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Clinical research

A novel *CHSY1* gene mutation underlies Temtamy preaxial brachydactyly syndrome in a Pakistani family

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ABSTRACT

Temtamy preaxial brachydactyly syndrome (TPBS) is an autosomal recessive rare disorder characterized by hyperphalangism of digits, facial dysmorphism, dental anomalies, sensorineural hearing loss, delayed motor and mental development, and growth retardation. Loss of function mutations have been recently reported in the *CHSY1* gene to cause the TPBS. Here, we report a novel missense mutation (c.1897 G > A) in the *CHSY1* gene in two TPBS patients from a consanguineous Pakistani family. The mutation predicted substitution of a highly conserved aspartate amino acid residue to asparagine at position 633 in the protein (D633N). Polyphen analysis supported the pathogenicity of D36N mutation. Our finding extends the body of recent evidence that supports the role of CHSY1 as a potential mediator of BMP signaling.

1. Background

Brachydactyly is a category of hand malformation defined by short finger or toe produced by absent or short metacarpus or metatarsus and/or phalanges. There are many different forms of brachydactyly that have been identified so far and were included as one of the dysostosis groups affecting the limbs in the latest international nosology and classification of genetic skeletal dysplasias. Brachydactyly can occur either as an isolated malformation or as a part of a complex malformation syndrome. Eleven phenotypes of isolated brachydactyly have been identified with minimal degrees of phenotypic overlap, while the number of syndromic brachydactyly is extensive. In isolated brachydactyly, the inheritance is mostly autosomal dominant with variable expressivity and penetrance. The genetic defects underlying the majority of isolated brachydactyly and some syndromic forms of brachydactyly have been identified at the molecular level. Mutations in the components of Bone Morphogenetic Protein (BMP) signaling pathway or its modulators have been reported to cause brachydactyly phenotypes. Mutations leading either to loss or to gain of BMP signaling may cause brachydactyly. Mutations in the GDF5 (a BMP ligand) or BMPR1B (GDF5 high-affinity receptor) may cause loss of BMP signaling leading to reduced bone formation and brachydactyly type A2 (BDA2) or brachydactyly type C (BDAC) [1]. Loss of function mutations in Noggin (a BMP inhibitor) may cause gain of BMP signaling leading to symphalangism (SYM1) and/or multiple synostosis syndrome (SYNS1) [2]. Mutations in the components of Hedgehog pathway, *IHH*, *SHH* and *PTHLH* may result in brachydactyly type A-1 (BDA1), triphalangeal thumb–polysyndactyly syndrome and brachydactyly type E (BDE), respectively. Mutations in *HOXD13* may also lead to BDE [3–6].

Loss of function mutations have been recently identified in the *CHSY1* gene on chromosome 15q26-qterm to cause the Temtamy preaxial brachydactyly syndrome (TPBS; MIM 605282) [7,8,20]. TPBS is a rare, autosomal recessive congenital syndrome, mainly characterized by bilateral, symmetric preaxial brachydactyly and hyperphalangism of digits, facial dysmorphism, dental anomalies, sensorineural hearing loss, delayed motor and mental development, and growth retardation [9]. As the causative gene (*CHSY1*) is a potential mediator of BMP signaling pathway, the TPBS phenotype overlaps with that produced by mutations in the BMP, GDF and Hedgehog pathways [1]. The *CHSY1* is ubiquitous, with the highest levels found in placenta and low levels in brain, heart, skeletal muscle, colon, thymus, spleen, kidney, liver, adrenal gland, mammary gland, stomach, small intestine, lung and peripheral blood leukocytes [10,11]. The *CHSY1* gene contains 3 exons and spans more than 40 kb. It encodes chondroitin synthase 1, an evolutionarily conserved sugar-synthesizing enzyme of 802 amino acids. It synthesizes chondroitin sulfate (CS), which is a glycosaminoglycan (GAG) composed of alternating glucuronic acid (GlcUA) and N-acetyl galactosamine (GalNAc) residues [10]. CHSY1 has both the glucuronyltransferase II and N-acetylgalactosaminyl-transferase II

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activities required for the synthesis of the repeating disaccharide units of CS. CHSY1 maintains the balance of sulfated chondroitin proteoglycan, which regulates bone growth and joint formation. Recently it was shown that *chsy1*−/− mutant mice had brachypodism, a particular abnormality in distal phalanges, chondrodysplasia and decreased bone density [12]. Decreased bone density has also been shown in mice null for chondroitin and dermatan sulfate proteoglycans showing that these play important role in skeleton development [13–19].

Until now, ten mutations in the *CHSY1* gene have been reported [7,8,20] in Temtamy preaxial brachydactyly syndrome patients from Egypt, Turkey, Jordan, Sri Lanka and Pakistan (Table 1). In the current study, we describe a novel missense mutation in the *CHSY1* gene in a consanguineous Pakistani family affected with TPBS.

2. Subjects and methods

The patients were from a consanguineous Pakistani family living in a remote village in Khyber Pakhtunkhwa province. The patients and their family members were clinically investigated at the Leady Reading Hospital, Peshawar, Pakistan. Before the start of the study, approval was obtained from the Quaid-i-Azam University institutional review board. In addition, informed consent was obtained from all the family members (III-2, III-3, IV-1, IV-2 and IV-3) who participated in the study.

Venous blood samples were obtained from 5 family members and genomic DNA was extracted from whole blood following a standard protocol. Microsatellite markers tightly linked to the *CHSY1* gene (D15S1515, D15S966 and D15S87) were polymerase chain reaction (PCR) amplified from genomic DNA. The PCR was carried out according to a standard procedure in 50-μl reaction volume containing 100 ng of genomic DNA, 20 pmol of each primer, 200 μM of each deoxynucleoside triphosphate, 2.5 units of Taq DNA polymerase (MBI Fermentas) and 1 × PCR buffer. The thermal cycling conditions used were: 95 °C for 5 min, followed by 40 cycles of 95 °C for 1 min, 53–58 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 10 min. PCR was performed by use of the Palm-Cycler gradient thermal cycler (Corbett Life Science, Australia). The PCR products were resolved on 8% non-denaturing polyacrylamide gel and genotypes were assigned by visual inspection.

To screen for the mutation, each of the exons and adjacent exon/intron boundaries of the *CHSY1* gene were PCR amplified from genomic DNA using primers designed from intronic sequences of the gene. The sequences of the primers are available upon request.

Table 1
Summary of mutations in the *CHSY1* gene reported so far.

	Position	Sequence change	Mutation type	Protein change	Origin	Reference
1.	Exon 1	c.14delG	Deletion	Frameshift	Egypt	(Li et al., 2010)
2.	Exon 1	c.55-84del	Deletion	G19_L28del	Egypt	(Li et al., 2010)
3.	Exon 1	c.205C > T	Nonsense	Q69X	Turkey	(Li et al., 2010)
4.	Exon 1	c.96del	Deletion	Frameshift	Jordan	(Tian et al., 2010)
5.	Intron 1	c.321-3C > G	Splice site	Skipping of exon 2	Sri Lanka	(Li et al., 2010)
6.	Exon 2	c.664G > T	Missense	G222W	Egypt	(Temtamy et al., 2012)
7/8.[a]	Exon 3	c.1075C > T/ c.1763G > A	Missense	P359S/ R588T	Egypt	(Temtamy et al., 2012)
9.	Exon 3	c.1616C > G	Missense	P539R	Pakistan	(Li et al., 2010)
10.	Exon 3	c.2251T > C	Missense	C751R	Egypt	(Temtamy et al., 2012)
11.	Exon 3	c.1897G > A	Missense	D633N	Pakistan	This study

[a] Compound heterozygous.

PCR products were purified using the Gene JET PCR purification kit (Fermentas Life Sciences) and were sequenced in Applied Biosystems automatic sequencer 3730XL, using the BigDye terminator cycle sequencing kit V3.1 (PE Applied Biosystems, Foster City, Calif., USA) following purification by ethanol precipitation. Sequence variants were identified using BioEdit Sequence Alignment Editor version 7.0.5.3.

3. Results

3.1. Clinical features

Clinical features of our patients (Fig. 1) included abnormal faces: round face, mild hypoplasia, wide eyed look, short pointed nose,

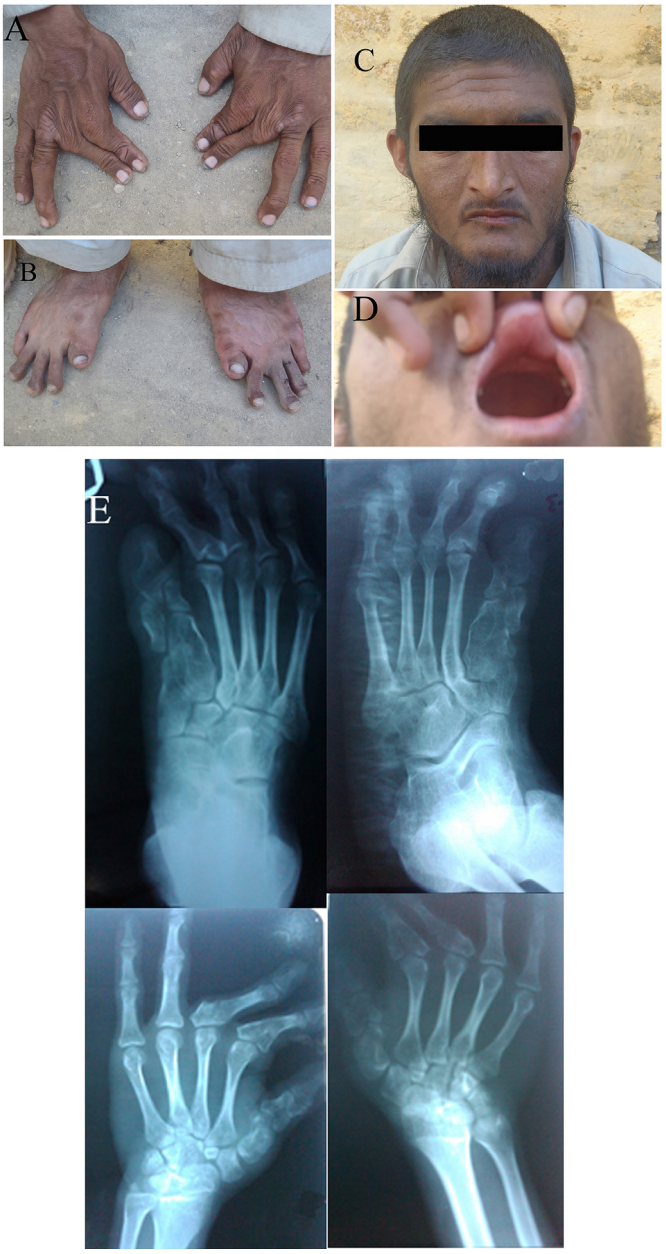

Fig. 1. Clinical presentation of patients with TPBS. (A & B) Bilateral malformations in hands and feet. (C) Facial dysmorphology. (D) Dental anomalies. (E) X-rays showing duplicated metatarsals and proximal phalanges in the big toes and the duplication of proximal phalanges in fingers 1–3 in the patient IV-1.