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## A novel *CHST3* allele associated with Spondyloepiphyseal dysplasia and hearing loss in Pakistani kindred

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### Abstract

Skeletal dysplasias are highly heterogeneous disorders composed of 40 clinical subtypes that are part of 456 well-delineated syndromes in humans. Here, we enrolled consanguineous kindred from a remote area of Sindh province of Pakistan, with fourteen affected individuals suffering with short stature, kyphoscoliosis, joint dislocations, clubfoot, heart valve anomalies and progressive bilateral mixed hearing loss. To identify pathogenic variants in this family, whole exome sequencing (WES) was performed in one affected and one normal individual, which revealed a novel transversion mutation (c.802G>T; p.Glu268\*) in *CHST3* associated with the phenotype. *CHST3* encodes a chondroitin 6-O-sulfotransferase-1 (C6ST-1) enzyme that is essential for the sulfation of proteoglycans found in cartilages. Previously, mutations in *CHST3* have largely been reported in sporadic cases of skeletal dysplasia, primarily with severe spinal abnormalities, joint dislocations, joint contractures, and clubfoot. Clinical and radiological examination of the affected individuals in this family provides new insights into phenotypic spectrum of *CHST3* alleles and disease progression with age.

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Conflict of Interest:

No conflict to declare.

### Web Resources

1000 Genomes: <http://www.1000genomes.org> NHLBI\_EPS: <http://evs.gs.washington.edu/EVS/> ExAC: <http://exac.broadinstitute.org>

## Introduction

Genetic disorders that result in deficits of skeletal systems are collectively termed as skeletal dysplasias (SDs) or Osteochondrodysplasias. Their prevalence has been estimated to be 1:5000 (1). However, in children with congenital birth defects, SD prevalence is approximately 5% (2). SD has been classified into 40 sub-groups primarily on the basis of clinical, molecular, biochemical and radiological findings (3). In some cases, SD leads to lethality due to small chest, pulmonary hypoplasia, and respiratory problems (4).

Genetically, SD can manifest under a variety of inheritance models including autosomal dominant or recessive, and X-linked dominant or recessive (2, 3). Hundreds of genes have been found that subserve a variety of functions in bone development, mineralization and growth in humans. Deciphering the genetic causes of SDs often help in comprehending basic bone biology, disease prognosis and treatment options. For instance, in some cases of monogenic SDs, novel targeted therapies including Cathepsin K inhibitors and anti-sclerostin monoclonal antibodies are now in late phases of clinical trials (5–7).

Here, we describe a six-generation Pakistani family (LUAB01) with autosomal recessively inherited congenital skeletal dysplasia and hearing loss. Whole exome sequencing (WES) revealed a nonsense (p.Glu268\*) mutation in the *CHST3* (OMIM 603799) gene segregating with the phenotype. C6ST-1 enzyme, encoded by *CHST3*, belongs to the carbohydrate sulfotransferase family of fifteen enzymes that is involved in the transfer of sulfate groups to carbohydrates in glycoprotein and glycolipids. C6ST-1 catalyzes sulfation of chondroitin containing proteoglycan, an essential constituent of connective tissues (8). Previously, mutations in *CHST3* have been associated with a rare phenotype of skeleton dysplasia, known as Spondyloepiphyseal dysplasia with congenital joint dislocations (OMIM 143095) (9, 10). The phenotypic presentation of the disease varies among ethnically and geographically divergent populations (9–12). Common features include large joint contractures, progressive kyphosis, clubfoot, and dislocation of hip joints (9–12). Hearing loss incidence in patients with *CHST3* mutations has been anecdotal. Intriguingly, all the affected individuals of the family LUAB01 have bilateral symmetric mild-to-severe hearing impairment and thus provided statistically significant evidence of linkage of hearing loss phenotype with the *CHST3* allele.

## Subject and Methods

### Enrollment and clinical evaluation

This study was approved by the Ethical Review Committees of the participating institutes. After obtaining written informed consent, blood samples were collected from the participating members of the family LUBA01. Anthropological measurements and earlier developmental milestone were recorded. Detailed clinical investigations including X-rays, echocardiograms, and audiometry were performed on available affected individuals.

### Whole exome sequencing (WES)

WES was performed using genomic DNA samples from one affected and one normal individual and NimbleGen SeqCap EX Exome v2.0 kit (Roche Diagnostics, San Francisco,

CA). Sequencing data analysis, alignment, variants calling, and filtration were performed as described previously (13).

### Sanger sequencing and LOD score calculation

Sequencing reaction was carried out by using a Big Dye Terminator v3.1 (Applied Biosystems) as described previously (14). To confirm the association with the phenotype, two point LOD score for the *CHST3* variant was calculated with Superlink by EasyLinkage 5.02 v GUI (15). An autosomal recessive inheritance and a disease allele frequency of 0.001 were used for linkage analysis.

### Molecular modeling

Molecular modeling for *CHST3* was performed using Phyre2 (16) with the Sulfotransferase domain from the Curacin biosynthetic pathway as the template (Protein Data Bank (PDB), 4GBM).

## Results

### Clinical findings

We enrolled a six-generation family (LUAB01) from Sindh province of Pakistan with fourteen individuals in ten sibships who were affected with recessively inherited congenital skeletal dysplasia (Fig. 1a). The neonatal clinical records of the affected individuals were not available at the time of enrollment. According to the family history, the affected individuals exhibited restricted movement at the elbow and knee joints at birth and progressively developed kyphoscoliosis and joint contractures in adulthood (Fig. 1b). The mean age of the patients at the time of enrollment was 13.4 years ranging from 5 to 25 years (Table 1). All affected family members were of short stature for age (Table 1). The mean height of the four adult males examined was  $84 \pm 2.27$  cm (SEM). Examination of the 5 pre-pubertal male children indicated that stature was significantly greater in childhood (mean height  $101.2 \pm 1.16$  cm;  $p$ -value 0.0016). This suggests that the short stature phenotype is progressive with age.

### Radiological findings

X-ray analysis of available affected individuals (Fig. 2a–f) revealed kyphosis and scoliosis along with bilateral fixed flexion deformities at knee, elbow, hip and shoulder joints. Vertebrae were fused, and distinct widening between T12 and L1 was observed (Fig. 2). Ribs were flattened and distorted (Fig. 2a–b). Femoral head was hypoplastic and femoral neck was short. Epiphysis (under-mineralization) was found in upper tibial and femoral bones. Hip joints were dislocated and dysplastic.

### Audiometry

Six individuals (VI:5, VI:8, VI:6, V:16, V:3 and V:10), underwent pure tone air and bone conduction audiometry (Fig. 2g). All of them demonstrated bilateral symmetric mild-to-severe hearing impairment (Fig. 2g). Additionally, three (VI:5, V:16 and V:10) of the six individuals tested had mixed hearing impairment (Fig. 2g). Median threshold values from all

audiograms indicated moderate sensorineural hearing impairment at 2–4 kHz and moderate mixed hearing loss at the low frequencies (Fig. 2g). These results indicated that inner ear function at all frequencies was affected, with greater loss of function at low frequencies (Fig. 2g; Table 1). We did not observe any obvious correlation between age and hearing thresholds (Fig. 2g).

### Echocardiograms

Echocardiography in seven affected individuals revealed multiple heart valve anomalies (Table 1). Mitral valve regurgitation and tricuspid valve regurgitation was observed in all patients. In addition, mild mitral stenosis, mild mitral prolapse and mild tricuspid prolapse were noticed in four patients (Table 1). Mild to moderate aortic regurgitation was found in two patients and one patient had bicuspid aortic valve (Table 1).

### Molecular Analysis

WES followed by segregation analysis through Sanger sequencing (Supplementary Table 1) revealed a novel homozygous single base substitution (c.802G>T;p.Glu268\*) in *CHST3*, segregating with the skeletal dysplasia phenotype in family LUAB01 (Fig 1a, 1c). This variant is present in an evolutionary conserved region (Fig. 1d) and absent in the 1000 Genomes (see web resource), the NHLBI 6500 exome variants, and the ExAC databases as well as in 140 ethnically matched chromosomes. Parametric linkage analysis was performed using the *CHST3* variant genotype, which resulted in a two-point LOD score of 10.5 at  $\theta=0$ . In the ExAC database, 10 loss-of-function (truncating) alleles are present, of which none are homozygous. The carrier frequency is approximately 1 in 3800, meaning that the likelihood of bi-allelic loss-of-function mutations in *CHST3* is less than 1 in 14 million individuals based on Hardy-Weinberg calculations. The *CHST3* gene consists of two coding exons and encodes a 479 amino acid long chondroitin 6-O-sulfotransferase-1 (C6ST-1) enzyme. The stop-gained mutation is predicted to remove 211 residues from the encoded protein, which would result in truncation of sulfotransferase domain (Fig. 1e).

### Discussion

Mutations in *CHST3* gene have been associated with skeleton dysplasia, primarily among patients with short stature, joint dislocation and kyphoscoliosis (12). Other associated phenotypes include microdontia and cardiac valve anomalies (10, 12). As of August 2015, 30 disease-associated mutations in *CHST3* have been found in 45 patients, including familial cases from Oman and Tanzania, with congenital dislocations and vertebral changes (9, 12, 17).

Here, we described a large Pakistani consanguineous family, LUAB01, segregating *CHST3*-associated skeleton dysplasia and hearing loss. The prominent phenotypic features, consistent with spondyloepiphyseal dysplasia with congenital joint dislocations syndrome, were progressive short stature likely due to progressive kyphoscoliosis and congenital joint contractures including clubfoot and restricted movement at elbow and knee joints (Table 1). The severe adult short stature, likely related to progressive spinal curvature, is worse in this family (average 84 cm) than typically reported (110–130 cm). Notably, children in this

family were on average significantly taller than adults. Within the group of male children (age range 5–11), heights were essentially the same, and as such, older children exhibited further deviation from normative stature than their younger relatives. Thus, there may be a halting of growth that occurs sometime in childhood. However, we do not have access to individual heights across time, thus we will be following these children prospectively to understand whether the growth deficits during childhood are attributable to long bone length, spinal curvature, or endocrinologic etiologies. Importantly, we will determine whether their post-pubertal height is less than their pre-pubertal height, as suggested by comparison to the adult average male height.

The affected individuals also had bilateral mixed hearing loss. Hearing loss incidence in patients with *CHST3* mutations has been anecdotal. One of two Turkish siblings, described previously, had unilateral mixed hearing loss (18). Similarly one of two Somalian half siblings with mutation in *CHST3* had conductive hearing loss (8). However, hearing loss observed in these two individuals was attributed to environmental cause, especially for Somalian proband who had frequent ear infections requiring surgical intervention (8). To date, limited data are available about the association of sensorineural hearing loss with skeleton dysplasia. So far, only one patient with Larsen syndrome (OMIM 150250) has been documented in detail with presentation of mixed hearing loss with dominant sensorineural portion (8, 11, 18, 19). In this study, six affected individuals evaluated through audiometry demonstrated mixed hearing loss. We did not observe any obvious co-relation of age with hearing loss severity segregating in family LUAB01 (Fig. 2g). The conductive portion of the hearing loss may be due to ossicular malformation; however, the sensorineural hearing loss requires further exploration. In summary, our results support a role of *CHST3* in the middle and inner ear functioning. The identification of p.(Glu268\*) nonsense mutation in *CHST3* expands the repertoire of the known genetic causes of hearing loss and the skeletal dysplasia phenotypic spectrum of *CHST3* alleles.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

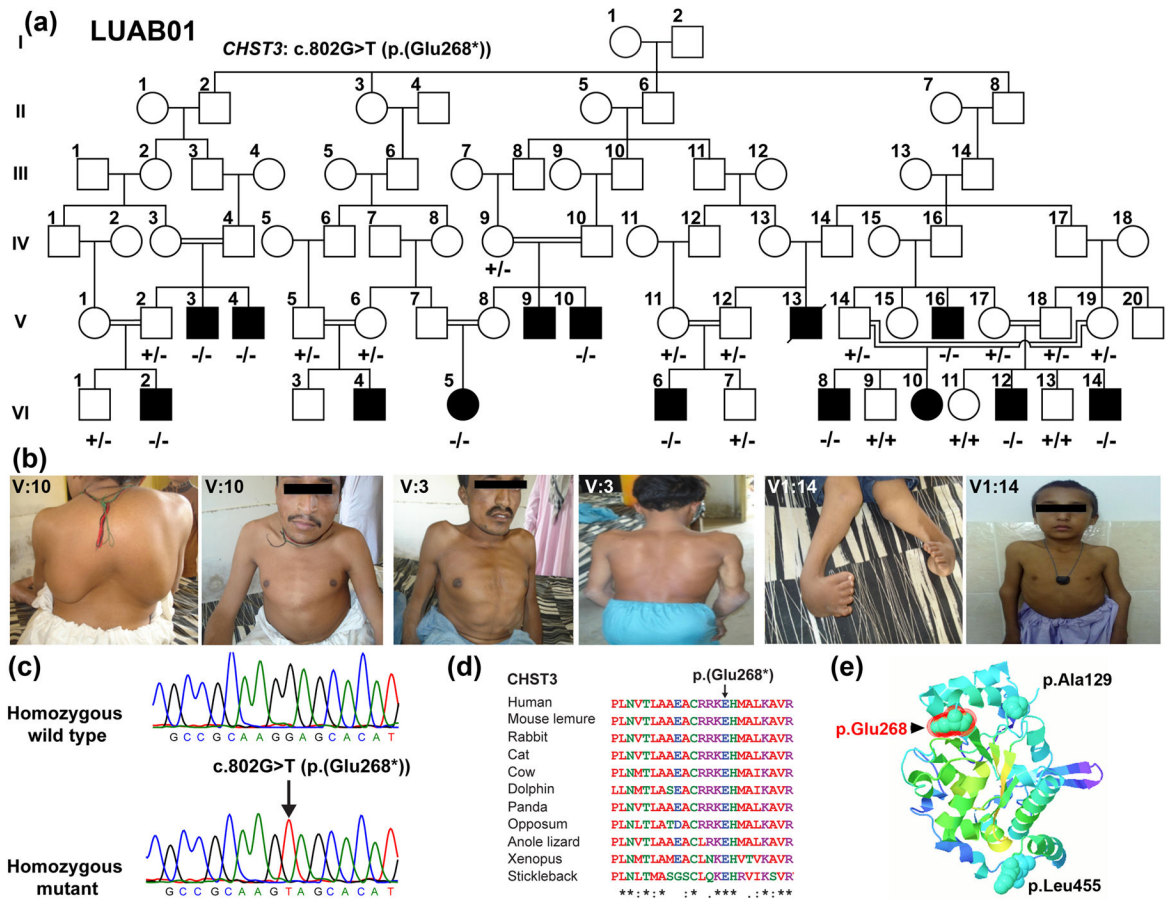
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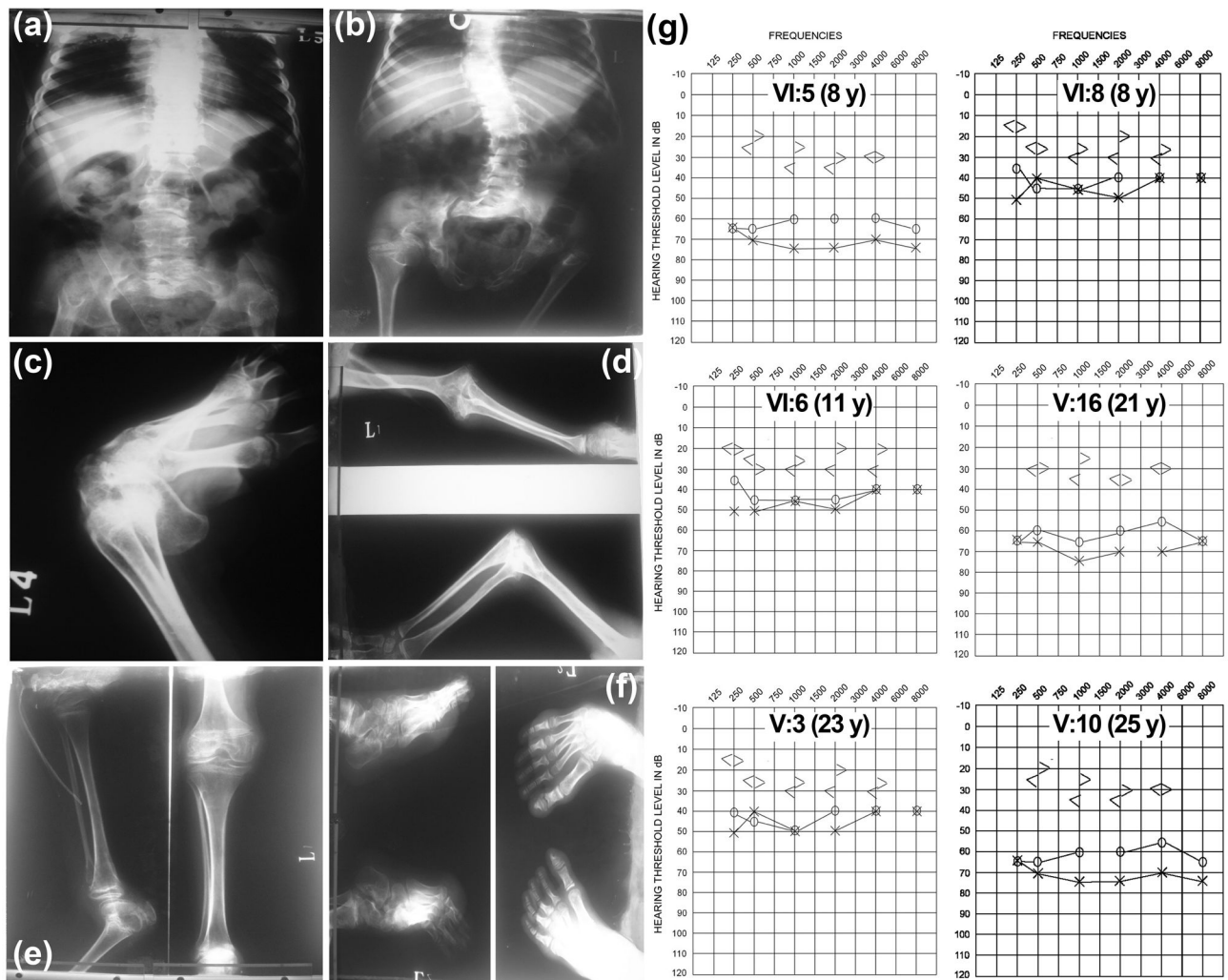
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**Fig. 1.** Congenital skeletal dysplasia and hearing loss phenotypes in family LUAB01 co-segregate *CHST3* mutation. **(a)** Family LUAB01 pedigree. Filled and empty symbols represent affected and unaffected individuals, respectively. A double line uniting two individuals represents consanguineous marriage. Genotypes of all the participating individuals are shown. **(b)** Photographs of individuals homozygous for *CHST3* allele. **(c)** Wild type and homozygous mutant nucleotide sequence chromatograms of exon 2 of *CHST3* illustrating homozygosity for the c.802G>T (p.Glu268\*) mutation. **(d)** ClustalW multiple sequence alignment of the 23 amino acids of *CHST3* shows that p.Glu268 and downstream residues are conserved across species. Amino acids are numbered with reference to GenBank Accession number NP\_004264.2. **(e)** A homology model of the sulfotransferase domain of *CHST3* constructed using Phyre2 and c4gbmA as a template. The location of p.Glu268 residue (red) is shown along with the terminal amino-acids.



**Fig. 2.** X-Ray and audiometric findings of affected individuals of LUBA01. **(a)** Patient V: 10 and **(b)** patient V: 3 imaging revealed severe scoliosis, fused vertebra, Coxa vara and dislocated hips. **(c–d)** Patient V: 3 imaging revealed clubfoot, Pes cavus, equinus and ankylosed elbow. **(e–f)** Patient VI: 4 had sub-luxated knee and clubfoot. **(g)** Representative air and bone conduction audiograms of patients with *CHST3* mutation.



**Table 1**

Clinical findings of affected members of LUAB01

ID	Age	Sex	Height (cm)	Height (SD)*	Weight (kg)	Weight (SD)*	Audiological findings	Radiological Findings	Echo cardiograms
V:10	25	M	80	---	30	---	Mild to moderate	Severe scoliosis, kyphosis, fused vertebra, coxa vara and dislocated hips, club foot	Mitral valve and tricuspid valve regurgitation
V:3	23	M	81	---	29	---	Mild to moderate	Scoliosis, fused vertebra, coxa vara and dislocated hips	Mitral valve and tricuspid valve regurgitation
V:16	21	M	85	---	28	---	Mild to moderate	Scoliosis, kyphosis, fused vertebra, dislocated hips, Club foot	Mitral valve and tricuspid valve regurgitation, mild mitral stenosis and prolapse, mild tricuspid prolapse
V:4	20	M	90	---	29	---	Not available	Not available	Not available
VI:6	11	M	105	-5.98	27	-1.49	Mild to moderate	Kyphosis, fused vertebra, coxa vara and dislocated hips, club foot	Mitral valve and tricuspid valve regurgitation
VI:5	8	F	99	-4.65	20	-1.43	Mild to moderate	Kyphosis, pes cavus, equinus hip and ankylosed elbow, dislocated hips, club foot	Mitral valve and tricuspid valve regurgitation, mild mitral stenosis and prolapse, mild tricuspid prolapse
VI:8	8	M	101	-4.88	23.5	-0.61	Mild to moderate	Kyphosis, fused vertebra, coxa vara and dislocated hips, club foot	Mitral valve and tricuspid valve regurgitation, mild mitral stenosis and prolapse, mild tricuspid prolapse
VI:12	7	M	102	-3.75	22	-0.36	Not available	Not available	Not available
VI:2	6	M	98	-3.55	18.5	-0.97	Not available	Not available	Not available
VI:14	5	M	100	-2.07	19	0.16	Not available	Kyphosis, coxa vara and dislocated hips, Club foot	Mitral valve and tricuspid valve regurgitation, mild mitral stenosis and prolapse, mild tricuspid prolapse

\* Based on Growth Calculator 2.01 (<http://growthcalc.chip.org/GrowthCalc> (ages 0–20)).