessive manner. The *asterisks* indicate individuals who were available for this study. For individuals with genotype data, haplotypes of the closely linked microsatellite markers on chromosome 4q22.3 are shown beneath each symbol. The mutation found in present family is shown beneath each individual sequenced. (a) Clinical and radiological features of two affected members (V-1 and V-4). Hands of affected individual showing short fingers (b) and short toes (c). (d) An affected member (V-4) along with one of the authors (left to right). € Bowed forelegs of affected member (V-4). (f) Affected individuals V-1 and V-4 (right to left) showing short stature. Radiographs of affected member V-4 showing bowed radius/ulna, short metacarpals and phalanges, radial deviation of all fingers in both

hands (g), dislocated knee, bowed tibia (h), short metatarsal and phalanges of toes, and tibial deviation of distal phalanges (i) [Colour figure can be viewed at wileyonlinelibrary.com]

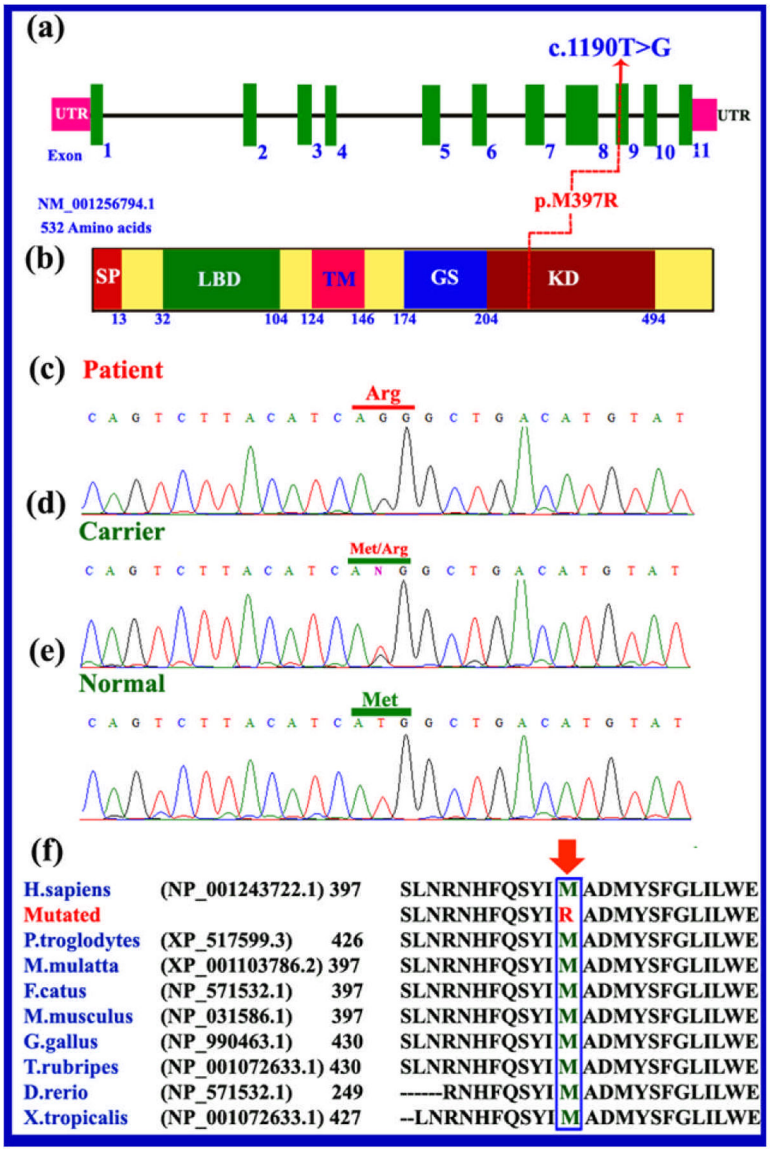

FIGURE 2.

Schematic representations showing the coding regions of *BMPR1B* (a) and predicted protein with predicted domains and amino acids sequence. (b) *Red arrow* highlights the missense variant (c.1190T > G, p.Met397Arg) found in the present study. (c–e) Sequence analysis of exon 9 of *BMPR1B* (5′−3′) in the patient, carrier, and unaffected individual. (f) Comparison of partial amino acid sequence of human *BMPR1B* across different species. Methionine *(M)* within the box with *arrow* indicates conserved residue across different species. UTR, Untranslated region; SP, signal peptide; LBD, ligand-binding domain; TM, transmembrane domain; GS, glycine-serine rich-box; KD, kinase domain; Met, Methionine; Arg, Arginine [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1

Clinical description of the two affected individuals (V-1 and V-4)

Clinical examination	V-l	V-4	Normal population
Status	Affected	Affected	Normal
Age	30	24	49
Married	Yes	No	Yes
Sex	Male	Male	Male
Total height (cm)	141	140	165–175
Head circumference (cm)	53	53	53–54
Total arm length (cm)	46	38	65–69
Total humerus length (cm)	25	17	31–33
Length of radius/ulna (cm)	21	21	24–26
Radii bowing	Yes	Yes	No
Wrist size (cm)	14	16.5	13–15
Total leg length (cm)	71	60	83–93
Length of femur (cm)	36	31	42–47
Length of tibia/fibula (cm)	36	29	41–46
Tibia/fibula bowing	No	Yes	No

TABLE 2

List of mutations reported in the *BMPR1B* gene resulting in acromesomelic dysplasia phenotypes

S. No	Amino acid change	Nucleotide change	Mutation type	Reported phenotype	Reference
1	p.Arg31Cys	c.91C> T	Missense	Chondrodysplasia, DuPan type	Stange etal. (2015)
2	p.Cys53Arg	c.157T> C	Missense	Acromesomelic chondrodysplasia, Grebe type	Graul-Neumann et al. (2014)
3	p.Trp219Term	c.657G > A	Stop codon	Acromesomelic chondrodysplasia, Grebe type	Graul-Neumann et al., 2014
4		c.361_368delGGACCTAT	Small deletions	Acromesomelic chondrodysplasia, genital anomalies	Demirhan et al., 2005
5	p.Met397Arg	c.1190T> G	Missense	Acromesomelic dysplasia Hunter-Thompson type	Present study