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The impact of spermine synthase (*SMS*) mutations on brain morphology

Shelli R. Kesler,

Center for Interdisciplinary Brain Sciences Research, Stanford University School of Medicine, 401 Quarry Road, MC5795, Stanford, CA 94305-5795, USA, skesler@stanford.edu

Charles Schwartz,

Greenwood Genetic Center, Greenwood, SC, USA

Roger E. Stevenson, and

Greenwood Genetic Center, Greenwood, SC, USA

Allan L. Reiss

Center for Interdisciplinary Brain Sciences Research, Stanford University School of Medicine, 401 Quarry Road, MC5795, Stanford, CA 94305-5795, USA

Abstract

Snyder–Robinson syndrome (SRS) is a form of X-linked mental retardation resulting from mutations in spermine synthase (*SMS*), which impact neurodevelopment and cognitive outcome. We obtained cerebral, cerebellum, hippocampus, and red nucleus volumes from two males with SRS and 24 age- and gender-matched typically developing controls using volumetric neuroimaging analyses. Total brain volume was enlarged in males with SRS while cerebellum, hippocampus, and red nucleus volumes tended to be reduced compared to controls. Mutations of the X chromosome may modulate the risk for mental retardation through altered early neurodevelopment, disruption in receptor function, and ongoing neural organization and plasticity. Disruption of *SMS* function may negatively affect regional brain volumes that subserve cognitive and motor abilities. This research provides valuable insight into the effects of polyamine function on brain development.

Keywords

SMS; Spermine synthase; Snyder–Robinson syndrome; MRI; Red nucleus; Cerebellum; Hippocampus; Cerebrum

Introduction

Snyder–Robinson syndrome (SRS) was initially reported as a nonsyndromic X-linked mental retardation (XLMR) condition present in a single family [1]. However, upon reevaluation, the affected males were found to have a thin habitus, kyphoscolioses, and long great toes. Distinctive facial features were observed: asymmetry, prominent lower lip, and a high or cleft palate [2]. Additional clinical features including unsteady gait, seizures,

nonspecific movement disorder, and abnormal EEG were noted in a follow-up evaluation [3].

The syndrome was mapped to Xp21.2–p22.2 [2] and subsequent studies identified a splice mutation (c.329+5 G>A) in the spermine synthase (*SMS*) gene which caused low level of intracellular spermine in lymphocytes and fibroblasts and elevated spermidine/spermine ratios in these cells [3]. Recently, a second family with SRS and a novel mutation in *SMS* was identified [4]. This family presented with severe epilepsy and cognitive impairment.

SMS encodes an enzyme that is widely expressed in the brain and is responsible for the conversion of spermidine to spermine. Spermine, a polyamine, is known to be involved in cell growth and maintenance [3,5]. Thus, it is likely that mutations in this gene may result in altered neurodevelopment. Based on this hypothesis, we examined brain morphology in SRS in an effort to provide further understanding of the relationship between *SMS*, brain development/function, and cognition. We obtained whole and regional brain volumes, including tissue-specific, gray and white matter measurements, from magnetic resonance brain images (MRI) acquired from two males with SRS. We then compared these volumes descriptively with those of age- and gender-matched typically developing controls. Regional volume measurements included cerebellum and red nucleus as abnormalities in these areas have been suggested based on the SRS phenotype [3] and hippocampus as this is a key region involved in the learning and memory functions that tend to be impaired in individuals with MR [6].

Materials and methods

Clinical reports

Patient V-2, [3] age 13.28 years, was born after a 32-week gestation and intrauterine growth retardation. He weighed 1,860 g and was delivered by cesarean section. Development was delayed with sitting at about 1 year, pulling up at 16 months, and walking at 2 years. He had myoclonic seizures from infancy until age 11 years. His forehead was prominent and the face tall and narrow with the left side larger than the right. There was general decrease in muscle mass; deep tendon reflexes were normal. IQ was 36. See Table 1 for further clinical findings.

Patient IV-8, [3] age 39.88 years, was born after an uncomplicated pregnancy and delivery. His birth weight was 3,180 g. At 17 months, he was not walking or talking. He was in special education and received speech therapy. He has never had any seizures. At age 20 years, he was found to have osteoporosis. This was detected on X-ray examination following a sledding accident, which resulted in multiple thoracic vertebral compression fractures. He works in a sheltered workshop.

His face was somewhat coarse with a large mouth and full lower lips with facial asymmetry. Palate was normal but with a thick alveolar ridge. He was tall and thin with mildly diminished muscle bulk and kyphoscoliosis. His strength was normal. He had long hands and mildly hyperextensible fingers and long great toes. His speech was thick and slow. He could follow commands and repeat names but had poor performance on simple calculations. His neurologic examination was normal. IQ was 54. See Table 1 for further clinical findings.

MRI acquisition

All MRI scans used in this study were obtained with 1.5 T GE Signa scanners (GE medical systems, Milwaukee, WI) at Stanford and Greenwood. Coronal brain images were acquired with a 3D volumetric radio frequency spoiled gradient echo pulse sequence using the

following scan parameters: TR=35 ms, TE=6 ms, flip angle=45°, NEX=1, matrix size=256×192, field of view=24 cm, slice thickness=1.5 mm, 124 contiguous slices.

MRI analysis

MRI scans were obtained from V-2 and IV-8 as well as from 24 age- and gender-matched typically developing controls from our structural neuroimaging database. Participants were excluded from MRI contraindications (e.g., orthodontia). Typically developing controls were excluded for any history of neurological, cognitive, or psychiatric disorders. Institutional Review Boards at both sites approved this study. MRI scans were imported into *BrainImage* (<http://cibsr.stanford.edu/tools>) for semiautomated whole brain segmentation and quantification [7,8] (Supplementary Figure 1). The hippocampus [9] and red nucleus (Supplementary Figure 2) were manually delineated for each participant.

Corrected volumes were computed using the ratio of regional volumes to total brain volume in order to determine differences that were disproportionate to overall brain volume. Brain volume z scores were calculated for participants with SRS, with $z \pm 1.6$ being considered “significant” [10]. Outlier status was determined using Tukey’s method boxplot [11] in *SPSS 16.0* (<http://www.spss.com>).

Results

Total brain volumes of both patients were somewhat enlarged compared to controls (Fig. 1; Table 2). Total brain volume enlargement appeared to affect gray, white, and cerebral spinal fluid volumes equally (Table 3). Cerebellar volume appeared to be disproportionately as well as absolutely decreased in V-2 (Fig. 1; Table 3). Hippocampal volumes in both patients were disproportionately smaller compared to controls, particularly in IV-8, the adult patient (Fig. 1; Table 3). Absolute brainstem volumes were enlarged in both patients (Table 2) but only V-2’s volume was disproportionately enlarged (Fig. 1; Table 3). Red nucleus volumes in both patients were disproportionately reduced respective to both total brain and brainstem volumes (Fig. 1; Table 3). Tukey’s boxplots indicated that the proportional cerebellum volume and absolute and proportional brainstem volumes of V-2, and the proportional hippocampus and red nucleus volumes (to total brain volume) of IV-8 were outliers (Fig. 2).

Discussion

This report is the first morphometric imaging study of neurodevelopment associated with SRS. Though the imaging results should be considered preliminary, these case studies offer a novel opportunity to explore the potential impact of particular X-linked genes on neurodevelopment. Although our sample of controls was relatively small, our volume findings are highly similar to those of other much larger studies of normative brain development [12].

Spermine and other polyamines are vital for cell proliferation and differentiation [13]. Spermine has neuroprotective effects following brain damage, particularly in the hippocampus and cortex [14,15]. However, over-accumulation of spermine can induce cell death [16] suggesting a dual role for spermine in cell function and maintenance [5]. Thus, spermine deficiency may result in an imbalance between cell growth and pruning mechanisms, potentially resulting in aberrant neurodevelopment.

The finding of disproportionately reduced cerebellar (corrected for total brain volume) and red nucleus volumes (corrected for both total brain and brainstem volumes) in V-2, the younger patient, suggests that *SMS* expression has differential effects on the forebrain and hindbrain during early neurodevelopment. The cerebellum and red nucleus are involved in

the circuitry subserving motor, speech, and cognitive functions—abilities that tend to be negatively affected by SRS [3]. Both V-2 and IV-8 were noted to have significant developmental motor delays and cognitive deficits (Table 1). Cognitive impairments, including intellectual and executive function, are related to cerebellar abnormalities in fragile X syndrome, a more common form of XLMR [17] as well as a large number of other conditions [18]. Accordingly, the cerebellar and red nucleus abnormalities observed in V-2 may be associated with his cognitive and motor deficits. In the clinical realm, neuroimaging findings suggesting relatively small cerebellar and/or red nucleus volumes in the context of a large cerebrum might also signal the possible diagnosis of SRS in males with cognitive and motor dysfunction and a family history of XLMR.

Cerebellar volume in IV-8 was similar to that of controls potentially suggesting that aberrant cerebellar development is attenuated with age and/or is associated with individual cognitive outcome. Specifically, while both individuals with SRS demonstrated IQ scores in the significantly impaired range, V-2, who demonstrated significantly reduced cerebellar volume, received an IQ score that was over a standard deviation lower than IV-4. However, red nucleus volume was reduced in both patients and, thus, continued cognitive-behavioral disabilities in adults with SRS may be related more to red nucleus morphology. Red nucleus volumes corrected for brainstem volume were reduced more than 1.6 standard deviations in both patients but were not significant Tukey outliers. However, total brainstem was relatively enlarged in both subjects with SRS (a Tukey outlier in IV-4) suggesting that red nucleus is likely reduced irrespective of other brainstem volumes. These hypotheses will require further study, ideally involving longitudinal assessments of individuals with SRS.

Spermine deficiency may be associated with cognitive dysfunction resulting from disrupted receptor processes. Spermine has been shown to block or inhibit inward rectifying potassium (IRK) channels [19], which modulate neuronal activity [20]. Overexpression of certain IRKs may disrupt gamma-aminobutyric acid (GABA) function, affecting the balance between inhibitory and excitatory processes in the hippocampus [21]. Spermine also inhibits *N*-methyl-D-aspartate and AMPA/Kainate receptors, which mediate the excitatory effect of glutamate [22]. Glutamate and GABA are essential for long-term depression and potentiation-synaptic plasticity mechanisms involved in learning and memory [23].

The gene product implicated in SRS may be directly or indirectly involved in synaptic plasticity-based learning mechanisms [24,25]. Thus, alteration of signaling pathway function as a result of genetic mutations in the *SMS* gene may disrupt the mechanisms necessary for the development and maintenance of new synapses. As such, the finding of disproportionately decreased hippocampal volume in the context of overall increased cerebral volume could be related to disrupted synaptic plasticity in SRS. As well, this finding could be related to long-term deficits in hippocampus neuronal excitability resulting from spermine deficiency [26] similar to that seen in individuals with Alzheimer's disease who interestingly show decreased spermine in certain brain regions [27].

Mutations of the X chromosome may modulate the risk for MR through altered early neurodevelopment in combination with disruption in receptor function and ongoing neural organization and plasticity. Future studies focusing on associations among genetic factors, basic neurobiology, neuroanatomy and cognitive-behavioral outcome in larger numbers of individuals with SRS are required to more fully elucidate potential relationships between structure and function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Scatterplot results for MRI volumes. Compared to controls, total brain volume was enlarged while proportional hippocampus and red nucleus volumes are reduced in both individuals with SRS. Proportional cerebellar volume was reduced and proportional brainstem volume was enlarged in the child with SRS. (*X axis*=age in years, *y axis*=brain volume in cubic centimeters; *TBV*=total brain volume)

Fig. 2.

Tukey boxplot results for regional volumes corrected for total brain volume. Proportional cerebellar and brainstem volumes were significant outliers (*circle*) in the child with SRS, being reduced and enlarged, respectively, compared to controls. Proportional hippocampal and red nucleus volumes were significant outliers in the adult with SRS, being reduced compared to controls

Table 1

Clinical findings in V-2 and IV-8 at two different ages

Pedigree #, [2]	V-2		IV-8	
Age (years)	2	13	28	39
Height, cm (centile)	82 (5)	149 (20)	187 (95)	188 (>97)
Weight, kg (centile)	9.1 (<3)	26 (<3)	54 (<3)	81.4 (50)
Head circumference, cm (centile)	49 (40)	53.6 (50)	59 (90)	59.5 (95)
Facial asymmetry	Mouth (when crying)	+	Orbital and mouth	Face
Thin habitus, with muscle hypoplasia	+	+	+	+
Kyphoscoliosis	-	-	+	+
Narrow chest			+	
Long hands (centile)	- (20)	- (25)	+ (>97)	+ (85)
Hyperextensible fingers			+ (mild)	+ (mild)
Long great toes	Hammer toe		+	+
IQ/development	Delayed development	36	60	54
Overall status	Preschool		Works as a janitor	Works in sheltered workshop
Other	Testes not descended, myoclonic jerks	Myoclonic seizures	Thoracic vertebra fractures, colectomy for ulcerative colitis	
EMG/nerve conduction				Increased insertional activity, fibrillations, and occasional sharp wave in muscles of left foot
EEG	Abnormal	Normal		Normal
AAS		Normal		Normal
Ophthalmologic		Myopia and astigmatism		Hyperopia and astigmatism
EKG		Normal		Normal
Serum cholesterol		Normal		Normal

Table 2

Brain volumes for V-2, IV-8, and controls (shown in cubic centimeters)

Volume	V-2 volume (z score)	Child control mean (SD)	IV-8 volume (z score)	Adult control mean (SD)
Total brain	1,670.30 (1.6) ^a	1,506.17 (103)	1,725.90 (1.8) ^a	1,412.33 (175.66)
Cerebral gray	804.80 (2.0) ^a	697.83 (54.16)	705.69 (1.1)	589.24 (105.07)
Cerebral white	545.00 (1.5)	473.38 (48.22)	651.85 (1.3)	478.80 (132.68)
Cerebral CSF	119.60 (−.20)	123.91 (21.50)	142.58 (−.32)	160.83 (57.61)
Subcortical gray	50.60 (1.6) ^a	45.04 (3.43)	50.22 (2.0) ^a	39.80 (5.19)
Subcortical white	56.00 (1.9) ^a	48.05 (4.25)	67.90 (2.5) ^a	49.50 (7.31)
Cerebellum	124.20 (−1.6) ^a	143.43 (12.39)	150.02 (1.3)	127.94 (16.83)
Hippocampus	6.42 (−.65)	7.06 (.98)	6.81 (−.63)	7.26 (.72)
Brainstem	51.50 (3.63) ^{a, b}	35.73 (4.3)	48.90 (2.07) ^a	35.57 (6.43)
Red nucleus	.32 (−1.3)	.36 (.03)	.25 (−3.0) ^a	.34 (.03)

SD standard deviation, *CSF* cerebral spinal fluid^a*Z* score ≥ 1.6^bTukey's method outlier

Table 3

Proportional brain volumes (cubic centimeters)

Volume	V-2 volume (z score)	Child control mean (SD)	IV-8 volume (z score)	Adult control mean (SD)
Cerebral gray/TBV	.48 (.50)	.47 (.02)	.41 (.13)	.40 (.08)
Cerebral white/TBV	.33 (.50)	.32 (.02)	.37 (.14)	.36 (.07)
Cerebral CSF/TBV	.07 (−1.0)	.08 (.01)	.08 (−1.0)	.10 (.02)
Subcortical gray/TBV	.03 (0.0)	.03 (.001)	.029 (.50)	.026 (.006)
Subcortical white/TBV	.034 (1.0)	.032 (.002)	.039 (.75)	.036 (.004)
Cerebellum/TBV	.074 (−2.3) ^{a,b}	.097 (.01)	.09 (0.0)	.09 (.008)
Hippocampus/TBV	.003 (−2.5) ^a	.005 (.0008)	.003 (−5.0) ^{a,b}	.005 (.0004)
Brainstem/TBV	.031 (2.82) ^{a,b}	.024 (.002)	.028 (.69)	.025 (.004)
Red nucleus/TBV	.00019 (−1.7) ^a	.00024 (.00003)	.0001 (−3.5) ^{a,b}	.00024 (.00004)
Red nucleus/brainstem	.019 (−2.54) ^a	.028 (.003)	.02 (−1.80) ^a	.029 (.005)

SD standard deviation, *TBV* total brain volume, *CSF* cerebral spinal fluid^a*Z* score ≥ 1.6^bTukey's method outlier