

Novel *HSPG2* mutations causing Schwartz-Jampel syndrome type 1 in a Chinese family: A case report

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Abstract. Schwartz-Jampel syndrome type 1 (SJS1) is a rare autosomal recessive disease caused by mutations in the gene heparan sulfate proteoglycan 2 (*HSPG2*; also known as basement membrane-specific heparin sulfate). In the present study, a 10-year-old female SJS1 proband from a Chinese family, who was diagnosed by X-ray and physical examination, was recruited. The key clinical features of the patient with SJS1 included short stature, joint contractures, pigeon breast, and myotonia that led to progressive stiffness of the face and limbs; barely discernible kyphosis was also noted. Genetic testing using whole exome sequencing and Sanger sequencing was performed for the proband and family members. A total of 2 novel mutations (c.8788G>A; p.Glu2930Lys and c.11671+5G>A) in the *HSPG2* gene were identified in the proband. The family members harboring 1 heterozygous mutation in *HSPG2* did not exhibit any skeletal abnormalities. The results of the present study suggested that the compound heterozygous mutations in *HSPG2* may be responsible for the induction of SJS1, and demonstrated the genotype-phenotype associations between mutations in the *HSPG2* gene and clinical characteristics of SJS1.

Introduction

Schwartz-Jampel syndrome type 1 (SJS1; Online Mendelian Inheritance in Man #255800) is a type of autosomal recessive skeletal dysplasia characterized by permanent myotonic

myopathy and skeletal dysplasia, which result in short stature, dystrophy of epiphyseal cartilages, joint contractures, blepharophimosis, unusual pinnae, myopia and pigeon breast. In addition, myotonic (leading to progressive stiffness of the face and limbs), persistent bowing of the limbs, and severe kyphoscoliosis may develop. Electromyograms exhibit continuous discharges at rest (1-3). Furthermore, walking becomes increasingly more difficult and secondary contractures of the large joints may develop in patients with SJS1; by late adolescence, severely affected patients may be confined to a bed and a wheelchair (2). Adult height varies from 140 to 170 cm, and ~25% of patients with SJS1 present with mental retardation (4,5). In addition, procainamide therapy has been shown to help muscle function (6).

SJS1 is caused by mutations in heparan sulfate proteoglycan 2 (*HSPG2*; also known as basement membrane-specific heparin sulfate). The *HSPG2* gene encodes the perlecan protein, a ubiquitous HSP, which serves essential roles in multiple biological activities, including forming the vascular extracellular matrix, maintaining endothelial barrier function, promoting growth factor and being a potent inhibitor of smooth muscle cell proliferation (7,8). Alternative splicing of this gene results in multiple transcript variants, which may cause SJS1, Silverman-Handmaker type dyssegmental dysplasia, and tardive dyskinesia (9).

In the present study, one SJS1 proband from a Chinese family, who was diagnosed by X-ray and physical examination, was recruited and genetic sequencing was performed. Our study suggested that the compound heterozygous mutations in *HSPG2* were responsible for SJS1 and demonstrated the genotype-phenotype relationship between mutations in the *HSPG2* gene and characteristics of SJS1.

Case report

The Ethics Committee of Nanjing Drum Tower Hospital (Jiangsu, China) approved the present study. Written informed consent was obtained from all subjects or their parents/guardians. The 10-year-old female proband was recruited to the present study for genetic testing between December 2016 and June 2017. The proband was the only child born to healthy parents of Chinese Han descent; there was no

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SJS1 or other skeletal diseases in the proband's family history. Upon physical examination of the proband, a peculiar facial appearance with pursed lips was noted. A small mouth, blepharophimosis, unusual pinnae and myopia were not presented (Fig. 1A); however, severe kyphoscoliosis, and progressive stiffness of the face, limbs and hands were observed in the proband (Fig. 1B-D). In addition, the patient did not exhibit anterior hypoplasia of cervical bodies with cervical kyphosis. At the age of 2, the proband exhibited hypotonia with poor muscle bulk and proximal leg weakness. Myotonic features, which resulted in progressive stiffness, were noticed during the proband's early childhood. At the age of 8, the proband began to suffer from contractures of the large joints, including the elbow, knee and hip, and walking became difficult; a lordotic gait was also present at this time. The results of neurological and other examinations were within the normal range, and the proband's height was 85, 3.5 cm below than the average height of Han Chinese girls of the same age.

X-ray imaging performed when the patient was 10 years of age revealed micrognathia and a narrow upper thoracic inlet (Fig. 2). No flattened vertebral bodies with regular end-plates were noted (Fig. 2). There was significant kyphoscoliosis, progression of epiphyseal changes and generalized osteopenia. Bowed femora and large round capital femoral epiphyses were also observed (Fig. 2).

These radiographic results of the patient were similar to those described in previous reports of SJS1 (1,10,11); however, due to the clinical and radiographic similarities of skeletal abnormalities, patients with other skeletal diseases, such as Stuve-Wiedemann syndrome or focal femoral hypoplasia-unusual facies syndrome, may be misdiagnosed with SJS1. Therefore, the present study performed molecular analysis of the patient and parents.

Once written informed consent was obtained, genomic DNA was extracted from the peripheral blood of the patient and family members using a Qiagen DNA Mini kit (Qiagen GmbH, Hilden, Germany). Whole exome sequencing (WES) of the proband was conducted. Briefly, genomic DNA was divided into smaller fragments of 200-250 bp using an ultrasonic instrument (Covaris LE220; Covaris, Inc., Woburn, MA, USA). Subsequently, purification with Ampure Beads (Beckman Coulter, Inc., Brea, CA, USA) was performed to add poly A/joint reaction to the end of the purified DNA fragments. The gene-trapping chip (Roche NimbleGen, Madison, WI, USA) was hybridized with the purified DNA fragments of the proband. Following hybridization, captured DNA was sequenced with an Illumina HiSeq2500 Analyzer (Illumina, Inc., San Diego, CA, USA) and read using Illumina Pipeline software (version 1.3.4; Illumina, Inc.). The present study used BWA v0.59 (12) to align sequence reads to the human genome reference (build 37) and removed duplicated reads from subsequent analyses. Sequence variants were identified via comparisons with the NCBI reference sequence NM 005529.5 and annotated by the current version of ANNOVAR (2017 Jul 16; annovar.openbioinformatics.org/en/latest/). The 20X coverage for the RefSeq coding region was 98.31% (Fig. 3).

A novel missense mutation (c.8788 G>A; p.Glu2930Lys) in exon 67 of *HSPG2* and a mutation in intron 85 (c.11671+5G>A) the splicing region, were detected in the proband (Fig. 4A). One guanine ribonucleotide was altered to an adenine ribonucleotide

in codon 8788, which caused a change in the reading frame from glutamine to lysine. The Polyphen-2 tool (13) predicted the mutation as likely to cause damage to the *HSPG2* protein function (Table I). To the best of our knowledge, the mutations c.8788G>A and c.11671+5G>A have not been reported in SJS1 previously. The mutation c.8788G>A; p.Glu2930Lys was located in the domain Immunoglobulin_2 in *HSPG2*, while the mutation c.11671+5G>A was present in the splicing region near the Epidermal Growth Factor Calcium-binding domain. These 2 mutations may result in the loss-of-function of *HSPG2*. In the 1,000 Genomes database (www.international-genome.org), dbSNP database (www.ncbi.nlm.nih.gov/SNP/), Exome Sequencing Project 6500 database (evs.gs.washington.edu/EVS/) and Exome Aggregation Consortium EAS database (exac.broadinstitute.org), the allele frequencies of the c.8788G>A mutation were 0, 0, 0 and 0.000155, respectively, while the allele frequencies of c.11671+5 G> A mutation were 0, 0, 0.000077 and 0.01684, respectively (Table I).

In the present study, Sanger sequencing was also performed for the proband's family, which confirmed the compound heterozygous mutation in the proband and the heterozygous status of the father and mother (Fig. 4A); there was no evidence of SJS1 or of other skeletal diseases in the proband's parents. Furthermore, the mutations c.8788G>A; p.Glu2930Lys and c.11671+5G>A were highly conserved among a diverse range of species (Fig. 4B). Thus, the present study identified 2 novel *HSPG2* mutations in a child case of SJS1.

Discussion

SJS1 is a rare, autosomal recessive progressive disorder that is characterized by clinical features including short stature, myotonic myopathy, dystrophy of epiphyseal cartilages, joint contractures, blepharophimosis, unusual pinnae, myopia, pigeon breast, and progressive stiffness of the face and limbs (14-16). This disorder can be further divided into the subtypes: SJS1A, a milder phenotype with an onset during infancy to early childhood and relatively milder chondrodysplasia; and SJS1B, a more severe phenotype with neonatal onset and significant chondrodysplasia (17). Mask-like facies typically manifest as blepharophimosis, with a pursed mouth and a fixed face with a sad appearance. Chondrodysplasia consists of metaphyseal widening, slender diaphysis and kyphoscoliosis (18).

Recessive inheritance is consistent with the loss-of-function nature of the 2 mutations (c.8788 G>A; p.Glu2930Lys, and c.11671+5G>A) and the previously reported *HSPG2* mutations in SJS (14-18). In total, <40 patients with SJS have been reported and no genotype-phenotype correlation is apparent. Although the splicing variation c.11671+5G>A is a non-coding variant, it is highly conserved among diverse species and several splicing-site variants in *HSPG2* have been reported to cause the aberrant splicing in the exon region (14,15,18), which form a considerable proportion of the reported variants. In addition, the splice variation c.11671+5G>A in intron 85 of the *HSPG2* gene could highly affect the 5' splice site, the base 5 nucleotides downstream of intron 85, increasing the likelihood of reducing the content of effective mRNA that codes for the correct protein. Finally, by combining the specific clinical information and genetic evidence, the present study confirmed the diagnosis of SJS1 in the proband.

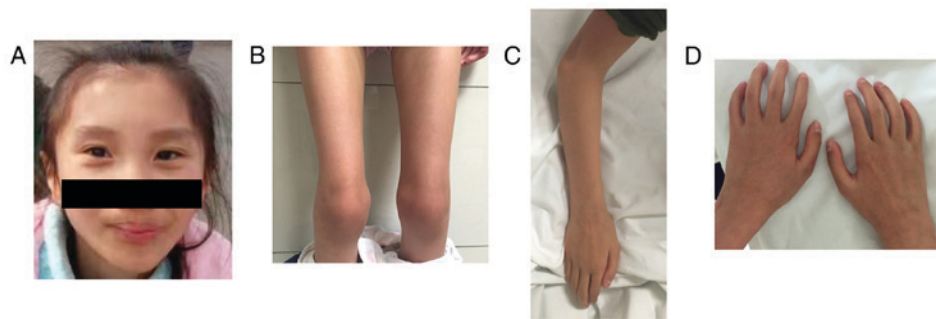


Figure 1. Clinical features of the patient with Schwartz-Jampel syndrome type 1. (A) Peculiar facial appearance with pursed lips and blepharophimosis. (B) Bowed legs. (C) Hand of patient at age 10. The interphalangeal joints exhibit contracture. (D) Progressive stiffness and joint contractures were presented.

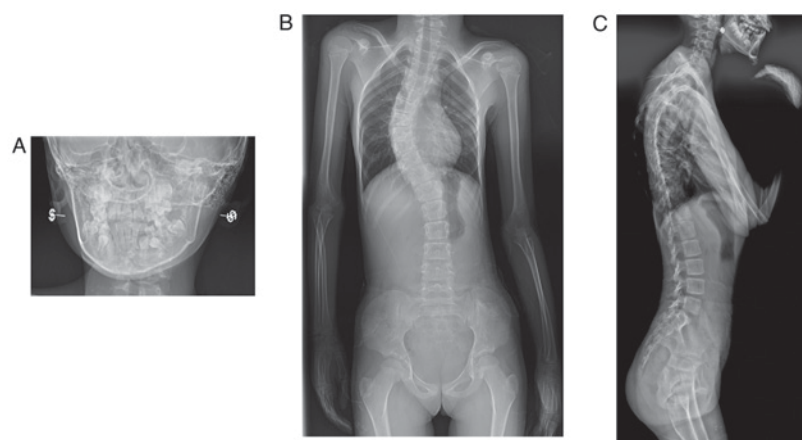


Figure 2. Radiographic features of the 10-year-old female patient (the proband) with Schwartz-Jampel syndrome type 1. (A) Micrognathia, and the (B and C) Pectus and Pelvis. A narrow upper thoracic inlet was noted, as well as severe kyphoscoliosis, bowing of the long bones, epiphyseal, metaphyseal and hip dysplasia. In addition, a bowed femora and large round capital femoral epiphyses were observed.

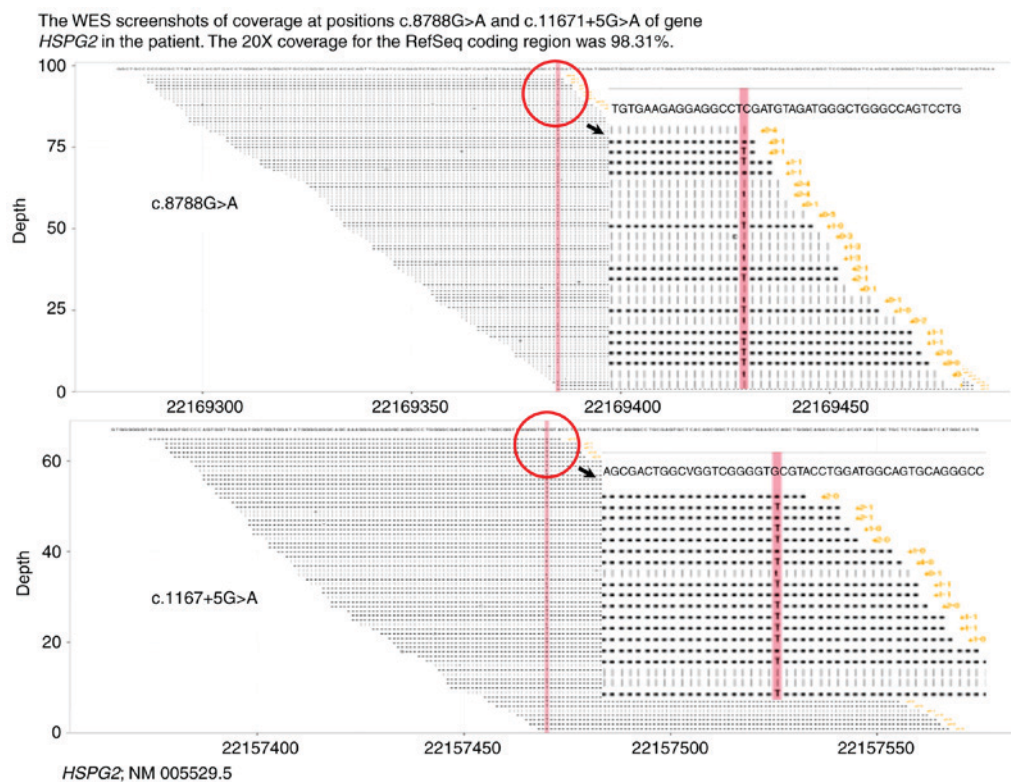
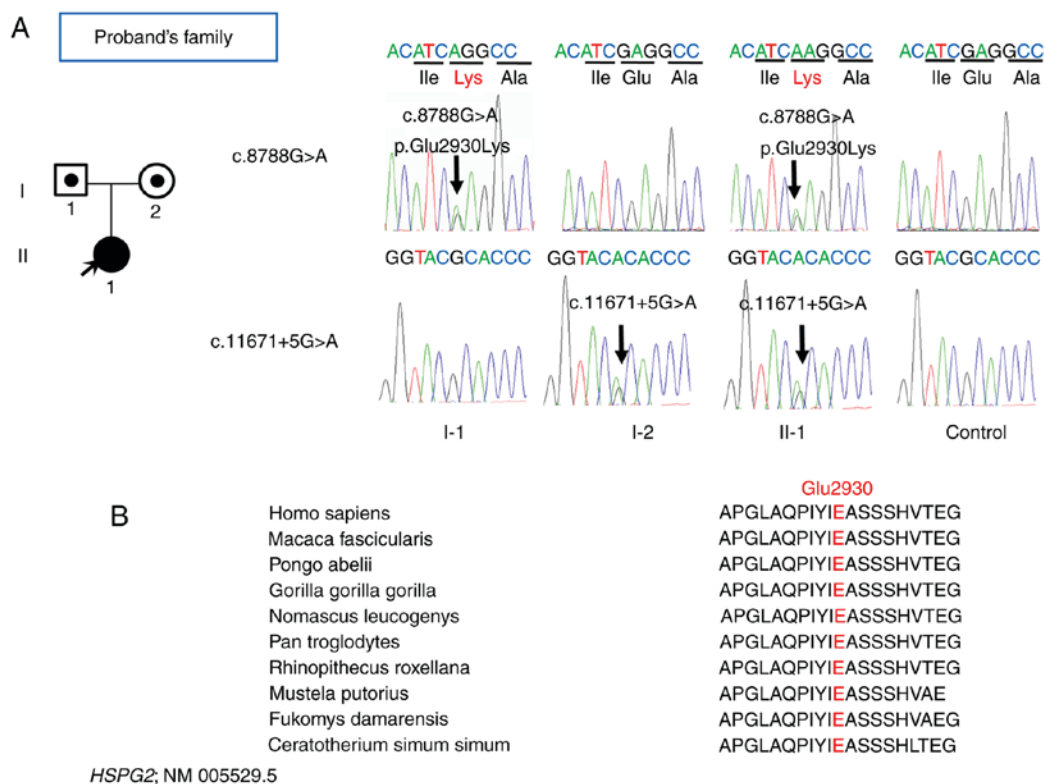


Figure 3. The WES screenshots of coverage at positions c.8788G>A and c.11671+5G>A of gene *HSPG2* in the patient. The 20X coverage for the RefSeq coding region was 98.31%.

Variable	c.11671+5G>A mutation	c.8788G>A mutation
Chromosome	1	1
Position	22,157,470	22,169,385
Predicted protein variants	-	p.Glu2930Lys
Variants type	Het	Het
Gene region	Splicing	Exon
Ref Seq	NM_005529.5	NM_005529.5
SNP ID	rs77527456	rs368020528
1000g_chbs	-	-
dbSNP	0	-
ExAC_EAS	0.01684	0
ESP6500	0.000077	0.000155
Polyphen	-	‘Probably damaging’

1000g_chbs, the allele frequencies included in the 1,000-genome project database; dbSNP, the allele frequencies included in the dbSNP database; ExAc_EAS, the allele frequencies in East Asian included in the Exome Aggregation Consortium EAS database; ESP6500, Exome Sequencing Project 6500; Het, heterozygous mutation; '-', the database is not covered; Polyphen, Polyphen database prediction results.



The present study utilized WES, which allows molecular results to be obtained faster, particularly when traditional specific diagnosis takes a longer time to sequence a large gene. *HSPG2* is a very large gene, consisting of 97 exons, and encodes for the protein Perlecan, which is known to serve an

important role in maintaining cartilaginous tissue integrity and regulating muscle excitability (2). Exome sequencing provides the ability to identify rare variants, analyze the candidate gene and to check for the presence of mutations in the genes at the same time (19,20). In the present study, the *HSPG2* mutation

spectrum was further expanded, thus contributing to the earlier detection of the disease, which may provide significant benefit to the patient and their family. In addition, it may increase awareness of the growing number of SJS1 patients for future clinical diagnosis.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JD, XX, DS, DC, ZX and QJ performed the physical examination and diagnosis of the patients. WY, XH and HT analyzed and interpreted the WES data, and were major contributors to writing and revising the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Ethics Committee of Nanjing Drum Tower Hospital (Jiangsu, China) approved the present study. Written informed consent was obtained from all subjects or their parents/guardians.

Consent for publication

Written informed consent was obtained from the proband and their parents for the publication of the data and images presented in the present study.

Competing interests

The authors declare that they have no competing interests.

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