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Mutation update for the *SATB2* gene

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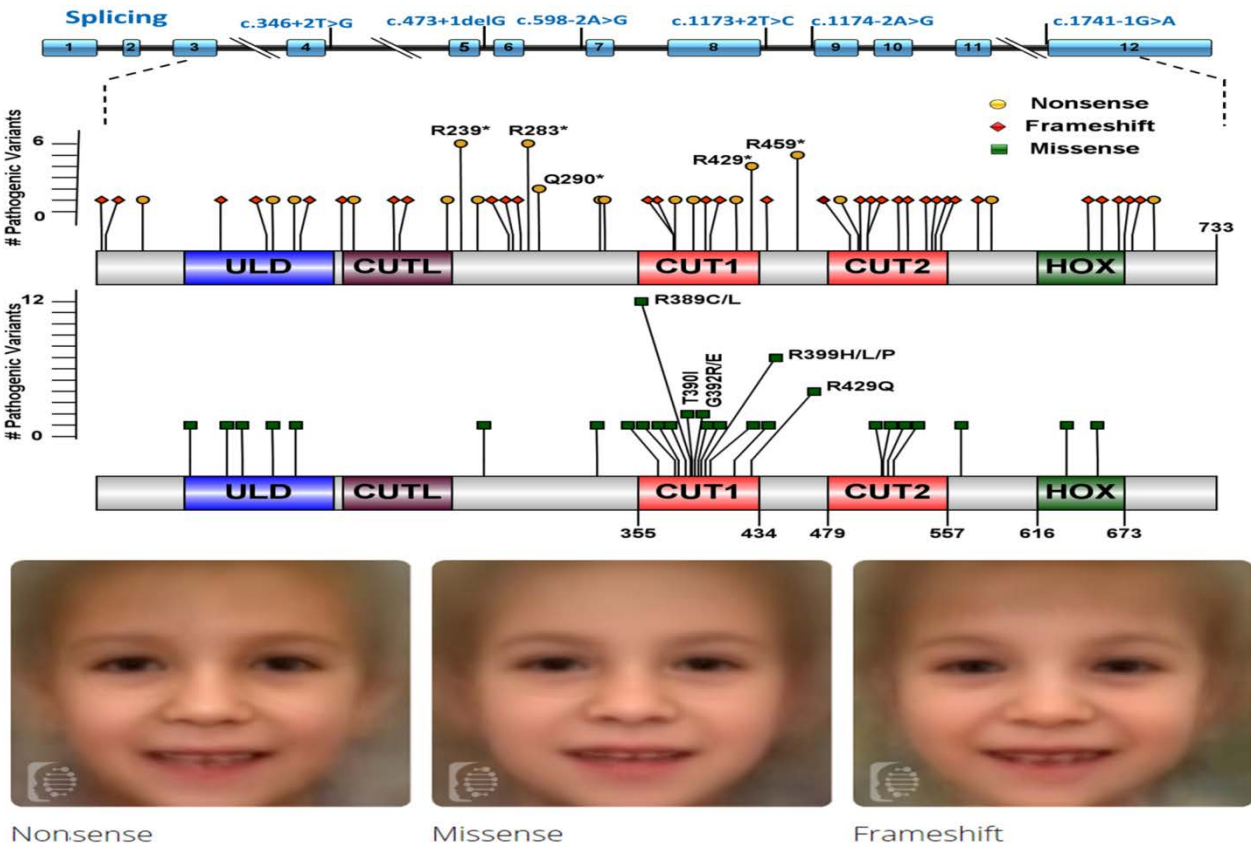
ABSTRACT

SATB2-associated syndrome (SAS) is an autosomal dominant neurodevelopmental disorder caused by alterations in the *SATB2* gene. Here we present a review of published pathogenic variants in the *SATB2* gene to date and report 38 novel alterations found in 57 additional previously unreported individuals. Overall, we present a compilation of 120 unique variants identified in 155 unrelated families ranging from single nucleotide coding variants to genomic rearrangements distributed throughout the entire coding region of *SATB2*. Single nucleotide variants predicted to result in the occurrence of a premature stop codon were the most commonly seen (51/120=42.5%) followed by missense variants (31/120=25.8%). We review the rather limited functional characterization of pathogenic variants and discuss current understanding of the consequences of the different molecular alterations. We present an expansive phenotypic review along with novel genotype-phenotype correlations. Lastly, we discuss current knowledge on animal models and present future prospects. This review should help provide better guidance for the care of individuals diagnosed with SAS.

Graphical Abstract

SATB2-associated syndrome (SAS) is an autosomal dominant neurodevelopmental disorder. In this mutation update we review the current state of our knowledge of SATB2 mutations and present 38 novel alterations. We examine the molecular and phenotypic findings and discuss the genotype-phenotype correlation.



Key Words: *SATB2*-Associated syndrome, *SATB2*, genotype-phenotype correlation, pathogenic variants, whole exome sequencing

1. BACKGROUND

SATB2-associated syndrome (SAS; Glass syndrome, MIM# 612313) is an autosomal dominant disorder first reported in 1989 in a 16-year-old male with severe intellectual disability and an interstitial deletion of 2q32.2-2q33.1 (Glass, et al., 1989). Clinically, SAS is characterized by developmental delay/intellectual disability with absent or limited speech development, palatal and dental abnormalities, feeding difficulties, behavioral problems, and dysmorphic facial features (Docker, et al., 2014; Zarate, et al., 2015; Zarate, et al., 2018a). Other supportive findings such as skeletal anomalies with low bone

density and abnormal brain neuroimaging have been described (Zarate, et al., 2018a; Zarate, et al., 2018b).

SAS is caused by alterations of *SATB2* that can include single nucleotide variants (loss-of-function as well as missense), intragenic deletions and duplications, contiguous deletions, and translocations with secondary gene disruption (Zarate and Fish, 2017; Zarate, et al., 1993). While haploinsufficiency of *SATB2* seems the most likely mechanism of disease, a dominant negative effect has been suggested in at least one instance in an individual with a nonsense variant (Leoyklang, et al., 2013). The *SATB2* gene maps to 2q32-q33 and has 3 transcripts (NM_001172509, NM_001172517, and NM_015265), codes for SATB2, a 82.6 kDa protein of 733 amino acids.

SATB2 binds to nuclear matrix-attachment regions (MARs) where it organizes chromatin to regulate tissue-specific gene regulatory networks (GRNs), and thus has critical roles in multiple developmental processes (Britanova, et al., 2006; Dobreva, et al., 2006; Dobreva, et al., 2003; Gyorgy, et al., 2008). The SATB2 protein has two CUT domains and a homeodomain (FitzPatrick, et al., 2003) that are highly conserved across vertebrate taxa (FitzPatrick, et al., 2003; Sheehan-Rooney, et al., 2010). The CUT domains and homeodomains are both DNA-binding motifs, which may bind DNA independently or cooperatively.

Clinically, SAS has been characterized through two large cohort studies (Bengani, et al., 2017; Zarate, et al., 2018a). We recently presented the common clinical features and natural history of 72 individuals with SAS due to a variety of molecular mechanisms. In this study, we review the previously described individuals with SAS and present 57

additional individuals that expand the mutation spectrum seen in this condition and describe novel genotype-phenotype correlations. All families reported for the first time were enrolled under a research clinical registry protocol approved by the Institutional Review Board of the University of Arkansas for Medical Sciences.

2. VARIANTS

All *SATB2* variants are described according to current HGVS mutation nomenclature guidelines based on Genbank accession number NM_015265 (den Dunnen, et al., 2016). Novel variants are interpreted using ACMG classification recommendations (Richards, et al., 2015). This report excludes larger deletions and duplications that encompass *SATB2* along with adjacent genes.

Tables 1 and 2 detail all 101 previously published *SATB2* intragenic alterations in the international peer-reviewed literature (PubMed database) and the Human Gene Mutation Database (HGMD professional 2018.3) (Asadollahi, et al., 2014; Balasubramanian, et al., 2011; Baptista, et al., 2008; Bengani, et al., 2017; Boone, et al., 2016; Bowling, et al., 2017; Brewer, et al., 1999; Cherot, et al., 2018; Deciphering Developmental Disorders Study, 2017; Farwell, et al., 2015; Gilissen, et al., 2014; Kaiser, et al., 2015; Kikuiri, et al., 2018; Lee, et al., 2016; Leoyklang, et al., 2007; Lieden, et al., 2014; Lv, et al., 2018; Rainger, et al., 2014; Rauch, et al., 2012; Rosenfeld, et al., 2009; Schwartz, et al., 2017; Scott, et al., 2018; Talkowski, et al., 2012; Tegay, et al., 2009; Trakadis, et al., 2014; Vissers, et al., 2017; Zarate, et al., 2017; Zarate, et al., 2015; Zarate, et al., 2018a; Zarate, et al., 2018b). In this study, we also report 57 additional individuals with 47 *SATB2* alterations (Tables 1 and 2) that have been submitted to the LOVD database: https://databases.lovd.nl/shared/genes/SATB2.

Overall, including our data and those of the literature, a total of 120 unique variants were found in 158 individuals from 155 unrelated families. While all types of pathogenic variants were found, single nucleotide variants that are predicted to result in the occurrence of a premature stop codon were the most commonly seen (51/120=42.5%). Missense variants were also frequently found (25.8%), followed by intragenic deletions (18.3%), translocations (5%), splice site alterations (5%), intragenic duplications (2.5%), and a single in-frame alteration (0.8%) (Supp. Figure S1).

2.1 Point pathogenic variants

Eighty-nine distinct point variants including single base substitutions and small deletions/insertions were found in 127 individuals from 125 families (Figure 1) (Liu, et al., 2015). Most molecular diagnostics were performed by whole exome sequencing (WES) (105/127=82.7%) with the remaining individuals obtaining the diagnosis through a different next generation sequencing (NGS) platform (epilepsy, intellectual disability or Angelman syndrome dedicated panels, 11.8%), *SATB2* Sanger sequencing (3.1%), or whole genome sequencing (WGS, 2.4%). *De novo* status was confirmed in almost all instances when parental testing was performed (98.1%, 105/107 families), including a pair of monozygotic twins. The remaining two instances correspond to a pair of siblings found to have the same variant indicating germline mosaicism and a case of low level blood mosaicism in a father of a single SAS-affected individual previously reported. Here, we present individual (SATB2-135) with a *de novo* mosaic pathogenic variant (c.1498delG) as determined by WES (32/143 reads) and presenting with the common phenotypic features.

The 89 pathogenic variants were distributed along the entire coding sequence of *SATB2*, and while variants were present in every coding exon (exon 3-12), the distribution was not uniform (Supp. Figure S2A). Unique pathogenic variants found in single individuals were most common (Figure 1). However, 7.9% of the pathogenic variants (7/89) were seen in two to four different families and 4.5% (4/89) were present in five or more families. Nearly half (41/89=46.1%) of the pathogenic variants were found in exons 8 and 9. There was a clear overrepresentation of exon 9 in particular when adjusting by size of each individual exon. This suggests a hotspot of pathogenic variants (Supp. Figure S2B).

2.1.1 Missense variants

Thirty-one unique missense variants were found in 49 individuals. Most missense variants were located within exons 8 and 9 (19/31=61.3%, Supp. Figure S2A) and located within the CUT1 domain (17/31=54.8%) of the SATB2 protein. Missense variants were often shared by multiple individuals with alterations in codons 389 (12 individuals) and 399 (7 individuals) being particularly common, suggesting hotspots. Most missense variants were confirmed to be *de novo* (44/45=97.8%). To assess the predicted pathogenicity of the 11 novel variants reported in this study, Polyphen2, SIFT, Provean, Mutation Taster, and CADD prediction programs were used (Supp. Table S1). Ten variants were interpreted as deleterious by all 5 programs, the remaining (p.Gln514Arg) with 4/5 programs predicting damaging effects.

Functional studies have been performed for three missense variants: c.1165C>T, p.(Arg389Cys), c.1543G>A, p.(Gly515Ser), and c.1696G>A p.(Gln566Lys) (Bengani, et

al., 2017). The p.Arg389Cys change located in the CUT1 domain led to a marked increase in the proportion of soluble fraction of the protein while the p.Gly515Ser and p.Gln566Lys variants located within the CUT2 domain and the region between CUT2 and the HOX domains respectively, had the opposite effect. These experiments suggested a role of the CUT1 domain in initiating interaction with chromatin and a requirement for the CUT2 domain to facilitate dissociation of SATB2 from bound chromatin. These alterations in the kinetics of chromatin association resulting from missense variants have been postulated to functionally result in alterations that resemble complete loss-of-protein function (Bengani, et al., 2017).

2.1.2 In frame insertion

A single *de novo* in frame insertion of 12 nucleotides not predicted to alter the reading frame has been reported in *SATB2*: c.929_930insTTGTAAGGCAAC, p.(Q310delinsHCKAT). The individual had a history of moderate to severe intellectual disability, autism, macrocephaly, frontal bossing, and deep-set eyes (Gilissen, et al., 2014).

2.1.3 Predicted truncating variants

Fifty-one *SATB2* variants reported were predicted to be disruptive to protein production resulting in loss of function, including 5 nonsense and 11 frameshift novel variants from this study. The variants were distributed throughout the reading frame but were frequently found within exons 8 to 12 (38/51=74.5%). Stop-gain pathogenic variants located within the last 2 exons might be expected to escape nonsense-mediated decay and result in a shorter protein. In keeping with this hypothesis, a single variant located within

the last exon of *SATB2* (c.2074G>T; p.Glu692*) was shown to result in a shorter protein consistent with the predicted 692aa protein product of the mutant cDNA (Bengani, et al., 2017). Two additional predicted loss-of-function variants have been studied in greater detail: c.715C>T (p.R239*) and c.847C>T (p.R283*) both found in six individuals each. The c.715C>T variant located in exon 8, was documented at the RNA level in two previously described unrelated individuals, indicating that the RNA was stable enough to escape nonsense mediated decay (NMD) (Docker, et al., 2014; Leoyklang, et al., 2007). Further, the translated truncated protein retained the SATB2 dimerization domain. Through luciferase assays using a MAR sequence binding domain, it was documented to interfere with the repressive MAR-regulated transcriptional activity of the wild-type SATB2, suggesting a dominant negative effect for this mutation (Leoyklang, et al., 2013). Conversely, the c.847C>T variant, also located in exon 8, was studied from tooth mesenchymal cells from an affected individual. Diminished SATB2 expression by Sanger sequencing and reduced SATB2 mRNA compared to control was demonstrated for this variant, suggesting NMD of the mutant RNA transcript (Kikuiri, et al., 2018).

2.1.4 Splice site variants

Six variants (1 novel from this study) disrupted an essential splice site consensus sequence: three affecting the donor (5') site at the end of exons 4, 5, and 8, and three affecting the acceptor (3') site at the start of exons 7, 9, and 12. Splice-prediction programs (MaxEntScan and Human Splicing Finder) predicted abnormal splicing for all variants and a potential activation of cryptic donor site or acceptor site for c.473+1delG and c.1741-1G>A, respectively. No RNA analysis has been performed to confirm the impact on splicing for any of these variants.

2.2 Large intragenic rearrangements

Twenty five *SATB2* exonic (1 to 12 inclusive) rearrangements including 22 deletions (10 from this report) and 3 duplications have been described (Table 2). For the 10 individuals that underwent parental testing, d*e novo* status was confirmed. A pair of siblings (SATB2-02 and SATB2-128) with an intragenic deletion from phenotypically unaffected parents is also reported here for the first time suggesting germline mosaicism. Except for one individual (SATB2-92) identified through WGS and another as part of subtelomere multiplex ligation-dependent probe hybridization (MLPA) analysis (SATB2-140), all other copy number variations were initially identified by chromosomal microarray performed as part of the clinical evaluation with sizes ranging from 10 to 317kb for deletions and between 32 and 54kb for duplications (Table 2). For all individuals, *SATB2* was the only gene affected with no involvement of adjacent genes. Multi-exonic rearrangements were more common (19/25=76.0%) than single exon involvement (Figure 1). Abnormalities involving at least exon 7 were present in half of these individuals (14 total from 13 unrelated families). No deletions have been documented at the cDNA level and only 2 refined by MLPA. A single duplication was studied at the cDNA level (Kaiser, et al., 2015). In this female individual, an initial chromosomal microarray (Cytoscan HD, Affymetrix) revealed an 84-kb duplication within chromosomal region 2q33.1 (200,256,583–200,340,204) encompassing a part of the *SATB2* gene. Reverse transcription PCR analysis with cDNA primers flanking the duplicated exon and subsequent Sanger sequencing of the extracted cDNA fragments showed the tandem in-frame duplication consistent with co-expression of transcripts with a duplicated exon and wild-type transcripts.

2.3 Large chromosomal rearrangements including 2q33.1

Thirty-five individuals with larger chromosomal alterations that include 2q33.1 (33 deletions, 2 duplications) have been reported and reviewed in the past (Zarate and Fish, 2017; Zarate, et al., 2018a). For deletions, some of these include dozens of adjacent genes and are as large as 26.3 Mb (Rifai, et al., 2010). While overlapping features with SAS are present in most individuals with larger deletions, other less common abnormalities such as genitourinary anomalies, cardiac defects, and ectodermal changes (other than dental) appear to be more common (or exclusively present) when compared to intragenic molecular alterations (Zarate and Fish, 2017). For this group of individuals with larger rearrangements of 2q33.1, it remains difficult to establish genotype/phenotype correlations given the potential phenotype contribution of other genes besides *SATB2*.

2.4 Translocations

The original report of two *de novo* apparently balanced autosomal translocations t(2;7)(q33;p21) and t(2;11)(q32;p14) allowed the recognition of *SATB2* as the causative gene for this syndrome

(Brewer, et al., 1999). High resolution mapping of the t(2;7) translocation showed disruption of the coding region of the *SATB2* gene between exons 2 and 3 (FitzPatrick, et al., 2003), whereas t(2;11) disrupted the long-range *cis* regulatory elements located in the centromeric gene desert 3' of *SATB2* (Rainger, et al., 2014). A few additional individuals with *SATB2* disruption secondary to *de novo* chromosomal translocations were subsequently described to result in SAS providing further supporting evidence for this

molecular mechanism of disease (Baptista, et al., 2008; Rainger, et al., 2014; Talkowski, et al., 2012; Tegay, et al., 2009).

3. BIOLOGICAL RELEVANCE

SATB2 pathogenic alterations are distributed throughout the coding regions of the gene but exons 8, 9, and 11 are most commonly involved. For missense variants, most (23/31=74.2%) are located within the CUT1, CUT2 or HOX DNA protein domains. While the joint function of these DNA binding domains is not fully clear, it is suggested that missense variants

within the core of the CUT domain are likely to result in loss of DNA-binding activity; CUT1 being required to initiate interaction with chromatin and CUT2 (and the region between CUT2 and HOX) required to facilitate dissociation of SATB2 from bound chromatin (Bengani, et al., 2017). The vast majority of pathogenic alterations of *SATB2* are null variants (frameshift, nonsense, canonical splice site, and single or multiexon deletions) and while predicted to result in a loss-of-function and haploinsufficiency, limited functional studies have suggested a potential dominant negative effect for some. Of note, an increasing number of missense pathogenic variants have been described with functional alterations that resemble the complete loss-of-protein function.

4. CLINICAL AND DIAGNOSTIC RELEVANCE

A summary of clinical features of all 158 individuals previously reported and from this report, excluding those with larger deletions and duplications that include *SATB2* and surrounding genes, is presented in Table 3. With rare exceptions, SAS diagnosis was not clinically recognized a priori. However, while the diagnosis of SAS still relies on

molecular confirmation of a pathogenic variant in *SATB2*, a distinctive phenotype can often be identified. Speech delay is present in all individuals older than 2 years of age.

Severe expressive language delay is common with 84.3% (102/121) of individuals older than 4 years of age having 10 or fewer words in their expressive vocabulary, with 42.1% (51/121) demonstrating completely absent verbal communication (Supp. Figure S3). Other areas of neurodevelopment can also be compromised as evidenced by an average age at first steps of 25.5 months. Intellectual disability has been reported in several individuals old enough to undergo cognitive evaluations and often in the moderate to severe range (Zarate, et al., 2018a). Dental abnormalities are present in all individuals and include delayed development of the mandibular second bicuspids or the roots of the permanent teeth, severely rotated or malformed teeth, taurodontism, and multiple odontomas (Kikuiri, et al., 2018; Scott, et al., 2018). Behavioral difficulties, feeding issues, abnormal brain neuroimaging, low bone density, suggestive facial features, and cleft palate complete the characteristic phenotype of SAS (Table 3). Through previous reports and the evaluation of dozens of patients by at least a single examiner (Y.A.Z.), broad thumbs and/or halluces appear to be another distinctive feature, present in a third of individuals evaluated (16/47=34%), that could raise the clinical suspicion of this diagnosis (Figure 2A).

Since the identification of *SATB2* as the gene responsible for SAS, the number of described individuals has continued to grow over the last few years. Most current molecular cytogenetic platforms should be able to detect exon level intragenic deletions involving *SATB2*. Likewise, *SATB2* is part of several commercially available panels targeting broad phenotypes such as developmental delay, autism, or seizures. As families

receive counseling, it is important to discuss the potential recurrence risk considering a few instances of suspected germline mosaicism documented. With a previous report of coding variants in siblings and the sibling pair with intragenic deletions reported here, we estimate a 1-2% germline mosaicism risk (2/155 families=1.3%). Of note, paternal blood mosaicism has also been documented in one occasion. Lastly, management and surveillance guidelines for SAS have been proposed and typically need the participation of a multidisciplinary team with heavy emphasis on pediatric dentistry and speech therapy (Zarate and Fish, 2017; Zarate, et al., 1993).

5. GENOTYPE/PHENOTYPE CORRELATIONS

Supplementary table S2 presents detailed clinical features present in each of the 158 individuals (114 individuals enrolled in the SAS registry and 44 additional reported in the literature). We identified differences in clinical characteristics by molecular mechanism. Specific changes to the gene were compared to all other changes, using either Chi-square or Fisher's exact tests (when at least one cell had an expected count of less than 5) for categorical variables, and t-tests for continuous variables. While there were no differences in the average age at walking or talking, other clinically relevant distinctions were identified (Supp. Table S3). The proportion of individuals with no verbal words to communicate older than 4 years of age was lowest for nonsense variants (8/29=27.6%) and highest for missense pathogenic variants mutations (20/39=51.3%), (p=0.0496). Individuals with missense pathogenic variants were less likely to have cleft palate (11/49=22.5% vs 59/105=56.2% for other groups, p<0.0001) but more likely to have clinical seizures (14/46=30.4% vs 15/97=15.5% for other groups, p=0.0375). Conversely, individuals with nonsense variants had significantly fewer clinical seizures (3/35=8.6%

vs 26/108=24.1% for other groups, p=0.0474). Lastly, individuals with frameshift variants were more likely to have feeding difficulties (25/26=96.2% vs 64/104=61.5% for other groups, p=0.0007). If this difference in the prevalence of feeding difficulties is the result of a true biologically different mechanism for frameshift variants compared to nonsense variants or merely the result of an statistical anomaly, is unclear.

To determine if facial dysmorphisms were different enough among the most common molecular mechanisms, 102 2D photographs from 69 individuals (19 nonsense, 31 missense, and 19 frameshift variants) were analyzed using Face2Gene (FDNA Inc., Boston, MA) analytic tool vs.18.2.0. No statistically significant differences were found among the three composite images (Figure 2B). However, binary comparisons for each of the three groups against a respective age and gender matched cohort of typical individuals revealed statistically significant differences for all three groups (nonsense, p=0.04; missense, p=0.018; frameshift, p=0.019).

6. ANIMAL MODELS

Much of what we know about SATB2 function in human development has come from studies in animal models. In mice, *Satb2* is expressed in tissues that are affected in SAS patients. During development, *Satb2* is expressed in upper layer neurons, neural crest progenitors of the jaw, osteoblasts, odontoblasts and other dental progenitor cells (Britanova, et al., 2006; Dobreva, et al., 2006; He, et al., 2017). In adults, *Satb2* continues to contribute to bone and brain function through its expression in osteoblasts and pyramidal neurons of the cerebral cortex and hippocampus (Huang, et al., 2013; Jaitner, et al., 2016; Wei, et al., 2012).

In general, Satb2 functions as a transcriptional regulator that is important for tissue-specific functions. In mice bone progenitors, *Satb2* regulates osteogenic differentiation genes (Dobreva, et al., 2006), while in post-mitotic neurons *Satb2* regulates expression of genes involved in upper layer identity, synaptic transmission, and axon guidance (Jaitner, et al., 2016; Li, et al., 2017; Whitton, et al., 2018). The consequence of loss of Satb2 in bone progenitors is reduced bone size and density due to increased cell death and reduced differentiation potential (Britanova, et al., 2006; Dobreva, et al., 2006).

In murine brain development, Satb2 is required to specify upper layer cortical neurons that project axons across the corpus callosum to the contralateral hemisphere (Alcamo, et al., 2008; Britanova, et al., 2008). Loss of Satb2 also results in reduced branches and spine density in basal dendrites of hippocampal neurons (Li, et al., 2017). Similar to SAS patients, *Satb2*$^{+/-}$ heterozygous mice have no reported corpus callosum defects (Alcamo, et al., 2008; Zarate, et al., 2018a). However, *Satb2*$^{+/-}$ heterozygous mice suffer from impaired working and spatial memory. This deficit is exacerbated in mice where Satb2 has been deleted postnatally in hippocampal neurons (Li, et al., 2017). These mice also have difficulties in locomotion, short-term novel object recognition memory, and long-term contextual fear memory (Jaitner, et al., 2016; Li, et al., 2017).

Taken together, research in animal systems has provided molecular and cellular mechanisms underlying SAS pathogenesis. These data help outline expectations of long-term care and provide medical practitioners with guidance about the potential spectrum of defects to manage in SAS patients. However, current treatments are symptom-guided,

and do not specifically target pathogenic mechanisms. Future research is needed to further explore potential therapies that directly target SAS pathology.

7. FUTURE PROSPECTS

A key avenue of future research is the use of human induced pluripotent stem cells (hiPSCs) to model disease and evaluate potential treatments. hiPSCs can be generated from patients with defined clinical phenotypes, thus enabling *in vitro* cellular phenotypes to be linked to individual clinical presentation. Use of hiPSCs allows the pathogenic mechanisms of different types of mutations to be evaluated. The potential to have hiPSCs from an unaffected parent provides a control for genetic background, allowing molecular and cellular outcomes, including the effect of different mutations on *SATB2* mRNA and protein levels to be efficiently compared. Importantly, this *in vitro* system allows for reasonably high throughput testing of pharmacological agents.

To facilitate patient treatment, it will be important to elucidate how variability in individual disease pathogenesis contributes to SAS phenotypes. This variability may derive from the type of molecular alteration to the *SATB2* locus, differences in genetic background, or even differences in lifestyle. Although therapies attempting to exogenously supplement reduced protein levels have achieved little success (Dietz, 2010), it may be possible to increase the endogenous amount of active protein in several ways. For example, in individuals with nonsense pathogenic variants, low levels of SATB2 are thought to result from nonsense mediated decay of prematurely terminated mRNA transcripts. Several pharmacological agents are now being used to promote “read-through” of stop codons, thus increasing levels of full-length mRNA (Baradaran-Heravi, et al., 2017; Landfeldt, et al., 2018; Roy, et al., 2016). This is an especially attractive

avenue for treatment; however, not all nonsense mutations may be equally amenable to these treatments as sequences around the mutation may affect read-through activity (Bolze, et al., 2017). The power of testing multiple different nonsense mutations in an hiPSC *in vitro* system has great potential to elucidate the details of this potential therapeutic mechanism. Of note, loss of function mutations may be amenable to some treatment types that would not alleviate the effects of dominant negative mutations. Also important to consider, knowing that SATB2 has an important role in early neurodevelopment as it has been demonstrated in mice models, the degree of correction or reversal of cognitive and speech deficits even if achieving normalization of SATB2 protein levels in potential human patients treated at later ages could be limited.

SATB2 function is affected by both post-transcriptional regulation by microRNAs (miRNAs) and post-translational modification via sumoylation (Deng, et al., 2013; Dobreva, et al., 2003; Wei, et al., 2012). While miRNAs regulate amounts of SATB2 protein, sumoylation affects SATB2 activation potential and association with endogenous MARs *in vivo* (Dobreva, et al., 2003). Sumoylation targets SATB2 to the nuclear periphery (Dobreva, et al., 2003). Therefore, inhibiting sumoylation may increase the amount of active SATB2 in association with MARs.

Another promising approach for treatment of SAS is to focus on modifiers that buffer or compensate for reductions in protein function (Chen, et al., 2016). In mice, loss of *Satb2* dysregulates the expression of multiple miRNAs involved in memory and synaptic plasticity (Jaitner, et al., 2016). The effects of dietary supplementation with phospholipidic concentrates of krill oil and buttermilk on the expression of miRNAs in hippocampal neurons have been studied in murine models (Crespo, et al., 2018). Finally,

neurological defects in SAS patients have been reported to share molecular and cellular mechanisms with other neurodevelopmental or neurodegenerative diseases such as schizophrenia and Alzheimer's disease (Whitton, et al., 2018). Therefore, similar treatments could be explored for these diseases where shared molecular mechanisms are identified.

8. CONCLUSION

In this mutation update, we present data from 158 SAS individuals and review the current state of knowledge and future prospects on human *SATB2* alterations. The 120 unique variants from 155 unrelated families range from single nucleotide variations to complex genomic rearrangements involving the entire coding region of *SATB2*. While germline mosaicism has been found in some instances, most pathogenic variants have been confirmed to be *de novo*. Missense pathogenic variants are often found, and for those studied functionally, are predicted to act as loss-of-protein function pathogenic variants. Almost invariably, the diagnosis of SAS is made after molecular investigations are performed and for individuals with genomic coding variants, next generation sequencing technologies are most often used. Through our extensive review of individuals with SAS, we present an emerging phenotype that appears more recognizable with age. Our broad molecular and clinical descriptions of individuals with *SATB2* should help clinicians and families establish the diagnosis of SAS and develop future therapies.

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FIGURES

Figure 1. Schematic representation of the spectrum of *SATB2* variants previously described and from this study. **A.** Splicing and coding exonic pathogenic variants. Splicing variants are represented along the genomic structure of *SATB2* gene according to NM_015265.3 including 12 exons (boxes) and introns (black horizontal lines). Exonic pathogenic variants are illustrated according to changes at the protein level (p.) by corresponding mutation types as follows: green squares for missense variants, red diamonds for frameshift variants, and yellow circles for nonsense variants. Codons 239 and 283 for nonsense (6 each), and 389 (12 individuals) and 399 (7 individuals) for missense variants are affected by the highest number of pathogenic variants. Diagrams were constructed using Illustrator for Biosequence (IBS1.0.1). **B.** *SATB2* intragenic rearrangements. Full boxes correspond to deletions while lighter rectangles to duplications. In case of recurrence of the same exon being involved, number of occurrence is indicated next to the rearrangement.

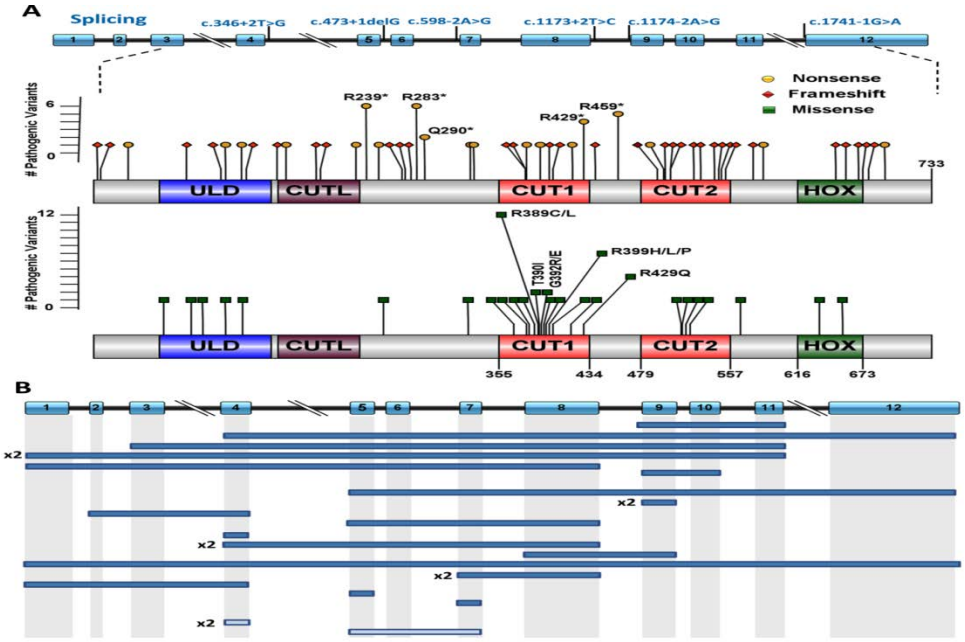

Figure 2. **A.** Broad halluces from 4 different individuals. **B.** Composite images of individuals with nonsense, missense, and frameshift variants. Across all 3 images a flat philtrum with thin vermillion of the upper lip can be recognized.

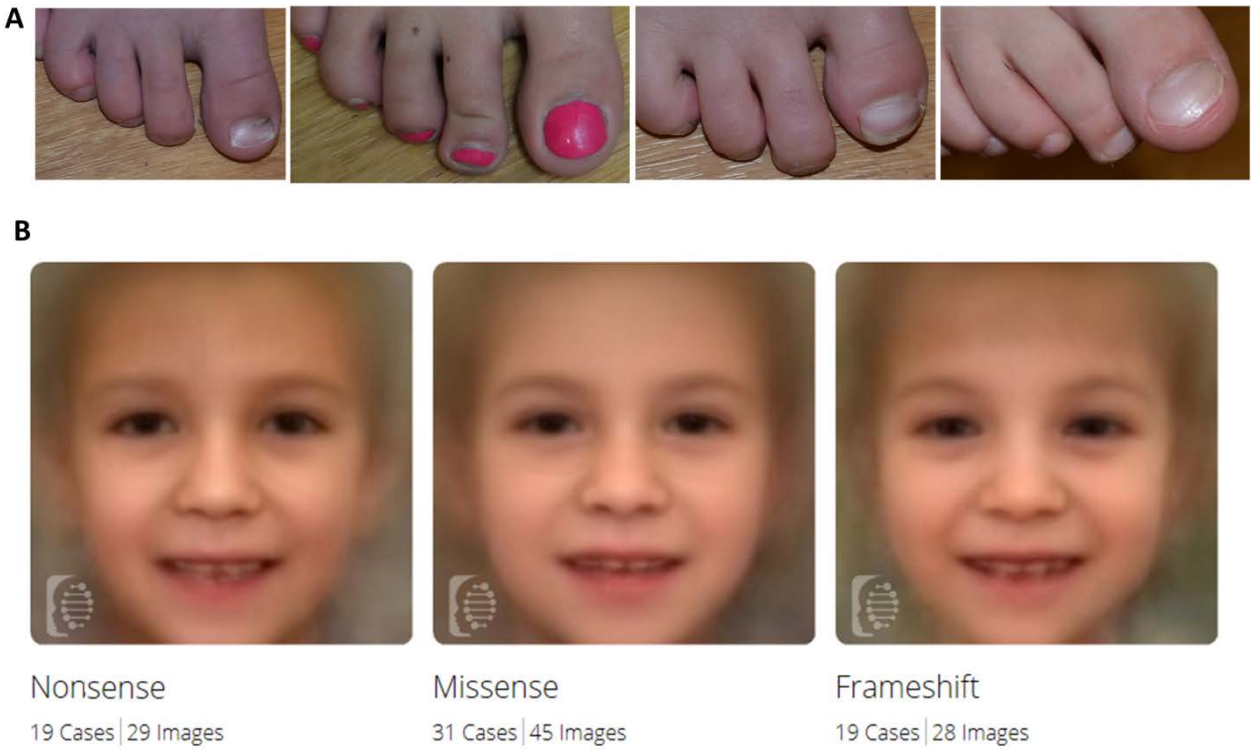

Table 1. *SATB2* variants found in individuals with *SATB2*-associated syndrome previously reported and from this study (Numbering is according to the cDNA sequence (GenBank entry NM_015265.3).

Variant type	Position	DNA variant	Predicted effect on protein	Unrelated families in our cohort	Unrelated families in the literature	Variant Reference (dbSNP, ClinVar)	References
Nonsense	Exon 3	c.124G>T	p.Gly42*		1	RCV000486598.1; rs1064793947	Zarate et al., 2017; Zarate et al., 2018a
	Exon 4	c.346G>T	p.Gly116*		1		Zarate et al., 2018a; Scott et al.
	Exon 5	c.390T>A	p.Tyr130*		1		Bengani, Zarate et al., 2018a
	Exon 6	c.505C>T	p.Gln169*		1		Zarate et al., 2018a; Scott et al.
	Exon 7	c.688A>T	p.Lys230*		1		Visser s et al.

	Exon 8	c.715C>T	p.Arg239*	3	3	RCV000002627.6; RCV000256175.2; rs137853127	Leoyklang et al.; This study
	Exon 8	c.748C>T	p.Gln250*		1	RCV000708552.1	Zarate et al., 2015
	Exon 8	c.847C>T	p.Arg283*	2	4	RCV000190685.3; RCV000254800.2; RCV000686152.1; rs797044874	Zarate et al., 2015; Kikuiri et al.; This study
	Exon 8	c.868C>T	p.Gln290*	2		RCV000413829.1; RCV000624904.1; rs1057518496	This study
	Exon 8	c.988C>T	p.Gln330*	1			This study
	Exon 8	c.997C>T	p.Gln333*	1		RCV000599413.1	This study
	Exon 8	c.1135C>T	p.Gln379*	1			This study
	Exon 8	c.1171C>T	p.Gln391*		1	RCV000708554.1	Zarate et al., 2018a; Scott et al.
	Exon 9	c.1255C>T	p.Gln419*		1		Zarate et al., 2017, Zarate

							et al., 2018a, Scott et al.
	Exon 9	c.1285C>T	p.Arg429*	1	3	RCV000307104.1; rs886041847	Bengani et al.; This study
	Exon 9	c.1375C>T	p.Arg459*	1	4	RCV000625170, RCV000680089.2, RCV000719613.1	Bengani et al.; This study
	Exon 10	c.1495A>T	p.Lys499*		1	RCV000224980.1; rs878853163	Zarate et al., 2018a, Bowling et al., Scott et al.
	Exon 11	c.1756C>T	p.Gln586*	1			This study
	Exon 12	c.2074G>T	p.Glu692*		1	RCV000708559.1	Bengani et al.
Missense	Exon 4	c.185T>A	p.Val62Asp		1	RCV000479021.1; rs1064796649	Zarate et al., 2018a
	Exon 4	c.257T>G	p.Leu86Arg	1			This Study
	Exon 4	c.287T>G	p.Leu96Arg		1	RCV000485845.1; rs1064795247	Zarate et al., 2018a

	Exon 4	c.346G>C[+]	p.Gly116Arg		1	RCV000494383.1; rs1131691672	Zarate et al., 2017; Zarate et al., 2018a
	Exon 5	c.392T>A	p.Val131Glu		1	RCV000485195.1; rs1064794638	Zarate et al., 2018a
	Exon 8	c.760C>T	p.His254Tyr	1			This study
	Exon 8	c.983C>A	p.Ala328Asp		1		Farwell et al.
	Exon 8	c.1102G>T	p.Val368Phe	1			This study
	Exon 8	c.1136A>C	p.Gln379Pro	1			This study
	Exon 8	c.1142T>G	p.Val381Gly		1	RCV000708553.1	Rauch et al.
	Exon 8	c.1157C>T	p.Ala386Val		1		Bengani, Zarate et al., 2018a
	Exon 8	c.1165C>T	p.Arg389Cys	5	5	RCV000430827.1; RCV000623230.1; RCV000656508.2; rs1057521083	Bengani et al.; This study
	Exon 8	c.1166G>T	p.Arg389	1	1		Bengani et

			Leu				al.; This study
	Exon 8	c.1169C>T	p.Thr390 Ile	1	1	RCV000199456.1; rs863224917	Zarate et al. 2018a, 2018b; This study
	Exon 9	c.1174G>C	p.Gly392 Arg		1		Zarate et al. 2018a, 2018b
	Exon 9	c.1175G>A	p.Gly392 Glu	1		RCV000489186.1; rs1085308028	This study
	Exon 9	c.1181T>C	p.Leu394 Ser		1		Bengani et al.
	Exon 9	c.1186G>C	p.Glu396 Gln		1	RCV000708555.1	Lee et al.
	Exon 9	c.1196G>A	p.Arg399 His	2	2	RCV000413401.1; rs1057518190	Bengani, Zarate et al., 2018a; Scott et al.; This study
	Exon 9	c.1196G>T	p.Arg399 Leu	2			This study
	Exon 9	c.1196G>C	p.Arg399 Pro		1	SCV000847591.1; rs1057518190	Zarate et al. 2018a, 2018b

	Exon 9	c.1204G>A	p.Glu402Lys		1		Bengani et al. ; Zarate et al., 2018a
	Exon 9	c.1253T>G	p.Met418Arg	1			This study
	Exon 9	c.1286G>A	p.Arg429Gln	1	3	RCV000300452.2; RCV000708556.1; rs886041516	Zarate et al., 2017; Zarate et al., 2018a; This study
	Exon 10	c.1541A>G	p.Gln514Arg	1			This study
	Exon 11	c.1543G>A	p.Gly515Ser		1	SCV000778507.1	Bengani et al.
	Exon 11	c.1554T>G	p.Cys518Trp	1			This study
	Exon 11	c.1564C>T	p.Arg522Cys	1			This study
	Exon 11	c.1696G>A	p.Glu566Lys		1	RCV000482629.1; RCV000656509.1; rs1064795530	Bengani et al.
	Exon 12	c.1903G>T	p.Asp635Tyr	1			This study
	Exon 12	c.1964C>T	p.Pro655		1	RCV000502290	Zarate et al.,

			Leu			.1	2017; Zarate et al., 2018a
Frameshift	Exon 3	c.9_16del	p.Arg4Glufs*68	1			This study
	Exon 3	c.19dupA	p.Ser7Lysfs*68	1		RCV000519084.1; RCV000709933.1;	This study
	Exon 4	c.245_263del	p.Ala82Glyfs*30	1			This study
	Exon 4	c.334delG	p.Ala112Profs*6	1			This study
	Exon 5	c.400delG	p.Ala134Hisfs*17		1		Zarate et al., 2017; Zarate et al., 2018a
	Exon 6	c.482delA	p.Lys161Serfs*19		1	RCV000479959.1; rs1064793820	Zarate et al., 2017; Zarate et al., 2018a
	Exon 6	c.583dupT	p.Cys195Leufs*14		1		Zarate et al., 2017; Bengani et al.; Zarate et al., 2018a, 2018b

	Exon 6	c.594_595delCC	p.Gln199Glufs*9		1		Bengani et al.
	Exon 8	c.808_809delCA	p.Gln270Valfs*33		1	RCV000623347.1	Zarate et al., 2018a; Scott et al.
	Exon 8	c.816delT	p.His273Thrfs*21		1		Zarate et al., 2017; Zarate et al., 2018a
	Exon 8	c.832delC	p.His278Thrfs*16	1			This study
	Exon 8	c.1131_1132delGT	p.Ser378Profs*18		1	RCV000209866.1; rs875989830	Zarate et al., 2018a
	Exon 8	c.1132_1133insAC	p.Ser378Tyrfs*36	1			This study
	Exon 9	c.1196delG	p.Arg399Leufs*14	1		RCV000624362.1	This study
	Exon 9	c.1203dupA	p.Glu402Argfs*35		1		Bengani et al.
	Exon 9	c.1311_1314dupGGAG	p.Arg439Glyfs*38		1	SCV000845949.1	Zarate et al., 2018a
	Exon 10	c.1478_1479delAG	p.Gln493Argfs*19		1		Kikuiri et al.

	Exon 10	c.1498delG Mosaic	p.Val500 Cysfs*46	1			This study
	Exon 10	c.1511_1512ins CAAGCCT	p.Phe505 Lysfs*10	1			This study
	Exon 10	c.1515delT	p.Phe505 Leufs*41		1	RCV000255520 .1; rs886039740	Zarate et al., 2018a; Scott et al.
	Exon 11	c.1574delA	p.Glu525 Glyfs*21		1		Bengani et al.
	Exon 11	c.1592dupA	p.Asn531 Lysfs*21		1	RCV000533352 .1	Zarate et al., 2018a
	Exon 11	c.1627delC	p.Arg543 Alafs*3		1	RCV000496200 .1; rs1135401803	Cherot et al.
	Exon 11	c.1639_1642deli nsTTT	p.Leu547 Phefs*77	1			This study
	Exon 11	c.1645delC	p.Gln549 Serfs*75		1		Bengani et al.
	Exon 11	c.1657delG	p.Asp553 Metfs*71	1			This study
	Exon 11	c.1728delT	p.Glu577 Serfs*47		1		Zarate et al., 2017; Zarate et al., 2018a

	Exon 12	c.1945dupT	p.Ser649Phefs*40		1	SCV000837674.1	Zarate et al., 2015; Zarate et al., 2018a
	Exon 12	c.1972_1973delAC	p.Thr658Hisfs*30		1		Zarate et al., 2018a
	Exon 12	c.2005dupC	p.His669Profs*20		1		Zarate et al., 2018a, 2018b
	Exon 12	c.2018dupA	p.His673Glnfs*16		1	RCV000708558.1	Boone et al.
	Exon 12	c.2028delG	p.Glu678Serfs*18		1		Zarate et al., 2018a
In frame insertion	Exon 8	c.929_930insTTGTAAGGCAAC	p.Q310delinsHisCysLysAlaThr		1		Gilissen et al.
Splice	Exon 4	c.346+2T>G	p.?		1	SCV000837664.1	Zarate et al., 2015
	Exon 5	c.473+1delG	p.?	1		RCV000598568.1	This study
	Exon 7	c.598-2A>G	p.?		1		Bengani et al.
	Exon 8	c.1173+2T>C	p.?		1		Bengani et

							al.
	Exon 9	c.1174-2A>G	p.?		1		Zarate et al., 2018a, Scott et al.
	Exon 12	c.1741-1G>A	p.?		1		Kikuiri et al.
Translocation	t(2;11) cis regulatory	c.?	p.?		1		Rainger et al.
	t(2;3) cis regulatory	c.?	p.?		1		Rainger et al.
	t(2;7) intron 3	c.?	p.?		1		Brewer et al.
	t(2:14) intron 10	c.?	p.?		1		Tegay et al.
	t(2;10) Intron 8-to 11	c.?	p.?		1		Baptista et al.
	t(2;6)	c.?	p.?		1		Talkowski et al.

†This variant is also predicted to affect splicing if not resulting in a missense substitution

Table 2. Intragenic *SATB2* chromosomal abnormalities previously reported and from this report

Abnormality	Position	Min sequence coordinates hg19	Size (kb)	Methodology	Reference
Intragenic Deletions	Exons 9-11	chr2:200,168,993-200,203,730	35	105K Oligo Agilent	Balasubramanian et al.
	Exons 4-12	chr2:200,128,960-200,312,555	183.6	105K Oligo Agilent	Rosenfeld et al.
	Exons 3-11	chr2:200,151,982-200,325,064	173.1	105K Oligo Agilent	Rosenfeld et al.
	Exons 1-11	chr2:200,151,782-200,336,956	185.2	105K Oligo Agilent	Rosenfeld et al.
	Exons 1-8	chr2: 200,199,481-200,340,178	141	SNP Affymetrix	Zarate et al., 2018a
	Exons 4-8	chr2: 200,198,963-200,314,171	115	SNP Affymetrix	This report
	Exons 9-10	chr2: 200,180,939-200,200,560	19.6	OGT 60k oligo	Zarate et al., 2018a
	Exons 1-11	chr2:200,151,782-200,336,956	185	105K Oligo Agilent	Zarate et al., 2018a
	Exons 5-12	chr2: 200,018,395-200,246,466	228	60K Oligo Agilent	Zarate et al., 2018a
	Exon 9	chr2:200,190,560-200,200,832	10	Affymetrix 750k	Zarate et al., 2018a
	Exons 2-4	chr2:200,280,770-200,325,182	45	OGT 8x60k	Zarate et al., 2018a

	Exons 5-8	chr2:200,212,737-200,256,585	44	Affymetrix Cytoscan HD	Zarate et al., 2018a
	Exon 4	chr2:200,292,552-200,302,732	10.2	Affymetrix Cytoscan HD	Zarate et al., 2018a
	Exons 4-8	chr2:200,195,604-200,315,398	120	CombiSNP	This report
	Exons 8-9	chr2:200,192,250-200,230,824	39	Affymetrix Cytoscan HD	This report
	Exons 1-12	chr2:200,133,428-200,450,474	317	Agilent 8x60K	This report
	Exons 7-8	chr2:200,197,135-200,242,625	45.5	Affymetrix Cytoscan HD	This report
	Exons 7-8	chr2:200,194,727-200,240,771	46	180k Cytosure ISCA v2	This report
	Exon 5	chr2:200,246,407-200,296,329	50	Agilent 4x180K, PCR	This report
	Exons 1-4	chr2:200,275,266-200,344,231	69	Oligo array + SNP	This report
	Exon 7	chr2:200,222,207-200,243,660	21.4	Research WGS (MLPA confirmed)	This report
	Exon 9	chr2:200,193,421-200,193,634	0.213 †	MLPA	This report
Intragenic Duplications	Exon 4	chr2:200,278,502-200,310,272	32	Affymetrix Cytoscan HD, MLPA	Asadollahi et al.
	Exon 4	chr2:200,256,546-200,310,885	54	Affymetrix Cytoscan HD, PCR, MLPA	Kaiser et al.

	Exons 5-7	chr2:200,233,354-200,255,458	35	180K Oligo Agilent, MLPA	Lieden et al.

†The exact location of the breakpoints of this deletion is unknown as the MLPA kit used only covered limited exons.

Table 3. Demographic and phenotypic features of 158 individuals with *SATB2*-associated syndrome

Characteristic (N with data)	**All patients (n = 158)**
Demographics	
Male (152)	90 (59.2%)
Mean age, years (152)	9.5±7.5
Molecular mechanism	
Missense	49 (31.0%)
Nonsense	38 (24.1%)
Frameshift	32 (20.3%)
Splicing	7 (4.4%)
In frame insertion	1 (0.6%)
Intragenic deletion	22 (13.9%)
Translocations	6 (3.8%)
Intragenic duplication	3 (1.9%)
Phenotype	
Neurodevelopmental abnormalities	
Developmental delay (157)	157 (100%)
No words for speech (152)	66 (43.4%)
Autistic behavior (145)	29 (20.0%)
Dental anomalies (137)	135 (98.5%)
Sialorrhea (109)	96 (88.1%)

Facial dysmorphism (127)	107 (84%)
Low BMD (46)	33 (71.7%)
Feeding difficulties (130)	89 (68.5%)
Hypotonia (114)	67 (58.8%)
Sleeping difficulties (109)	55 (50.5%)
Abnormal Neuroimaging (108)	50 (46.3%)
Sleeping difficulties (145)	66 (45.5%)
Cleft palate (154)	70 (45.5%)
Strabismus (143)	51 (35.7%)
Agitation/Aggressive (145)	45 (31.0%)
Growth Retardation (144)	44 (30.6%)
Clinical Seizures (143)	29 (20.3%)
Hyperactivity (145)	29 (20.0%)