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A missense mutation, p.V132G, in the X-linked spermine synthase gene (*SMS*) causes Snyder-Robinson syndrome

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Abstract

Snyder-Robinson syndrome (SRS, OMIM 309583) is a rare X-linked syndrome characterized by mental retardation, marfanoid habitus, skeletal defects, osteoporosis and facial asymmetry. Linkage analysis localized the related gene to Xp21.3-p22.12, and a G-to-A transition at point +5 of intron 4 of the spermine synthase gene, which caused truncation of the SMS protein and loss of enzyme activity, was identified in the original family. Here we describe another family with Snyder-Robinson syndrome in two Mexican brothers and a novel mutation (c.496T>G) in the exon 5 of the *SMS* gene confirming its involvement in this rare X-linked mental retardation syndrome.

Keywords

X-linked mental retardation; osteoporosis; Snyder-Robinson syndrome; SMS gene

INTRODUCTION

In 1969, Snyder and Robinson reported a family of eleven males in 4 generations affected by mental retardation, hypotonia, and unsteady gait [Snyder and Robinson, 1969]. In a reevaluation of the family, Arena *et al.* [1992] defined the disorder as a specific X-linked syndrome with mental retardation, a characteristic marfanoid-like habitus, diminished muscle bulk, skeletal changes caused by the osteoporosis, facial asymmetry with a prominent lower lip, nasal voice, high, narrow or cleft palate, and long, thin fingers and toes. They also concluded that carrier females were clinically normal. Linkage analysis localized the related gene to Xp21.3-p22.12 [Arena *et al.*, 1996]. Cason *et al.* [2003] further delineated the clinical features in members of the same family, noting that some affected males had an unsteady gait, a nonspecific movement disorder, and seizures. Additionally, they identified a G-to-A transition at position +5 of the 5' splice site of intron 4 of the spermine synthase gene (*SMS*, HGNC ID:11123). This base change caused truncation of the SMS protein and loss of

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enzyme activity. The mutation segregated with affected status in the family [Cason *et al*, 2003]. Recently, de Alencastro *et al*. [2008] reported a new family of Brazilian origin showing a missense mutation, p.G56S, in the *SMS* gene that greatly reduces the SMS activity. Here we describe another family with Snyder-Robinson syndrome (SRS) in two Mexican brothers with mental retardation, osteoporosis, multiple fractures, and facial asymmetry. A novel missense mutation, p.V132G (c.496T>G), has been identified in the *SMS* gene clearly establishing its involvement in this rare X-linked mental retardation syndrome.

CLINICAL REPORT

Patient 1

The proband (II-2 pedigree, Figure 1A), is a 28-year-old male, who was the product of the second pregnancy of a healthy nonconsanguineous 47-year-old mother and 60-year-old father. Uneventful gestation and no history of any adverse intrauterine exposure were recorded. Birth weight and length were 2,950 g (-1 SD) and 49 cm (-0.5 SD) respectively, OFC was 34 cm (-0.5/1 SD), and the Apgar score was 8. Lactose intolerance was diagnosed at 6 months of age. Several fractures were recorded at 9, 11 and 14 years of age in the right femur, and at 17 years in the left femur; all of the fractures occurred during everyday activities. A generalized delay in psychomotor milestones was observed early: sitting at 4 years, first words at 5 years, walked at 5 years, and sphincter control at 6 years. He received special education by 8 years without psychomotor improvement. An IQ evaluation, at age of 18, reported a score of 40. Currently he is able to obey simple orders and helps take care of his younger affected brother. Physical examination (Figure 2) showed his weight was 48 kg (-2 SD), height 165 cm (-2/-1.5 SD), OFC 57 cm (50-75 centile), arm span 174 cm, lower segment 89.7 cm and his upper/lower segment ratio was 0.83. A thin habitus and poor muscular development was observed. Other findings included brachycephaly, a single central hair whorl, asymmetric face (due to hypoplastic right side), slanted upper palpebral fissures (outer canthal distance 8.3 cm, inner canthal distance 2.8 cm, interpupillary distance 5.5 cm), sparse eyebrows, discrete synophrys, right palpebral ptosis, high nasal bridge, bulbous nasal tip, anteverted nares, smooth philtrum, prominent lower lip, oral cavity with high palate, overcrowded teeth, asymmetrical tongue (hypoplastic right side), and coarse voice. The ears were dysplastic and asymmetric (right length 7.4 cm, left length 6.6 cm) and the neck was short and webbed. His thorax examination revealed a wide inter nipple distance, pectus excavatum, dorsal kyphosis and right scoliosis. His cardiac evaluation was normal and his abdominal examination showed no visceromegaly. The external male genitalia showed pubic Tanner score 4-5 with testicular volumes of 6 cc on the right and 5 cc on the left. Long and thin upper limbs with right hand measurements: total length 17.3 cm (3-25 centile), palm length 9.8 (3-25 centile) and middle finger length 7.5 (3-25 centile), palm/finger ratio 1.3 and left hand measurements were total length 17.5 cm (25 centile), palm length 10.3 cm (25-50 centile) and middle finger length 7.2 cm (3-25 centile), palm/finger ratio 1.43. He also had long and thin lower limbs with shortening of the right limb, broad and long hallux, and hypoplastic 2-5 toenails. The patient showed patchy skin hyperpigmentation on the forehead, at the right inguinal groove and in the lumbar area. X-ray examination (Figure 3) disclosed a normal skull, thickened calvarium, thoracic kyphoscoliosis and vertebral compression. The pelvis showed diminished bone density (-3.22 SD) and the tubular bones were long and with thin cortices. The long bones of the lower extremities were asymmetric due to a bend of the right femur. The left one has a plaque and screws because of the most recent fracture. The tibia showed a posterior bend and the fibula was thin.

Screening tests for metabolic defects, including analysis of urinary glycosaminoglycans, oligosaccharides and aminoacids, endocrinological studies (thyroid and hypophysis-testis

profiles) were normal. Thyroid testing was initially performed as a standard endocrinological workup and repeated later as part of a protocol to rule out Allan-Herndon-Dudley syndrome in one of the centers (Greenwood Genetic Center) with normal results. Karyotype (GTG banding technique at 550-650 band resolution) was also normal. Molecular analysis for fragile-X gave 34 CCG repeats. Routine hematological analyses reported a low platelet count (127,000) on one occasion with no relevant clinical bleeding events observed during evaluations. Further follow-up analysis revealed platelet counts within the normal range.

Patient 2

Patient 2 (II-3, Figure 1A), at 21 years of age was similarly affected as the proband. Postmaturity was observed and birth weight was 3,000 g (-0.5/-1SD), his length was 51 cm (0.5 SD). His birth OFC and Apgar scores were not recorded. Neonatal anomalies were not recorded. A single fracture was recorded at 10 years of age in the right clavicle. Similar to patient 1, a generalized delay in psychomotor development milestones was observed early but an IQ evaluation was not performed.

Physical examination (Figure 4) showed his weight was 53 kg (-2 SD), height 171 cm (-0.5/-1 SD), OFC 56.6 cm (50-75 centile), armspan 171.3 cm, lower segment 91.5 and his upper/lower segment ratio was 0.87. A thin habitus and poor muscular development was observed. He had brachycephaly, a single central hair whorl, asymmetric face (due to hypoplastic right side), slanted upper palpebral fissures (outer canthal distance 8.2 cm, inner canthal distance 2.8 cm, interpupillary distance 6 cm), sparse eyebrows, right palpebral ptosis, high nasal bridge, bulbous nasal tip, anteverted nares, smooth philtrum, prominent lower lip, oral cavity with high palate, overcrowded teeth, asymmetrical tongue (hypoplastic right side) and a coarse voice. His ears were dysplastic and asymmetric (right length 7.6 cm, left length 6.9 cm) and his neck was webbed. The thorax had a wide inter nipple distance, pectus excavatum, and left scoliosis. His cardiac evaluation was normal as well as his abdomen. The external male genitalia displayed pubic Tanner score 4-5 with testicular volumes of 6 cc bilaterally. His upper limbs were long and thin with right hand measurements of total length 17.5 cm (3-25 centile), palm length 9.8 (3-25 centile) and middle finger length 7.7 (25-50 centile), palm/finger ratio 1.27, single palmar crease was observed and left hand measurements of total length 17.5 cm (3-25 centile), palm length 9.9 cm (3-25 centile) and middle finger length 7.6 cm (3-25 centile), palm/finger ratio 1.3. The patient had long and thin lower limbs, shortening of right limb, broad and long hallux, and hypoplastic 2-5 toenails. A patchy skin hyperpigmentation in the left thigh was observed. X-ray examination (Figure 5) disclosed a normal skull, thickened calvarium, mild thoracic scoliosis, a pelvis with low bone density (-3.22 SD) and tubular bones that were long and with thin cortices.

Screening tests for metabolic defects, including analysis of urinary glycosaminoglycans, oligosaccharides and aminoacids. Endocrinologic studies (thyroid and hypophysis-testis profiles) were normal. Karyotype (GTG banding technique at 550-650 band resolution) was normal. Molecular analysis for fragile-X gave 34 CCG repeats. Hematological analyses reported a low platelet count (109,000) in one occasion.

Both patients were managed by the Endocrinology service and received calcium, calcitriol and alendronate under medical supervision with clinical improvement and absence of new fractures over a period of at least two years. Mineral bone density has revealed minimal improvement in measurements and is still under follow-up by the Endocrinology service.

Family data

The family history was remarkable for one spontaneous abortion in the first trimester in the mother of the proband (II-5, Fig. 1A). Careful examination of both parents and two brothers revealed no evidence of mental retardation, osteoporosis, multiple fractures or facial asymmetry.

Mutation Analysis

Sequencing—All 11 exons of the *SMS* gene were amplified separately. Primer sequences, annealing temperatures and amplicon sizes are available upon request (C.E.S.). Primers had either the standard M13 forward or reverse primer added to the 5' end to facilitate sequencing. With the exception of exon 1, which was amplified with MasterAmp Buffer G (Epicentre), all exons were amplified in a PTC-200 thermocycler (MJ Research), in a total volume of 30 µl containing 1 X PCR buffer with 1 µM of each primer, 0.1 mM dNTP's, and 1.5U of GoTaq DNA polymerase (Promega). The following PCR conditions were used: 95°C for 5 minutes, 95°C for 30 seconds, specified annealing temperature for 30 seconds, 72°C for 30 seconds for a total of 30 cycles and a final extension at 72°C for 5 minutes. PCR reactions were analyzed on a 1.5% agarose gel.

Amplified products were purified by GFX columns (GE Healthcare) and sequenced in both directions on the MegaBACE™ (Amersham Biosciences) using the DYEnamic™ dye terminator kit (Amersham Biosciences) according to the manufacturer's protocol. Sequences were analyzed by the DNASTAR program (Lasergene).

BsaI digestion—The c.496T>G alteration creates a *BsaI* enzymatic cut site in the mutant allele. Genomic DNA was amplified in a final reaction volume of 30 µl containing 1 X PCR buffer with 1 µM of *SMS* exon 5 F (5' ttgtcaaaagtcggcagtcac 3') and *SMS* exon 5 R (5' tctcaaaaaccagcagtcgac 3') primer pair and, 0.1 mM dNTP's, and 1.5 U of GoTaq DNA polymerase (Promega). The following PCR conditions were used: 95°C for 5 minutes, 95°C for 30 seconds, 50.9°C for 30 seconds, 72°C for 30 seconds for a total of 30 cycles followed by a final extension at 72°C for 5 minutes. 10 µl of the amplified PCR product was digested with *BsaI* (New England Biolabs) and incubated at 60°C. The digested product was loaded on 1.5% agarose gel to separate the fragments.

Analysis of spermine synthase activity—*SMS* activity was determined in Epstein-Barr virus (EBV) - transformed lymphoblastoid cell lines by measuring the production of [³⁵S] methylthioadenosine from [³⁵S]decarboxylated AdoMet in the presence of 0.5 mM spermidine as described previously [Cason *et al*, 2003].

Analysis of intracellular polyamine content—Cellular polyamine content in the lymphoblastoid cell lines was determined using an ion-paired reverse phase HPLC separation method and post-column derivatization with o-phthalaldehyde as described previously [Cason *et al*, 2003].

Western blot analysis—Lymphoblastoid cell pellets were resuspended in 50 mM sodium phosphate buffer, pH7.2, containing 0.3 mM EDTA and 10 mM betamercaptoethanol. Three freeze/thaw cycles were carried out and the suspension was centrifuged at 17000 × g for 20 min. at 4°. Proteins present in the supernatant were resolved by SDS-PAGE using a 12% gel. Electrotransfer to polyvinylidene fluoride membrane (PALL Life Sciences, Pensacola, FL) was followed by hybridization with purified *SMS* antibody (25 ng/ml), and detection using the LumiGLO®* chemiluminescent Western Blot Detection System (Cell Signaling Technology™, MA). An amino-link column (Pierce Chemical Company, Rockford, IL) to

which purified spermine synthase had been crosslinked was used to purify the polyclonal rabbit antiserum to (histidine)₆-tagged human SMS [Ikeguchi *et al*, 2003].

Results

Sequence analysis revealed a T to G change at position 496 (c.496T>G) in exon 5 in the proband (Figure 1B). This alteration resulted in the substitution of valine with a glycine at the highly conserved amino acid at position 132 (p.V132G). The alteration created a *Bsa*II restriction endonuclease site, which was utilized for segregation analysis in family K9482. The mutation (g.496T>G) was found to segregate with the phenotype in the family (Figure 1C). The g.496T>G mutation was not present in 549 normal X chromosomes indicating it is not likely to be a polymorphism.

Further investigation of the family found the p.V132G mutation significantly reduced the activity of spermine synthases in the two affected males while their mother's spermine synthase retained normal activity (Table 2). Spermine levels were also significantly reduced in the 2 affected males (Table 2). A reduced level of spermine synthase protein was seen on Western blot analysis of protein isolated from lymphoblasts (Figure 1D).

DISCUSSION

The clinical and radiological findings in the patients reported here are similar to those reported for Snyder-Robinson syndrome (SRS, OMIM 309583) [OMIM, 2008], a rare syndromic X-linked mental retardation (XLMR) entity characterized by a thin habitus, facial asymmetry, mental retardation, and osteoporosis (Table 1) [Snyder and Robinson, 1969]. Clinical and molecular evaluations in this family expanded the phenotype to include an unsteady gait, a nonspecific movement disorder, abnormal EEG and seizures in affected males [Arena *et al*, 1992; Arena *et al*, 1996; Cason *et al*, 2003]. Recently, de Alencastro *et al*. [2008] reported a second family, from Brazil, with three affected males displaying a novel missense mutation, p.G56S, located in the N-terminal region of the *SMS* gene. The authors also described new phenotypic features such as: short philtrum, mandibular prognathism, ear abnormalities, high myopia and *pectus carinatum*. More importantly, the psychomotor and cognitive development was more severely impaired in the three patients. The Mexican patients here reported did not display any of these clinical features. In addition to the SRS phenotype, our cases showed hyperchromic skin lesions and low platelet count with no apparent explanation. These findings could be just stochastic or could reflect a genotype-phenotype effect. Future patient reports will help to clarify this possible clinical association.

By linkage analysis, SRS was located to Xp21.3-p22.12, distal to the 3' end of the *DMD* gene [Arena *et al*, 1996]. In 2003, Cason *et al*. identified a G-to-A transition at position +5 of the 5' splice site of intron 4 of the spermine synthase (*SMS*) gene in affected males. This mutation caused a truncation of the *SMS* protein and loss of the carboxyl terminal end containing the active site [Cason *et al*, 2003]. Not surprisingly, *SMS* activity and cellular levels of spermine were significantly reduced in the affected males. The second mutation, p.G56S, found in the Brazilian family introduces a larger side chain and impairs *SMS* dimerization and function. This mutation leads to a severe reduction in spermine levels and alteration of polyamine levels that would explain the notably severe impairment in neuronal development of the affected patients [de Alencastro *et al*, 2008]. Sequence analysis of the *SMS* gene in the present family identified a missense mutation, p.V132G, in exon 5. The V132 residue is a highly conserved residue in spermine synthases down to *Xenopus* (Figure 1E). Even in spermine synthases from fish, insects and sea urchins, this residue has a highly conserved hydrophobic side-chain (leucine, isoleucine or methionine). This mutation is

located in the region between the N-terminal domain and the linker domain prior to the C-terminal domain that contains the active site of spermine synthase [Wu *et al*, 2008]. This might be expected to have an effect on the ability of the SMS protein to form a homodimer which is necessary for enzymatic activity and could also affect the active site directly by altering the positioning of the linker domain which forms a lid to the active site. Additional bioinformatic analysis using PolyPhen (<http://tux.emblheidelberg.de/ramensky/polyphen.cgi>) predicted the p.V132G alteration to be probably damaging and SIFT (Sorting Intolerant From Tolerant) analysis (<http://blocks.fhcrc.org/sift/SIFT.html>) predicted that the change would not be tolerated. The predicted pathogenicity of the p.V132G alteration was confirmed by a significant reduction in enzymatic activity (Table 1) and a significant reduction in SMS protein in lymphoblastoid cells from the affected brothers (Figure 1D).

Differential diagnosis for SRS included syndromes displaying mental retardation (MR) and thin habitus such as: Lujan-Fryns syndrome (OMIM 309520) [van Buggenhout and Fryns, 2006]; Allan-Herndon-Dudley syndrome (OMIM 300523) [Allan *et al*, 1944; Bialer *et al*, 1992; Schwartz *et al*, 2005], Fragoso syndrome (OMIM 248770) [Fragoso and Cantu, 1984] and Martin-Bell syndrome (OMIM 309550) [Mattei *et al*, 1981].

Particular attention was paid to XLMR syndromes in which the affected males have a relatively asthenic habitus, reduced muscle mass and mental retardation: Lujan or Lujan-Fryns syndrome (OMIM 309520) [van Buggenhout and Fryns, 2006] and Allan-Herndon-Dudley syndrome (AHDS, OMIM 309520) [Allan *et al*, 1944; Bialer *et al*, 1992; Schwartz *et al*, 2005]. For the latter syndrome, there is now a biomarker available as males with AHDS have elevated plasma free T3 and abnormally low plasma free T4 [Schwartz *et al*, 2005]. For the Lujan syndrome, a specific mutation (p.N1007S) has been identified in the *MED12* gene [Schwartz *et al*, 2007]. However, two families with a diagnosis of Lujan-Fryns syndrome have been found to have mutations in another gene, *UPF3B* [Tarpey *et al*, 2007]. Based on these recent findings, it would appear that a male with MR and an asthenic habitus might be tested for spermine synthase activity and levels of free T3. If these are negative, analysis of the *MED12* or *UPF3B* genes might be considered. Clearly, SRS is a distinct XLMR syndrome and should be considered in males with MR and an asthenic habitus

In summary, we describe an additional mutation in the *SMS* gene that is associated with the Snyder-Robinson syndrome. The clinical findings in the present family are almost identical to those initially reported by Snyder and Robinson [Snyder and Robinson, 1969] and further elaborated by Arena *et al*. [1996]; they also share a clinical spectrum with the patients recently reported by de Alencastro *et al*. [2008] (See Table 1); it is interesting that each family, with different ethnic background, is displaying a particular mutation that would help to explain the subtle clinical differences now observed in addition to differences in dietary habits (that modify the levels of polyamine intake) suggested by de Alencastro *et al*. [2008]. Once a clinical suspicion is made, measurement of spermine/spermidine enzyme activity and further complete sequencing of the gene should be considered to confirm Snyder-Robinson syndrome.

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Figure 1.

A) Pedigree of family with clinical features of Snyder-Robinson syndrome. B) DNA electropherograms of the proband (II-2) and a normal male. The normal V132 residue is indicated in red and the G132 mutation residue is indicated in blue. C) *BsaI* digestion of a PCR product of exon 5 from the family. The 445 bp band represents the normal allele (V132) and the 242 bp and 203 bp bands represent the mutant allele (G132). D) Western blot of protein isolated from lymphoblastoid cell lines. The filter was hybridized with purified SM antibody. Lane 1 is the proband, II-2; Lane 2 is the mother, I-2; Lane 3 is the affected brother, II-3; Lane 4 is the proband from the original SRS family and Lane 5 is

purified His-tagged spermine synthase protein. E) Protein alignment for spermine synthase across multiple species; the V132 residue is highlighted in yellow.

Figure 2. Patient 1 (II-2); A) frontal view, note thin habitus, wide internipple distance, pectus excavatum, shortening on the right pelvic limb, and patchy pigment of skin; B) facial asymmetry, left eyelid ptosis, slightly prominent lower lip, and dysplastic ears; C) dorsal view of hands; D) feet with broad and long halluces, and hypoplastic toenails; E) and F) dorsal kyphosis and right scoliosis.

Figure 3.

X-ray images of patient 1; A) lateral view of skull, note lower bone density and thick diploe, and large sella turcica; B) long, thin tubular bones, more severe on cubitus; C) note right scoliosis and dorsal vertebral compression secondary to osteoporosis; D) right shortening and bent of femur, and on the left a recent fracture with a plaque and screws; E) positional alteration on pelvis secondary to femoral shortening, diminished bone density; F) long and thin long bones.

Figure 4.

Patient 2 (II-3); A) frontal view, note thin habitus, wide internipple distance, pectus excavatum, and patchy pigment of skin (right side of forehead, right inguinal groove and on lumbar region); B) facial asymmetry, left palpebral ptosis, prominent lower lip, and ears are dysplastic; C) dorsal view of hands; D) feet with broad and long halluces, and hypoplastic 2-5 toenails; E) and F) mild right scoliosis.

Figure 5.

X-ray images of patient 2; A) lateral view of skull, note lower bone density and thick diploe, and large sella turcica; B) note mild right scoliosis; C) diminished bone density on pelvis bones; D) upper limbs with long and thin tubular bones; E) lower limbs with long and thin long bones.

Table 1

Clinical features in the reported affected families with SRS

Physical features	Family 1 [Snyder and Robinson, 1969; Arena <i>et al.</i> , 1996]	Family 2 [de Alencastro <i>et al.</i> , 2008]	Patient 1	Patient 2	Total
Age	19-41	6, 12, 23	28	21	-
Mental retardation	5/5	3/3	+	+	10/10
Asthenic body build	5/5	3/3	+	+	10/10
Diminished muscle bulk	5/5	3/3	+	+	10/10
Facial asymmetry	4/5	0/3	+	+	6/10
High myopia	0/5	2/3	-	-	2/10
Short philtrum	0/5	3/3	-	-	3/10
Prominent lower lip	5/5	3/3	+	+	10/10
High, narrow, or cleft palate	3/5	1/1	+	+	6/8
Mandibular prognathism	1/5	3/3	-	-	4/10
Pectus excavatum	3/5	0/3	+	+	5/10
Pectus carinatum	0/5	3/3	-	-	2/10
Kyphoscoliosis	4/5	3/3	+	-	8/10
Long and thin fingers and toes	5/5	1/3	+	+	8/10
Osteoporosis	4/4	1/1	+	+	7/7
Speech abnormalities	5/5	3/3	+	+	10/10
Unsteady gait	1/5	3/3	+	+	6/10
Seizures	3/6	3/3	-	-	6/11

Table 2

Spermine synthase activity and Spermine levels in the present family

Sample	SMS Activity	Putresine	Spermine	Spermidine	Spd/Spm
	(pmol/h/mg protein)	(nmol/h/mg protein)			
Normal control	500	0.51	6.81	3.54	0.52
Mother (I-2)	750	0.87	9.18	6.11	0.67
Patient 1 (II-2)	4	0.15	2.54	8.82	3.47
Patient 2 (II-3)	<1	0.18	3.26	11.12	3.41

Spd = spermidine, Spm = spermine