RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

Further Supporting Evidence for the *SATB2*-Associated Syndrome Found Through Whole Exome Sequencing

Yuri A. Zarate,[1]* Hazel Perry,[2] Tawfeg Ben-Omran,[3] Elizabeth A. Sellars,[1] Quinn Stein,[4] Mariam Almureikhi,[3] Kirk Simmons,[5] Ophir Klein,[2] Jennifer Fish,[6] Murray Feingold,[7] Jessica Douglas,[7] Michael C. Kruer,[4] Yue Si,[8] Rong Mao,[8] Dianalee McKnight,[9] Federica Gibellini,[9] Kyle Retterer,[9] and Anne Slavotinek[10]

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

[2]Division of Craniofacial Anomalies, University of California San Francisco Department of Orofacial Sciences, San Francisco, California

[3]Clinical and Metabolic Genetics, Department of Pediatrics, Hamad Medical Corporation, Doha, Qatar

[4]Divisions of Pediatric Neurology & Genetics, Sanford Children's Specialty Clinic, Sanford Children's Hospital, Sioux Falls, South Dakota

[5]Deparment of Dentistry, University of Arkansas for Medical Sciences, Little Rock, Arkansas

[6]Department of Orthopaedic Surgery, University of California San Francisco, San Francisco, California

[7]Division of Genetics and Genomics, Feingold Center, Boston Children's Hospital, Boston, Massachusetts

[8]Molecular Genetics and Genomics, ARUP Laboratories, Salt Lake City, Utah

[9]GeneDx, Gaithersburg, Maryland

[10]Division of Genetics, Department of Pediatrics, University of California San Francisco, San Francisco, California

Manuscript Received: 25 August 2014; Manuscript Accepted: 27 September 2014

The *SATB2*-associated syndrome (SAS) was recently proposed as a clinically recognizable syndrome that results from deleterious alterations of the *SATB2* gene in humans. Although interstitial deletions at 2q33 encompassing *SATB2*, either alone or contiguously with other genes, have been reported before, there is limited literature regarding intragenic mutations of this gene and the resulting phenotype. We describe five patients in whom whole exome sequencing identified five unique de novo mutations in the *SATB2* gene (one splice site, one frameshift, and three nonsense mutations). The five patients had overlapping features that support the characteristic features of the SAS: intellectual disability with limited speech development and craniofacial abnormalities including cleft palate, dysmorphic features, and dental abnormalities. Furthermore, Patient 1 also had features not previously described that represent an expansion of the phenotype. Osteopenia was seen in two of the patients, suggesting that this finding could be added to the list of distinctive findings. We provide supporting evidence that analysis for deletions or point mutations in *SATB2* should be considered in children with intellectual disability and severely impaired speech, cleft or high palate, teeth abnormalities, and osteopenia.

Key words: *SATB2*; 2q33.1 deletion; cleft palate; whole exome sequencing

How to Cite this Article:
Zarate YA, Perry H, Ben-Omran T, Sellars EA, Stein Q, Almureikhi M, Simmons K, Klein O, Fish J, Feingold M, Douglas J, Kruer MC, Si Y, Mao R, McKnight D, Gibellini F, Retterer K, Slavotinek A. 2015. Further supporting evidence for the *SATB2*-associated syndrome found through whole exome sequencing.
Am J Med Genet Part A 167A:1026–1032.

INTRODUCTION

The Special AT-rich sequence-binding protein 2 (*SATB2*; OMIM 608148) is a highly conserved gene located at 2q33.1 that spans 191 kb [Leoyklang et al., 2007]. It functions as a transcription factor regulating gene expression through chromatin modification and

Conflict of interest: The authors do not declare any conflict of interest.
*Correspondence to: Yuri A. Zarate, M.D., Arkansas Children's Hospital, 1 Children's Way; Slot 512-22, Little Rock, AR 72202.
E-mail: yazarate@uams.edu
Article first published online in Wiley Online Library (wileyonlinelibrary.com)
DOI 10.1002/ajmg.a.36849

interaction with other proteins and ligand receptors [Leoyklang et al., 2007; Rosenfeld et al., 2009; Balasubramanian et al., 2011; Docker et al., 2013; Zhao et al., 2014].

Animal models have shown high expression in the developing brain, branchial arch derivatives, and sites of bone formation in mice as well as branchial arches of zebrafish and *Xenopus* [Britanova et al., 2005; Britanova et al., 2006; Dobreva et al., 2006; Alcamo et al., 2008; Ahn et al., 2010; Sheehan-Rooney et al., 2010; Fish et al., 2011]. Its relevance in mammalian development has also been documented with its critical role in palatogenesis, neuronal differentiation and migration, and skeletal development [FitzPatrick et al., 2003; Britanova et al., 2005; Leoyklang et al., 2007; Alcamo et al., 2008; Balasubramanian et al., 2011; Docker et al., 2013].

It has been recently proposed that regardless of the mechanism that results in loss of *SATB2* function in humans (contiguous deletions, intragenic deletions, translocations with secondary gene disruption or point mutations), the result is a clinically recognizable syndrome termed—the *SATB2*-associated syndrome (SAS). The characteristic features are intellectual disability (ID) with absent or limited speech development, behavioral problems, craniofacial abnormalities, dysmorphic features, cleft or high palate, and dental abnormalities [Docker et al., 2013].

We describe five patients in whom whole exome sequencing (WES) identified de novo previously unreported mutations in the *SATB2* gene. The five patients had overlapping features that confirm SAS as a recognizable syndrome. Patient 1 also had other features not previously described that could represent an expansion of the phenotype.

MATERIALS AND METHODS

Approval for the review and reporting of these cases was given by the Institutional Review Board of the University of Arkansas for Medical Sciences.

Patient 1

Exome sequencing was performed using the sample from the proband. Parental specimens were used for Sanger confirmation of the targeted mutations/variants. Genomic DNA was extracted from peripheral blood using Gentra Puregen Blood Kit (QIAGEN NV, Hilden, the Netherlands). Exons were captured with Agilent SureSelect kit (XT All Exon V4 kit, Agilent Technologies, Santa Clara, CA) and sequenced with 2×100 bp paired end reads on an Illumina HiSeq 2500 according to the manufacturer's recommendations (Illumina, San Diego, CA). Sequence was aligned to hg19 using the Burrows–Wheeler Aligner (0.5.11) and variants were called with Genome Analysis Toolkit (v.1.6). More than 93% of bases sequenced had a quality score greater than 10 and variants with a quality score <10 were removed to avoid false positives. Variants with an allele frequency of 1% in dbSNP, 1000 Genomes Project, and the NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS/) were filtered out. Further filtering removing synonymous variants, deep intronic variants, 5′- and 3′-UTR resulted in 2,316 variants. Short tandem repeat markers were used to confirm family relationships. An amplicon containing the mutation in *SATB2* (NM_001172517.1) was amplified and the variant was confirmed by Sanger sequencing using BigDye ® Terminator Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA) on an Applied Biosystems 3730 DNA Analyzer. Primer sequences are available upon request.

Data analysis. For autosomal recessive variant analysis, variants with a population frequency higher than 0.01 and frequency higher than 0.03 in an ARUP internal database, generated following the same methods, were filtered. For autosomal dominant variant analysis, sample frequency higher than 0.001 in the general population or frequency higher than 0.005 in the ARUP internal database were filtered. Variants within the exon and the exon and intron junctions ($+/-10$ bp) were used for further analysis. In silico computational prediction software NetGene2 Berkeley NNSplice was used for splice site predictions; PolyPhen2, SIFT, and MutationTaster were used to predict the possible disruption of protein functions (http://www.fruitfly.org/seq_tools/splice.html; http://genetics.bwh.harvard.edu/pph2/index.shtml;http://sift.jcvi.org/www/SIFT_enst_submit.html; http://www.mutationtaster.org/).

Patients 2–5

GeneDx Exome sequencing was performed on exon targets isolated by capture using the Agilent SureSelect XT2 Human All Exon V4 (50 Mb) kit (Agilent Technologies, Santa Clara, CA). DNA was extracted from peripheral blood with QiaSymphony BioRobot (QIAGEN). One microgram of extracted DNA was sheared into 350–400 bp fragments, which were then repaired, ligated to adaptors, and purified for subsequent PCR amplification. Amplified products were then subjected to capture by biotinylated RNA library baits in solution, following the manufacturer's instructions. Bound DNA was isolated with streptavidin-coated beads and re-amplified. The final isolated products were sequenced using the Illumina HiSeq 2500 sequencing system with 100-bp paired-end reads (Illumina, San Diego, CA). Data was aligned to hg19 using the BWA Burroughs Wheeler Aligner (v0.6.2). Local realignment was performed using the Genome Analysis Toolkit Indel Realigner (v1.6). Variants were called simultaneously on the proband and parents using SAMtools (v0.1.18). The amplicon containing a mutation in the *SATB2* (NM_001172517.1) gene was amplified using standard PCR protocol. The variant was confirmed by Sanger Sequencing using BigDye® Terminator Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA) and AxyPrep™ Mag Dye-Clean Kit (Axygen Biosciences, Union City, CA) on an Applied Biosystems 3730xl DNA Analyzer. Primer sequences are available upon request.

CLINICAL REPORTS

Patient 1

The patient was born at term after an uncomplicated prenatal history. At 2 weeks of age, a routine check-up demonstrated cleft palate that was repaired at one year of age. He continued with normal health until 5 years of age, when he contracted viral encephalitis and had a seizure. A brain magnetic resonance imaging (MRI) scan at this time showed mild cerebral volume loss and he did not have subsequent seizures. Developmental history revealed the boy to have delays in communication and language. His early gross

motor skills, such as crawling, sitting, and walking, were within the normal range. He was able to speak single words, but had difficulty with articulation and combining words. He repeated kindergarten, but then progressed to first grade in both mainstream and resource classes. He has been described as a very friendly and affectionate child. Family history was not significant.

At 7 years of age, he was referred to genetics clinic because of his developmental delays and dysmorphic facial features. On physical exam, he was noted to be short and obese (BMI of 24.7, 99th centile, Table I). In addition to having dysmorphic features, he was noted to have a cleft in his ear-lobe, generalized hirsutism, and tapered fingers (Fig. 1). He had primary macrodontia and dental crowding with evidence of bruxism. A skeletal survey showed diffuse osteopenia.

Patient 2

The proband was the first child of nonconsanguineous parents and was conceived by in-vitro fertilization with intracytoplasmic sperm injection. Two embryos were implanted and at 8 weeks, a vanishing twin was noted. The proband was born at 40 weeks gestation via normal spontaneous vaginal delivery. Cleft palate and micrognathia were noted at birth. At the 7-month assessment, he had epicanthal folds, a prominent sulcus on the tongue, restricted range of motion of hips and knees, and a mild pectus deformity. He did not babble and was unable to sit independently. At 20 months, he had global developmental delays and hypotonia. He was unable to stand or walk, use a pincer grasp, and feed himself. He had undergone surgery for alternating esotropia and wore glasses. At 3 years of age, the proband spoke no words and was diagnosed with severe speech delay. He began walking independently at 4 years of age and required orthotics. Macrodontia was diagnosed at 5 years of age and his lateral incisors were removed because they grew anterior to the central incisors (Fig. 1). Family history was negative for ID and orofacial clefting.

Patient 3

The 32-year-old patient was the first child born to his nonconsanguineous parents. He was delivered after an uneventful pregnancy at term. He was noted postnatally to have a cleft of the soft palate that was repaired. Strabismus surgery was also needed during childhood. Other medical problems include grand mal seizures and staring spells since age 12 years that continue to require medical management, severe osteopenia diagnosed at 26 years of age treated by calcium supplementation, and hypothyroidism diagnosed at 24 years of age managed with hormonal replacement. Developmental delay was noted during infancy with predominant deficits in speech. He walked at 20 months of age, but continues to be nonverbal. He was able to use signs but this ability regressed and he currently uses gestures for communication. He was dependent on caregivers for his daily routines and lived in a group home with 24/7 staffing. He was diagnosed with leg length discrepancy in childhood and was recently prescribed orthotics. During his most recent examination, he was noted to have dysmorphic features and mild kyphosis (Fig. 1). The family history was negative for orofacial clefting or ID.

Patient 4

The proband was born at 36 3/7 weeks gestation via uncomplicated vaginal delivery, the third child of nonconsanguineous parents. The pregnancy was complicated by oligohydramnios, mild left pyelectasis, and an increased nuchal fold. After an uncomplicated delivery, he experienced postnatal feeding difficulties and was subsequently discovered to have an incomplete bilateral cleft palate with micrognathia. Surgical repair of the cleft was completed at 12 months of age. During infancy, he was delayed in achieving early motor and language milestones and had generalized hypotonia. He began walking at age 2 years. At age 3 years, he was referred to the Neurogenetics Clinic for further evaluation of his global developmental delay, dysmorphic features, and cleft palate. Examination revealed dental crowding, micrognathia, broad thumbs and great toes, and 5th finger clinodactyly (Fig. 1). He also had generalized hypotonia, night terrors, sialorrhea, bilateral esophoria, and a dyspraxic gait. He was able to complete 1-step directions and would gesture and use 4–5 signs to communicate, but he did not have any expressive speech. He had a good-natured disposition, with interest in peers and the ability to pretend play. Currently, at 4 years of age, he uses technology systems in addition to other modalities to assist in expressing his wants and needs. The family history was negative for orofacial clefting or ID.

Patient 5

This 4-year-old boy was the second child of healthy consanguineous parents (first cousins) of Qatari origin. Pregnancy and perinatal history were uncomplicated. At 7 months of age, he presented with hypotonia and gross motor with episodic vomiting and fever. On examination, there was a prominent upper jaw, malocclusion teeth, joint hypermobility, and increased skin elasticity. He was hypotonic, but with normal deep tendon reflexes, head control, cranial nerve function, and eye movements. Vision and hearing evaluations were normal. At age 2 years, developmental delay persisted with hypotonia and bilateral intermittent squinting. However, he showed some improvement with physiotherapy and speech therapy. He started walking at 21 months with a waddling gait, but had no clear words. He currently has mild hypotonia. No dysmorphic features were noted on exam. A brain MRI scan performed at 14 months showed delayed myelination with interval improvement on a follow-up study at 2 years of age. Family history was noncontributory.

RESULTS

Patient 1

Exome sequencing revealed one pathogenic, de novo splice site mutation in intron 6 of the *SATB2* gene, c.346 + 2T > G (p.Gly115fs*15) (Supplementary Figure A). This mutation is predicted to abrogate a splice donor site, potentially leading to loss of expression and/or function of the SATB2 protein. Three variants of unknown significance were also detected. Two heterozygous mutations in the alpha-aminoadipic semialdehyde synthase gene (*AASS* gene; NM_005763) were noted: a c.323T > C (p.Phe108Ser) change of uncertain significance and a c.1678C > T (p.Pro560Ser) change predicted to be benign. One paternally inherited variant of

TABLE I. Clinical Features of Described Patients With Mutations Affecting the *SATB2* Gene

	Leoyklang et al.	Döcker et al.	Rauch et al.	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Mutation	c.715C > T (p.R239X)	c.715C > T (p.R239X)	c.1142T > G (p.V381G)	c.346 + 2T > G (p.G115fsX15)	c.1945dupT (p.S649Ffs*40)	c.748C > T (p.Q250X)	c.1171C > T (p.Q391X)	c.847C > T (p.R283X)
Origin	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo
Age, years	36	3	NR	7	5	32	3	4
Gender	Male	Female	Female	Male	Male	Male	Male	Male
Birth measurements								
Weight, kg (centile, WHO)	NR	3.32 (58th)	NR	3.6 (69th)	3.74 (78th)	2.92 (18th)	3.22 (40th)	3.7 (76th)
Length, cm (centile, WHO)	NR	52 (94th)	NR	50 (52nd)	53 (95th)	53 (95th)	53.8 (98th)	N/A
Current anthropometrics								
Weight, kg (centile)	40 (<3rd)	12 (10th)	NR	26.2 (70th)	16.7 (17th)	70.8 (51st)	14.7 (57th)	11 (10–25th)
Height, cm (centile)	165 (25th)	94.5 (25th)	NR	102.9 (<3rd)	104.1 (11th)	179 (62nd)	98 (74th)	87 (50th)
Head circumference, cm (centile)	56 (50th)	49 (40th)	NR	54 (93rd)	N/A	60 (>99th)	51 (50th)	N/A
Developmental delay/ Intellectual disability	Profound	Severe	Severe	Mild	Severe	Severe	Moderate	Moderate
Speech development	Limited	Absent	NR	Limited	Absent	Absent	Absent	Absent
Behavior and sleep	Happy mood	Behavior and sleep problems	NR	Very friendly	Jovial personality	Skin picking	Normal	Normal
Brain imaging	No intracranial abnormality	Normal	NR	Mild cerebral atrophy	Borderline cerebellar tonsilar ectopia, Mild ventriculomegaly	Normal	Normal	Decreased myelination
Seizures	Yes	No	NR	Yes (single episode)	No	Yes	No	No
Dysmorphic features	Hypertelorism	Downslanted palpebral fissures, anteverted nares	NR	Hypertelorism, short palpebral fissures, synophrys	Epicanthal folds	Deeply set eyes, convex nasal ridge	Low-set and protruding ears, tall forehead	None
Cleft palate	Yes	Yes	Bifid uvula	Yes	Yes	Yes	Yes	No
Micrognathia	Yes	Yes	NR	Yes	Yes	No	Yes	No
Teeth abnormalities	Anterior-pointing incisors, oligodontia	Crowded, irregularly shaped	NR	Macrodontia, crowded	Macrodontia, crowded	Crowded	Macrodontia, crowded	malocclusion
Other craniofacial	Bilateral asymmetric mandibular hypoplasia, small maxillary sinuses	None	NR	None	None	Hypoplastic frontal sinuses	None	None
Skeletal abnormalities	Osteoporosis	None	NR	Osteopenia	None	Osteopenia, leg length discrepancy	Broad thumbs and halluces	None
Ophthalmologic findings	None	Astigmatism	NR	None	Strabismus	Strabismus	Strabismus, hyperopia	Squinting
Additional findings	None	None	NR	Body hirsutism	None	Hypothyroidism	None	Hypotonia
Prior normal or unremarkable genetic testing	NR	AS, FX, CMA	NR	Metabolic studies, FX, CMA, lysosomal screening	Metabolic studies, FX, CMA	Metabolic studies, FX, CMA, urine MPS	CMA, AS	VLCFA, CDG, CMA, SMA

AS, Angelman syndrome; CDG, Congenital disorders of glycosylation screen; CMA, chromosomal microarray; FX, fragile X; MPS, mucopolysaccharidosis; N/A, not available; NR, not reported; SMA, spinal muscular atrophy; VLCFA, very long chain fatty acids; WHO, world health organization.

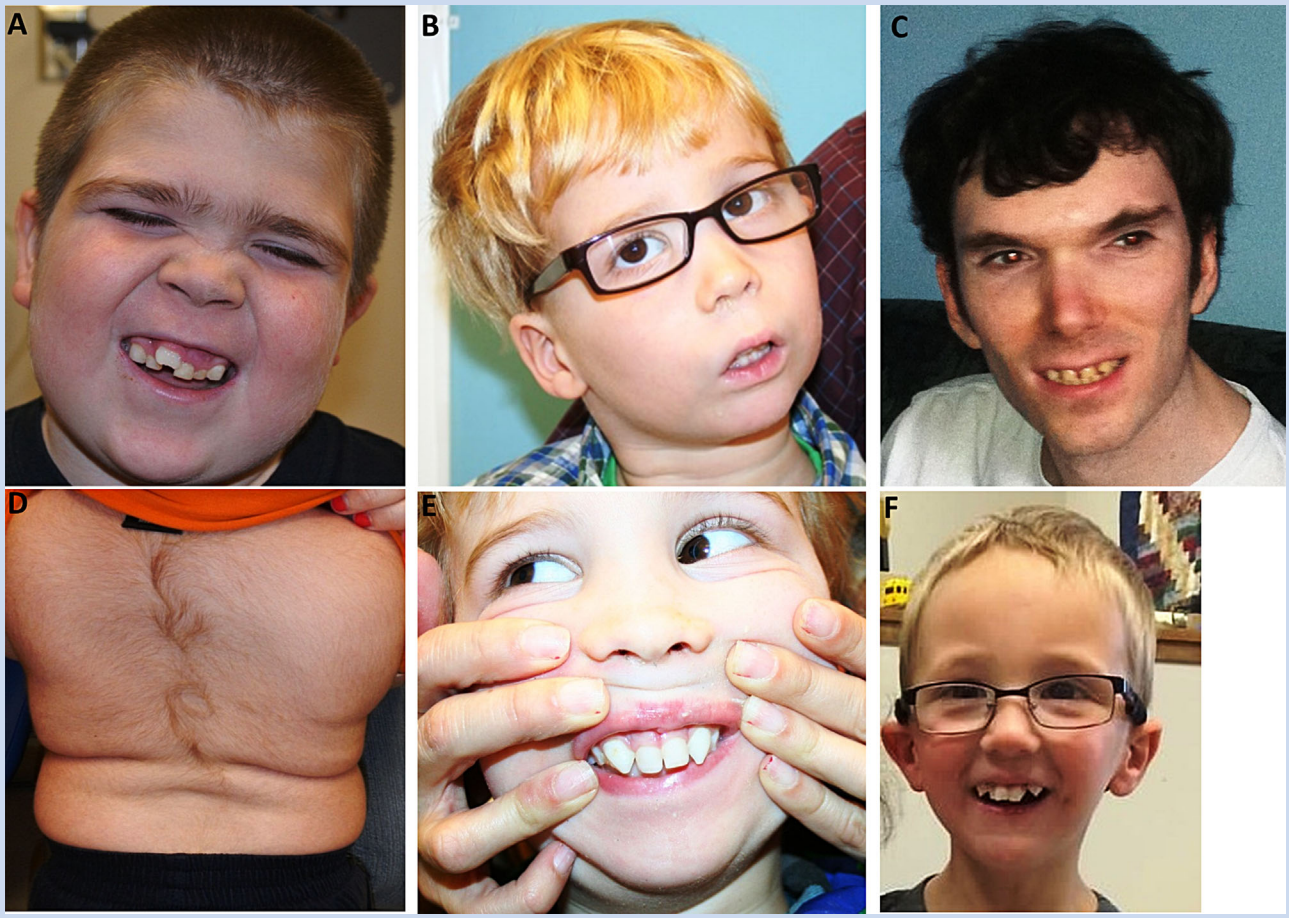

FIG. 1. A,D: Patient 1 at 7 years of age. Dysmorphic features include widow's peak, synophrys, hypertelorism, macrodontia, and crowded dentition. Note body hirsutism. B,E: Patient 2 at 5 years of age. Patient has long and smooth philtrum with macrodontia (lateral incisors have been removed). C: Patient 3 at 30 years of age. Dysmorphic features include prominent supraorbital ridges, deep-set eyes, and a triangular-shaped face. F: Patient 4 at 3 years of age. Note low-set and protruding ears, tall forehead, long and smooth philtrum with macrodontia.

unknown significance, c.2105G > A (p.Arg702Gln), was present in the kinesin family member 7 gene (*KIF7*; NM_198525) and a mutation of unknown significance, c.1055G > A (p.Ser352Asn), was noted in the WD repeat-containing protein 81 gene (*WDR81*; NM_001163809).

Patient 2

Prior genetic workup included an oligonucleotide microarray study (Agilent Human Genome CGH 105 k Chip©) that revealed a 294 kb maternally inherited duplication at Xp22.31 that was considered to be of unknown clinical significance (hg19: ChrX: 7,837,474–8,131,811). Exome sequencing revealed a de novo mutation in exon 12 of *SATB2*, c.1945dupT (p.Ser649Phe*fs**40) (Supplementary Figure B). This mutation is predicted to cause a frameshift, creating a premature stop codon at position 40 of the new reading frame. This mutation is predicted to cause loss of normal protein function through premature protein truncation and to disrupt the DNA-binding homeobox domain (residues 615–674; Uniprot Q9UPW6).

Patient 3

An oligonucleotide microarray study (Athena WholeGenome microarray© 105K) identified two separate changes of unknown significance that were inherited from his phenotypically normal father: a 170 kb deletion at 2q21.3 (hg19: 135,908,052–136,078,392) and a 13 kb duplication at 8q24.3 (hg19: 124,259,891–124,272,915). A de novo mutation in exon 8 of the *SATB2* gene was identified by WES: c.748C > T (p.Gln250*) (Supplementary Figure C). This mutation is predicted to cause loss of normal protein function either through protein truncation or nonsense-mediated mRNA decay.

Patient 4

WES identified a de novo mutation in exon 8 of the *SATB2* gene: c.1171C > T (p.Gln391*) (Supplementary Figure D). This mutation is predicted to cause loss of normal protein function either through protein truncation or nonsense-mediated mRNA decay.

Patient 5

Liver function tests, creatine kinase, endocrine workup, acylcarnitine profile, lysosomal studies, very long chain fatty acids, phytanic acid in blood, congenital disorders of glycosylation screen, array CGH, molecular testing for spinal muscular atrophy, and urine organic acid analysis, all were normal. His lactate level was slightly elevated at 2.87 mmol/L (normal 1–2.4) and fasting plasma amino acids disclosed mildly elevated levels of citrulline 36 μmol/L (normal 3–35), valine 335 μmol/L (normal 64–294), and isoleucine 111 μmol/L (normal 31–86), but normal leucine. Alloisoleucine was not detected. WES identified a de novo nonsense mutation in exon 8 of the *SATB2* gene: c.847C > T (p.Arg283*) (Supplementary Figure E). Furthermore, the patient was found to be homozygous for a c.685G > T (p.Gly229Cys) sequence variant in exon 9 of the dihydrolipoamide dehydrogenase gene (*DLD*; NM_000108.3). His parents were heterozygous for this mutation as well. This mutation is a non-conservative amino acid substitution of a non-polar Glycine with a neutral, polar Cysteine at a residue that is conserved across species and has been previously reported to lead to DLD deficiency (DLDD) [Shaag et al., 1999].

DISCUSSION

We report on WES using a trio-based approach (probands and their unaffected biological parents) to identify five de novo, and previously unreported mutations in the *SATB2* gene in patients that shared similar features. Studies of murine *Satb2* show that this gene is highly expressed in the developing upper and lower jaw, including the palatal shelves and teeth buds, osteoprogenitor cells, and developing cerebral cortex [FitzPatrick et al., 2003; Britanova et al., 2005; Leoyklang et al., 2007; Docker et al., 2013]. In these tissues, Satb2 appears to have an important role in palatogenesis, regulation of the expression or activity of several target genes that are critical components of osteoblast differentiation and skeletogenesis (through *Hoxa2* repression and *Runx2*/*Atf4* enhancement), and in neuronal migration and differentiation [Britanova et al., 2005; Dobreva et al., 2006; Leoyklang et al., 2007; Alcamo et al., 2008; Zhang et al., 2011]. As expected, murine mutants show variable incisor hypodontia and/or adontia, hyoid and craniofacial malformations, shortening of the hindlimbs, and skeletal malformations [Britanova et al., 2006; Dobreva et al., 2006; Ahn et al., 2010].

As in mice, defects or haploinsufficiency of *SATB2* in humans are predicted to potentially impair multiple systems, resulting in a broad phenotype that includes cleft palate, osteoporosis, and ID [Zhao et al., 2014]. This gene has also been considered as a candidate gene for Toriello–Carey syndrome (OMIM 217980) [Tegay et al., 2009]. A variety of different molecular mechanisms can result in *SATB2* alterations. It can be contiguously deleted, as in the recently described 2q32q33 microdeletion syndrome (OMIM 612313, Glass syndrome), disrupted through smaller, interstitial deletions limited to *SATB2* or by translocations involving 2q33.1, or altered through intragenic *SATB2* mutations. Regardless of the mechanism, a recognizable phenotype has emerged with core features that include: developmental delay (with pronounced impairment of speech development) or ID, cleft or high palate, and dental malformations (broad teeth, missing teeth, abnormally shaped teeth, maloclussion, and diastema). Other common manifestations reported include: osteoporosis or osteomalacia, feeding difficulties with growth delay, behavioral problems, thin and sparse hair, thin and transparent skin, and dysmorphic features with a long and narrow face, high forehead, downslanting palpebral fissures, a high nasal bridge with overhanging nasal tip, low-set ears, a small mouth, and micrognathia [Leoyklang et al., 2007; Rosenfeld et al., 2009; Urquhart et al., 2009; Balasubramanian et al., 2011; Rauch et al., 2012; Docker et al., 2013; Leoyklang et al., 2013; Rainger et al., 2014; Zhao et al., 2014].

There are only three previously reported patients with intragenic *SATB2* point mutations (Table I). The first case was described by Leoyklang et al. [2007] and comprised an adult male ascertained through a cohort of 59 patients with craniofacial dysmorphism with or without ID [Leoyklang et al., 2007]. The second case was diagnosed using WES in a 3-year-old girl with craniofacial malformations, severe speech impairment, dental abnormalities, and behavioral issues [Docker et al., 2013]. Of interest, in both cases, the same de novo nonsense mutation in *SATB2* was identified (c.715C > T; predicting p.Arg239*). The last case was that of a female patient with a bifid uvula that was diagnosed by WES in a cohort of patients with severe ID and a novel missense mutation predicted to be probably damaging [Rauch et al., 2012]. While the exact pathomechanism of point mutations remains unknown, it has been speculated that the relatively severe phenotype could be caused by a dominant negative effect of the truncated or altered SATB2 protein when compared to the phenotype that results from haploinsufficiency of 2q32–q33 deletions and translocations [Leoyklang et al., 2007; Docker et al., 2013; Leoyklang et al., 2013]. However, other heterozygous *satb2* mutant species show extreme variation in their mandibular length, indicating that other mechanisms are also possible [Fish et al., 2011].

Patient 1 was noted to have coarse facial features and hirsutism that were not previously described in patients with *SATB2* alterations. He underwent more extensive evaluation for other possible underlying conditions, with unsuccessful results. Of the three additional genes with sequence variants of unknown significance that were detected by WES, none seem to explain these additional phenotypic features. The *AASS* gene catalyzes the first two steps in the lysine degradation pathway. Individuals with disease typically have elevated lysine levels, but our patient demonstrated normal levels of serum lysine. The *KIF7* gene encodes a cilia protein in the kinesin family and can cause a broad range of clinical findings in an autosomal recessive pattern. However, based on the fact that his healthy and developmentally normal father was found to carry this same genetic change and that only one change was identified, we concluded that this sequence variant was unlikely to contribute to his phenotype. Lastly, in the *WDR81* gene, autosomal recessive mutations can cause cerebellar ataxia and ID. Our patient was found to have one sequence variant in this gene but he does not exhibit the cerebellar ataxia nor the cerebellar hypoplasia typical of affected patients.

Patient 5 was found to be homozygous for a pathogenic mutation in the *DLD* gene. This gene encodes for dihydrolipoamide dehydrogenase, a house keeping enzyme that is part of other complex enzyme systems such as the pyruvate dehydrogenase complex,α-ketoglutarate dehydrogenase complex, and the branched chain ketoacid dehydrogenase complex. DLDD is an autosomal recessive metabolic disorder with a broad phenotype that ranges from lactic acidosis with early onset neurologic presentation to adult-onset isolated liver involvement [Shaag et al., 1999]. The c.685G > T mutation found in this

patient has been commonly found in patients with DLDD and it has an estimated carrier frequency of 1:94 in the Ashkenazi Jewish population [Shaag et al., 1999]. The delayed myelination on brain MRI scan, the elevated lactate level, and his abnormal plasma branched chain amino acids may be explained by this diagnosis. On the other hand, the prominent upper jaw and malocclusion teeth are most likely related to the pathogenic change in *SATB2*. This case draws attention to the importance of recognizing the possibility of two disorders in the same child particularly in complex consanguineous families. Moreover, it provides a unique opportunity to highlight the implications of the coexistence of two genetic disorders on patient care and genetic counseling of the family.

Microcephaly or anatomical brain malformations on cranial imaging do not seem to be a consistent feature in reported patients with 2q33.1 microdeletion syndrome [Balasubramanian et al., 2011]. However, in two patients described here with no second molecular pathogenic changes, cerebral volume loss was documented on brain MRI (Table I), suggesting that routine imaging should be considered.

In accordance with Docker et al. [2013], a clinically recognizable syndrome termed the *SATB2*-associated syndrome (SAS) has been proposed and herein we have added five more cases, supporting the characteristic features: ID with absent or severely limited speech development, craniofacial abnormalities including cleft palate, dysmorphic features, and dental abnormalities. Osteopenia and skeletal anomalies were not only seen in Patients 1 and 3 in this report, but also in some of the previous reported cases. Because of the critical role of *SATB2* in skeletogenesis, we therefore suggest adding these features to the list of distinctive findings. The only large population screening for alterations in the *SATB2* gene by Fitzpatrick et al. [2003] did not reveal mutations in children with only isolated cleft palate. We support the previous recommendation by Docker et al. [2013] that analysis for deletions or point mutations in *SATB2* should be performed in children with ID and absent or severely impaired speech, cleft or high-arched palate, dental abnormalities, and skeletal anomalies.

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