Clinical Transcriptome Sequencing Confirms Activation of a Cryptic Splice Site in Suspected *SYNGAP1*-Related Disorder

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Established Facts

- Pathogenic variants in the brain-specific RasGAP *SYNGAP1* cause a relatively common neurodevelopmental disorder.
- The interpretation of novel variants identified through next generation sequencing technologies represents an increasing challenge for clinicians.

Novel Insights

- Intronic variation in *SYNGAP1* outside the canonical splice site may represent an underrecognized etiology of the related neurodevelopmental disorder.
- We demonstrate the utility of functional confirmation of intronic variants using clinically available transcriptome analysis.

Keywords

Intronic variation · RNA sequencing · *SYNGAP1* · Transcriptome · Variant interpretation

Abstract

SYNGAP1 encodes a brain-specific Ras GTPase activating protein (GAP) that regulates synaptic strength in glutamatergic neurons. Pathogenic variants in this gene are associated with a neurodevelopmental disorder characterized by intellectual and developmental disabilities, generalized epi-

lepsy, hypotonia, and autism spectrum disorders. We describe a young male with suspected *SYNGAP1*-related disorder given clinical overlap and identification of an intronic variant of uncertain significance; clinical transcriptome analysis demonstrated activation of a cryptic acceptor splice site resulting in frameshift and introduction of a stop codon. This report highlights the utility of functional studies newly available to clinical practice in confirming a suspected genetic diagnosis, which can directly impact medical management and preclude the need for additional diagnostic testing.

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Loss-of-function variants in *SYNGAP1* are emerging as one of the more common causes of genetic intellectual disability, with estimated frequencies of 0.7–1% in selected populations [Mignot et al., 2016]. As affected individuals are increasingly identified, a consistent syndromic presentation has been described. *SYNGAP1*-related disorder is characterized by variable presentations of intellectual and developmental disabilities, generalized epilepsy (with near universal EEG abnormalities), hypotonia, autism spectrum disorders, and maladaptive behaviors. Many affected individuals are ataxic with a wide-based and unsteady gait. Brain imaging is typically normal or reveals nonspecific findings. In some, seizure control is achieved with one or more antiepileptic drugs; in others, epilepsy remains pharmacoresistant [Parker et al., 2015; Mignot et al., 2016; Weldon et al., 2018].

The *SYNGAP1* gene encodes a brain-specific Ras-GAP that is abundant in the glutamatergic postsynaptic density, where it acts as a negative regulator of excitatory synapse strength [Araki et al., 2015]. In transgenic animal models, *Syngap1* is highly expressed at critical periods of synaptogenesis; expression is spatially restricted to the forebrain, with strong and persistent staining in the hippocampus [Porter et al., 2005]. *SYNGAP1* haploinsufficiency results in a hyperexcitable cortical state and subsequent imbalance of excitatory/inhibitory processes; this mechanism is hypothesized to account for neurologic phenotypes observed in humans and animal models [Clement et al., 2012, 2013; Ozkan et al., 2014; Zeng et al., 2017]. Additional perturbations to excitatory/inhibitory homeostasis include accelerated maturation of glutamatergic synapses [Clement et al., 2012, 2013], aberrant mitogen-activated protein kinase signaling leading to reduced surface expression of AMPA receptor [Rumbaugh et al., 2006], and disruption of cortical pyramidal neuron development with diminished dendritic spine dynamics and plasticity [Aceti et al., 2015]. In addition, recent evidence also implicates *SYNGAP1* haploinsufficiency in the formation and activity of GABAergic synapses [Berryer et al., 2016]. Heterozygote animals accurately model the behavioral features of *SYNGAP1*-related disorder and consistently demonstrate significant deficits in working and reference memory, hyperactivity, anxiolysis, altered social interactions, and generalized epileptiform discharges on EEG [Guo et al., 2009; Clement et al., 2012; Muhia et al., 2012; Ozkan et al., 2014; Berryer et al., 2016]. Importantly, *SYNGAP1*-related disorders appear to result from perturbations to a critical neurode-

velopmental time point, as conditional restoration in adulthood does not rescue behavioral phenotypes [Clement et al., 2012].

We have recently diagnosed a young boy with *SYNGAP1*-related disorder caused by an intronic variant of uncertain significance outside the canonical splice site; this variant was subsequently shown to be *de novo*. Transcriptome analysis was critical in establishing pathogenicity, demonstrating the utility of functional studies in clinical genomics. We also draw attention to other reports of pathogenic intronic variants in *SYNGAP1*, a relatively common etiology in this neurodevelopmental disorder.

Clinical Report and Results

We describe a 4-year-old male with mild global developmental delay and generalized epilepsy due to a *de novo* intronic variant in *SYNGAP1*. The patient first presented at 31 months of age, with his parents reporting mild delays in reaching language and motor milestones. He is described as sitting independently at 6 months, walking independently at 17 months, and using his first word at 12 months. At present, he speaks in short phrases that are often supplemented with gestures. Shortly after presentation, the child experienced an episode of full-body shaking and increased tone, which lasted approximately 4 s. In retrospect, the family recalled prior history of brief staring spells with behavioral interruption. Continuous video EEG identified brief generalized 3-Hz spike and slow waves with clinical correlate of behavioral arrest and staring as well as some instances of eye fluttering or eyes rolling upward. Of note, a brain MRI was read as normal. The seizure semiologies are best characterized as absence seizures with myoclonic and atonic components. He continues to have multiple brief seizures per day and appears to be treatment refractory as lamotrigine and the ketogenic diet did not reduce seizure frequency. The family has also trialed cannabidiol, which has not provided adequate seizure control.

A developmental and behavioral pediatric evaluation at 40 months was consistent with delays in language and motor skills, with borderline scores noted across all domains of the Vineland Adaptive Behavior Scales (communication, daily living skills, socialization, motor skills, adaptive behavior; scores between 3rd and 4th percentile). Although he did not meet criteria for a diagnosis of autism spectrum disorder per the ADOS-2, delays in social communication were documented. At 52 months of

Table 1. Clinical phenotypes reported with pathogenic *SYNGAP1* variants at intron 16/exon 17 junction

	Age at writing	Development	Epilepsy	EEG findings	Other	<i>SYNGAP1</i> variant
This report	4 years	Mild GDD	Onset at 31 months; frequency >1/h; LTG, CBD, and ketogenic diet ineffective; myoclonic and atonic absences	Ictal: generalized 3-Hz spike and polyspike and slow waves with occipital and frontocentral predominance Background: mild diffuse slowing	Developmental coordination disorder, behavioral difficulties, poor sleep	c.3583–9G>A (p.V1195Rfs*27), de novo
Personal communication with J. Propst	2.5 years	Moderate GDD	None	Normal overnight video EEG at 10 months	Wide-based gait, hand stereotypies, hypotonia, dysmorphic features	c.3583–6G>A (p.V1195Afs*27), de novo
Okazaki et al., 2017	4 years	Absent speech, severe GDD	Onset at 17 months, frequency >50/day, combination of ESX and LTG effective, childhood absence epilepsy, eyelid myoclonia with absences	Ictal: diffuse slow or spike-wave activity with occipital to central predominance Interictal: bilateral frontal spikes	Hypotonia, sialorrhea, clumsy gait	c.3583–6G>A (p.V1195Afs*27), inheritance unknown
Redin et al., 2014	NA	Moderate ID	NA	NA	NA	c.3583–6G>A (p.V1195Afs*27), de novo

CBD, cannabidiol; ESX, ethosuximide; GDD, global developmental delay; ID, intellectual disability; LTG, lamotrigine; NA, not commented on in report.

age, a FSIQ of 86 was documented, consistent with low average abilities; he performed better on verbal than non-verbal reasoning tasks, although there was not a significant discrepancy between scores. On neurologic exam, psychomotor slowing or encephalopathy with variable processing speed was observed, as were deficits in language production. The exam was also significant for an unsteady gait and difficulty with fine motor movements. Head circumference is normal.

An epilepsy gene panel identified a novel intronic variant of uncertain significance in the gene *SYNGAP1* (c.3583–9G>A). This sequence variant is predicted by the in silico model Human Splicing Finder to alter the wild-type acceptor splice site in intron 16 and was subsequently found to be de novo [Desmet et al., 2009]. Although this variant remained a strong candidate to explain the boy's presentation, the significance of an intronic variant outside of the canonical splice site remained unclear. Thus, transcriptome analysis was pursued through the CLIA- and CAP-certified MNG Laboratories to assist in establishing pathogenicity. A prior report of RT-PCR for characterization of a de novo *SYNGAP1* variant suggested the gene was adequately expressed in blood [Prchalova et al., 2017]. Further, sufficient expression was observed in an internal database and in the Human Protein Atlas (www.proteinatlas.org) [Uhlén et al., 2015]. RNA was isolated from whole blood collected with a Tempus™ Blood RNA

Collection tube (Thermo Fisher Scientific). Following ribosomal RNA depletion using Ribo-Zero Human (Illumina), cDNA libraries were generated using TruSeq Stranded Total RNA LT Sample Prep (Illumina). Reads were aligned by Hisat2 [Kim et al., 2015], followed by transcript reconstruction by StringTie [Pertea et al., 2015], resulting in greater than 50 million uniquely mappable reads. Aligned reads were manually inspected to assess splicing events between exons 16 and 19 of the *SYNGAP1* transcript. Five of 13 sequenced *SYNGAP1* transcripts were incorrectly spliced, resulting in frameshift and a predicted truncated protein product. The resulting amino acid sequence can be expressed as p.Val1195Argfs*27.

Discussion

Although genomic next generation sequencing technologies are widely used in modern medicine, transcriptome analysis has only recently become available clinically. In this report, a de novo candidate variant in *SYNGAP1* was identified outside the canonical splice site, with uncertain impact on the mature *SYNGAP1* transcript. Transcriptome analysis demonstrated activation of a cryptic acceptor splice site located 9 bp upstream of exon 17. As a result, 7 bp of intron 16 are included in the

transcript, disrupting the reading frame and leading to a prematurely truncated product. Interestingly, splice site variants impacting this junction at intron 16 and exon 17 have been previously reported [Redin et al., 2014; Okazaki et al., 2017]. Although our case represents a milder clinical presentation, all those described have a neurodevelopmental phenotype consistent with a *SYNGAP1*-related disorder (Table 1). Reported features in these individuals are variable and include moderate-to-severe developmental and intellectual disabilities as well as infantile-onset generalized epilepsy [Redin et al., 2014; Ozkan et al., 2017]. We could speculate that perhaps in our patient, the cryptic acceptor site is inconsistently used and that wild-type mature transcript is generated, but at lower efficiencies. Although both wild-type and mutant sequences were identified at near equal fractions in blood, the ratio in brain tissue where *SYNGAP1* is more abundantly expressed may be different. The post-transcriptional modification landscape in the central nervous system is complex, with established neuron-specific alternative splicing events and unique isoforms of transcriptional machinery [Porter et al., 2018]. We expect the identified splice variant to alter expression of *SYNGAP1* in the central nervous system; however, the outcome cannot be precisely inferred from what is observed in blood.

Although the presumed impact of an intronic variant is protein truncation through reading frameshift, the application of this dogma to variants outside the canonical splice sites is less certain. This report illustrates the utility of clinical transcriptome sequencing, particularly in the interpretation of candidate variants of uncertain significance in proximity of intron-exon junctions. Although limited to those genes that are constitutively expressed in an accessible tissue, transcriptome analysis represents a streamlined and accessible opportunity to assess the functional consequences of DNA variation. The potential impact cannot be understated; confirming a presumed diagnosis can directly influence medical management and family planning, and eliminates the need for additional diagnostic work-up.

Statement of Ethics

This case report was produced in accordance with institutional policies; informed consent was obtained from the family for all clinical studies and for publication.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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