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Yuri A. Zarate

Katherine A. Bosanko

Mary Ann Thomas

David T. Miller

Kristina Cusmano-Ozog

See next page for additional authors

Authors

Yuri A. Zarate, Katherine A. Bosanko, Mary Ann Thomas, David T. Miller, Kristina Cusmano-Ozog, Antonio Martinez-Monseny, Cynthia J. Curry, John M. Graham Jr., Lea Velsher, Mir Reza Bekheirnia, Veronica Seidel, Demitrios Dedousis, Anna L. Mitchell, Amy M. DiMarino, Angelika Riess, Meena Balasubramanian, Jennifer L. Fish, Aisling R. Caffrey, Nicole Fleischer, Tyler Mark Pierson, and Ronald V. Lacro

Growth, development, and phenotypic spectrum of individuals with deletions of 2q33.1 involving *SATB2*

Short title: 2q33.1 deletions and *SATB2*-Associated syndrome

Yuri A. Zarate[1], Katherine A. Bosanko[1], Mary Ann Thomas[2], David T. Miller[3], Kristina Cusmano-Ozog[4], Antonio Martinez-Monseny[5], Cynthia J. Curry[6], John M. Graham Jr[7], Lea Velsher[8], Mir Reza Bekheirnia[9], Veronica Seidel[10], Demitrios Dedousis[11], Anna L. Mitchell[11], Amy M. DiMarino[12], Angelika Riess[13], Meena Balasubramanian[14], Jennifer L. Fish[15], Aisling R. Caffrey[16], Nicole Fleischer[17], Tyler Mark Pierson[18], Ronald V. Lacro[19]

[1]Section of Genetics and Metabolism, University of Arkansas for Medical Sciences, Little Rock, AR, USA

[2]Departments of Medical Genetics and Pediatrics, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada

[3]Division of Genetics and Genomics, Boston Children's Hospital, Boston, MA, USA

[4]Department of Pathology, Stanford University Medical Center, Stanford, CA, USA

[5]Department of Clinical Genetics and Rare Disease Paediatric Unit, Hospital Sant Joan de Déu, University of Barcelona, Barcelona, Spain

[6]Genetic Medicine, Department of Pediatrics, University of California, San Francisco/Fresno, Fresno, CA, USA

[7]Medical Genetics, Department of Pediatrics, Cedars-Sinai Medical Center, Los Angeles, CA, USA

[8]North York General, Toronto, Canada

[9]Departments of Pediatrics and Molecular & Human Genetics, Baylor College of Medicine, Houston, TX, USA

[10]Clinical Genetics, Department of Pediatrics, HGU Gregorio Marañón, Madrid, Spain

[11]University Hospitals Center for Human Genetics, Cleveland, OH, USA

[12]UH Rainbow Babies and Children's Hospital, Cleveland, OH, USA

[13]Institute of Medical Genetics and Applied Genomics,Tuebingen, Germany

[14]Sheffield Clinical Genetics Service, Sheffield Children's NHS Foundation Trust, UK

[15]Department of Biological Sciences, University of Massachusetts Lowell, Lowell, Massachusetts

[16]Health Outcomes, College of Pharmacy, University of Rhode Island, Kingston, RI, USA

[17]FDNA Inc, Boston, MA, USA

[18]Departments of Pediatrics and Neurology & The Board of Governors Regenerative Medicine Institute, Cedars Sinai Medical Center, Los Angeles, CA, USA

[19]Department of Cardiology, Boston Children's Hospital and Department of Pediatrics, Harvard Medical School, Boston, MA, USA

Correspondence To:
Yuri A. Zarate, M.D.
Arkansas Children's Hospital
1 Children's Way; Slot 512-22
Little Rock, AR 72202
Telephone: 501-364-2971, yazarate@uams.edu
Fax: 501-364-1564

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ABSTRACT

SATB2-Associated syndrome (SAS) is an autosomal dominant, multisystemic, neurodevelopmental disorder due to alterations in *SATB2* at 2q33.1. A limited number of individuals with 2q33.1 contiguous deletions encompassing *SATB2* (ΔSAS) have been described in the literature. We describe 17 additional individuals with ΔSAS, review the phenotype of 33 previously published individuals with 2q33.1 deletions (n=50, mean age=8.5±7.8 years), and provide a comprehensive comparison to individuals with other molecular mechanisms that result in SAS (non-ΔSAS). Individuals in the ΔSAS group were often underweight for age (20/41=49%) with a progressive decline in weight (95% CI=-2.3 to -1.1, p<0.0001) and height (95% CI=-2.3 to -1.0, p<0.0001) Z-score means from birth to last available measurement. ΔSAS individuals were often noted to a broad spectrum of facial dysmorphism. A composite image of ΔSAS individuals generated by automated image analysis was distinct as compared to matched controls and non-ΔSAS individuals. We also present additional genotype-phenotype correlations for individuals in the ΔSAS group such as an increased risk for aortic root/ascending aorta dilation and primary pulmonary hypertension for those individuals with contiguous gene deletions that include *COL3A1*/*COL5A2* and *BMPR2*, respectively. Based on these findings, we provide additional care recommendations for individuals with ΔSAS variants.

Key Words: *SATB2*-Associated syndrome, *SATB2*, 2q33.1 deletion, facial recognition technology, genotype-phenotype correlation, Glass syndrome

INTRODUCTION

SATB2-Associated syndrome (SAS; Glass syndrome, OMIM 612313) is a multisystemic disorder caused by variants in *SATB2* at 2q33.1.[1] While numerous coding variants have been reported and are thought to be the most common molecular etiology, other documented molecular mechanisms include intragenic deletions and duplications, translocations interrupting *SATB2* or its regulatory elements, and contiguous deletions.[2-4]

Since the original description of a 16-year-old male with an interstitial deletion of 2q32.2-2q33.1[5], several other individuals with deletions encompassing *SATB2* (henceforth referred to as ΔSAS) have been reported. This has led to the separate designation of 2q33.1 microdeletion syndrome or 2q32-q33 deletion syndrome. Nevertheless, as more individuals with SAS were reported, the most consistent features of SAS continue to consist of neurodevelopmental disabilities, behavioral abnormalities, and craniofacial anomalies, regardless of the molecular mechanism.[1,2,6,7] A recent review of 17 individuals with ΔSAS revealed a phenotypic spectrum: developmental delay with significantly impaired speech, dental abnormalities (mainly with abnormal teeth shape/size and crowding), behavioral anomalies (hyperactivity and agitation/aggression being most frequent), feeding difficulties, hypotonia, cleft palate, and abnormal neuroimaging.[1] Other features such as facial dysmorphism, clinical seizures, and skeletal anomalies were also reported at variable frequencies.

Despite the limited number of described individuals with ΔSAS, there have been some attempts to identify genotype-phenotype correlations. Pre- or post-natal growth retardation, hypotonia, genitourinary anomalies, congenital cardiac defects, and some ectodermal manifestations such as thin skin or reduced subcutaneous fat were reported to

be more prevalent or exclusively seen in individuals with ΔSAS as opposed to intragenic

SATB2 abnormalities. Interestingly, all have had consistently similar issues with speech

development.[1,6,8]

In this report, we describe 17 additional individuals with 2q33.1 contiguous

deletions and provide a review of all previously described cases. We present a

comprehensive overview with more detail about the frequency of the main phenotypic

features of ΔSAS and how they compare to other molecular mechanisms such as

intragenic *SATB2* alterations.

MATERIALS AND METHODS

Individuals with 2q33.1 deletions that include *SATB2* entirely or with breakpoints near

the 3' or 5' end of *SATB2* were included. All new reported individuals agreed to share

clinical information and were enrolled under a research clinical registry protocol

approved by the Institutional Review Board of the University of Arkansas for Medical

Sciences and comforming with the Declaration of Helsinki. For all individuals, medical

records including laboratory results were reviewed. Supplementary information was also

obtained by parental report through a REDCap™ questionnaire. All families who shared

photographs signed consent for publication. Individuals SATB2-85 and SATB2-168 were

briefly presented in previous publications.[9,10]

Molecular cytogenetic studies

Several different chromosomal microarray platforms were used over time to analyze

genomic DNA extracted from peripheral blood over time. Earlier patients were tested

with oligonucleotide-based platforms (Agilent Human Genome 44K and 180K

oligonucleotide arrays, Agilent Technologies, Santa Clara, CA; Oxford Gene Technology

CytoSure™ 60K Oligonucleotide array, Oxford, UK), while more recent cases were

evaluated using the whole genome SNP arrays (Affymetrix, Santa Clara, CA).

Furthermore, microarray findings were confirmed with FISH analysis whenever possible.

Coordinates for older studies were converted to the GRCh37/h19 build.

Anthropometric values and growth charts

Available height, weight, and head circumference measurements were extracted from

medical records. Reference data and Z-scores for height, weight, and head circumference

were obtained from 2006 World Health Organization (WHO) international growth

standards for individuals aged 0 to 24 months and adjusted by gestational age

accordingly, and the 2000 CDC growth curves for children and adolescents from 2 to 20

years of age.[11]

Statistical analysis

Individuals' demographic and clinical milestones were characterized using medians and

means according to the genetic alteration type. For genotype-phenotype correlation, the

categorical analysis was performed using Fisher´s exact test. A paired t-test was used for

prospective analysis of anthropometric measurements over time. Analyses were

performed using SAS 9.4 software (SAS Institute, Cary NC).

RESULTS

Detailed clinical information from 17 novel individuals with deletions that included

SATB2 entirely (6 males and 11 females, mean age of 6.8 years, age range of 6 months to

34 years), along with the cumulative prevalence of clinical features of all 50 individuals

(17 from this cohort and 33 individuals previously reported in the literature) are presented

in Table 1.[6,12-21] Two individuals from the literature with 2q33.1 deletions that did not

include *SATB2* but had breakpoints near it were included in the ΔSAS group given the

known presence of regulatory elements at least on the 3' end of *SATB2* and their

overlapping phenotypic features with those reported in SAS.[12] Clinical information for all

50 individuals is described in the following sections with particular emphasis on

phenotypic features that are unique or have not been explored in detail for individuals

with ΔSAS.

Cytogenetic alterations

Through a variety of different molecular cytogenetic platforms utilized in clinical

settings, the mean deletion size for all individuals was 11Mb (range 600kb to 26.3Mb),

with 16 individuals having deletions greater than 15Mb (Figure 1). Patient 1 and patient 6

reported by Balasubramanian et al. had deletions falling 20kb and 220kb from the 5' and

3' end of *SATB2* respectively.[12] Segregation analysis in several families revealed *de novo*

status in 17/18 (94%) individuals, with a single case resulting from a balanced maternal

chromosomal rearrangement involving chromosomes 2 and 21.

Phenotypes

Individuals with ΔSAS, similar to non-ΔSAS, had the core clinical features of

developmental delay with severe speech delay, behavioral abnormalities, and craniofacial

anomalies (Table 1, Table 2). Developmental delay was present in all individuals with

delayed acquisition of early milestones and speech limited to 10 words or less in 69%

(22/32 with reported data) of individuals 4 years or older. Speech ability was not

correlated with the deletion size (data not shown). The frequency of other features was in

concordance with the reported prevalence in non-ΔSAS individuals: behavioral

abnormalities (85%), hypotonia (67%), abnormal neuroimaging (57%) ophthalmologic

anomalies (56%), cleft palate (50%), and epilepsy (28%).[3] Dental anomalies in the ΔSAS

cohort were frequently documented (96%) ofen with distinctive large and abnormally

shaped upper central incisors. Several subjects underwent bone density scans with low

bone density reported in 44% (4/9).

Perinatal history, growth, and nutrition

The mean gestational age was 38 weeks (range 32 to 41 weeks). The mean Z-scores

(adjusted for gestational age) for birth weight, length, and head circumference were: -

0.47 (n=49; -4.0 to +2.9), +0.22 (n=33, -2.4 to +4.4), and -0.32 (n=20, -3.8 to +2.83),

respectively. Feeding difficulties at any age were reported in 80% (40/50) of individuals,

with 24% (8/33) requiring a gastrostomy tube. At their most recent visit, almost half of

the individuals were underweight for age (49%; 20/41), often with short stature (41%;

15/37) and microcephaly (36%; 12/33). Detailed analysis of postnatal growth revealed

that for individuals with birth and postnatal values available for review (at least 6 months

apart), there was a tendency for decreased Z-score values with age for weight and height.

From birth until the latest measurement, there was a decrease in Z-score means for

weight and height of -1.7 (95% CI=-2.3 to -1.1, p<0.0001) and -1.7 (95% CI=-2.3 to -1.0,

p<0.0001), respectively (Figure 2). A higher frequency of low weight was seen for

individuals with deletions larger than 5Mb (62% vs 21%, p=0.02). Only one individual

(SATB2-147) received growth hormone supplementation briefly with no improvement

noted before discontinuation.

Cardiovascular and respiratory

Several individuals had cardiovascular defects (26%; 11/42) with five individuals having

septal defects alone or in combination with other findings. Other less common findings

included patent ductus arteriosus persisting beyond the neonatal period, vascular ring,

and valve dysplasias that were documented beyond the neonatal period.

Deletions involving BMPR2

Fifty percent (11/22) of ΔSAS individuals with deletions that included *BMPR2* had

echocardiograms performed and 27% (3/11) had pulmonary hypertension diagnosed

during the first few weeks of life (one individual had a ventricular septal defect that

required surgical repair and it is unclear whether pulmonary hypertension persisted after

repair of the septal defect, the other two lacked related severe congenital heart defects)

and were diagnosed with pulmonary hypertension. We highlight two individuals from the

new cohort below.

Individual SATB2-099 is a 2-year-old female diagnosed with a 2q32.1q33.3

deletion at 2 weeks of age. Her large deletion includes *COL3A1*, *COL5A2*, and *BMPR2*.

After an initial normal echocardiogram at 1 month of age, a repeat study at 9 months of

age revealed pulmonary hypertension that was persistent on her most recent evaluation at

2 years of age (moderate based on echocardiogram). To date, she has not required

medical or surgical treatment for her pulmonary hypertension. Because bronchiectasis

has not been reported to be associated with SAS, further clinical genetic testing was

conducted identifying a single variant (p.Phe508del) in *CFTR* thought to be insufficient

to explain the pulmonary phenotype.

Individual SATB2-193 is a 7-month old male, former 36-week infant who

presented with desaturation, cyanosis, hypoglycemia, and feeding difficulty. His deletion

includes *BMPR2*. Postnatal echocardiogram on day of life 1 showed right ventricular

pressures estimated to be about 2/3 systemic consistent with pulmonary hypertension. He

required ventilator support, dopamine for persistently low systemic blood pressures, and veno-arterial extracorporeal membrane oxygenation (ECMO) for the first 9 days of life. At 12 days of age, despite his clinical improvement, his persistent pulmonary hypertension required treatment with sildenafil that continued until his discharge from the NICU at 2 months of age. Over the following months, he had persistent episodes of cyanosis, arching, and desaturation consistent with pulmonary hypertensive spells and confirmed by right heart catheterization. Bosentan and amlodipine were added to his pulmonary hypertension management plan. His pulmonary hypertension is persistent to his current age.

Deletions involving COL3A1 and COL5A2

Of the 10 individuals reviewed in the present study with deletions that included both of these collagen genes, six had documented echocardiograms performed and two had aortic dilation. Individual SATB2-184 was noted to have mild aortic dilation at 2 years of age (Z-score +2.13) with no other structural heart defect. Individual SATB2-118 is a 9-year old female who was born full-term to a 26-year-old healthy mother. An echocardiogram on day 6 of life showed a patent foramen ovale versus small secundum atrial septal defect, a trivial, physiologic patent ductus arteriosus, aortic root dilation (Z-score +3.82), and normal ascending aorta dimension. Echocardiogram at age 16 months revealed aortic root dilation (Z-score +4.0) and normal ascending aorta dimension. Over time, she was diagnosed with tortuosity of craniocervical vasculature, severe aortic root dilation, and moderate ascending aorta dilation, with stable Z-scores over several years (+5 - 6 for the aortic root and +4 – 5 for the ascending aorta). She is currently on losartan and atenolol therapy without evidence of pulmonary artery hypertension.

Other systemic manifestations

Four individuals were reported to have pectus type deformity (3 excavatum, 1 carinatum)

and three others had scoliosis (only one of these seven individuals had a deletion

encompassing *COL3A1* and *COL5A2*). In addition to five individuals with inguinal

hernias, no other consistent genitourinary anomalies were described. Single instances of

colobomas, cataracts, metopic synostosis, and congenital diaphragmatic hernia were also

reported. Ectodermal manifestations such as thin skin, reduced subcutaneous fat, and

fine/sparse hair were reported in earlier cases but not consistently found in other recently

described individuals, including the 17 novel cases presented here.

Facial phenotype

Facial dysmorphic features were described in 90% of individuals (45/50) but with more

variability than previously reported in SAS.[3,6,7]Besides a thin vermilion of the upper lip,

long and flat philtrum, and deeply set eyes, other features noted include a prominent

forehead with a receding anterior hairline, a pointed chin, and triangular appearance of

the lower face with a narrow jaw (Figure 3). Of note, overlapping facial features to those

described in Sotos syndrome were seen in 2 inviduals (individual SATB2-093 and

individual SATB2-147). For additional objectivity, 2D photographs were analyzed using

Face2Gene image analysis (FDNA Inc., USA, v19.1.12), a tool that has been shown to

add value to phenotypic evaluations in clinical genetics.[22] Images from 24 individuals

with ΔSAS were noted to be significantly different from 24 age-, ethnicity-, and gender-

matched controls (area under the curve 0.89, p-value = 0.008). When compared to

similarly matched non-ΔSAS group (N=59), the ΔSAS cohort were very close to being

different in a statistically significant manner (area under the curve 0.706, p-value = 0.06)

(Figure 3).

DISCUSSION

SAS caused by contiguous gene deletions as compared to other molecular mechanisms

contributes to approximately 20% of SAS cases (https://satb2gene.com/pros-molecular-

data/). Through the comprehensive analysis of 50 ΔSAS individuals (17 that were

previously unreported), we determined that the cardinal findings include severe speech

delay, behavioral issues, cleft palate, dental anomalies, hypotonia, ophthalmologic

abnormalities, and epilepsy. Of note, we present additional clinical features that were

more prevalent or unique to our -ΔSAS cohort. These features are important in that they

require additional counseling, surveillance, or interventions.

Detailed anthropometric measurement analysis has not previously been conducted

in SAS. Approximately 2/3 of individuals with ΔSAS were found to have growth

retardation, which was twice as common when compared to non-ΔSAS individuals. This

growth retardation occurred despite the lack of any difference in the frequency of cleft

palate or reported feeding difficulties. Indeed, Z-score values for weight and height

showed a significant decrease over time in the ΔSAS cohort with individuals with larger

deletions having a higher frequency of low weight. This suggests that other genes in the

contiguous deleted segment may be influencing growth parameters in the ΔSAS cohort.

Generation of dedicated SAS growth charts may assist in predicting the expected growth

patterns in the ΔSAS population as compared to non-ΔSAS individuals. This information

will assist in determining whether aggressive feeding interventions (including

gastrostomy tubes) may be required to assist the child to attain a normalized weight for better growth and for airway protection.

Individuals with ΔSAS had variable facial dysmorphisms beyond the previously described features seen in non-ΔSAS. Some individuals in the ΔSAS cohort displayed changes in the lower face configuration which could be related to other contiguous genes included in their deletions or could be a reflection of a true *SATB2*-dosage effect that supports haploinsufficiency in ΔSAS individuals. In mice, jaw development is *Satb2*-dosage sensitive and although the loss of *Satb2* affects both upper and lower jaws, previous studies have shown a predominant compromise of the distal part of the mandible independently of the proximal region.[23,24]

The deletions encompassing the *SATB2* gene and involving the 2q32-q33 region were quite diverse in size and makeup. Some of the genes located in this region that are linked to potentially relevant dominant phenotypes and with high probability of being loss-of-function intolerant: *HSPD1* (pLI=0.99), *HECW2* (pLI=1), *COL3A1* (pLI=1), *COL5A2* (pLI=1), and *BMPR2* (pLI=1). The full impact that results from the haploinsufficiency of these genes in ΔSAS individuals, genes remains to be elucidated.

Hemizygous deletion of *COL3A1* with resulting haploinsufficiency has been associated with cardiovascular complications. Meienberg et al. described a family with a 3.4Mb deletion at 2q31.1q32.3 and that included both *COL3A1* and *COL5A2* (but not *SATB2*). All seven individuals displayed features of a connective tissue disorder characterized by some overlapping clinical signs of vascular and classic Ehlers-Danlos syndromes. In two of these subjects, fatal aortic dissections (thoracic and abdominal) occurred during adulthood and there was evidence of involvement of medium-sized

arteries.[25] Based on the individuals described in this report and the previously described

family by Meienberg et al., we recommend baseline echocardiographic evaluation for

those individuals with deletions that include the *COL3A1* and/or the *COL5A2*, with

consideration for ongoing yearly surveillance of the thoracic and abdominal aorta for

aortic aneurysms and risk of dissection. Furthermore, we recommend having a low

threshold to obtain detailed imaging of the vascular tree depending on echocardiographic

results, as well as clinical symptoms such as headache, stroke, or abdominal pain, or

physical findings such as decreased peripheral pulses, asymmetric blood pressure

measurements, or abdominal bruits.

Primary pulmonary hypertension (PPH) is a rare and often fatal disease

characterized by pulmonary vascular cell proliferation and a sustained elevation in mean

pulmonary artery pressure. Heterozygous mutations (including whole gene deletions)

resulting in haploinsufficiency in *BMPR2* are often found in children and adults with

idiopathic and familial cases of PPH.[26-29] Considering the known reduced penetrance and

variable expressivity of BMPR2-related PPH and based on the cases here described, we

suggest that ΔSAS individuals with deletions containing *BMPR2* should also have a

baseline echocardiogram at diagnosis and should then continue to follow published

expert guidelines to accurately identify PPH.[30]

In summary, in addition to the core features of SAS such as developmental delay,

behavioral issues, and craniofacial anomalies, Individuals with ΔSAS also displayed a

higher tendency for growth retardation, variable facial dysmorphism, and increased risk

for pulmonary hypertension and aortic dilation/dissection. Larger studies that look at the

impact of contiguous genes deleted are needed to better delineate the phenotypic

differences among ΔSAS individuals.

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FIGURE LEGENDS

Figure 1. Schematic representation and main phenotypic features of individuals with chromosomal deletions at 2q33.1 including (48 individuals) or near (2 individuals) *SATB2* based on GRCh37/hg19 assembly (Dashed lines only to help to identify individuals with their corresponding deletion). IDs/references are listed on the left side with "*" depicting novel individuals from this report. Stars next to ID on left side represent those individuals with current weight below 3rd centile. Individuals ID#11, #12, #13, #17, #19, #21, #30, #32, #37, #40, #44, #45, #50, #72, #74, and #77 were previously published.[6] Only some of the OMIM genes with associated autosomal dominant phenotypes are represented. Boxes highlight overlapping deletions with deleted *COL3A1*, *COL5A2*, *SATB2* or *BMPR2*.

Figure 2. Anthropometric changes over time for individuals with deletions involving *SATB2*. Birth and last available measurement pairs for weight (**A.**, 34 individuals) and height (**B.**, 21 individuals) were analyzed using paired t-test. Mean Z scores and standard deviations for each group are provided.

Figure 3.Facial features in individuals with deletions encompassing *SATB2* were variable. **A.** Individual SATB2-085. **B.** Individual SATB2-093. **C.** Individual SATB2-099. **D.** Individual SATB2-118. **E.** Individual SATB2-141. **F.** Individual SATB2-147. **G.** Individual SATB2-156. **H.** Individual SATB2-176. **I.** Individual SATB2-180.

Note thin vermilion of the upper lip with a flat and/or long philtrum in individuals A, B, C, E, and G; deeply-set eyes in individuals A and C; prominent forehead with a receding anterior hairline in individuals C, D, F, G and I; pointed chin in individuals E, F, G;

narrow jaw in individuals B and F; abnormal appearance of upper central incisors in

individuals D and F; and subtle myopathic face in individual H.

J., K., L. Composite facial features as generated by facial recognition technology as

provided by Face2Gene vs.19.1.2 for individuals with ΔSAS (24 individuals), Control

cohort (24 individuals), and non-ΔSAS (59 individuals).