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## Small intragenic structural variants in *SATB2*-associated syndrome

CAP TODAY and the Association for Molecular Pathology have teamed up to bring molecular case reports to CAP TODAY readers. AMP members write the reports using clinical cases from their own practices that show molecular testing's important role in diagnosis, prognosis, and treatment. The following report comes from Washington University School of Medicine in St. Louis. If you would like to submit a case report, please send an email to the AMP at [amp@amp.org](mailto:amp@amp.org). For more information about the AMP and all previously published case reports, visit [www.amp.org](http://www.amp.org).



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**Case.** A two-year-old female presented for a comprehensive plastic surgery evaluation for the repair of her cleft palate; this evaluation included a referral to medical genetics. The patient was born at full term to a 32-year-old woman with six total gestations and four live births. The pregnancy was uncomplicated, and the patient had an appropriate length, weight, and head circumference. A fully cleft palate was noted 24 hours after birth. Subsequently she missed several developmental, behavioral, and social functioning milestones. The family was concerned that she might have autism; therefore, a Modified Checklist for Autism in Toddlers (M-CHAT) screen was performed, on

which she scored a six (medium/borderline risk for autism). The patient takes no medication but receives occupational and speech-language therapy. She lives with her family, including three full siblings. There is no family history of cleft palate or autism (Fig. 1A, page 2). A review of systems was notable for dental problems, drooling, and behavioral problems (agitation, self-injury). On the physical exam, the patient has an underbite, full cheeks, and prominent veins on the eyelids and bridge of the nose. She is nonverbal, aside from the word “no.” Her feet are notable for long toes and left greater than right fourth toe clinodactyly.

About 10 percent of individuals with cleft lip and palate have a chromosome condition that can be diagnosed with a chromosomal microarray. Further, chromosomal microarray is the first-line diagnostic test for patients with intellectual disability/developmental delay and congenital anomalies per American College of Medical Genetics and Genomics recommendations.<sup>1</sup>

Genetic testing was arranged with informed consent. A buccal swab was collected from the patient, and DNA was extracted per standard protocols. Chromosomal microarray testing was performed to assess copy number variants using the CytoScan HD array (Thermo Fisher Scientific). It revealed an interstitial loss on the long arm of chromosome 2 at band

q33.1, spanning approximately 35 kb in length and consisting of 88 markers/probes,  $\text{arr}[\text{GRCh37}] \text{ 2q33.1 (200209972\_200245277)} \times 1$  (Fig. 1B, page 2). This copy number loss spans 200209972\_200245277 and includes exons 6–8 (NM\_015265.4, hg19) of the Special AT-rich sequence-binding protein 1 (*SATB2*, OMIM #608148) gene (Fig. 2, page 3).

*SATB2* is a DNA-binding protein involved in chromatin and transcriptional regulation. The deleted regions of the *SATB2* protein include the CUT1 and CUTL domains, which are known hotspots for pathogenic single nucleotide missense variants in *SATB2*-associated syndrome (SAS). Given the strong overlap between the patient's presentation with SAS and these molecular findings, the diagnosis of SAS was made based on the ACMG and Clinical Genome Resource joint consensus recommendations.<sup>1,2</sup> While cascade genetic testing has not been performed, given that the patient has three healthy full siblings and both parents are unaffected, this variant is likely de novo. SAS is almost exclusively caused by de novo variants and has a germline mosaicism risk of 1.3 percent.<sup>3</sup>

**Discussion.** *SATB2*-associated syndrome, also known as Glass syndrome, is an autosomal-dominant neurogenetic multisystemic disorder caused by variants in Special AT-rich sequence-binding protein 1 (*SATB2*). It was first described in 1989 and

mapped to the 2q32.2q33.1 region based on a report of an individual with severe developmental delay.<sup>4</sup> The *SATB2* gene encodes a nuclear matrix DNA-binding protein that participates in transcription regulation and chromatin remodeling by specifically binding to genomic nu-

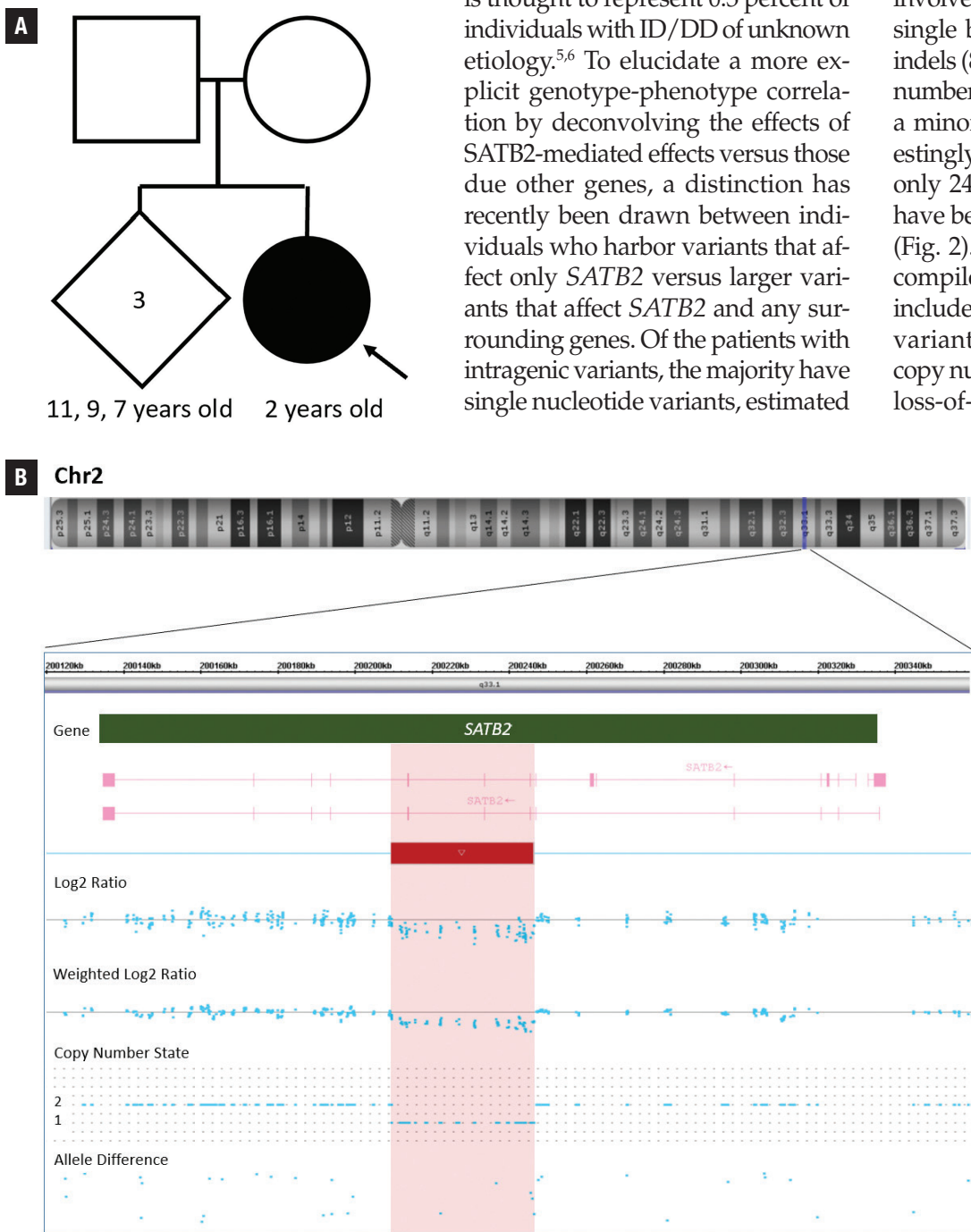
clear matrix attachment regions. In 2003, the gene *SATB2*, which resides within the 2q33.1 region, was uncovered as causing cleft palate in two individuals with intellectual delay. Subsequent studies have confirmed that the dominant presenting symptom of SAS is intellectual disability/developmental delay (ID/DD). SAS is thought to represent 0.3 percent of individuals with ID/DD of unknown etiology.<sup>5,6</sup> To elucidate a more explicit genotype-phenotype correlation by deconvolving the effects of *SATB2*-mediated effects versus those due other genes, a distinction has recently been drawn between individuals who harbor variants that affect only *SATB2* versus larger variants that affect *SATB2* and any surrounding genes. Of the patients with intragenic variants, the majority have single nucleotide variants, estimated

to be approximately 68.3 percent. By contrast, intragenic deletions in *SATB2* have been described much less frequently.<sup>3</sup>

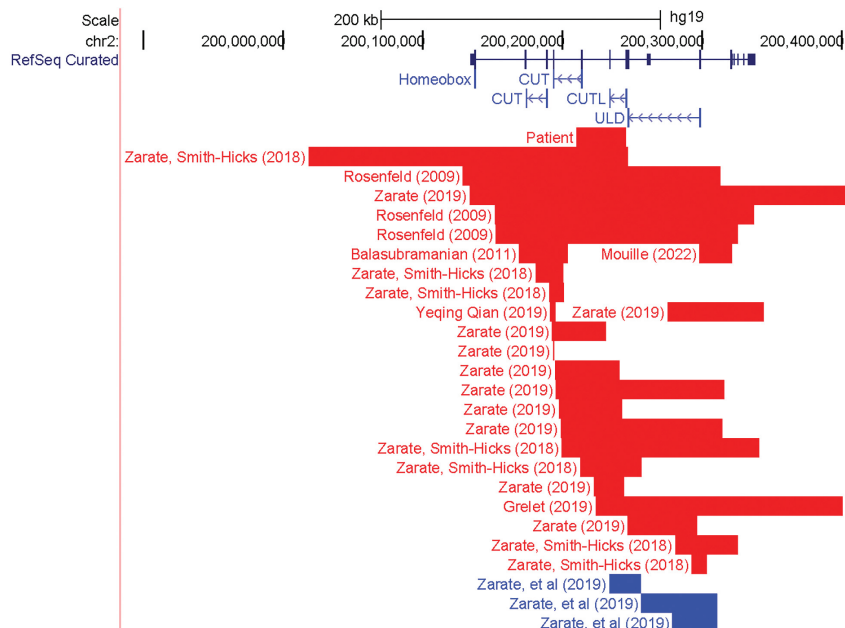
*SATB2*-affecting variants are either large deletions encompassing *SATB2* and surrounding genes, or smaller variants that involve only *SATB2*.<sup>3,7</sup> Among the variants that involve only *SATB2*, the majority are single base substitutions and small indels (82.7 percent).<sup>3</sup> Intragenic copy number variants of *SATB2* constitute a minority of reported cases.<sup>3</sup> Interestingly, in reviewing the literature, only 24 unique intragenic deletions have been reported to cause SAS<sup>3,8-13</sup> (Fig. 2). In fact, some resources that compile variants in *SATB2* do not include an option for copy number variants. Nevertheless, intragenic copy number variants are considered loss-of-function variants and have

been associated with the same phenotypic presentation as single nucleotide variants.<sup>3</sup>

The most common phenotypic features of SAS—intellectual disability with limited speech, dental abnormalities, and behavioral issues—can easily overlap with other conditions, such as Angelman syndrome, mitochondrial disorders, or cerebral palsy.<sup>14-16</sup> The lack of specificity in the clinical patient presentation can lead to a delay in diagnosis; in fact, the mean age of diagnosis in SAS is roughly 6.6 years.<sup>5</sup> Overlap with known disease-causing genes can be used as supportive evidence for the pathogenicity of newly detected variants. Therefore, combining different molecular genetic tests, such as sequencing and deletion/duplication analysis to identify the



**Fig. 1. A.** A pedigree of the family with the children's ages shown. Squares are male, circles female, diamond indicates unknown gender. Filled-in shapes are affected individuals. The arrow indicates the proband. **B.** A novel 35kb 2q33.1 deletion detected by chromosomal microarray. Affymetrix CytoScan chromosomal microarray analysis of chromosome 2 detects a 35kb deletion at 2q33.1 arr[GRCh37] 2q33.1(200209972\_200245277)x1, including three exons of gene *SATB2*. The deletion (represented by a red bar) is indicated by log2 ratio, weighted log2 ratio, copy number state, and allele difference.



**Fig. 2.** Summary of previously reported intragenic deletion and duplication as shown in the UCSC Genome Browser. *SATB2* gene is shown in dark blue. The Pfam protein domains are indicated in blue: ULD, CUTL, CUT1, and CUT2. Red segments indicated deletions; blue segments correspond with insertions.

potential intragenic copy number changes as well as SNVs and indels, may help clinicians make the final diagnosis in a timely fashion.

In conclusion, SAS is a rare neurogenetic disease with fewer than 300 reported cases that relies heavily on molecular testing for diagnosis. Most pathogenic intragenic variants are single nucleotide variants; however, increasingly small disease-causing deletions and duplications are being reported. We report an additional case of a two-year-old female with developmental delay and cleft palate who had a deletion spanning exons 6–8 detected on chromosomal microarray. In reviewing the literature, we noted that intragenic variants most frequently overlap with the CUT1, CUTL, and ULD protein domains, which is a similar distribution to the missense variants found in SAS. Intragenic deletions, particularly in exons 4 and 6–8, are a reoccurring disease-causing variant type in *SATB2*-associated syndrome, demonstrating the clinical utility of chromosomal microarray in detecting disease-causing variants in SAS. □

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See 'Test yourself' questions, next page.

## Test yourself

*Here are three questions taken from the case report. Answers are online now at [www.amp.org/casereports](http://www.amp.org/casereports) and will be published next month in CAP TODAY.*

- 1.** What is the first-line diagnostic test for patients with intellectual disability and/or developmental delay?
  - a. Chromosomal microarray
  - b. Exon sequencing
  - c. M-Chat
  
- 2.** Chromosomal microarray is designed to detect which type of variants?
  - a. Unbalanced chromosomal gains or losses
  - b. Low-level mosaicism
  - c. Single nucleotide variants
  
- 3.** What is the most frequent variant described in *SATB2*-associated syndrome?
  - a. Missense variants
  - b. Large deletions
  - c. Internal tandem duplication