



SATB2 Gap and Landscape Analysis

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Scope of Work: The goal of this document is to provide concrete recommendations to the SATB2 Gene Foundation about how best to direct their research efforts for SAS. To this end, we have reviewed published material and public databases, interviewed key stakeholders (including families, clinicians, and researchers), and integrated learnings from other related fields and disorders to inform our recommendations. The activities we are suggesting should be considered alongside the organizational priorities, available funding, and bandwidth or resources needed to pursue these activities. SAS is a multifaceted disease that affects multiple biological systems at once. Priorities may shift over time as scientific understanding of the disease progresses and as therapeutic technologies mature. We recommend revisiting strategic plans and community priorities yearly to ensure your foundation's goals are aligned with your research activities. This first half of the document identifies key gaps in understanding and associated recommendations, while the second half of the document reviews SAS and SATB2 biology. The SAS and SATB2 review may be useful for researcher or clinicians that are new to the field or would like a more in-depth understanding. Due to the length of the document a number of sections repeat content since we assume the reader might skip around and we do not want key information to be missed.

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Table of Contents

SATB2-ASSOCIATED SYNDROME OVERVIEW	5
UNDERSTANDING THE BIOLOGY OF SAS	5
THERAPEUTIC CONSIDERATIONS.....	5
RESEARCH PRIORITIES.....	7
SUMMARY RECOMMENDATIONS	7
KEY SATB2 BIOLOGICAL QUESTIONS (ORDERED BY PRIORITY):.....	8
IMPORTANT RESEARCH AREAS OR TOOLS FOR ADDRESSING BIOLOGICAL QUESTIONS	11
<i>Neuroimaging</i>	11
Electroencephalogram (EEG)	11
Functional magnetic resonance imaging (fMRI)	12
<i>iPSCs</i>	13
Characterization	13
Screening.....	13
<i>Animal models</i>	14
Mouse model	14
** COMMENTARY ON SATB2'S VIABILITY AS A TARGET FOR GENE THERAPY:.....	14
SYMPTOMS WITH HIGHEST IMPACT ON QUALITY OF LIFE	15
RESOURCES AND TOOLS:.....	15
AVAILABLE RESOURCES AND TOOLS:.....	15
RESOURCES AND TOOLS NEEDED:	16
SUGGESTED ADDITIONS TO COMMUNICATION:	16
GENETICS	16
SATB2 GENE OVERVIEW	16
SATB2 GLOBAL RNA AND PROTEIN EXPRESSION	17
SATB2 STRUCTURE, FUNCTION, AND ANIMAL MODELS OF DISEASE.....	21
<i>Molecular function overview:</i>	21
<i>SATB2 protein functional domains</i>	23
<i>Development Expression Pattern</i>	24
<i>Adult Expression Pattern</i>	24
<i>SATB2 in specification of cortical neurons and projections</i>	25
<i>SATB2's role in cognition and memory</i>	26
<i>SATB2 Mouse Models:</i>	27
<i>Cellular models of SATB2:</i>	28
SATB2 MOLECULAR MECHANISMS IN DISEASE.....	28
DISEASE OVERVIEW	28
AGE AT ONSET.....	28
INITIAL SYMPTOMS	28
COMMON CLINICAL PHENOTYPES ASSOCIATED WITH SATB2 MUTATION	29
<i>Symptom prevalence in SAS population:</i>	29
<i>Clinical Findings overview [30, 31, 33]:</i>	30
• Speech and Language deficits	30
• Developmental delay/intellectual disability	31
• Abnormal brain MRI and EEG	31
• Craniofacial dysmorphism	33
• Behavioral issues.....	33
• Palate anomalies	33
• Dental anomalies	34

• Skeletal anomalies	34
• Decreased sensitivity to pain	34
• Other anomalies:	34
CLINICAL DIAGNOSIS.....	35
SYMPTOMS THAT LEAD TO SEEK DIAGNOSIS.....	35
GENETIC TESTING	35
GENETIC COUNSELING	37
GENETIC PANELS RESOURCE	37
DIAGNOSTIC OBSTACLES.....	38
SPECIALISTS SEEN.....	38
TYPES OF MUTATIONS	39
COMMON MUTATIONS	41
GENOTYPE-PHENOTYPE CORRELATIONS AND CLINICAL POPULATION SYMPTOM HETEROGENEITY	42
GENOTYPE-PHENOTYPE CORRELATIONS.....	42
CLINICAL POPULATION HETEROGENEITY.....	43
PROGRESSION	43
RATE OF DECLINE.....	43
POPULATION.....	43
INCIDENCE & PREVALENCE	43
ETHNIC OR GEOGRAPHICAL ENRICHMENT.....	44
CURRENT MANAGEMENT AND TREATMENT OPTIONS.....	44
MANAGEMENT RECOMMENDATIONS FOR DEVELOPMENTAL DELAY/INTELLECTUAL DISABILITY:.....	46
MANAGEMENT RECOMMENDATIONS FOR MOTOR DYSFUNCTION:.....	46
MANAGEMENT RECOMMENDATIONS FOR SOCIAL/BEHAVIORAL CONCERNs:	47
ALTERNATE THERAPY APPROACHES:.....	47
PATIENT DATA.....	47
REGISTRIES	48
CITATIONS.....	49

SATB2-associated Syndrome Overview

SAS (SATB2-associated syndrome), previously referred to as Glass syndrome, is the constellation of symptoms, or the condition, associated with mutations in the SATB2 gene. SAS is an autosomal dominant disorder in which the presence of one mutated allele (or one copy) leads to clinical symptoms of the disorder. Glass Syndrome was first described in 1989 through the report of a male with a cytogenetically visible 2q32.2-q33.1 deletion, and it was later discovered that SATB2 was the causative gene responsible for the syndrome [1]. More recently, SAS was proposed as a clinically recognizable syndrome with defining characteristics of developmental delay/intellectual disability, reduced or absent speech, and dental and palatal anomalies; however, symptoms are varied and are part of a spectrum of severity.

Understanding the biology of SAS

SATB2 plays an important role in prenatal development. To determine symptoms or aspects of SAS that are treatable or preventable it is important to understand the biological function of SATB2 after birth, when most patients are diagnosed. A number of genome-wide associate studies (GWAS) identify an association of sequence variants in the SATB2 genome region with changes in bone density, Schizophrenia, anxiety, IL-8 levels in the CSF, and autism, [2–8]. Importantly, these studies are not likely to include SAS patients, so these findings imply that the SATB2 gene may play a role in other disorders. Most of these studies involve adults or include disorders with onset later in life, leading to the consideration that SATB2 plays a role in adult-onset disorders. Links to these conditions should be explored to determine if there are similarities in the disease biology of these conditions that are applicable to SAS disease biology, as well as, whether treatments for these conditions might be useful in treating aspects of SAS.

Therapeutic considerations

Developing a diverse portfolio of therapeutic approaches for SAS can increase the likelihood of finding an effective treatment, but patient groups can't spread funding resources too thin. In order to establish objective funding and research goals, we advise making prioritization decisions based on feasibility and biological considerations.

Below is a general list of different therapeutic modalities or approaches to drug development:

- Small molecules: when screening compounds already approved by the FDA this is called drug repurposing. Examples include Tylenol and Zyrtec. While cost effective and sometimes the most efficient path to find a drug, this approach is what we call a “fishing expedition.” You don’t know what you will find or if you will find something. If there is a potential drug identified, it isn’t always clear what the mechanism of action is. And small molecules usually don’t target the root cause of the disease.
- Biologics (antibodies, soluble proteins, hormones): These are large protein-based molecules that usually must be injected into the patient and target circulating targets. Because of their size they do not usually penetrate into cells easily and therefore they must exert their effects outside of the cell. This limits their utility.
- Cell therapies (bone marrow transplants, or iPSC delivery): The injection or replacement of cells into the body is call cell therapy. Sometimes a functional cell is put into the body when the

previous cells die off or are dysfunctional, and sometimes cells are taken from the body and treated with a gene therapy and then put back into the body.

- Gene therapies and genome editing (AAV, lentivirus, CRiSPR): These approaches deliver genetic material (such as a functional gene) or to directly edit the mutation in a genome.
- RNA therapeutics (siRNAs, ASOs, aptamers, etc): Targeting RNA is sometimes easier than delivering DNA, especially when the aim is to decrease the expression or function of a protein.

From our initial review, there are two primary research areas that can help assess therapeutic approaches for SAS:

Small molecule screen: The most cost-effective place to begin therapeutic discovery is often with a repurposing screen. Once there is a cellular phenotype (discussed in more detail under iPSCs), researchers can use libraries of small molecules to test if any are effective in changing that phenotype, or if the small molecule targets a known SATB2 pathway (such as SATB2 expression or activity of another protein that is thought to affect SAS). This can be done unbiased, meaning all compounds are treated equally, or you can prioritize testing certain classes of compounds that are suggested to work from the biology of SAS. The drawback to small molecule screens is understanding how the small molecule works. While more cost effective and often cheaper, this approach can lead to dead-ends; however, it could help with identifying seizure and/or sleep medication that works best for SAS patients.

Alternatively, focusing research on targeting the underlying genetic cause of the disease is a more direct approach with fewer unknowns. That being said, sometimes these approaches are much more costly to fund, such is the case with gene therapy, and there are biological questions that need to be answered before deciding on a genetic therapy. The mutation in SATB2 occurs in only one copy of the gene, leaving the other copy to be normal. For SAS, understanding the role of the mutated SATB2 allele and the normal SATB2 allele in the same patient may provide clues to determine if genetic approaches are appropriate for SAS.

Mutated SATB2 may cause disease in one of two ways:

- 1) the mutated SATB2 is no longer able to perform its normal function so that function is decreased in the cell
- 2) the mutated SATB2 takes on a new and harmful function, while also decreasing the normal function by at least 50% (loss of one of the two normal copies)

In scenario one, gene replacement therapies may be a simple way to provide the missing function. In scenario two, the harmful function may need to be blocked, which could improve symptoms but it is likely that you will still need to increase normal function. Understanding the difference between one and two will be helpful in evaluating therapeutic options for SAS.

The following sections will outline the key biological questions remaining for SATB2 and give recommendations on priorities for the SATB2 Foundation.

Research Priorities

The goal of this document is to provide concrete recommendations to the SATB2 Gene Foundation about how best to direct their research efforts for SAS. To this end, we have reviewed published material and public databases, interviewed key stakeholders (including families, clinicians, and researchers), and integrated learnings from other related fields and disorders to inform our recommendations.

A part of the review process involves identifying key gaps in biological understanding. This helps us to prioritize the recommendations we provide, principally based on 1) the potential for impact (often driven by unmet need), and 2) the potential for success. The activities we are suggesting should be considered alongside the organizational priorities, available funding, and bandwidth or resources to pursue these activities. SAS is a multifaceted disease that affects multiple biological systems at once. Priorities may shift over time as scientific understanding of the disease progresses and as therapeutic technologies mature. We recommend revisiting strategic plans and community priorities yearly to ensure your foundation's goals are aligned with your research activities.

Summary Recommendations

1. **Key Gaps that can be addressed with future research:** We recommend using an open call for grant applications with a peer review process to address priority questions. There are also vendors that can sometimes provide adequate and efficient services to address straightforward questions.
 - Does SATB2 mutations result in haploinsufficiency or does the mutated allele also take on an added deleterious function?
 - What is the postnatal function of SATB2, and what does this suggest about the progression of SAS over time, and which symptoms might be treatable or even reversible?
 - What aspects of SAS are treatable? From what age?
 - Does replacement of the functional gene (or higher expression of the wildtype allele) ameliorate symptoms or cellular phenotypes? Which symptoms or phenotypes are treatable after birth and at different points in development?
 - Which aspects of SAS are interconnected (i.e. are behavioral symptoms primarily driven by sleep deficits or frustration cause by limitations in communication?)
 - What are the seizure types and seizure geneses for SAS patients? Are there higher numbers of SAS patients with undiagnosed seizures or seizure-like brain activity? Do these contribute to sleep disturbances seen in the population?
2. Highest Unmet needs in the community (discussed in [Symptoms with highest impact on quality of life](#))
 - Speech and language deficits
 - Developmental delays
 - Behavioral issues
 - Sleep issues
3. Investment in research tools
 - Fully characterized patient iPSCs
 - SATB2 antibodies
 - Patient and family data collection

Key SATB2 Biological Questions (ordered by priority):

After reviewing the published research and interviewing families and researchers, we have assembled a prioritized list of key questions pertaining to SATB2 and SAS. This list is prioritized based on potential impact on standard of care for patients or future therapeutic research directions. A large portion of these questions rely on strong iPSC development, and as such that should be a priority.

1. Sleep disturbances- What is the underlying, biological cause of sleep disturbances? Is this due to seizure activity or over activation of certain pathways? Are there available sleep aids that are more effective for this population.
 - ◆ We recommend using iPSC and neuroimaging-based experiments to assess. EEG data already collected can also be re-analyzed.
2. Seizure genesis- What seizure types do SAS patients have? Which types are absent in the population? What portion of the SAS population has undiagnosed seizure activity? What portion of the SAS population has abnormal brain activity? Are there potential treatments that are effective at controlling overt seizures and potentially aiding in the regulation of abnormal activity and related downstream dysregulation (potentially, learning, sleep, memory)
 - ◆ iPSCs and organoids may help in understanding the underlying biology, and EEG analysis may help identify types and genesis of seizures. EEG data previously collected can be re-analyzed as well.
3. Postnatal function of SATB2- Most proteins have multiple functions, and proteins that are important during embryonic development can have a completely different function later in development. The cellular function of SATB2 after birth is more likely to respond to treatment than cellular functions of SATB2 that occurred during embryonic development, the results of which may be permanent. Studies suggest that SATB2 plays a role in memory and cognition and different than the embryonic role of SATB2. Also, the SATB2 locus (i.e., the area where SATB2 is located in the genome) is associated with a number of diseases with later onset than SAS (i.e., Schizophrenia, bone mineral density, etc.). If SATB2 is the relevant gene in these studies, then these data suggest that changes in SATB2 function or expression can cause dysfunction in adolescents and adults, suggesting strongly that SATB2 plays an important role postnatally. Understanding SATB2's role on a deeper molecular and functional level will help in identifying treatable symptoms of SAS.
 - ◆ Recommendation: fund a grant to fully characterize post-natal SATB2 expression (Which cell types is it expressed in? Which isoform? What are the levels? Compared to prenatal- What is the effect of turning SATB2 back on postnatally?)
4. Understanding the regulation of expression of SATB2 from the mutated allele compared to the non-mutated/wildtype allele. Is there true haploinsufficiency (half the protein) or is there an auto feedback loop that alters the normal regulation once the cell recognizes there is not sufficient functional protein, or potentially too much mutated protein sticking around too long. Are there ways of downregulating mutant allele and upregulating the wildtype allele? Do certain types of mutations lead to altered binding efficiency of the SATB2 protein, affecting function? Are there detrimental dominant negative effects?

- ◆ We suggest starting with iPSC lines to answer this question then moving into mouse model if applicable
 - ◆ Suggested methods of SATB2 RNA modulation include:
 - i. Readthrough technology for patients with nonsense mutations (cause premature termination codons)
 - ii. Suppression of mutant allele—ASOs, microRNA,
5. Speech and language deficit manifestation- is apraxia of speech the main cause of language deficits? Could there be other modes of speech delay or absence outside of apraxia? Some data suggests that apraxia is the main cause, however this is hard to assess in non-verbal SAS patients. Full understanding of speech deficits can suggest alternatives to language therapy currently being suggested.
 - ◆ We suggest further conversations with SLPs, and possible fMRI analysis for identifying speech deficit manifestations.
 6. SATB2's interaction with hormones: mentioned in conversation with Dr. Zarate that behavioral issues seem to worsen near puberty. Is there a connection between SATB2 and sex hormones?
 7. Are there further genotype-phenotype correlations that can be discerned from available data? Understanding how the different SATB2 mutations affect symptom onset and severity may help with planning treatments, integrating therapies, and understanding the molecular mechanism of SATB2 pathogenesis.
 8. Can SATB1 compensate for SATB2 function? Both proteins are structurally very similar. Endogenous upregulation strategies can be used to assess- small molecule screening,
 - ◆ Use iPSCs to identify cellular phenotypes that can be screened against
 - ◆ Conduct small molecule screens against these phenotypes
 - ◆ Does delivery of satb1 revert? Satb2? To the same extent?—in cell lines and mouse models
 9. Jaw muscle innervation changes- is there a difference in jaw muscle innervation in SAS patients that manifests as feeding and speech difficulty?
 - ◆ Suggest assessing this in animal model if model can recapitulate feeding issues well
 10. Are any aspects of the disease progressive after initial onset of that symptom? What symptoms get worse? What symptoms could be prevented or ameliorated with treatment?
 11. If SAS could be diagnosed before the onset of symptoms and treated, how would the trajectory of disease be affected?

The table below summarizes data and recommendations from the above list.

Priority	Key Biological or Therapeutic Question	Recommendations	Research Tool
1	Sleep disturbances	We recommend using iPSC and neuroimaging based experiments to assess. EEG data already collected can also be re-analyzed.	Patient iPSCs and neuroimaging
2	Seizure genesis	iPSCs and organoids may help in understanding the underlying biology, and EEG analysis may help with identifying types and genesis of seizures. EEG data previously collected can be re-analyzed as well.	Patient iPSCs, organoids, and neuroimaging
3	Postnatal function of SATB2	Recommendation: fund a grant to fully characterize post-natal SATB2 expression (Which cell types is it expressed in? Which isoform? What are the levels? Compared to prenatal- What is the effect of turning SATB2 back on postnatally?)	Mouse model of SATB2
4	Understanding the regulation of expression of SATB2 from the mutated allele compared to the non-mutated/wildtype allele	We suggest starting with iPSC lines to answer this question then moving into mouse model if applicable. Methods of SATB2 RNA modulation include: Readthrough technology, ASOs, miRNAs	Patient iPSCs, mouse model
5	Speech and language deficit manifestation	We suggest further conversations with SLPs, and possible fMRI analysis for identifying speech deficit manifestations.	Neuroimaging, SLP assistance
6	SATB2's interaction with hormones		
7	Are there further genotype-phenotype correlations that can be discerned from available data?	Recommend re-evaluation of any correlations and use of cell lines	iPSCs and data evaluation
8	Can SATB1 compensate for SATB2 function?	Use iPSCs to identify cellular phenotypes that can be screened against. Conduct small molecule screens against these phenotypes. Does delivery of satb1 revert? Satb2? To the same extent?—in cell lines and mouse models.	iPSCs and animal model
9	Jaw muscle innervation changes	Suggest assessing this in animal model if model can recapitulate feeding issues well	Animal model
10	Are any aspects of the disease progressive after initial onset of that symptom	Suggest more detailed longitudinal studies of patients and centralized database for this information	Form patient portal and database for keeping details of symptoms
11	If SAS could be diagnosed before the onset of symptoms and treated, how would the trajectory of disease be affected?		

Important research areas or tools for addressing biological questions

Neuroimaging

Neuroimaging, usually MRI or EEG, has been conducted in many SAS patients in some capacity. It is recommended that neuroimaging be considered as part of standard assessment in patients with SAS---to include baseline MRI and EEG at diagnosis and follow up as determined by care team.

Electroencephalogram (EEG)

EEG measures electrical activity in the brain and can be used as a tool to understand complex brain functions. EEGs that have previously been collected can be re-evaluated to further investigate the functional changes that occur in SAS patients, also, EEGs have the potential to offer insights into SATB2's role in post-birth brain function.

SATB2 is required for upper layer cortical neuron differentiation and neural projections, specifically corticocortical projections through the corpus callosum. From knockout mice we know that in the absence of SATB2, neurons that were supposed to cross the midline through the corpus callosum no longer make corticocortical projections, but instead make cortical-subcortical connections. This altered connectivity seen in the mouse model may be recapitulated in the patients. Although seizures are only reported in a portion of SAS patients, many report findings of abnormal brain activity, particularly while sleeping. While common in neurodevelopmental disorders, abnormal brain activity in the absence of overt seizures is not often treated. More research into this area may lead to a better understanding of the frequency of abnormal EEG findings, as well as potential insights into SAS treatments.

For patients that have had EEGs and MRIs, scans should be collected and centralized for further research. One concern with the SAS population is the actual procedure of the EEG may be hard because of the age and intellectual level of the patients. However, there are EEG caps designed to be worn during exercise now, that may aid in the ability of conducting an EEG on the patients while awake. EEGs are relatively inexpensive, and the data collected from each one can be analyzed in a multitude of ways.

Areas EEG can be utilized:

- EEGs that have already been collected can be analyzed to find frequency tag signals that will aid in understanding the functional brain changes seen in SAS patients. This data can also be used to generate hypotheses to test in formal studies.
 - Anecdote from the Aniridia community: Aniridia is a rare disorder caused by heterozygous mutations to the PAX6 gene, also a transcription factor. Much like SATB2, PAX6 is required for normal development and maintenance of the central nervous system and likely plays unique roles in each. One of the symptoms that had the greatest impact on patient lives was the difficulty children had in school. Findings from an EEG [9] and fMRI [10] study allowed the families to understand the differences in how their children processed sound, leading to a change in school environment (to a less noisy room) which from anecdotal accounts, greatly improved academic performance and concentration.

- EEG can be used as a way of understanding patients' responses to sleep aids and epileptic drugs. Patient EEG results can be compared to known sleep aid responders and epilepsy drug responders to determine the best drug for these patients. This could be done in partnership with sleep institutes that have the normal responder EEG data.
- EEGs early in the patient's life/soon after diagnosis can be used to identify abnormal or seizure-like activity and help aid how the patient is managed.
- EEG pre- and post-different medications can show how well tolerated and efficacious that medicine is.
- EEG can give hints as to which cell types (inhibitory or excitatory) are important for the phenotypes. Perhaps there are differences we cannot discern from models or even our understanding of iPSCs.

Functional magnetic resonance imaging (fMRI)

Functional MRI measures brain activity and may help answer key questions that could lead to better therapies. Families most often cite communication as the symptom that impacts quality of life most. An inability to communicate not only can be hard on its own, but can compound behavioral issues, increasing frustrations in the SAS patient. In published clinical cases studies, it is often noted that the nonverbal aspect of SAS are a result of speech apraxia, or difficulty making the movements necessary for speech, but it is possible that there are other components contributing to the speech deficits [11]. Within that same vein, some family members have mentioned their concern that patients are unable to understand speech, and there is some evidence that language comprehension is lower in SAS patients. These two ideas—speech articulation and language comprehension—are hard to fully disentangle when patients have difficulty communicating. Standard BOLD fMRI or arterial spin labeling (ASL) fMRI can assist in uncovering which part of speech/communication is the main cause. Speech based testing (playing natural language, asking patients to try to say certain words, etc.) can be used to gain insight into the speech and language comprehension. In neurotypical patients you expect to see activation of Wernicke's area when language is understood and Broca's area when speech is produced. It is possible that the deficiency of SATB2 in could cause deficits in these areas and/or incomplete or inaccurate connections that affect speech and language. Additionally, if apraxia is truly the root cause of the verbal deficits, that should be able to be seen through activation of the motor cortex. Learnings from fMRI data on patients could help inform better/more specified types of speech and motor therapies that may lead to better outcomes.

Questions remaining for SAS patients addressable by neuroimaging:

1. Are more patients experiencing seizures and/or seizure-like activity that is undiagnosed?
 - ◆ Recommendation: Re-analyze previously recorded EEGs for new signature tags that suggest how brain activity is changing in SAS patients compared to neurotypical individuals
2. Could the seizure activity or abnormal brain activity seen in SAS patients be due in part to misrouted organization of the brain? And if so, could this also be affecting other symptoms such as speech and language anomalies, sleep disturbances, and behavioral issues?
 - ◆ Recommendation: EEG, fMRI analysis could lead to better plans for speech and language therapy

3. Is apraxia of speech the main speech deficit in SAS patients? Are there more language comprehension concerns?
 - ◆ fMRI analysis and further investigation by SLPs

iPSCs

Characterization

iPSCs can be important tools to explore the molecular and cellular basis of disease in cell types that are more closely related to the relevant tissue. They can also be used to screen for therapeutic effects.

To enable characterization as well as identification of potential therapeutics, the iPSCs should be differentiated into cell types relevant for disease, including cortical neurons and osteoblasts, and others dependent upon the organ system. Initial characterization should focus on identifying phenotypes common to the SATB2 deficient cells that differ from the control cells. This should be explored in different cell types given the diversity in affected systems in SAS. Ideally, a phenotype could be reversed when SATB2 levels are normalized, which can be most closely simulated through AAV delivery of the SATB2 gene. AAV should be used in order to deliver sufficient amounts of SATB2 to the cells. Using a tagged SATB2 will also allow the investigator to visualize which cells have been transduced and therefore which cells should show a change in the phenotype. Once a phenotype is identified that can be ameliorated by replacing SATB2, this phenotype can be used for drug screening.

Screening

Once robust phenotypes are identified, these phenotypes can be used as to measure if a potential therapeutic can ameliorate the phenotype associated with SATB2 deficiency.

Patient iPSCs can be a robust tool for screening prioritized drug panels (i.e. current treatments on the market for closely related symptoms) or large, unbiased panels. Medications used to treat sleep irregularities, seizures, bone health, or behavioral disorders related to those seen in SAS should be prioritized for initial screening [12]. For example, sleep disturbances have been reported by patient families to be one of the hardest aspects of SAS to deal with, and likely contributes to many other aspects. Additionally, not all sleep aids may work the same with each patient. Using differentiated patient iPSCs to screen sleep aids may provide significant information about which drugs could be the most useful for patients.

iPSC screens to consider:

1. Increased SATB1 expression- will increasing SATB1 expression compensate for loss of SATB2 function?
2. Micro RNAs (miR) that upregulate SATB2 expression, with the aim of identifying miRs that upregulate expression of the SATB2 functional allele which should increase SATB2 levels overall. This will need to be tested in an animal model as well, as miRNAs are known to have multiple targets within organism (see microRNA table in [Molecular Function Overview](#) for preliminary list).
3. Small molecule screening: identify drugs that impact the phenotype. Treatments for GWAS related disorders (Schizophrenia, low bone mineral density, anxiety). Interestingly, a GWAS of

- IL8 levels in CSF found an association with the SATB2 locus. It would be interesting to explore this association and to determine if IL8 has any role in SAS [4–8].
4. Organoid screening: If SATB2 haploinsufficiency is thought to strongly impact network of cells, rather than operating in a cell-autonomous function, organoids can be used to test the findings of #1-3

Animal models

Mouse model

Both mouse models discussed within this document in [SATB2 Mouse Models](#) have been useful in understanding the symptoms of SAS and the molecular function of SATB2; however, there are limitations to what a mouse model can show. For these models, the heterozygous mutant mice, which are the genetic mimic of patients, do not show the same severity or number of symptoms as the patients. In the heterozygous state, the models show palate abnormalities in approximately 25% of animals and recapitulate some of the bone and skeletal abnormalities. The homozygous mutant mouse model shows more severe brain defects, like an absent corpus callosum, but this is not something seen in the patients. The two symptoms in patients that have the biggest impact on daily life are the speech deficits and behavioral issues, both of which are nearly impossible to assay in a mouse model. It should also be noted that the human brain is much more complex than a mouse brain, and as such it can be difficult to use the mouse as a model of complex neurodevelopmental syndromes such as SAS. A more detailed investigation of the behavioral changes in the heterozygous mouse could be performed to try to identify subtle changes. There is utility in using the mouse model for further molecular understanding and therapeutic testing. Alternative ways of screening the mouse model for changes after therapy will have to be developed due to the lack of similar symptoms of patients.

*** Commentary on SATB2's viability as a target for gene therapy:*

A better understanding of the role and regulation of mutant SATB2 and the normal or wildtype SATB2 is needed before moving into gene replacement strategies along with a strong decision on which tissue and what time point it is necessary to target. Once that understanding has been developed, gene replacement therapy proof-of-concept data can be addressed in the animal model.

Important considerations for a gene therapy in SATB2:

- *cDNA size*: for transcript variant 1 of SATB2, the coding region is within the size constraints of current AAV vectors.
- *Inheritance pattern*: autosomal dominant inheritance provides some difficulties for gene therapy programs because of the presence of wildtype message and protein. Additionally, for most transcription factors such as SATB2, there is a very specific level of protein tolerated and any more or any less (as in the disease) could lead to additional issues. This can be a difficult thing to overcome with traditional gene therapy approaches which provide a means to increase expression but cannot be 'finely tuned'.
- *Well characterized animal model*: While there is an animal model for SATB2, it does not recapitulate the same clinical phenotypes seen in the human population within the heterozygous animal which makes it difficult to use as a readout of efficacy in a gene therapy study—efficacy could be shown with other metrics though. Alternate ways of assessing efficacy

- may include: expression-based analysis, possible behavioral testing (learning and memory testing), analyzing aspects of the model that do mimic SAS symptoms (e.g. bone health)
- *Expression characterization*: The expression of SATB2 is fairly well characterized both during development and adulthood. However, because SATB2 is expressed in multiple tissues, it is difficult to decide on the appropriate route of administration (ROA). If SATB2's expression in one tissue, for instance the CNS, is leading to most of the symptoms, then that tissue would be the primary target for the ROA.
- *Isoform characterization*: do not yet fully understand the role of transcript variants- may make choosing correct isoform more difficult
- *Window for treatment* (age and rate of onset): because SATB2 has a large developmental role, finding a treatable window post-birth may prove challenging for this population
- *Proof-of-concept data*: no POC data on gene therapy in SATB2 has currently been generated with the animal model
- *Restorative potential*: until we have more information on the basic biology of SATB2, its role in postnatal development and maintenance, and a better understanding of disease mechanisms, it is difficult to determine the restorative potential of a gene therapy for this population.

Symptoms with highest impact on quality of life

The SATB2 Gene Foundation conducted a survey of caregivers with 83 respondents that assessed areas of the disorder that impacted patient and caregiver life the most . They found that the following symptoms impacted life the most for both groups, but especially for caregivers. The following are in order from most impact to least impact:

1. Speech and Communication
2. Developmental Delays
3. Behavioral Issues
4. Craniofacial and Dental anomalies
5. Sleep Issues
6. Bone Density
7. Seizures

Resources and Tools:

We suggest continuing this conversation with your scientific advisors about which resources are needed to move research forward. In our research and discussions with patient families and SATB2 researchers, these are our recommendations on areas to expand.

Available Resources and Tools:

- iPSCs: SATB2 Gene Foundation has established iPSC lines and familial controls, ensuring these cell lines are maintained and accessible to anyone (non-profit, or for-profit) is important for enabling research. We suggest openly discussing
- Clinical registry
 - SAS Patient Registry is discussed in more detail in the [Patient Data Section](#)
- Mouse disease/knockout lines available

- Dobreva, 2006 [13]: LacZ reporter insert
 - <http://www.informatics.jax.org/allele/MGI:3692768>
- Britanova, 2006 [14]: Satb2 null
 - <http://www.informatics.jax.org/allele/key/51609>

Resources and Tools needed:

- Antibodies are critical for understanding disease and developing therapeutics. Ensuring that there are robust SATB2 antibodies should be a top priority for the research field.
 - N-terminal antibody is needed- to differentiate between truncated or absent protein
- RNA phasing: Developing sequencing methods to determine how SATB2 mRNA expression differs from the mutant allele compared to the wildtype allele will help elucidate molecular aspects of SAS.
- Fully characterized patient cell lines ([discussed under iPSCs](#))
- One of the things patient families asked for was a centralized way of “tracking important medical information, lab results, treatment guidelines, behavioral challenges, cognitive/speech progression”- it is important to centralize data for individual families and for researchers if families want their information to be shared. We recommend discussing different options with registry providers such as AllStripes and Invitae.

Suggested additions to communication:

Adding a list of research tools and resources on your website can help researchers quickly identify what tools are available and where they can order them from. By facilitating this process, there are less obstacles in the way of a researcher conducting SATB2 research. Additionally, if there are tools that are commonly used across SATB2 researchers, it is easier to compare results and findings directly. Overall, this improves the quality and speed of research and often stimulates collaboration.

Suggested resource list:

- Antibodies
- Cell lines
- Animal models
- Overview of clinical research centers
- Registry information (an overview of what has been collected and how to access data)

Genetics

SATB2 Gene Overview

SATB2 is a homeobox DNA binding protein that specifically binds to nuclear matrix attachment regions (MARs) and is involved in transcriptional regulation and chromatin remodeling. The SATB2 protein is 733 amino acids and contains two CUT domains and a homeodomain. These functional domains of SATB2 are highly conserved across vertebrates—human protein shares 100% identity with mouse, 98-100% with chicken, and up to 96% with zebrafish [15].

Naturally occurring sequence and isoforms:

- SATB2 gene <https://www.genecards.org/cgi-bin/carddisp.pl?gene=SATB2>
 - https://www.ncbi.nlm.nih.gov/nuccore/NM_001172509.2?report=GenBank
- Variant 1: 11 exons; Processed mRNA length of 5568 bp; CDS is 2201 bp for
 - There are two alternate transcripts: [NM_015265](#) and [NM_001172517](#), consist of 12 exons with processed mRNAs of 5306 bp and 5326 bp respectively
- There are 2 human isoforms that are alternately spliced
 - Isoform 1, or canonical, is 733 amino acids-- identifier: Q9UPW6-1
 - Isoform 2, missing aa 116-233, is 615 amino acids-- identifier: Q9UPW6-2

SATB2 global RNA and protein expression

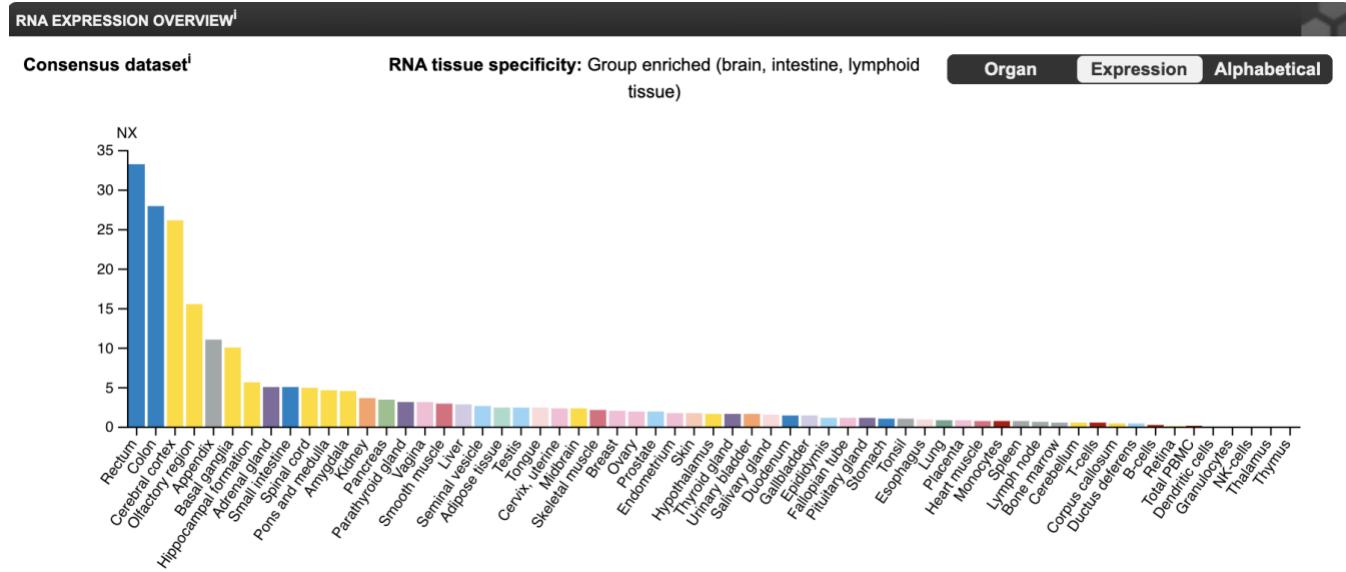
SATB2 is expressed in multiple tissues throughout development and adulthood, which will be discussed in more detail in the following sections on developmental and adult expression. Although different studies and databases show varied results, there is consensus that SATB2 is highly expressed in the developing and mature central nervous system, developing jaw and dental precursors, developing and adult bone, intestines, kidneys, and varied other tissues seen in the figures below. These are consensus data sets, and as evidenced in the figures, expression of mRNA is not perfectly replicated in protein expression data, suggesting there are differences in post-transcriptional regulation of SATB2. It is important to keep this in mind when choosing how expression of SATB2 is measured (by protein levels or mRNA) and which tissues or cell type experiments are conducted in as this may change the outcome significantly.

SATB2 mRNA expression overview from various public databases or publications:

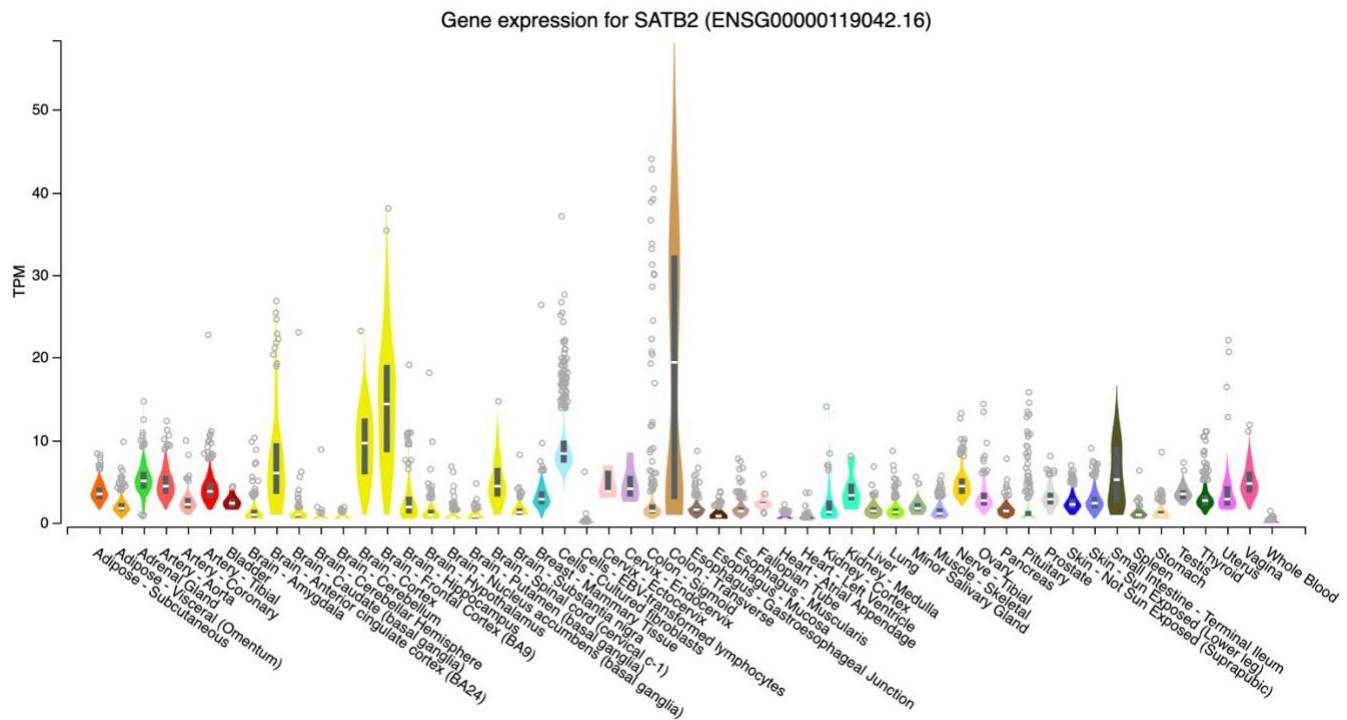
Below is a collection of SATB2 expression measurements. These databases look at the expression of SATB2 in various tissues in the human body through the measurement of the transcript, not the protein. The protein is eventually made from the transcript so it is important to keep in mind that the transcript levels can hint at protein, but it's not conclusively the same as measuring the protein levels.

Human RNA Expression: RNA expression from a consensus dataset shows highest expression in brain and intestinal tissues; however, low level expression exists in many tissues.

<https://www.proteinatlas.org/ENSG00000119042-SATB2/tissue>

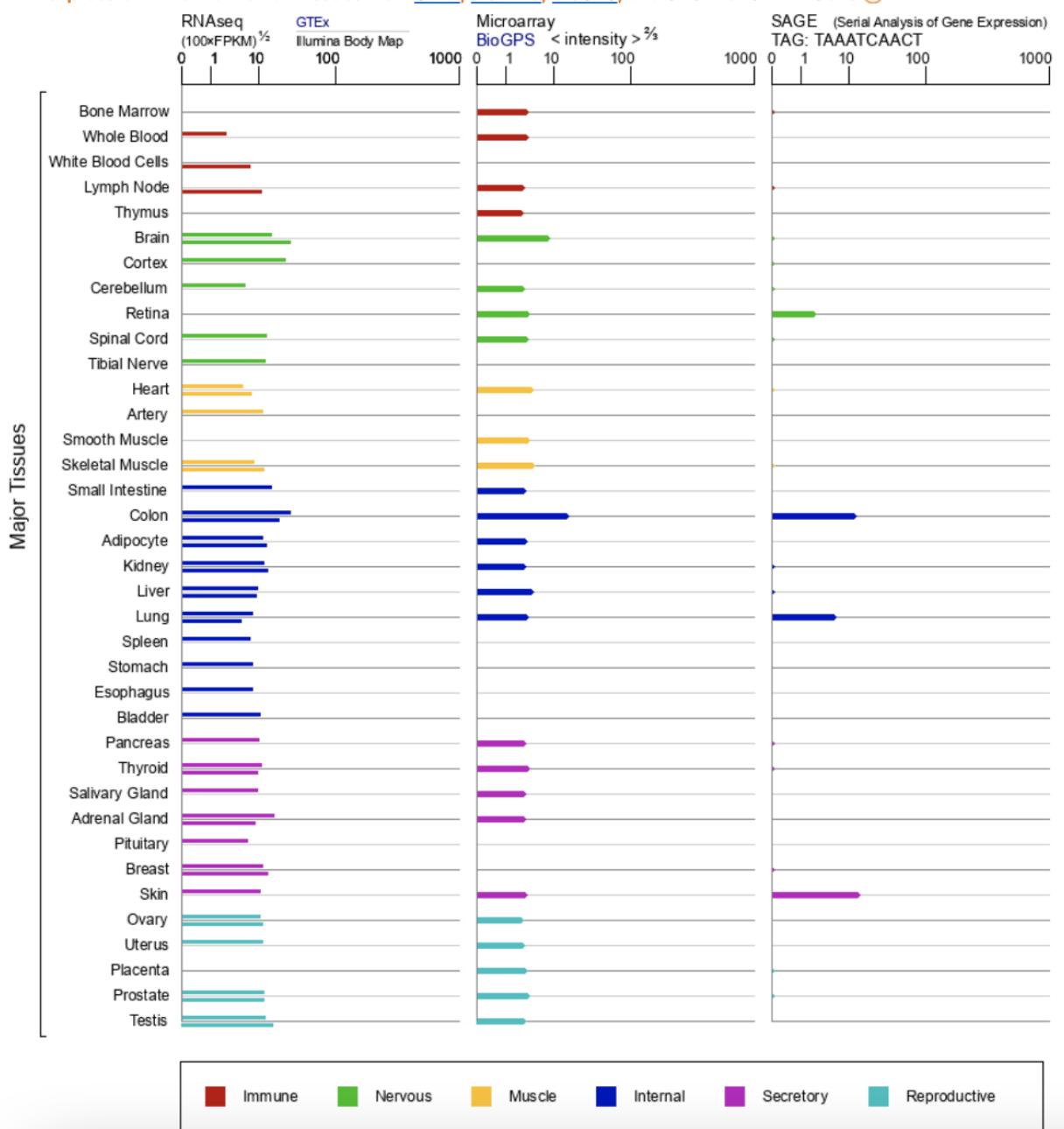


<https://gtexportal.org/home/gene/SATB2>



<https://www.genecards.org/cgi-bin/carddisp.pl?gene=SATB2>

mRNA expression in normal human tissues from [GTEX](#), [Illumina](#), [BioGPS](#), and [SAGE](#) for SATB2 Gene [?](#)



Mouse mRNA expression:

The figure below shows an expression profile of Satb2 (Satb2 with lowercase denotes mouse and SATB2 uppercase denotes human within this document) mRNA in mouse tissues and cells shows expression in multiple tissue types [16].

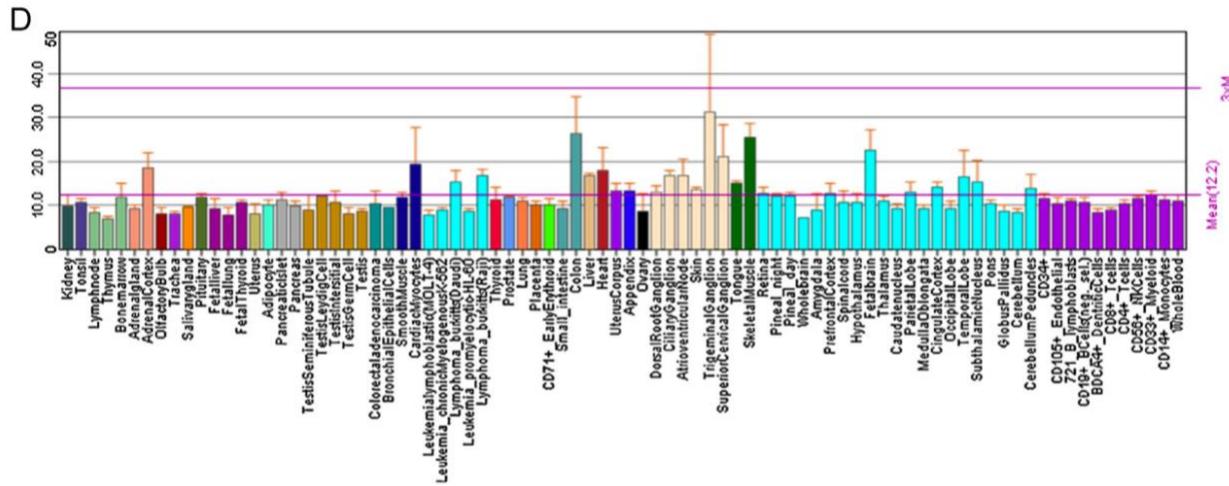
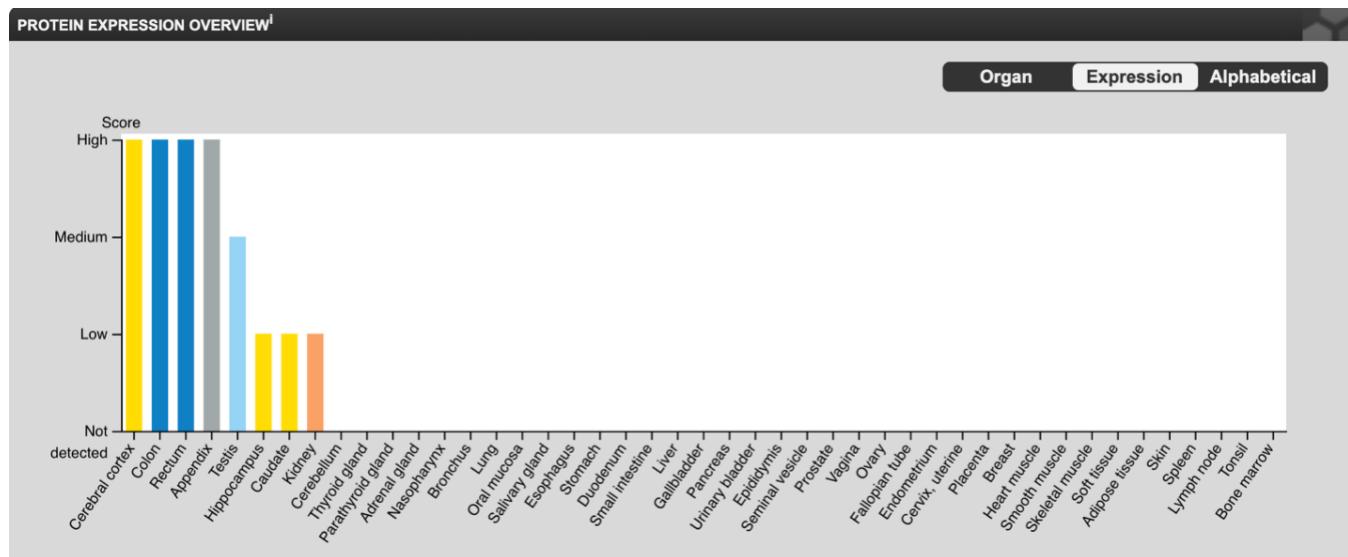


Fig. 1. (A) A schematic diagram showing the predicted molecular structure of SATB2 (generated using <http://smart.embl.de/smart/>). CUT domain is a DNA-binding domain found in combination with a downstream homeodomain. (B) Predicted 3D structure of the sub-domains of SATB2 (generated by <http://swissmodel.expasy.org/>). (C) Predicted 3D structure of SATB2 as a whole molecule (generated using <http://swissmodel.expasy.org/>). (D) Putative expression profile of Satb2 mRNA in mouse tissues and cells (generated using BioGPS, <http://biogps.gnf.org/>).

Human Protein Expression: Protein expression is important for identifying the tissues that SATB2 is likely active. These include the brain (cortex, hippocampus, and caudate), intestinal tissues, testes, and the kidney.

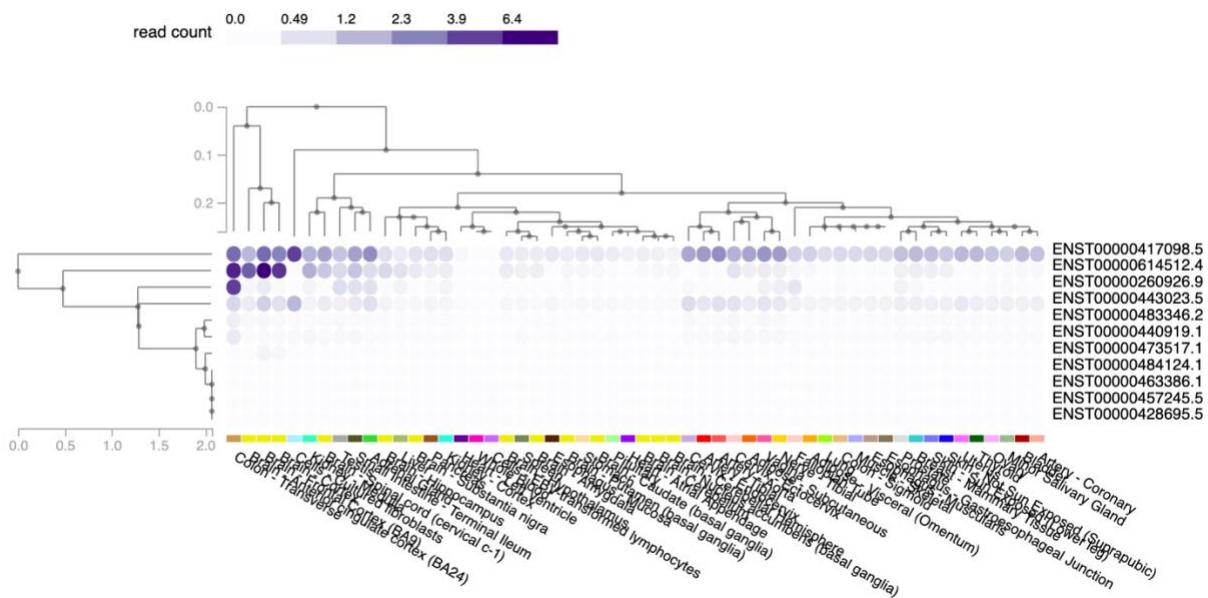
<https://www.proteinatlas.org/ENSG00000119042-SATB2/tissue>



Isoform Expression: the isoform expression data shown below suggest that there are two main isoforms of SATB2.

<https://gtexportal.org/home/gene/SATB2>

Isoform Expression of SATB2: ENSG00000119042.16 SATB homeobox 2 [Source:HGNC Symbol;Acc:HGNC:21637]



SATB2 Structure, Function, and animal models of disease

Molecular function overview:

For this document, and particularly this section, *Satb2* sequence or protein with lowercase denotes mouse and *SATB2* uppercase denotes human or non-specific species sequence or protein.

SATB2 is a transcription factor and DNA binding protein that controls gene expression by binding to DNA at nuclear matrix attachment regions or scaffold-associated regions. SATB2 can act as a docking site for several chromatin remodeling enzymes and can recruit corepressors or coactivators directly to promoters and enhancers. It binds to AT-rich DNA elements of nuclear matrix-attachment regions and modifies the chromatin structure through recruitment and interactions with HDAC1 and MAT2. In this capacity SATB2 plays an important role in integrating genetic and epigenetic signals for gene transcription modulation [16]. SATB2 is a high-level regulator of multiple gene regulatory networks, and as such, plays as a critical role in many developmental processes including cortical development, palate formation, skeletal development, and osteoblast differentiation. In essence, SATB2 acts as both a transcription factor to modulate gene expression in multiple tissues and as a molecular tool for opening up chromosomal DNA to allow for transcription or gene expression.

SATB2 is necessary for the development of many different tissues, and is also regulated by other transcription factors and multiple microRNAs including miR-34b and c. Both of these microRNAs regulate osteoblast differentiation during the postnatal period. SATB2's function in osteoblastogenesis

and bone formation has been heavily studied, and it is known that within this role SATB2 is regulated by multiple cytokines and growth factors, as seen in the figure below [16]. SATB2 is thought to drive osteoblast differentiation through modulation of Runx2 and Osx expression [7]. During development, SATB2 normally inhibits expression of the Hoxa2 gene, which represses bone formation. In SATB2 patients, there is less repression of Hoxa2, and therefore results in abnormal craniofacial development and abnormal bone formation [13]. Satb2 also plays a role in enhancing the activity of two other transcription factors, Runx2 and ATF4, which both play a role in regulating osteoblast differentiation [13]. This highlights Satb2's role in bone differentiation, growth, and patterning and explains the bone defects that have been observed in patients. In addition to SATB2's role in osteoblastogenesis, it plays a critical role in other diverse and conserved developmental pathways-- proper jaw growth and patterning, upper layer neuron specification [13, 17]. Satb2's role in cortical patterning, differentiation, and projections is discussed further in the section below.

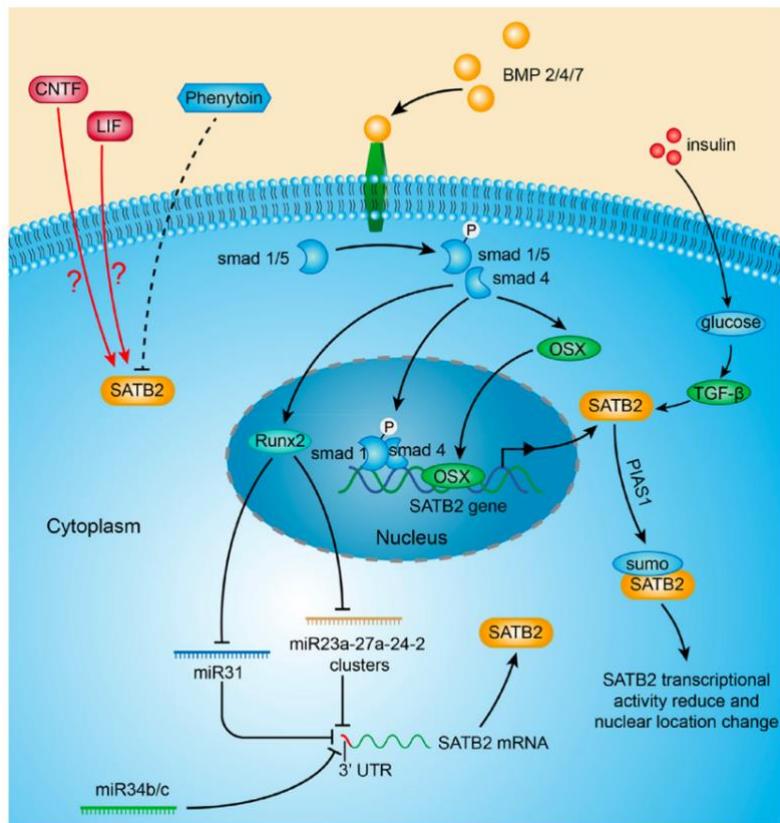


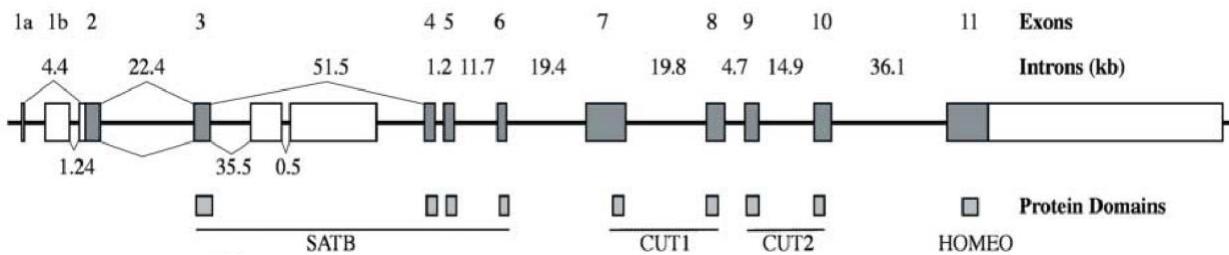
Table of microRNAs known to regulate SATB2:

microRNA	Tissue or Cell Type	Outcome	SATB2 Modulation	Citation
miR-35b	postnatal osteoblast differentiation and maturation	inhibit osteoblast proliferation and differentiation	Downregulates SATB2	[18]
miR-35c	postnatal osteoblast differentiation and maturation	inhibit osteoblast proliferation and differentiation	Downregulates SATB2	[18]
miR-31	dental	reduced proliferation	Downregulates SATB2	[19]
miR-483-5p	MC3T3-E1 (mouse-derived osteoblast)	Inhibiting miR-483-5p can increase bone mineral density	Downregulates SATB2; activates PI3K/AKT	[20]
miR-449b-5p	Bone marrow stem cells (BMSC)	inhibiting osteogenic differentiation	Inhibits SATB2	[21]

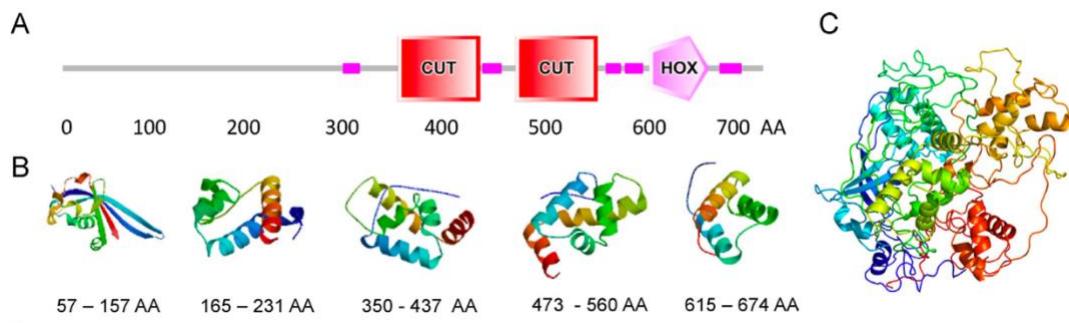
SATB2 protein functional domains

The two CUT domains in SATB2 (aa352–aa437 and aa482–aa560) bind to central nuclear matrix attachment region (MAR). The homeodomain's (aa614–aa677) role is not fully determined but might play a role in interactions with other proteins or protein complexes, much like other homeobox proteins. Another highly conserved part of the SATB2 protein is the large region (SATB2 residues 57–231) corresponding to the Pfam-B_10016 domain, which shows high conservation with SATB1. In SATB1 this part of the protein is known to be required for dimerization, and it may play a similar role in SATB2 [16]. Missense point mutations have been reported to be highly severe in SAS patients, particularly in the CUT domains. This might be due to changes in binding efficiency and/or specificity in the mutated protein.

The figure below provides a nice schematic of the structure of both the gene and corresponding protein domains of SATB2 [15].



The figure below shows predicted molecular structure of SATB2 [16].



Development Expression Pattern

SATB2 plays a clear role in development of the palate, teeth, bones, and portions of the central nervous system. In developing mouse embryos, Satb2 is expressed in a site- and stage-specific pattern with initial expression starting mid development at E10.5 in the first pharyngeal arch, regions that develop into the primary palate, and maxillary process. By E14.5 (mid/late development) expression has dropped dramatically [15].

In a Satb2 knockout mouse model, Satb2 expression was seen earlier in the hindbrain at E8.5, hindgut diverticulum at E9.5, and branchial arches in the apical ectodermal ridge at E10.5. Additionally, expression was seen in the cortex at E12.5 and E14.5 in addition to sites of bone formation [16]. Expression of SATB2 in the developing central nervous system is discussed in more detail in the following section on cortical organization.

Adult Expression Pattern

In the adult tissue, Satb2 has been shown to be expressed in breast tissue, the spinal cord, kidneys, leukocytes, and the central nervous system [13, 22]. Mouse expression profiles for Satb2 showed diverse distribution of Satb2 in many tissues. Staining for Satb2 in 2-month-old mice showed intense expression in osteoprogenitor cells on the inner surface of the adult femur and surface of the trabecular bone at the growth plate. Additionally Satb2 was found to be strongly expressed in osteoblasts in newly formed bone at sites of mandibular bone defects [22].

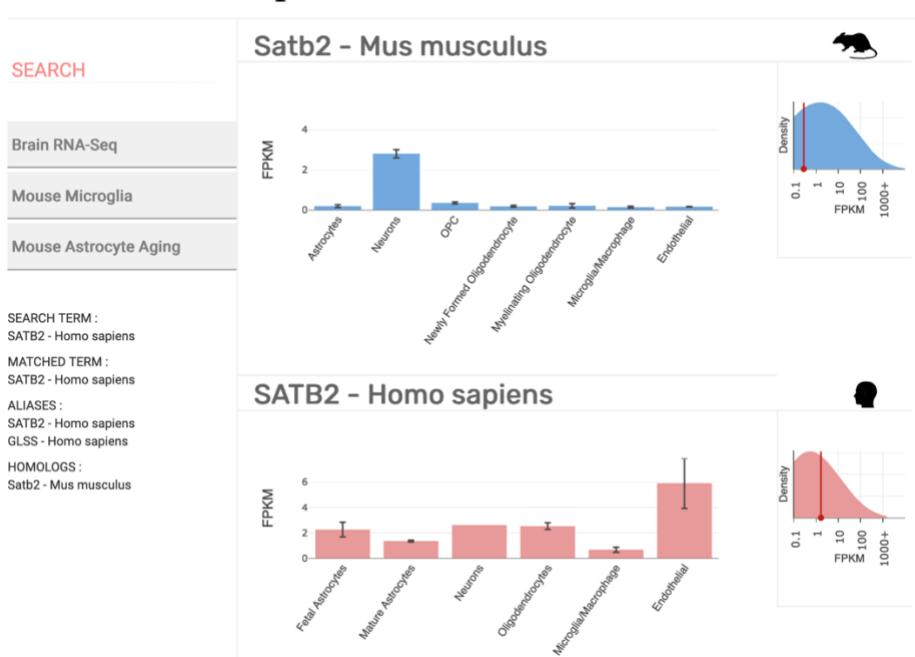
Satb2 mouse studies have shown expression in upper layer cortical neurons in development and more widely in adulthood in pyramidal neurons of the cortex. Outside of the cortex, Satb2 expression has

been found in the bed nucleus of the stria terminalis, horizontal limb of the diagonal band, lateral hypothalamic area, arcuate nucleus, hypothalamic paraventricular nucleus, ventral tegmental nucleus, latero-dorsal tegmental nucleus, dorsal raphe nucleus, rostral periolfactory region, and parabrachial nucleus. Immunostaining for neuronal cell type also showed that Satb2 is exclusively expressed in the excitatory neurons of the neocortex of mice [23]. High levels of Satb2 expression in the adult mouse brain show Satb2 in two brain regions important for memory formation—the cerebral cortex and the CA1-hippocampal regions [24].

In humans it was shown that SATB2 is expressed in normal tissues (through analysis of 65 cell types), which include: glandular cells of the gastrointestinal tract, appendix, colon, rectum, and a subset of neuronal cells from the cerebral cortex and hippocampus. Additional analysis of cancer tissues showed SATB2 was expressed, at a higher level, in all analyzed colorectal cancers [16]. Of interest, SATB2 shows different expression profiles in mice and humans within the brain, suggesting that there are differences between the two species that might limit the insights that can be gained from animal studies for the neuro-related phenotypes.

<https://www.brainrnaseq.org/>

Brain RNA-Seq



SATB2 in specification of cortical neurons and projections

The mammalian cerebral cortex has two major types of pyramidal neurons—corticocortical projection neurons which are concentrated in the upper layers (UL) of the cortex, and subcortical projection neurons, which are found in the deeper layers (DL). Upper layer (UL1) neurons, produced from progenitors in the subventricular zone, express SATB2 during differentiation while the UL2 neurons do not express SATB2. SATB2 is also required for the formation of axonal projections that connect the two hemispheres, and SATB2 expression seems to be maintained in adulthood in these corticocortical

pyramidal neurons. SATB2 promotes callosal projection neuron identity within the cortex through direct repression of Ctip2 expression. When SATB2 is no longer expressed in the cortex, the UL1 neurons lose their identity and take on DL and UL2 gene expression profiles. Additionally, UL1 neurons in a SATB2 mutant model fail to migrate to the superficial layers of the brain and no longer contribute to the corpus callosum, but rather these neurons now contribute to the corticospinal tract and project into subcortical targets, normally populated by the DL axons. SATB2 expression is also high during neuronal migration and axonal growth, suggesting SATB2 plays an important role in connectivity within the cortex [17, 25].

Cortical projections, specifically corticocortical projects are the most affected in Satb2 knockout animals. Britanova and colleagues found that in KO animals, axons that should extend through the corpus callosum no longer did, and that these axons may contribute to the anterior commissure, as it was thickened. Additionally, it was found that afferent and efferent cortical axon connections in the Satb2 mutants were misrouted. Loss of Satb2 also resulted in an increase in the number of neurons (from 37% in WT to 64% in mutant) that sent efferent connections to subcortical targets [17].

Research shows through comparisons between neonatal and adult SATB2 complexes, that there is a developmental shift in SATB2 molecular function from a transcription repressor towards a role in chromosomal superstructure and a regulator of neurotransmission and plasticity. Gene sets regulated by Satb2 in the neocortex of neonatal mice compared to adult mice show little to no overlap, supporting evidence that the function of satb2 changes as the animal ages. Through human GWAS data it is shown that “common variants associated with human cognitive ability are enriched within the genes encoding adult but not neonatal SATB2 interactors” [3]. This data supports a shift in the function of SATB2 from development to adulthood, and suggests SATB2 may contribute to cognitive function in the adult brain [3].

SATB2’s role in cognition and memory

In studies done on heterozygous Satb2 mutant mice and Satb2 conditional knockout mice it was found that spatial memory and working memory were significantly damaged in young and adult mice. In the Satb2 mutant mice, late phase long-term potentiation was greatly impaired functionally. Morphologically, CA1 neurons of the hippocampus were altered. They had decreased basal dendrite spine density and fewer branches of apical dendrites extended into the lacunar molecular layer. This study suggested that Satb2 plays a key role in both spatial and working memory by regulating hippocampal synaptic plasticity [26]. SATB2 common variants have also been found, through GWAS (genome-wide association studies), to be a risk factor for schizophrenia and educational attainment [2].

Anecdotally from conversations with parents and caregivers, some mention that their children can learn words and retain them but have difficulty keeping that information long-term. This difficulty with recall and memory could be due to SATB2’s role in the hippocampus as shown in the studies mentioned above. This is an area of research that should be explored further.

SATB2 Mouse Models:

Findings from *Satb2* mouse models have been discussed in the previous sections. Mutant mice confirm the role of *Satb2* in regulating craniofacial patterning, osteoblast differentiation, and cortico-cortical extension and projection. Mouse models have also demonstrated that SATB2 haploinsufficiency results in craniofacial defects that mimic those in humans and that full functional loss amplifies the phenotypes.

The mouse models available for *Satb2* show some phenotypes seen in patients with SATB2 mutations, but there are limitations in how the mouse model recapitulates the clinical conditions in patients. There has been evidence of cognitive changes in the tissue specific knockout mice, however long-term modeling in a full knockout is not possible due to immediate post-natal lethality. Additionally, the complexity of the human brain is not easily modeled in the mouse, making the mouse a difficult species for studying the neurodevelopmental changes that occur in patients.

- Summary of SATB2 Models and findings:

- LacZ reporter line [13] → Created SATB2 deficient mice through the insertion of a LacZ reporter construct downstream of the first ATG codon in exon 2, leading to inactivation of the SATB2 gene
 - The Heterozygous mice were phenotypically normal
 - For *Satb2*-/- animals, there was no obvious morphological abnormalities during development until E12.5. After E11.5 the mutants can be distinguished by their shorter lower jaws—craniofacial defects in these animals include significant truncation of mandible, shortening of nasal/premaxillary and amaxillary bones, malformations of the hyoid bone and squamous bone, and cleft palate
- *Satb2* null [14]: generated through homologous recombination and elimination of the second exon in the protein coding region and replacement with Cre recombinase sequence and a Neo expression cassette
 - These mice did have a phenotype: cleft palate (in 25% of cases), microcephaly, reduced oral aperture, hypoplasia of the premaxillae and nasal capsules, lower jaw micrognathia, and variable incisor hypodontia and/or adontia due to *Satb2* haploinsufficiency
 - In another study using this line, they did not find any differences in the position and number of *Satb2*-expressing neurons between wildtype and heterozygous embryos indicating that the expression level of *Satb2* is not affected significantly in the *Satb2*+/- brains.
 - The homozygous *Satb2*-/- animals did not have a corpus callosum, not shocking due to the role of *Satb2* in corticocortical projections. However, they did have an anterior commissure and hippocampal commissure [17].
- Dr. Fish's findings with a conditional Cre/lox line (non-inducible):
 - Outside of bone defects, these heterozygous animals do not seem to have the other phenotypes.
 - The knockout animal may also be a hypomorph (less protein but not fully gone) not a full knockout, but homozygous has no corpus callosum.

Cellular models of SATB2:

Studies using SATB2 transduced iPSCs transplanted into mice on silk scaffolds demonstrated that SATB2 facilitates the differentiation of iPSCs toward osteoblast-lineage by repressing HoxA2 and enhancing osteoblast determinants such as Runx2, BSP, and OCN [27]. In dental implants in mice, two different retroviral gene delivery systems (PBABE- Satb2 virus or RCAS-Satb2 virus) were used to show that local administration of SATB2 to bone defects significantly accelerated new bone formation around implantation site of dental implants and enhanced the osteointegration of the implant [28]. Additionally, in transplantation of SATB2-overexpressing adult stem cells into mandibular bone defects, it was found that SATB2 significantly promoted adult stem cells to regenerate new bone tissue [16, 22]. These studies show that there is promise in SATB2 being part of bone tissue engineering or gene therapy for bone loss.

SATB2 Molecular Mechanisms in Disease

In both mice and humans, mutations in SATB2 lead to a high level of variation in symptomology. In mouse studies Satb2 has been shown to act in a dose-dependent manner in which heterozygous mice exhibit significant variation in phenotypes such as micrognathia and cleft palate. Humans also exhibit gene dosage effects, suspected haploinsufficiency, where a deficiency of functional SATB2 protein is suspected to be responsible for the clinical features seen in SAS. The exact mechanism of SATB2 point mutations is not fully understood, however the possibility of dominant negative effects have been described [29]. This theory was tested with truncated SATB2, and it was shown that there was interference with the repressive function of wild-type SATB2 [29]. **These findings suggests that the variation in phenotypic penetrance in patients may be a result of genetic background as well as differences in SATB2 function related to the specific genetic mechanism disrupting the specific SATB2 locus [30].**

Disease Overview

Age at onset

In general, some of the symptoms of the disorder (speech delay, dental and palate abnormalities, and motor deficiencies) are noticed before the second year of life, while SAS patients born with a cleft palate are generally diagnosed more quickly as SATB2 is now part of cleft palate genetic screening panels. But correct diagnosis can take much longer.

Initial Symptoms

Caregivers usually notice concerning developmental delays such as speech delays, motor dysfunction, and dental abnormalities before the age of 2. The presence of a cleft palate from birth does not always come with a SAS diagnosis since cleft palates are not often associated with other SAS symptoms such as neurodevelopmental delays. For children with cleft palate, that is the first indicator that something may be wrong; however, it is not always diagnosed as SATB2 related. From discussions Odylia had with parents, it seems like many parents notice some anomalies with even infants, but it is incredibly hard to determine if these are worrisome. Symptoms seen very early can include difficulty feeding, intolerance to pain, a calm demeanor, a lack of crying, and delayed or absent motor milestones (like picking up finger food at a few months old). As the toddler develops,

other abnormalities start to present that can look like missed milestones such as an inability to form words, gross motor delays, trouble sleeping, choking and gagging risks, and dental anomalies. Although parents report expressing concern to their pediatrician, most are initially advised that it is common, and the child will catch-up eventually. Anecdotally, three years or older seems to be when doctors start to agree with these concerns and provide referrals to see specialists.

Common clinical phenotypes associated with SATB2 mutation

SAS is a multisystem disorder characterized most significantly by neurodevelopmental delays with limited to absent speech, behavior issues, and craniofacial anomalies. All individuals present with developmental delay or intellectual disability, with speech delay being the most common phenotype reported across the SAS community. Affected children can also have hypotonia (decreased muscle tone) and feeding challenges in infancy. The behavioral issues can include autistic features, hyperactivity, and aggressiveness. Craniofacial abnormalities can include palate abnormalities, micrognathia, and abnormal shape or size of the upper central incisors. The less common features include skeletal abnormalities, growth deficiencies, strabismus/refractive errors, congenital heart defects, genitourinary anomalies, and epilepsy [31].

Symptom prevalence in SAS population:

- Developmental Delay and Intellectual disability- 100%
- Speech delay prevalence in population- 95%
 - 72% of patients are fully nonverbal
 - Predominately caused by [Speech apraxia](#): when a child's brain has difficulty coordinating the complex oral movements needed to create sounds into syllables, syllables into words, and words into phrases
 - Speech symptoms also come with feeding difficulties—difficulty chewing, over stuffing mouth, and excessive drooling
 - Current options for managing symptoms—speech therapy, target receptive vocabulary skills, teach alternate communication early
- Behavioral issues prevalence in population (80%): autistic tendencies, hyperactivity, sleep disturbances, aggressiveness, frustration likely significantly due to inability to communicate.
 - There is some evidence of a progressive nature of behavioral issues in two cases (20yo and 34yo)—both required antipsychotic drugs [32]. Progression is not seen in many of the other aspects though. The behavioral issue worsening as the child ages may be due to hormonal changes and/or frustrations that come with the inability to communicate how one feels in social and emotional situations
- Seizure prevalence in the SAS population- 20% of population has diagnosed seizures but there could be undiagnosed seizure-like brain activity in a higher number of patients
- Palatal anomalies prevalence in population 45%: cleft palate, bifid uvula, or high-arched palate.
- Dental anomalies prevalence in population 98%: prominent upper incisors, other anomalies
- Low bone density prevalence in population- 70%

Table 2 from Zarate and Fish, 2017 shows the percentage of patients reporting each clinical phenotype [31].

Table 2.

Summary of the Most Common Clinical Findings in 76 Individuals with *SATB2*-Associated Syndrome

Finding	% of Affected Individuals ¹
Developmental delay / intellectual disability	100%
Speech delay	95%
Craniofacial dysmorphism	89%
Dental anomalies	72%
Behavioral issues	55%
Cleft palate	50%
Abnormal brain MRI	49%
Micrognathia	42%
Hypotonia	42%
Feeding difficulties	39%
Growth restriction	34%
Skeletal anomalies	32%

1. Complete information was not available on some individuals.

Clinical Findings overview [30, 31, 33]:

- [Speech and Language deficits](#)
 - Speech delay is seen in almost all patients, and during our interviews was mentioned as one of the most impactful symptoms affecting patient and family quality of life.
 - Patients are normally treated with aggressive speech therapy and learn alternate communication techniques such as signing and device usage. Signing can become difficult or limited in scope due to fine motor issues.
 - Evidence of speech apraxia: A portion of the evidence of speech apraxia in SAS patients is from a study on speech, language, and feeding in *SATB2* patients [11]. For patients who had the ability for mimic sounds, the researchers administered the “*LinguiSystems Articulation Test (LAT) - Apraxia Screening Summary*” assessment. This test has been used in other disorders and shows that inconsistent repetition of multisyllabic words is a sign of Childhood Apraxia of Speech (CAS).
 - This study revealed a very low ability for repeating multisyllabic words in 8 participants who were primarily verbal—high indicative sign of CAS
 - Speech language pathologist evaluations note that in children with SAS there is a high percentage that exhibit hallmarks of CAS.
 - It should be noted that in order to assess if CAS is the cause of speech deficits, the patient has to have enough verbal ability to make an assessment—this is not always a possibility with non-verbal SAS patients

- Because it is sometimes difficult to diagnose with CAS, there is a recommendation by SLPs to treat the patient with therapies consistent with CAS regardless
- Speech apraxia: Speech apraxia was also discussed in the letter found [here](#), which said that essentially all children with SAS who had some verbal ability (enough to participate in screening) showed symptoms consistent with childhood apraxia of speech. Additionally, children with SAS have a higher risk for hypernasal resonance- sound that resonates in the nasal cavity during production of voiced, oral sounds.
- Auditory comprehension: The letter linked [here](#) from the satb2gene.com website describes findings on speech deficits seen in SAS patients. It describes findings on auditory comprehension as significantly impacted in children with SAS. In a measure of receptive vocabulary skills, most of the SAS patients scored in the “severely” delayed range. Most children only exhibited single word receptive vocabulary skills, similar to a neurotypical child younger than 3 years old. Receptive vocabulary did seem to improve as SAS children aged; however, the gap in SAS and typically developing children increases with age.
- Developmental delay/intellectual disability
 - All reported cases of SAS have some degree of intellectual disability and more than half experience severe developmental delay or intellectual disability with absent speech
 - For patients with a heterozygous pathogenic variant in SATB2 (not a large deletion) the mean age for walking is 20.9 months and first word is 19.8 months; some patients never achieve verbal communication
 - Developmental regression or cognitive decline has only been described once in an adult female with an 8.6-Mb deletion of 2q32.2-q33.1. This patient progressed from mild to severe intellectual disability and from poor to absent speech between the ages of 6 and 12 years. It should be noted that this large deletion spanned 22 known genes including GLS, MYO1B, TMEFF2, PGAP1, and SATB2. It is thought that while loss of one copy of SATB2 did contribute to behavioral issues, the deletion of multiple genes led to the complexity of these issues [34].
- Abnormal brain MRI and EEG
 - Findings [30, 32, 33] show approximately 50% of patients have abnormal findings
 - Study 1 [33]:
 - 9 patients underwent MRI, 6 of which had abnormal findings
 - 3 had delayed myelination for their age
 - 3 had non-progressive white matter abnormalities
 - 5 patients had EEGs performed to evaluate for seizures
 - Only 1 individual was diagnosed with clinical seizures (type: absence) and successfully treated with valproic acid

- Another patient had EEG abnormalities, benign epilepsy with central temporal spikes in sleep—did not receive antiepileptic treatment
- Study 2 [32]:
 - reports findings from 55 patient given MRIs
 - Mean age of 2.8 years (range 1-7)
 - Abnormalities were detected in 26/55 individuals (47%)
 - White matter abnormalities were the most common in almost 30% of patients
 - Delayed myelination and enlarged cerebral ventricles were also seen
 - Abnormal myelination for age and/or non-progressive white matter abnormalities appear to be common (26%) in those with nonsense, frameshift, and missense variants
- (c) **Brain imaging anomalies**

Anomaly	Percentage
Abnormal white matter	~30%
Delayed myelination	~22%
Ventriculomegaly	~8%
small corpus callosum	~8%
Cerebellar ectopia	~2%
- EEG preformed on 29 patients
 - 7 individuals showed clinical abnormalities including slow background, abnormal wakefulness, or epileptiform discharges; however, none were categorized as clinical seizures
- Study 3 [35]: retrospective review of 101 patients with SAS who had reported to have had an EEG; aimed at identifying abnormal EEG findings; 41 identified as having at least one prior abnormal EEG
 - **Showed considerable activation of epileptic like activity during sleep**
 - 38 of that 41 individuals (93%) had epileptiform discharges
 - 28 (74%) had central localization
 - Sleep stages were included in 31 individuals and epileptic activity was recorded during sleep for all of those individuals
 - 17 (42%) of individuals were shown to have definite clinical seizures with a mean age of onset at 3.2 years (four months to 6 years)
 - Focal seizures were a common type of seizure seen (42%)
 - 6 subjects needed polytherapy (multi drug therapy)
 - Delayed myelination and/or abnormal white matter was seen through neuroimaging in 19 individuals (61%)
 - Treatment Findings:
 - Levetiracetam was the most tried medication, in 15 individuals—not well tolerated and only 2 people stayed on it. Common

- reasons for stopping this treatment included aggression, constipation, hallucinations, and rash
- Next most common drugs tried: oxcarbazepine (8 patients), valproate (8 patients), lamotrigine (6 patients), and clobazam (5 patients. These were mostly well tolerated with most patients continuing with the medication.
- Craniofacial dysmorphism
 - Most individuals have minor facial dysmorphic features. Thin vermillion of the upper lip (20%) and long and smooth philtrum (17%) are the most common for those with pathogenic variants within SATB2 [30, 33].
 - In individuals with larger deletions, the most common features include prominent forehead or high anterior hairline (53%), thin vermillion of the upper lip (35%), low-set ears (29%), and long face (24%) [30].
 - Often see of facial features become more apparent over time [32].
- Behavioral issues
 - There is a broad spectrum of behavioral anomalies seen which include: jovial or friendly personality, autistic tendencies, agitation or aggressive outbursts, hyperactivity, difficulty falling to fall asleep or maintaining sleep, and sensory issues. Additional less common behavioral issues can include high pain tolerance, obsessive tendencies, skin picking, and anxiety
 - In Odylia's discussions with parents of older children, it seemed that behavioral issues were one of the top two symptoms that affected families the most, only second to verbal communication. Finding ways to cope with or alter behavioral issues is one of the top priorities for most families. There is concern that the behavioral issues will continue and as the child grows older it will be harder to manage, especially with normal growth and an increase in strength or size.
 - From conversations with Dr. Zarate it seems that the behavioral issues follow certain patterns of change over time. First, there seems to be a buildup of frustration as communication struggles progress. This likely leads to behavioral issues seen in some patients with SAS. Additionally, there is anecdotal evidence that behavioral issues tend to worsen near puberty—this may have to do with new and changing hormone levels but has yet to be proven. Of note, not all patients have behavioral issues. Given the potential association of SATB2 with Schizophrenia, there could be adult-onset behavioral issues that are worth exploring in more detail with a clinical psychiatrist or psychologist, especially as it pertains to identifying potential treatments for severe forms.
- Palate anomalies
 - Occur in 76% of patients and is likely partially to blame for feeding issues seen during infancy and beyond

- Dr. Fish hypothesizes that the feeding issues seen in some patients may be in part due to incorrect innervation of muscles (nerves to muscle connection), and that this may affect the ability to chew or manipulate food. During interviews, one parent commented that they didn't think their child could feel or sense food in the same way which led them to overstuff and have difficulty swallowing.
- Cleft palate seen in 50%, high-arched palate (23%), and bifid uvula (3%). Micrognathia (reduced lower jaw), diagnosed in 42%, has not required surgical correction.
- Dental anomalies
 - Most common finding is abnormal shape or size of the upper central incisors (36%); however other abnormalities can include crowding (36%), hypodontia (16%), delayed primary dentition (6%), and/or diastema (4%). Other issues seen include sialorrhea, malocclusion, and fused incisors [33]
- Skeletal anomalies
 - Variety of skeletal anomalies seen including: Pectus deformities, kyphosis/lordosis, scoliosis, tibial and femoral bowing, osteopenia
 - Osteopenia and osteoporosis: "While numbers are still very limited, this type of bone health abnormality appears to be particularly common in patients with point mutations." [30]
- Decreased sensitivity to pain
 - Although not often reported in the literature, most parents we spoke with reported an abnormally high pain tolerance in their children. This included things such as issues with proprioception, delayed reaction to injuries, decreased distress in infants when hungry, bladder control, and other high pain tolerance reactions.
- Other anomalies:
 - Hypotonia
 - Clinical seizures
 - Gait, ataxia, spasticity
 - Growth restrictions,
 - Strabismus/refractive errors
 - Genitourinary
 - Cardiovascular
 - Ectodermal findings—seem to be more prevalent in patients with large deletions but not with other mechanisms [30]
 - Metabolic Dysfunction [36]

Clinical Diagnosis

Although some children are diagnosed in the first two years of life, it can take much longer for other children to receive a SAS diagnosis. This can be due to many things such as the rareness of the disorder, the overlap in symptoms with other neurodevelopmental diseases, access to genetic testing, and the wide range of how symptoms present. Currently, there are no formal diagnostic criteria for SAS; however, significant efforts have been made Dr. Zarate and colleagues to define diagnostic criteria and design a clinical scoring system to assist with better diagnosis. Early diagnosis would increase access to impactful care and reduce the stress on the patient and family that comes with a missing diagnosis.

Symptoms that lead to seek diagnosis

As discussed in the first section, there are different symptoms that lead parents or caregivers to see specialists. In most cases, a delay in motor skills (including difficulty feeding, picking up objects, sitting, or walking), difficulty with speech or no verbal communication, and dental or palate anomalies raise concerns by parents but don't always prompt the physician to take action (i.e., referral to a specialist or genetic testing). Increased awareness in the clinical community would aid in early diagnosis. A SAS acronym has been developed to help with diagnosis of the disorder.

SATB2 acronym can be used to help with diagnosis

- S: Severe speech anomalies
- A: Abnormalities of the palate
- T: Teeth abnormalities
- B: Behavioral issues with or without Bone and Brain anomalies
- 2: onset before age 2

Symptoms that should lead physicians to test for a SATB2 mutation, deletion, or translocation include the following [31]:

- Significant neurodevelopmental disorders in all affected individuals
- Infantile hypotonia and feeding difficulties (relatively common)
- Subsequent developmental delay and severe speech delay (including, in some, absence of speech)
- Behavioral issues: autistic tendencies, hyperactivity, and aggressiveness
- Palatal anomalies: cleft palate, bifid uvula, and high-arched palate
- Dental anomalies: prominent upper incisors and other anomalies

Genetic Testing

Genetic confirmation of SATB2-associated syndrome is established by the detection of one of the following [31]:

- A heterozygous intragenic *SATB2* pathogenic variant (61%)
- A heterozygous deletion at chromosome 2q33.1 which includes *SATB2* (22%)

- An intragenic deletion or duplication of SATB2 (9%)
- A chromosome translocation with a chromosome 2q33.1 breakpoint that disrupts SATB2 (8%)

Genetic testing is the only way to confirm SATB2-associated syndrome. This process can take quite a long time though, with some patients not getting a final diagnosis until late childhood. Genetic testing approaches can include a combination of chromosomal microarray analysis (CMA), multigene panel, comprehensive genomic sequencing, and exome array. Targeted genetic testing requires that a physician determine which gene or genes are likely involved. Because phenotypes of a lot of inherited disorders with developmental delay/intellectual disability overlap, it can be difficult to correctly diagnose a child with SAS. Comprehensive genomic sequencing is likely the best option because of the nearly indistinguishable phenotypes of SAS and other developmental disorder. The following recommendations are to assist with finding the correct diagnosis.

Genetic testing recommendations [31]:

- First tier genetic testing: chromosomal microarray analysis (CMA)
 - CMA using oligonucleotide of SNP arrays is the first recommended test because deletions/duplications are identified in ~25% of patients. The table below describes in more detail. The ability to find the size of the deletion/duplication will depend on the type of microarray and density of probes in the 2q33.1 region
- Second tier genetic testing: if change is not detected with CMA testing then options include the following:
 - Multi gene panel that includes SATB2 and other genes of interest. *"Note: (1) The genes included in the panel and the diagnostic sensitivity of the testing used for each gene vary by laboratory and are likely to change over time. (2) Some multigene panels may include genes not associated with the condition discussed in this GeneReview; thus, clinicians need to determine which multigene panel is most likely to identify the genetic cause of the condition at the most reasonable cost while limiting identification of variants of uncertain significance and pathogenic variants in genes that do not explain the underlying phenotype. (3) In some laboratories, panel options may include a custom laboratory-designed panel and/or custom phenotype-focused exome analysis that includes genes specified by the clinician. (4) Methods used in a panel may include sequence analysis, deletion/duplication analysis, and/or other non-sequencing-based tests. For this disorder a multigene panel that also includes deletion/duplication analysis is recommended"*
- Comprehensive genomic testing, when available, including exome sequencing and genome sequencing can be considered
 - Exome array may be considered if exome sequencing is not diagnostic

Other testing options include karyotyping for translocations. Single-gene testing (sequence analysis of SATB2, followed by gene-targeted deletion/duplication analysis) is rarely useful and not recommended.

Table 1.Molecular Genetic Testing Used in *SATB2*-Associated Syndrome

Gene ¹	Method	Proportion of Probands with a Genetic Alteration ² Detectable by Method
<i>SATB2</i>	Sequence analysis ³	46/76 (61%) ⁴
	Gene-targeted <u>deletion/duplication analysis</u> ^{5,6}	See footnote 7.
	CMA ⁸	24/76 (31%) ⁹
	Karyotype (to detect structural variants)	6/76 (8%) ¹⁰

[31]

Genetic Counseling

Almost all cases of SAS are caused by de novo *SATB2* mutations, meaning it was not inherited from a parent. Two reported families have shown some evidence of parental mosaicism given the recurrence of SAS in siblings and failure to detect the mutation in parental blood samples. Once a SAS-causing mutation, deletion, or translocation affecting *SATB2* has been identified in a patient, prenatal testing for at-risk pregnancies and preimplantation genetic testing are possibilities [31]. If both parents have no mutation, then the likely cause is a de novo mutation and siblings are at low risk. However, since there have been reports of parental mosaicism that is not detectable on first test. This puts siblings at a slightly higher risk than others for the same mutation.

Genetic Panels Resource

The Genetic Testing Registry lists 60 tests that include *SATB2* in their screening. Collectively, panels screen for a range of genetic mutation types including deletions, duplications, mutation scanning of the entire coding region, targeted variant analysis, methylation, and sequence analysis of the entire coding region. Outside of the USA, at least 9 additional countries have screening capabilities and utilize panels that include *SATB2*. The following countries have registered *SATB2* containing genetic panels in the Genetic Testing Registry:

- Canada
- Chile
- Denmark
- Estonia
- Finland
- Germany
- Portugal
- Spain
- Turkey

SATB2 is included on panels that are run for the following symptoms, diseases, or phenotypes:

- Glass Syndrome
- Microdeletion/Microduplicaton Syndromes
- Cleft lip, cleft palate and related disorders
- Seizure and epilepsy
- Developmental delay
- Intellectual disability
- Autism spectrum disorder
- Congenital hypotonia
- Mental retardation
- Microcephaly
- Non-syndromic intellectual disability
- Rett and Angelman related disorders
- Ataxia
- Mitochondrial disease
- Hereditary disease

Diagnostic obstacles

Identifying diagnostic obstacles is important for understanding the full patient population, best treatment options, and potential bottlenecks to clinical trial enrollment should a therapy be developed. Correct diagnosis in early infancy can be particularly difficult when developmental delay, hypotonia, feeding difficulties, and palate issues are the only observable features.

Angelman syndrome and related disorders (like KGB syndrome) are often tested for in children with SAS [31]. Additionally, in a case study of 2 subjects, the patients presented with Rett Syndrome-like phenotypes and were only found to have SAS after genetic testing [37]. A correct SAS diagnosis is more likely once dental issues and distinctive behavioral issues are seen along with lack of speech progression [31].

Time to diagnosis: In large study the average age of diagnosis was 6.6 years, ranging from 1 week to 34 years old [32]. Due to the presence of cleft palate at birth, many patients are diagnosed earlier if the patient has access to genetic testing. Efforts should be placed on discovering ways to speed up time to genetic diagnosis. Early therapies and correct symptom management may be key to helping with a significant number of the disorder's phenotypes.

Specialists seen

Documenting the specialists seen by a patient can provide a clearer understanding of the patient journey, as well as the burden of care for the patient and family. For SATB2, a developmental pediatrician is highly recommended from diagnosis. This specialist can help with referrals, transitions to adulthood, and each step of the disorder.

Recommended specialists include the following. Some specialists will not be required by all patients, and should be considered based on symptoms and the needs of the patient and family

- Developmental pediatrician
- Neurologists
- Sleep specialist
- Speech therapist
- Craniofacial specialists
- Geneticist
- Cardiologist
- ENT
- GI specialist
- Endocrinologist
- Nephrologist
- OT and PT therapists

Recommended evaluations and referrals after initial diagnosis are shown in the following figure [31]:

Table 4.

Recommended Evaluations and Referrals Following Initial Diagnosis of *SATB2*-Associated Syndrome

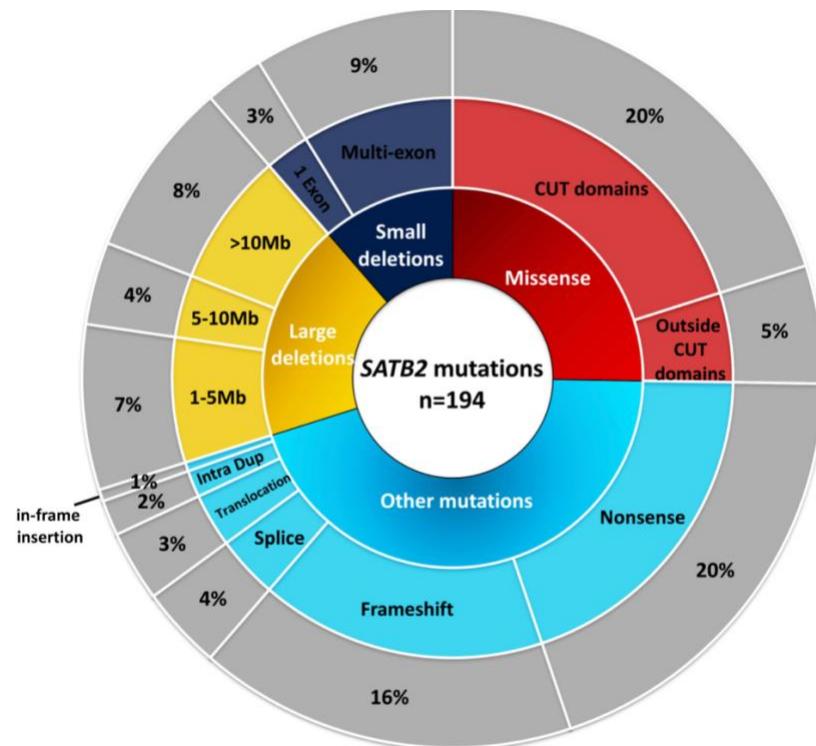
Organ System	Evaluation	Comment
Neurologic	Developmental	W/specific focus on nonverbal language ability
	Neuropsychological assessment	For behavioral problems
	EEG if seizures suspected	Referral to neurologist for seizure disorder management
	Consider head MRI if seizures present	
Oropharynx	Examination for palatal anomalies	Referral to craniofacial team or otolaryngologist as needed
	Dental, for abnormal tooth shape, number, & location	Referral to dentist
Gastrointestinal	Feeding	Consider videofluoroscopic swallowing study
	Growth (weight, length/height, growth velocity)	Consider referral to endocrinologist as needed
Musculoskeletal	Assessment for skeletal anomalies (e.g., scoliosis, kyphosis, tibial bowing)	Referral to orthopedist as needed
	Assessment for ↓ bone mineralization (e.g., recurrent fractures, ↑ alkaline phosphatase levels)	Consider bone mineral density scan
Genitourinary	For undescended testes, inguinal hernias, & hypospadias in males	
Eyes	Ophthalmology for strabismus & refractive errors	Incl visual acuity & dilated fundus examination
Cardiac	Consider echocardiogram	In those w/larger deletions incl <i>SATB2</i> & adjacent genes
Miscellaneous/ Other	Physical therapy	If hypotonia present
	Consultation w/clinical geneticist &/or genetic counselor	

[31]

Types of Mutations

There are many different types of mutations found in *SATB2* that are causal for SAS. The most commonly seen are single nucleotide variants that lead to a premature termination codon. Of the 194 reported *SATB2* mutations, missense mutations represent approximately 25% of all reported mutations--with 20% found in the CUT domains and 5% found in other parts of the gene. Other types of mutations, including nonsense, frameshift, splice, translocation, intragenic duplications, and in-frame insertions account for 46% of all mutations. Large insertions of varying sizes account for a total of 19% of mutations. Small deletions, within an exon, in the gene account for 3% of variants. Small, multi exon, deletions account for 9% of all variants. The

breakdown of all mutations reported can be found on the satb2gene.com website and in the figure below.



Mutation variants are present in every coding exon, from exon 3 to exon 12; however, the distribution of these variants is not uniform. Almost half of the pathogenic variants found are in exons 8 and 9, corresponding to portions of the CUT domains in the protein. When adjusting for exon size, exon 9 has significantly more variants within it. The figure below from satb2gene.com shows the distribution of mutations within the coding exons in addition to the type of mutation. A more detailed list of mutations can also be found on the website.

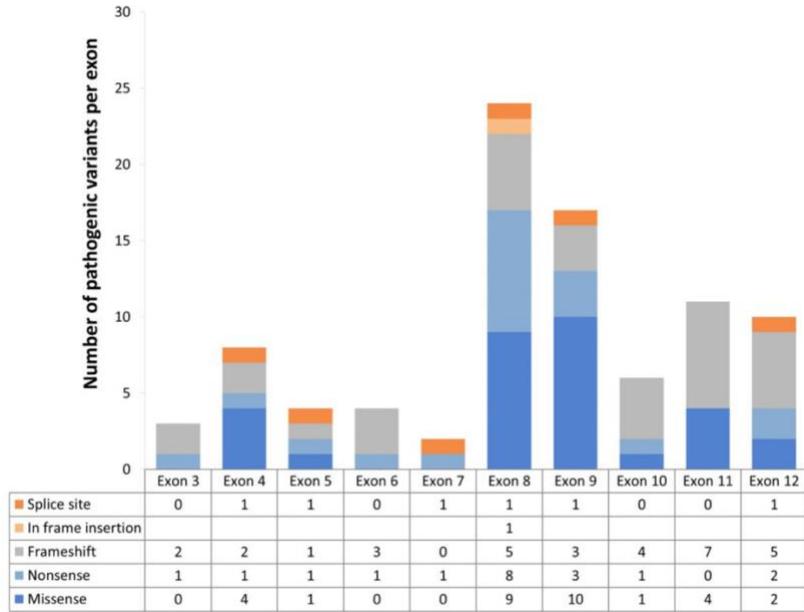
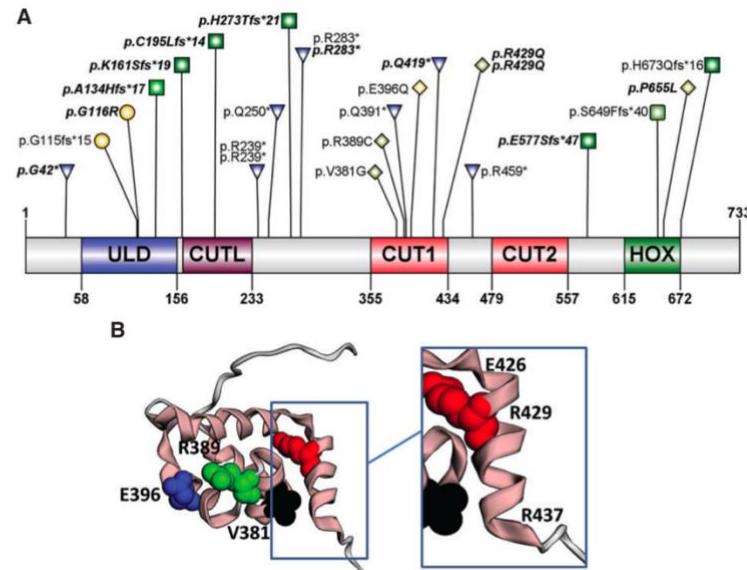


FIGURE 2 A, Schematic representation of SATB2 and its functional domains according to Pfam. Arabic numerals correspond to amino acid number. Diagrams were constructed using Illustrator for Biosequence (IBS1.0.1). Variants listed as black font were previously reported. Variants listed as italic bold font are from this report. Variants are represented with triangles (nonsense), circles (splice site), squares (frameshift) or diamonds (missense). Abbreviations: ULD, ubiquitin-like oligomerisation; CUTL, CUT1-like DNA binding; HOX: homeobox. B, Structural representation of the CUT1 DNA binding domain with the residues affected by missense variants. Previously reported variants include p. V381G (black), p.R389C (green), and p. E396Q (blue). The p.R429Q variant identified in 2 individuals in this cohort (red) is located in the α 5 helix. Starting and ending residues of the α 5 helix are also illustrated

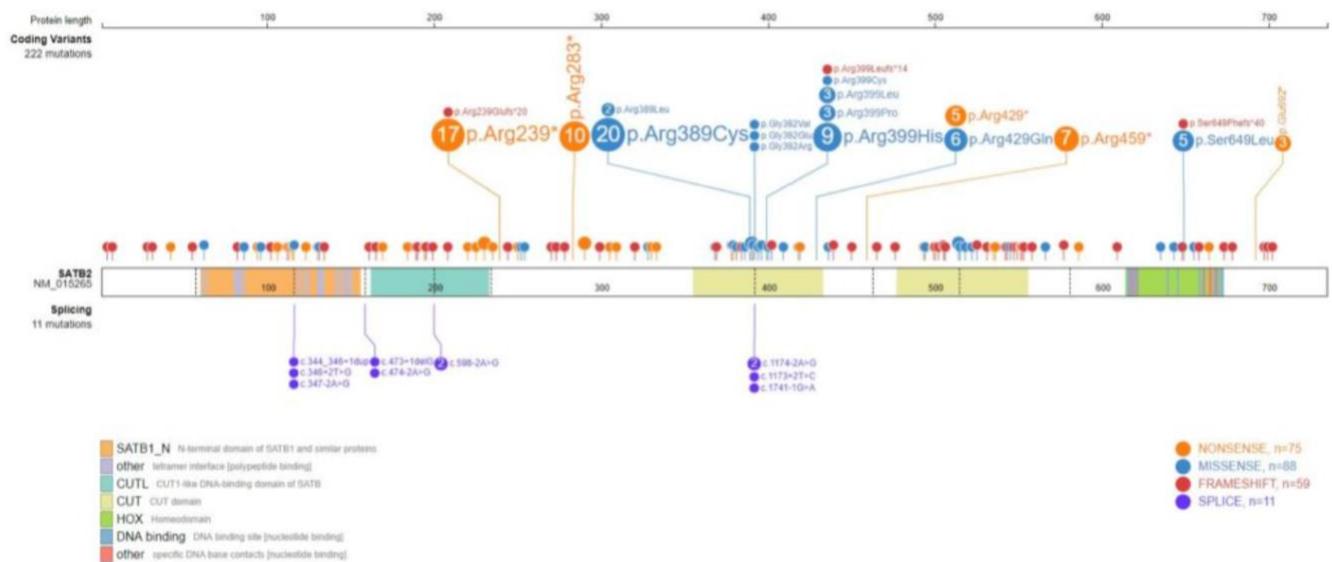


[33]

This figure shows the SATB2 protein domains and common mutations found in each domain. The two DNA binding CUT domains harbor a majority of the pathogenic variants found in the patient population [32]

Common mutations

The most up-to-date reports of genetic mutations for SATB2 are found on the satb2gene.com website. Below is an infographic from that website that illustrates where the most common mutations are found within the gene and protein. The most common mutation is seen with the blue bubble with a 20 in it and represents the c.1165C>T (p.Arg389Cys) variant and is found in the first CUT domain.



Genotype-Phenotype correlations and clinical population symptom heterogeneity

Genotype-Phenotype Correlations

As of now, few genotype-phenotype correlations for SATB2 variants have been formally established. Studies suggested that genitourinary anomalies, cardiac defects, and ectodermal changes are more common in individuals with large deletions involving SATB2 and adjacent genes. Individuals with large chromosomal deletions have also been generally diagnosed at earlier ages (mean of 2.5 years, $p \leq 0.0006$) and were more likely to have a history of growth retardation ($p=0.0033$) [32]. Additionally, patients with missense or disruptive pathogenic variants have been reported to have sialorrhea more often ($p=0.0115$) [32]. Of note, the number of reported individuals with SAS is still small, and more genotype-phenotype correlations may be discovered as more affected individuals are identified [31].

From conversations with Dr. Zarate, missense mutations occurring in the cut domains tend to have more severe phenotypes. There is thought that these types of mutations might lead to dominant negative effects, leading to worse phenotypes than a haploinsufficient model. It is also noted that these types of mutations sometimes lead to higher prevalence of seizures, likely due to the overall more severe phenotype seen. Nonsense mutation group has the lowest number of non-verbal individuals after 4 years of age and are less likely to have clinical seizures. Missense mutation group has the highest number of non-verbal individuals, are less likely to have cleft palate, and are more likely to have clinical seizures. And frameshift mutations tend to have the highest number of individuals with feeding difficulties (almost 97% of reported individuals).

Genotype-phenotype correlations in syndromes like SAS have been historically hard to predict and discover. The effects of modifier effects and environmental effects can lead to incredibly varied phenotypes even within a group with the same exact mutation. However, finding genotype-phenotype correlations can be very helpful for understanding the disease better and directing research efforts.

An extensive list of mutation type, location, and phenotype associated can be found [here](#).

Clinical Population Heterogeneity

The SAS clinical population has some phenotypes that are universal to the disorder including delayed or absent speech, intellectual disabilities, and dental abnormalities. Within these symptom categories though, there can be varied degrees of severity, or a spectrum of the disorder. Among other characteristics of the disease there is a high level of heterogeneity in the population, and each patient is a little different. Behavioral symptoms seem to be one area of high heterogeneity for the SAS community—some children have no behavioral issues while some can have a lot of issues. The behavior also seems to worsen over time, perhaps partially due to normal hormonal changes as children age into adolescence. Alternatively, studies of common genetic variation (also known as GWAS studies) have identified a potential link between genetic changes in SATB2 and psychiatric disorders such as Schizophrenia. It is possible that SATB2 also plays a role in behavioral regulation in young adults and that the SAS spectrum of symptoms may include behavioral challenges caused by lower levels of functional SATB2 in early adulthood.

Progression

Rate of decline

SAS is a neurodevelopmental disease and SATB2 is important for development of the craniofacial, dental, and central nervous system. Due to the necessity of SATB2 during development, many of the defining characteristics of the disease occur in early development, and tend to not worsen over time. SAS is a relatively static condition for most patients.

Although there are case studies and anecdotal accounts of worsening behavior or cognitive decline, this is not the normal progression for patients. A number of parents report that their children learned then lost words early on; however, for the most part the speech component to the disorder does not progress. Behavioral issues have been reported to worsen over time, but the cause of the worsening is not known.

Population

Incidence & Prevalence

Prevalence of a rare disease can be hard to determine, and there are likely more cases than the estimates provided here. Multiple families we spoke with mentioned their children were first diagnosed with Autism. The difficulty with diagnosis can lead to lower numbers of estimated population than there really is.

- SAS Prevalence:

- The prevalence of SAS is not known. However, two recent studies have estimated the frequency of SAS in large cohorts of individuals with undiagnosed intellectual disability/developmental delay at 0.24%-0.3% [32]
- It is estimated that there are over 200 cases of SAS worldwide

Ethnic or geographical enrichment

The is no known geographical or ethnic enrichment of SAS patients (higher than normal number of cases). This is expected given that SAS normally arises through de novo mutations which occur at an equally random chance throughout different ethnic groups

Current Management and Treatment Options

Treatment and management of symptoms is the current best practice for SAS patients. In early life this can include nutritional support for feeding concerns and management by a cleft/craniofacial team for those with palate anomalies. It is important to have early referral for developmental delay support and education [31]. Treatment right now is symptomatic and aims at ameliorating the major symptoms. There are currently no treatments for SAS. The table below lists symptom treatment options [31], and more detail is given on age specific recommendations in this section. It is important to note that each patient is unique, and treatment and care decisions should be made with that patient in mind. The recommendations in the following table are to be used as a guide of which specialists can help with certain disease aspects.

Table 5.Treatment of Manifestations in Individuals with *SATB2*-Associated Syndrome

Manifestation/Concern	Treatment	Considerations/Other
Developmental delay / intellectual disability	Early referral for developmental support / special education	See text following table
Dental anomalies	As per routine	
Cleft palate, bifid uvula, micrognathia	Management by cleft/craniofacial team; surgical correction of cleft palate	
Feeding difficulties	Nutritional support	Referral to gastroenterologist for those w/persistent issues
Sleep disturbance	Sleep hygiene healthy habits & potential medical management as needed	
Scoliosis, tibial bowing, joint contractures	Standard treatment per orthopedist	
Osteopenia	Treatment remains unclear	Denosumab used in 1 affected patient, pamidronate infusions used in 2 patients; long-term response to these treatments unknown [Boone et al 2016 , Zarate et al 2018]
Seizure disorder	Standard treatment per neurologist	
Undescended testes, inguinal hernia, hypospadias	Standard treatment per urologist	
Strabismus & refractive error	Standard treatment per ophthalmologist	
Congenital heart defects	Standard therapy per cardiologist	

[31]

Also important is surveillance of symptoms in patients, including evaluating nutritional status, growth, and developmental progress at every doctor visit. This should also include: monitoring by a neurologist for patients with epilepsy, annual sleep studies for those with sleep disturbances, evaluations for spine deformities or osteopenia, and routine checks by a dentist and ophthalmologist [31]. Table 6 below shows recommended surveillance for patients with SAS.

Table 6.Recommended Surveillance for Individuals with *SATB2*-Associated Syndrome

System/Concern	Evaluation	Frequency/Comment
Neurologic	Developmental assessments	Routine intervals to adjust therapies & adapt educational needs
	By neurologist	Per routine for individuals w/epilepsy
ENT/Mouth	Dentistry/orthodontics; audiology	Routine intervals
Growth	Evaluation of nutritional status & growth	At each visit
Respiratory	Sleep study (if history of sleep disturbance)	As needed
Musculoskeletal	Evaluate for scoliosis & spine deformities	At each visit
	Screening for osteopenia	
Eyes	Ophthalmology to screen for refractive errors & strabismus	Routine intervals

Management recommendations for developmental delay/intellectual disability:

2. **Age 0-3:** early intervention is highly recommended; referral to early access intervention programs allow access to occupational, physical, speech, and feeding therapists. The United States offers federally funded programs for this in all states.
3. **Age 3-5:** recommended public developmental pre-school. Evaluation made before placement to determine the services and therapies needed for each individual
4. **Age 5-21:** recommended that an individual education plan (IEP) is created based on the individual's level of function—this can be developed by the local public school district. Patients with SAS are permitted to remain in the public school district until the age of 21.
 - Transition plans that include financial planning, employment, and medical arrangements should start by 12 years—developmental pediatricians can assist with the transition to adulthood
5. **All ages:** consult with developmental pediatrician to ensure the correct involvement of different agencies to support the parents and maximize quality of life for the patient; can consider private support therapies based on needs
 - For US-based patients: Developmental Disabilities Administration (DDA) enrollment is recommended. Families with limited income may also qualify for supplemental security income (SSI) for their child

Management recommendations for motor dysfunction:

- **Gross motor dysfunction:** physical therapy to maximize mobility and reduce risk of late-onset orthopedic complications; consider use of medical equipment as needed such as wheelchairs, walkers, bath chairs, orthotics, and adaptive strollers
- **Fine motor dysfunction:** occupational therapy for difficulty with fine motor skills that can affect adaptive functions like feeding and dressing

- **Oral motor dysfunction:** if safe to eat by mouth, then recommended for affected patients who have a hard time feeding because of poor oral motor control—feeding therapy typically from an occupational or speech therapist
- **Communication Issues:** evaluation for alternative means of communication for patients with expressive language difficulties

[Management recommendations for social/behavioral concerns:](#)

- Developmental pediatrician can be helpful for giving guidance on the appropriate behavioral management strategies and/or medications. Some children may benefit from interventions used in autism spectrum disorder including applied behavioral analysis (ABA), which offers a more patient-specific approach.

[Alternate therapy approaches:](#)

From conversations with parents of SATB2 patients, we learned of a few alternate therapies that have been helpful. For younger children swimming lessons have been helpful for motor coordination and mood management. As the child ages continued swimming lessons, horseback therapy, gymnastics, and team sports like soccer have been well tolerated and enjoyed by SAS patients. These activities can help with concentration, a feeling of independence, and a means to express themselves, and an outlet for frustrations. Additionally, CBD oil and changes to diet have been used to help with SAS symptoms. CBD oil has been reported by parents to help with behavioral concerns, sleep issues, and seizure-like activity [35]. CBD derivatives have been used in other seizure, behavioral, or neurodevelopment disorders with success. Diet, specifically one low in carbohydrates and high in fats, is reported to decrease seizure activity and improve overall behavior, mood, and sleep [35].

[Patient Data](#)

Clinical researchers and patient families should be engaged to track natural history of the disorder in an effort to better characterize SAS and rates of change over time. If funding is not available, then a registry effort led by an investigator and/or direct to family questionnaires could be employed to better understand the disease, such as additional information added to the SAS patient registry.

Areas to consider tracking information

- Symptoms
- Medications taken
- Quality of Life (patient and family)
- Opt-in to future research and/or clinical trials
- Health economics
- Specialists seen

Registries

[SAS Patient Registry](#) is run by Dr. Zarate and colleagues at Arkansas Children's hospital. Information on how to become part of the registry is found in the linked website, and a PDF with instructions on registry enrollment can be found [here](#). It was founded in 2016 and has since enrolled 194 patients in a broad age range from 17 different countries. The registry aims to help identify problems caused by SAS and how these affect patients over time. It also serves as a unique resource for genotype-phenotype studies as it is an extensive list of mutations and associated symptoms.

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