Spondyloepimetaphysial Dysplasia with Joint Laxity in Three Siblings with *B3GALT6* Mutations

Pamela Trejo^a Frank Rauch^a Francis H. Glorieux^a Jean Ouellet^a
Thierry Benaroch^a Philippe M. Campeau^{a, b}

^aShriners Hospital for Children and McGill University, and ^bDivision of Medical Genetics, Department of Pediatrics, CHU Sainte-Justine Hospital, University of Montreal, Montreal, QC, Canada

Keywords

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Abstract

Spondyloepimetaphyseal dysplasia with joint laxity type 1 (SEMDJL1) is a rare entity with a recessive inheritance. In this report, we describe 3 affected members of the same family who present with short stature, hyperlaxity with secondary spinal malalignment, ulnar subluxation, developmental dysplasia of the hips, and craniofacial alterations; one member also had learning difficulties. DNA analysis showed compound heterozygous variants in the *B3GALT6* gene (c.901_921dup, c.511C>T) in all 3 patients, inherited from the parents. This family demonstrates the clinical variability of SEMDJL1.

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Skeletal dysplasias comprise a vast group of more than 400 diseases characterized by abnormalities of the skeleton during development and growth [Bonafe et al., 2015]. The spondyloepimetaphyseal dysplasias (SEMD) are characterized by abnormal growth of vertebral bodies,

epiphyses and metaphysis [Beighton and Kozlowski, 1980], and this group includes a condition with joint laxity with or without fractures. Type 1 (SEMDJL1; OMIM 271640), which has an autosomal recessive inheritance, is caused by homozygous or compound heterozygous mutations in the *B3GALT6* gene (OMIM 615291) in chromosome 1p36 [Malfait et al., 2013; Nakajima et al., 2013], and type 2 (SEMDJL2; OMIM 603546), with autosomal dominant inheritance, is caused by heterozygous mutations in the *KIF22* gene (OMIM 603213) in chromosome 16p11 [Boyden et al., 2011; Min et al., 2011].

Here, we report that several individuals in a single family affected with SEMDJL1 have compound heterozygous mutations identified in the *B3GALT6* gene.

Patients and Methods

Patients

All patients described were followed at the Shriners Hospital for Children and the CHU Sainte-Justine Hospital, in Montreal. Clinical details were obtained by retrospective chart review.

Genetic Analyses

For the *FLNB* gene, Sanger sequencing was done at Connective Tissue Gene Tests. An aCGH was performed for chromosomal analyses using a PerkinElmer CGX-HD™ 4x180K array analyzed

Fig. 1. Pedigree of the reported family. The father is of Hispanic origin (Guatemala) and the mother is Caucasian (Ireland/Holland). P, proband.

on an Agilent Bioanalyzer at the CHU Sainte-Justine Hospital. In order to sequence the glycosaminoglycan biosynthesis genes, for cost efficiency, we requested a predesigned next-generation sequencing panel for 161 skeletal dysplasia genes at Fulgent Diagnostics as well as the *B3GAT3* gene sequencing, given our clinical suspicion. Furthermore, we added an analysis for deletion and duplication of these genes by coverage analysis.

Measures

For assessing lumbar spine areal bone mineral density (BMD), dual-energy X-ray absorptiometry was performed at the lumbar spine (L1–L4) in the anteroposterior direction using a Hologic QDR Discovery device (Hologic Inc, Waltham, MA, USA). The results were expressed by age and gender matched Z-scores [Zemel et al., 2011; Kalkwarf et al., 2013].

BMD was also assessed by peripheral quantitative computed tomography of the radius at the metaphysis (4% of radius length) and diaphysis (65%) (pQCT; XCT-2000, Stratec Inc., Pforzheim, Germany) [for reference data, see Rauch and Schoenau, 2005, 2008].

Case Report

Proband (IV-5)

The proband is a female born from nonconsanguineous parents at 40 weeks of gestation with no complications by cesarean section for breech presentation (see Fig. 1). At birth, she presented with marked hyperlaxity, bilateral radioulnar dislocation, depressed nasal bridge, blue sclera, ulnar deviation of the fingers, and bilateral developmental dysplasia of the hips which required surgical correction at 1 year and at 5.3 years of age (Fig. 2; Table 1). Early in life, she developed severe scoliosis requiring spinal instrumentation with a growing device at 4.6 years, when the curvature

Table 1. Comparison of clinical features between the 3 affected individuals

	IV-2	IV-3	IV-5
Joint laxity	+	+	++
Blue sclerae	–	–	+
Radioulnar dislocation	++	++	++
Developmental dysplasia of the hip	+++	++	+++
Scoliosis	++	++	+++
Craniofacial dysmorphism	+	+	+
Pectus carinatum	–	–	+
Ulnar deviation of the fingers	–	–	+
Cognitive impairment	–	–	+
Hearing loss	+	+	–
Fractures	–	–	–
Final height, cm ^a	131.1	131.8	139.7
Final height Z-score	–4.6	–4.5	–3.3

+, mild; ++, moderate; +++, severe; –, not present. ^a Values of final height correspond to an age of 15 years in all 3 patients. Craniofacial dysmorphism in detail: Patients IV-2 and IV-3: depressed and wide nasal bridge and high-arched palate. Patient IV-5: dolicocephaly, depressed nasal bridge, shallow orbits, elongated face, high and narrow palate, mildly prominent maxilla and mandible.

had 110°, and had a final fusion when she was 12 years old (Fig. 2). Regarding her height, at 3 months she measured 57.9 cm (Z-score –0.5), but her growth was restricted with age, and at 15 years of age, she had a final height of 139.7 cm (Z-score –3.3; Table 1).

In terms of BMD, the lumbar spine areal BMD was 0.323 g/cm² at 3.4 years (Z-score –3.3). Trabecular BMD at radius metaphysis (4% site) measured by peripheral quantitative computed tomography was 156 mg/cm³ (Z-score –1.1) at 15 years of age. There was

Fig. 2. X-rays of the pelvis (**A–C**), upper extremities (**D–F**), and spine (**G–I**). **A** Developmental dysplasia of the hips at first evaluation, patient IV-2 at 11 months. **B** Patient IV-3 at 11 months. **C** Proband IV-5 at 1 month. **D** Radioulnar dislocation in patient IV-2 at 21.8 years of age. **E** Patient IV-3 at 21.8 years of age. **F** Proband IV-5 at 13.0 years of age. Progression of scoliosis in proband (IV-5): **G** 25° at 4 months. **H** 95° at 2.6 years. **I** After spinal fusion performed at 4.7 years of age with a residual scoliosis of 35°.

no history of fractures, and laboratory tests showed no alterations. Some difficulties in school because of a mild cognitive impairment were noted for this patient, but she showed no other psychological or social issues.

Individuals IV-2 and IV-3

These 2 older monozygotic female twins were born prematurely at 34 weeks of gestation (Fig. 1). At birth, both presented with severe hyperlaxity, depressed and wide nasal bridge, dolicocephalic skull, high-arched palate, bilateral radioulnar dislocation, and developmental dysplasia of the hips (Table 1).

Due to developmental dysplasia of the hips, patient IV-2 underwent 3 surgical corrections, the last at the age of 8.4 years. Patient IV-3 also needed surgical correction performed at the age of 2.4 years.

During growth they had short stature, and at 15 years of age, they reached a final height of 131.1 cm and 131.8 cm, respectively (Z-score -4.6 and -4.5). Both twins have scoliosis which measured 40 and 30°, respectively, after growth was completed (21 years old). Both patients also manifested dyslexia with no intellectual disability.

Lumbar spine areal BMD at the time they completed their growth (15 years old) was 0.610 g/cm² in patient IV-2 and 0.627

g/cm² in patient IV-3 (Z-score -3.4 and -3.2, respectively). Trabecular BMD at radius metaphysis (4% site) measured by peripheral quantitative computed tomography at 15 years of age was 153 mg/cm³ and 189 mg/cm³ (Z-score -1.2 and 0.1, respectively). Both individuals had no history of fractures, and the laboratory parameters showed no alterations.

Initially, Larsen syndrome was suspected because of joint dislocations, facial features (depressed nasal bridge), and short stature [Larsen et al., 1950; Girisha et al., 2016]. A heterozygous variant in the *FLNB* gene was found for patients IV-2 and IV-3, NM_001164317.1: c.4066G>C transversion in exon 24 (p.Ala1356Pro) reported once in ExAC in the Latino population (rs764743602, MAF 8.64e-05). Because DNA analysis for the proband (IV-5) did not show this change and the fact that it was present in the father (unaffected), it was disregarded as a cause for the phenotype.

On reevaluation, a glycosaminoglycan biosynthesis defect was suspected on a clinical basis, and testing was ordered for genes in this pathway as part of a panel (suspected genes were *B3GAT3*, *B3GALT6*, *KIF22*, and *CHST3*).

Results

DNA analysis for the proband (IV-5) and 2 affected siblings (IV-2 and IV-3) detected 2 heterozygous variants in the *B3GALT6* gene, a p.Lys301_Arg307dup (NM_080605.3:c.901_921dup) which results in a duplication of 7 residues, and a second heterozygous change, p.Arg171Cys (NM_080605.3:c.511C>T). The first variant is absent from ExAC, and the second (rs765337103) has a MAF of 0.0002371 in the Latino population.

DNA analysis of the parents revealed that one of the *B3GALT6* gene variants was inherited from the father (p.Arg171Cys) and the other from the mother (p.Lys301_Arg307dup). For the mutation from the father (p.Arg171Cys), the PhyloP score is 0.955 (predicted to be conserved); the SIFT score is 0.005 and PolyPhen2 HumVar 0.892 (the results are given between 0 and 1, a SIFT score closer to 0 is related to a change that causes damage and closer to 1 is more likely to be benign; the opposite happens with PolyPhen2).

Discussion

We report 3 patients within the same family affected with SEMDJL1 with variable clinical expression caused by heterozygous variants in the *B3GALT6* gene. SEMDJL was described by Beighton et al. [1983, 1984], Beighton and Kozlowski [1980], and Kozlowski and Beighton [1984] as a severe disorder associated with joint laxity, severe kyphoscoliosis, elbow deformities, and short stature.

The 3 patients described within this family developed scoliosis early in life, moderate for patients IV-2 and IV-3 and severe for the proband who required surgical correction at a young age. Non-axial skeletal deformities associated with this disease [Beighton et al., 1984; Bradburn and Hall, 1995; Malfait et al., 2013; Nakajima et al., 2013] were also present in these individuals who had bilateral radio-ulnar dislocation, developmental dysplasia of the hips, and ulnar deviation of the fingers.

None of the patients have bone fragility, none of them had fractures, and the lumbar spine areal BMD Z-scores were below the normal range in the context of short stature; supporting this, the trabecular BMD at the distal radius (4% site) was within the normal range in all individuals.

In terms of non-skeletal manifestations, all patients presented with craniofacial dysmorphisms, and the proband also had a mild cognitive impairment, all of these features having been previously described in patients with SEMDJL1 [Malfait et al., 2013; Nakajima et al., 2013]. Two of the reported individuals had hearing loss; this feature has not been described previously as part of SEMDJL1 caused by *B3GALT6* mutations. Although hearing loss was reported as part of the clinical symptoms in a patient with a homozygous *B3GAT3* mutation, which is also a gene involved in the synthesis of the linker region for glycosaminoglycans side chains to the protein core of the proteoglycans [Jones et al., 2015].

Interestingly, SEMD with joint laxity and spine malalignment was previously described by Bradburn and Hall [1995] in a patient with Guatemalan origin, as is the case of the father reported here, who has a family history of a grandmother with scoliosis, short stature, and limited extension of elbows.

Recently, SEMDJL1 has been associated with alterations in glycosaminoglycan biosynthesis [Malfait et al., 2013; Nakajima et al., 2013; Vorster et al., 2015; Alazami et al., 2016]. The clinical features of our patients are compatible with the phenotype described in patients with SEMDJL1 caused by *B3GALT6* gene mutations, which are short stature, elbow malalignment, hip dislocation, kyphoscoliosis, blue sclera, prominent eyes, and hyperlaxity among others [Malfait et al., 2013; Nakajima et al., 2013; Alazami et al., 2016]. Glycosaminoglycan biosynthesis defects comprise a rapidly expanding group of disorders now including 20 diseases caused by mutations in 16 genes [Sasarman et al., 2016]. The genes were first identified by next-generation sequencing for 5 of these. Clinically, different connective tissues are usually affected, such as the bone and cartilage (short bones, scoliosis), ligaments (joint laxity or dislocations) as well as skin and sclera.

DNA analyses in our family confirmed that one mutation comes from each parent. The variants identified correspond to a heterozygous change in *B3GALT6* p.Lys301_Arg307dup (NM_080605.3:c.901_921dup) which results in a duplication of 7 residues; this change has not been seen in the Broad ExAC database, indicating it is probably not a common benign variant. As for the second heterozygous change identified, p.Arg171Cys (NM_080605:c.511C>T), the allele frequency found in the Broad ExAC database is 0.02% in the Latino population. It is located in the glycosyl transferase family 31 domain; the PhyloP score for this change indicates the site is predicted to be conserved, and furthermore, the SIFT score and PolyPhen2 HumVar also predicted that the change could be damaging. The fact that the physicochemical difference between Arg and Cys is large further supports that it can have a pathogenic effect.

In conclusion, we present 3 cases of SEMDJL1 within a family caused by a *B3GALT6* compound heterozygous

mutation not described before. The variable phenotype even within a family with the same mutation illustrates the clinical variability of this condition.

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Statement of Ethics

Informed consent was obtained prior to genetic analyses and to publish clinical details of this study. The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors declare no conflicts of interest.

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