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SON-Related Zhu-Tokita-Takenouchi-Kim Syndrome With Recurrent Hemiplegic Migraine

Putative Role of *PRRT2*

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Abstract

Background and Objectives

Zhu-Tokita-Takenouchi-Kim (ZTTK) syndrome (OMIM 617140) is a recently identified neurodevelopmental disorder caused by heterozygous loss-of-function (LoF) variants in *SON*. Because the SON protein functions as an RNA-splicing regulator, it has been shown that some clinical features of ZTTK syndrome can be attributed to abnormal RNA splicing. Several neurologic features have been observed in patients with ZTTK syndrome, including seizure/epilepsy and other EEG abnormalities. However, a relationship between *SON* LoF in ZTTK syndrome and hemiplegic migraine remains unknown.

Methods

We identified a patient with a pathogenic variant in *SON* who shows typical clinical features of ZTTK syndrome and experienced recurrent episodes of hemiplegic migraine. To define clinical features, brain MRI and EEG during and after episodes of hemiplegic migraine were characterized. To identify molecular mechanisms for this clinical presentation, we investigated the impact of small interfering RNA (siRNA)-mediated *SON* knockdown on mRNA expression of the *CACNA1A*, *ATP1A2*, *SCN1A*, and *PRRT2* genes, known to be associated with hemiplegic migraine, by quantitative RT-PCR. Pre-mRNA splicing of *PRRT2* on *SON* knockdown was further examined by RT-PCR using primers targeting specific exons.

Results

Recurrent episodes of hemiplegic migraine in our patient typically followed modest closed head injuries, and recurrent seizures occurred during the most severe of these episodes. Transient hemispheric cortical interstitial edema and asymmetric EEG slowing were identified during episodes. Our siRNA experiments revealed that *SON* knockdown significantly reduces *PRRT2* mRNA levels in U87MG and SH-SY5Y cell lines, although a reduction in *CACNA1A*, *ATP1A2*, and *SCN1A* mRNA expression was not observed. We further identified that *SON* knockdown leads to failure in intron 2 removal from *PRRT2* pre-mRNA, resulting in a premature termination codon that blocks the generation of functionally intact full-length PRRT2.

Discussion

This report identifies recurrent hemiplegic migraine as a novel clinical manifestation of ZTTK syndrome, further characterizes this clinical feature, and provides evidence for downregulation of *PRRT2* caused by *SON* LoF as a mechanism causing hemiplegic migraine. Examination of the *SON* gene may be indicated in individuals with recurrent hemiplegic migraine.

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Glossary

PRRT2 = proline-rich transmembrane domain protein 2; **PTC** = premature termination codon; **RT-qPCR** = real-time quantitative PCR; **ZTTK** = Zhu-Tokita-Takenouchi-Kim.

Hemiplegic migraine represents a relatively uncommon manifestation of migraine headaches typically presenting with aura, motor weakness, sensory disturbances, seizures, and loss of consciousness.[1] Extensive evidence indicates that genetic factors play critical roles in the pathophysiology of this disorder.[1-3] Specifically, recent advances in genomic sequencing have provided insight into genetic causes of familial and sporadic hemiplegic migraine.[1-3] To date, pathogenic variants in 3 genes, *SCN1A*, *ATP1A2*, and *CACNA1A*, have been identified as major causes of familial hemiplegic migraine,[1-3] with preliminary evidence supporting the role of an additional gene, proline-rich transmembrane domain protein 2 (*PRRT2*).[4-8]

The *SON* gene, located on human chromosome 21, encodes a large nuclear protein with dual abilities to interact with both DNA and RNA. The SON protein functions as an RNA-splicing regulator[9] and a transcriptional repressor.[10] Recent studies have shown that pathogenic variants of *SON* are associated with a clinical syndrome (Zhu-Tokita-Takenouchi-Kim [ZTTK] syndrome or SON-related syndrome; OMIM 617140) consisting of intrauterine growth restriction, congenital malformation including brain anomalies, hypertonia/hypotonia, intellectual disability, and epilepsy and/or other EEG abnormalities.[11-16] However, a relation between *SON* loss-of-function (LoF) variants and hemiplegic migraines has only been recently reported in a single case with no mechanistic insight.[17]

Here, we describe a boy with a de novo LoF variant in the *SON* gene presenting with hemiplegic migraine in the setting of other clinical features consistent with ZTTK syndrome. Furthermore, we demonstrate that reduction of SON leads to incomplete intron removal from the *PRRT2* transcript, thereby decreasing *PRRT2* mRNA. Our findings on impaired splicing of *PRRT2* on *SON* knockdown support the hypothesis that SON dysfunction may represent another genetic condition predisposing to hemiplegic migraine.

Methods

Informed Consent for Using Clinical Data

The child's parents provided written informed consent permitting the publication of their child's case along with the full-face photographs shown in Figure 1.

Cell Culture and siRNA Transfection

Human-derived glioma cell line, U87MG, and human-derived neuroblastoma cell line, SH-SY5Y, were cultured in Dulbecco's modified Eagle's medium with 10% fetal bovine serum, penicillin/streptomycin, and L-glutamine. We used the Silencer Select siRNA (Life Technologies, Carlsbad, CA) directed against human *SON* (GCAUUUGGCCCAUCUGAGAtt) and a negative control siRNA (UAACGACGCGACGACGUAAtt). Cells were transfected using Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA) with 80–240 pmol of siRNA according to the manufacturer's instructions.

RNA Extraction, Reverse Transcription, PCR, and RT-qPCR

Total RNA was extracted from cell lines using RNeasy Mini Kits (Qiagen, Valencia, CA), and contaminating genomic DNA was removed with RNase-Free DNase (Qiagen). RNA was reverse transcribed using SuperScript III RT (Life Technologies, Carlsbad, CA). PCRs were performed in the following cycle conditions: 95°C for 3 minutes; followed by 30 cycles of 95°C for 30 seconds, 55°C for 40 seconds, and 72°C for 1 minute; and final extension of 3 minutes at 72°C. To detect splicing efficiency, the final PCR products were electrophoresed on 2% agarose gels. All real-time quantitative PCRs (RT-qPCRs) were performed using the CFX Connect real-time PCR system (Bio-Rad, Hercules, CA), and amplifications were performed using the iTaq Universal SYBR Green Supermix (Bio-Rad, Hercules, CA). RT-qPCR was performed under the following conditions: 1 cycle of denaturing at 95°C for 3 minutes, followed by 40 cycles at 95°C for 10 seconds and 60°C for 30 seconds. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or Tyrosine 3-Monooxygenase/Tryptophan 5-Monooxygenase Activation Protein Zeta (YWHAZ; a.k.a. 14-3-3-Zeta) was used as the internal control for normalization for qPCR data analysis. Relative quantification of gene expression was calculated according to the $2^{-\Delta\Delta Ct}$ method. All primer sequences are listed in Tables 1 and 2.

Statistics

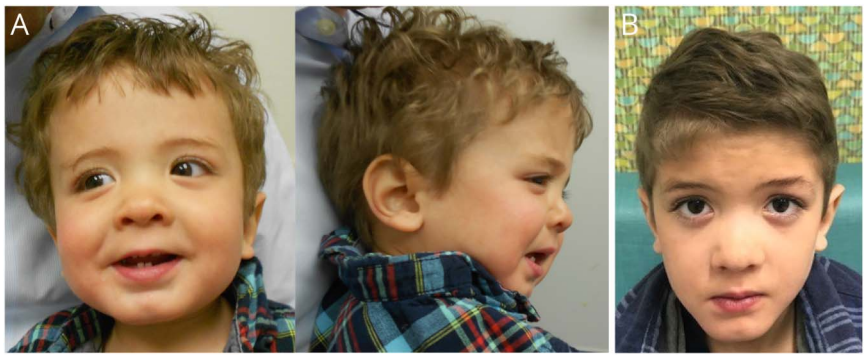
For RT-qPCR, most assays were performed in triplicate and independently repeated 3 times. Experimental data were plotted as mean values with SD using the Student *t* test.

Results

Case Report

This boy was the third pregnancy of healthy nonconsanguineous parents. Ultrasound at 20 weeks showed evidence of intrauterine growth restriction, with a length and weight of <2nd percentile. A normal head circumference was noted. He was delivered by cesarean at 39 weeks of gestation because of fetal decelerations. Birth weight was 2,230 g and length of 46 cm. He had thrombocytopenia. There are no first-degree relatives with developmental or congenital disorders. Numerous family members have had migraine headaches, and a paternal uncle has Joubert syndrome.

Figure 1 Photographs Showing the Facial Features of the Patient



(A) The propositus at 2 years of age. Note the prominent forehead, depressed nasal bridge, and down-slanted palpebral fissures. (B) The boy at 9 years.

In infancy, he experienced failure to thrive, respiratory syncytial virus infection, chordee repair at 6 months of age, chronic otitis media, and significant psychomotor delay. At age 6 months, he was unable to sit, and head lag was noted. Genetic consultation at 10 months of age identified weight and height below the fifth percentiles and an occipito-frontal circumference of 44 cm (fifth percentile). Distinctive findings included prominent forehead, ridging of the sutures, mild dolichocephaly, down-slanted palpebral fissures, depressed nasal bridge, bifid uvula, micrognathia, joint hyperextensibility, and hypotonia (Figure 1). Head CT demonstrated sagittal suture synostosis; surgical intervention was considered unnecessary. Initial brain MRI at 21 months of age showed mild ventriculomegaly with extensive subependymal gray matter heterotopia along the temporal and occipital horns of the lateral ventricles (Figure 2A). At the same age, formal assessment indicated that he was 4–5 months delayed in fine and gross motor skills. He experienced no developmental regression. At 2 years 8 months, he was able to put 2 words together and had a vocabulary of approximately 50 words.

At 2 years 11 months, he experienced the first of many episodes of transient altered mental status after minor closed head injury. These episodes were of variable severity and duration but consisted primarily of lethargy, encephalopathy, headache, and often transient right hemiparesis. Five of these episodes prompted emergency department visits, some with overnight observation. For most of these episodes, he was treated with a migraine cocktail consisting of diphenhydramine, an antiemetic, and a nonsteroidal anti-inflammatory agent (ibuprofen or ketorolac). With or without such treatment, most episodes were followed by profound sleep for a few hours, followed by resolution of symptoms within hours and return to baseline functioning by the next day. Brain CT and CT angiography during one such episode showed no new focal brain abnormalities or evidence of hemorrhage to suggest recent stroke. A hypoplastic left anterior cerebral artery A1 segment was demonstrated, with compensatory prominence of the right common carotid, internal carotid, and A1 segment of the anterior communicating artery. In addition, EEGs obtained during these episodes demonstrated persistent background asymmetry with high-amplitude delta predominant slowing over the left hemisphere (Figure 3, A and B).

One episode, occurring at 7 years of age, was more severe. In this instance, symptoms followed a suspected, but unwitnessed, head injury but failed to resolve with the usual intervention. The encephalopathy and right hemiparesis persisted, requiring hospitalization. Five days into this protracted episode, he developed hypoxemia and a partial seizure affecting the right side of his face and body progressing to status epilepticus. Status epilepticus resolved after treatment with lorazepam and fosphenytoin; however, the child required intubation and transfer to the intensive care unit. Brain MRI demonstrated extensive T2 signal abnormality in the left hemisphere consistent with interstitial edema (Figure 2, B and C), and EEG demonstrated asymmetric slowing similar to that noted in prior evaluations (Figure 3B). Shortly after the episode of status, periods of very regular delta activity appeared, possibly consistent with unusual ictal activity (Figure 3C). Treatment with lacosamide was instituted, and his encephalopathy and hemiparesis gradually resolved within 3 days. He has remained on lacosamide since that episode. Subsequent imaging 4 months later demonstrated full resolution of the left hemisphere edema (Figure 2D), whereas subtle asymmetry of EEG activity remained apparent 2 months after recovery (Figure 3D).

The last of these episodes occurred at 8.5 years of age and resolved within hours with sleep and the combination of medications described above.

Table 1 PCR Primer Sequences

PCR primers	Forward (5′-3′)	Reverse (5′-3′)
PRRT2 (exon 1–2)	CGGGAGCTGTCCGGAGG	CTTGGGACTCTCCTCAACCC
PRRT2 (exon 2–3)	ACCCAGAAACCTCGGGACTA	AGCGCCACGATGCTTAAGAG
PRRT2 (exon 3–4)	GCCGGGTAGCCAAGCTCTTA	GCAGAGCCCCTCACTTATACA

Table 2 qPCR Primer Sequences

qPCR primers	Forward (5′-3′)	Reverse (5′-3′)
PRRT2 (exon 2)	ATCGAATGAGAAGGGCACAC	GGATGGCAAGGATGATGTAGTC
GAPDH	GGCGCTGAGTACGTCGTGGAGTCCA	AAAGTTGTCATGGATGACCTTGG
YWHAZ	ACTTTTGGTACATTGTGGCTTCAA	CCGCCAGGACAAACCAGTAT
SCN1A	TCCGAGCATTGAAGACGATTT	GACAGAACACAGTCAGGATCATT
ATP1A2	TCCATCCTGCTGTGGATTG	CAGCACCACACCCAGATATAG
CACNA1A	ACAGTTGGGACGGAGTTTG	CGACTTCAGGACGACTTGTAA A

Abbreviations: ATP1A2 = ATPase Na+/K+ transporting subunit alpha 2; CACNA1A = calcium voltage-gated channel subunit alpha1 A; GAPDH = glyceraldehyde-3-phosphate dehydrogenase. SCN1A = sodium voltage-gated channel alpha subunit 1; YWHAZ = Tyrosine 3-Monooxygenase/Tryptophan 5-Monooxygenase Activation Protein Zeta.

Genomic analysis included normal cytogenomic microarray. Initial clinical exome sequencing consisting of a trio of proband and both parents and performed in a CLIA-accredited laboratory failed to identify a relevant pathogenic variant. However, reanalysis through the University of Utah's Penelope Undiagnosed Disease Program research pipeline identified a de novo frameshift variant in *SON* (NM_138927.4:c.1487del; NP_620305.3: p.Ala496GlyfsTer2) predicted to result in LoF of the SON protein. This variant is absent from gnomAD or the Exome Aggregation Consortium databases and has a pL1 score of 1.0. De novo frameshift variants in *SON* have previously been shown to cause nonsense-mediated mRNA decay, resulting in reduction of *SON* mRNA and SON protein in patients with ZTTK syndrome.[11,18] Although this novel variant (c.1487del) in *SON* has not been previously reported in association with ZTTK syndrome, it is predicted to result in SON LoF through this same mechanism. Thus, in the setting of typical clinical features of ZTTK syndrome, it is the likely cause of this child's clinical condition. Finally, neither the initial nor the subsequent exome sequencing identified any rare sequence variants or potential pathogenic variants in *PRRT2*, *ATP1A2*, *SCN1A*, and *CACNA1A* genes.

Molecular Study

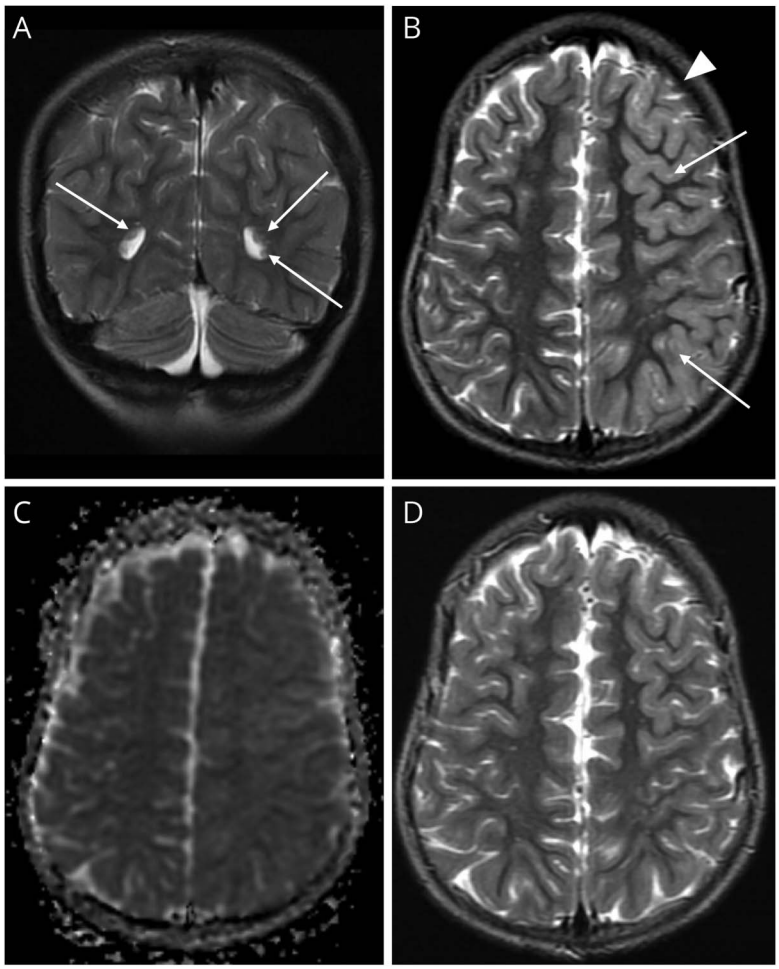
Although heterozygous LoF variants in *SON* lead to down-regulation of a set of genes associated with brain development and metabolism in individuals with ZTTK syndrome,[11] how *SON* LoF predisposes hemiplegic migraines is unknown. To identify whether *SON* LoF could be a causative factor of hemiplegic migraines, we first examined how *SON* LoF affects the mRNA expression of the *CACNA1A*, *ATP1A2*, *SCN1A*, and *PRRT2* genes, which are known to be associated with hemiplegic migraines.[2-8] We used U87MG and SH-SY5Y cells, which are a human-derived glioma cell line and a neuroblastoma cell line, respectively, because these are brain-originated cell lines with relatively high levels of *PRRT2* expression (proteinatlas.org/). On *SON* knockdown induced by small interfering RNA (siRNA), the relative mRNA expression of *CACNA1A* and *ATP1A2* remained unchanged (Figure 4A). *SCN1A* mRNA expression was increased on *SON* knockdown, indicating that SON potentially has a repressive effect on *SCN1A*. Of interest, we found that the *PRRT2* mRNA level was significantly decreased on *SON* knockdown in U87MG cells (Figure 4A), which was also reproducible in SH-SY5Y cells (Figure 4B).

The *PRRT2* gene located on human chromosome 16 contains 4 exons encoding a transmembrane protein. To date, there are 2 different models for PRRT2 topology in the literature; one model predicts that the long N-terminal portion is an intracellular domain,[19,20] whereas the other model predicts the N-terminal portion as an extracellular domain.[21,22] A recent study further characterized the PRRT2 protein topology using TMHMM (TransMembrane prediction using Hidden Markov Models), a transmembrane helix prediction program,[23] which supports the model of extracellular localization of the N-terminal region. Therefore, in this report, we adapted the gene/protein structure and the membrane topology of PRRT2 with a long N-terminal extracellular domain, 2 transmembrane domains (M1 and M2) separated by an intracellular (cytoplasmic) domain, and a short C-terminal extracellular domain (Figure 5, A and B).

So far, 3 different *PRRT2* transcript variants have been documented as potential protein-coding transcripts (Figure 5C); transcript variant 1 (NCBI Reference Sequence NM_145239; ENSEMBL ID ENST00000358758), transcript variant 2 (NM_001256442; ENST00000567659), and transcript variant 3 (NM_001256443; ENST00000300797). Although transcript variant 1 is generated by removal of all 3 introns, transcript variant 2 retains intron 3 as a coding sequence, resulting in additional 54 amino acids in the C-terminal short extracellular domain. Transcript variant 3 retains both intron 2 and intron 3, and this transcript is predicted to encode a truncated form of PRRT2 because intron 2 harbors a premature termination codon (PTC) (Figure 5C).

To further understand the underlying molecular mechanisms of SON regulation of *PRRT2*, we analyzed pre-mRNA splicing of *PRRT2* on *SON* knockdown. The locations of designed primers to detect different splice variants are shown in Figure 5C with a schematic of the *PRRT2* gene exons and introns. From our PCR-based splicing analysis, we found that

Figure 2 Imaging Features of the Patient With the *SON* Variant c.1487del and Hemiplegic Migraine



(A) T2-weighted image demonstrating periventricular nodular heterotopia. (B) T2-weighted image demonstrating extensive subtle T2 signal hyperintensity and edema is apparent throughout the cerebrocortical mantle of the left hemisphere (thin arrows). Note the effacement of sulci on the left, as evidenced by diminished subarachnoid fluid (arrowhead). (C) Apparent diffusion coefficient map obtained on the same day as (B), demonstrating a relative lack of corresponding diffusion restriction. (D) T2-weighted image obtained 4 months after hemiplegic migraine attack showing resolution of previous abnormalities.

intron 2 is removed in control conditions, whereas intron 3 is predominantly retained in both U87MG and SH-SY5Y cell lines, suggesting that transcript variant 2, with additional 54 amino acids at the C terminus, is the major form expressed in these cell lines. Of interest, we found that knockdown of *SON* significantly increased intron 2 retention in these cell lines (Figure 5D), suggesting that transcript variant 3–like form is increased. The potential protein translated from this intron 2–retained transcript lacks the cytoplasmic domain, the entire M2 transmembrane domain, and the C-terminal extracellular domain, indicating that intron 2 retention is detrimental to producing functional PRRT2. If this transcript bearing a PTC is recognized by nonsense-mediated mRNA decay machinery during translation, it will decrease the total *PRRT2* mRNA level, which we detected by qPCR (Figure 4). Taken together, our data indicate that reduction of SON in cells leads to decreased *PRRT2* mRNA expression and the failure of intron 2 removal from the *PRRT2* pre-mRNA, resulting in a decrease of functionally intact PRRT2.

Data Availability

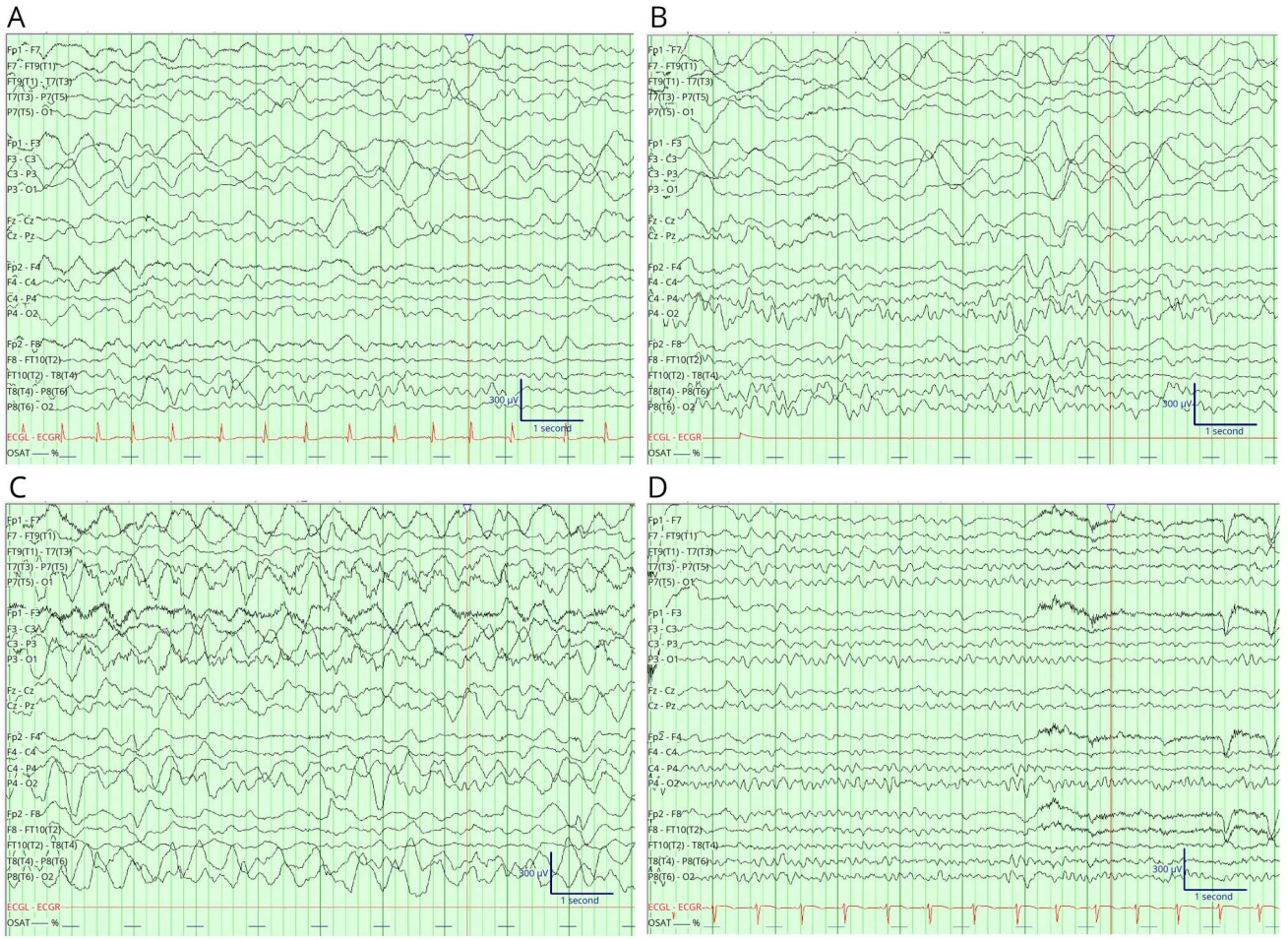
Data regarding the patient are not publicly available because of confidentiality pertaining to private health information. The data supporting the findings of the molecular study are available from the corresponding author (E.-Y.E.A.), on reasonable request.

Discussion

In this report, we describe a de novo frameshift variant in *SON* (NM_138927.4:c.1487del; NP_620305.3:p.Ala496GlyfsTer2) in a boy with clinical findings consistent with ZTTK syndrome or an *SON*-related disorder. His phenotype, including somatic features, clinical history, psychomotor delay, and imaging findings, is all consistent with typical findings of most persons with ZTTK syndrome.[11-14,16,24]

However, although our patient demonstrates characteristic features of ZTTK syndrome, he also presents a unique additional clinical feature that may represent a phenotypic expansion. Specifically, he has experienced repeated episodes of transient altered mental status with associated clinical and imaging features consistent with hemiplegic migraine. Familial and sporadic hemiplegic migraines are characterized by transient stroke-like episodes

Figure 3 EEG Features



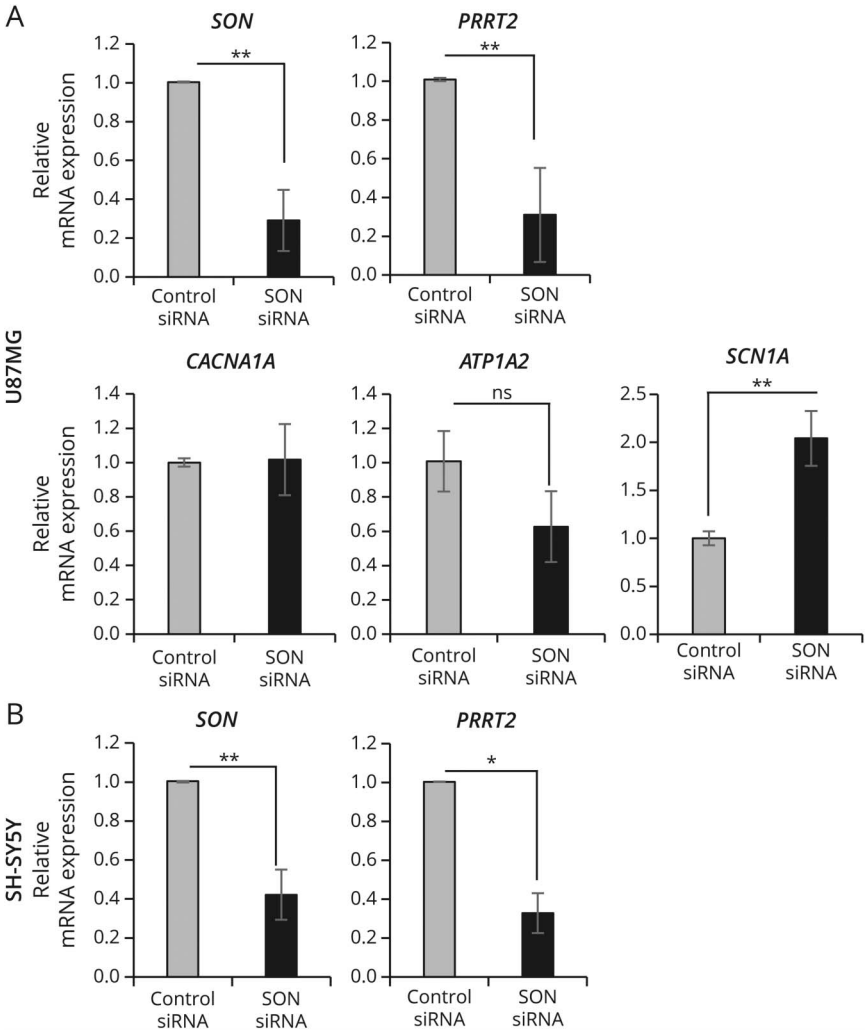
(A) Left hemisphere slowing is apparent during a typical episode of hemiplegic migraine at 6 years of age. (B) EEG during severe episode of hemiplegic migraine at 7 years of age obtained early in the course, before the episode of status epilepticus. Similar left hemisphere slowing is apparent. (C) A few days later, after resolution of status epilepticus. Unusual rhythmic delta activity is apparent diffusely, with sharply contoured fast activity over the left posterior hemisphere. The patient had altered responsiveness at this time. (D) 2.5 months after the episode of status epilepticus. Only subtle asymmetry persists.

involving aura, motor weakness, sensory disturbances, seizures, and loss of consciousness.[1,25,26] These attacks are generally reversible and last anywhere from hours to days often with complete resolution of symptoms.[1,26] Attacks are often associated with minor or trivial head traumas serving as a trigger for onset.[1,25-27] MRI studies obtained during hemiplegic migraines may show cortical edema.[1,25-27] EEG obtained during hemiplegic migraine attacks demonstrates polymorphic slow waveforms—typically over the hemisphere contralateral to the side with focal motor weakness—which can persist for up to several weeks.[1,26-28] With both MRI and EEG studies, complete resolution of acute findings (edema on MRI and slow waves on EEG) is typical once the migraine attack fully subsides.[1,2,25-28] The pathogenesis of familial hemiplegic migraine has been linked to multiple genes, including *SCN1A*, *ATP1A2*, *CACNA1A*, and *PPRT2*. Pathogenic variants in these genes have also been identified in some sporadic cases of hemiplegic migraine.[2,3,6]

Our patient's recurrent episodes of altered mental status are consistent with sporadic hemiplegic migraine in the setting of phenotypic and genetic findings consistent with ZTTK syndrome. His episodes, beginning at 2 years, typically followed minor closed head injuries and exhibited all the characteristic clinical, imaging, and electrographic features of hemiplegic migraine. Specifically, these episodes meet International Classification of Headache Disorder (ICHD)-3 criteria for hemiplegic migraine given the presence of headaches consistent with migraine with aura associated with fully reversible motor/sensory changes.[29] EEG and MRI studies obtained months after the most severe event demonstrated complete or near-complete recovery.

Although seizures are a commonly reported symptom of *SON*-related disorders, existing reports provide little detail regarding the semiology of these putative epileptic events.[11,14,16] In addition, a recent study aiming to establish the phenotypic spectrum of *SON*-related disorders shows that less than half of the patients with reported seizure events have identifiable epileptiform abnormalities.[16] In one study, 3 of all patients with seizures also showed developmental regression after seizure events resembling the clinical course experienced by our

Figure 4 *SON* Knockdown Leads to Decreased *PRRT2* mRNA Expression



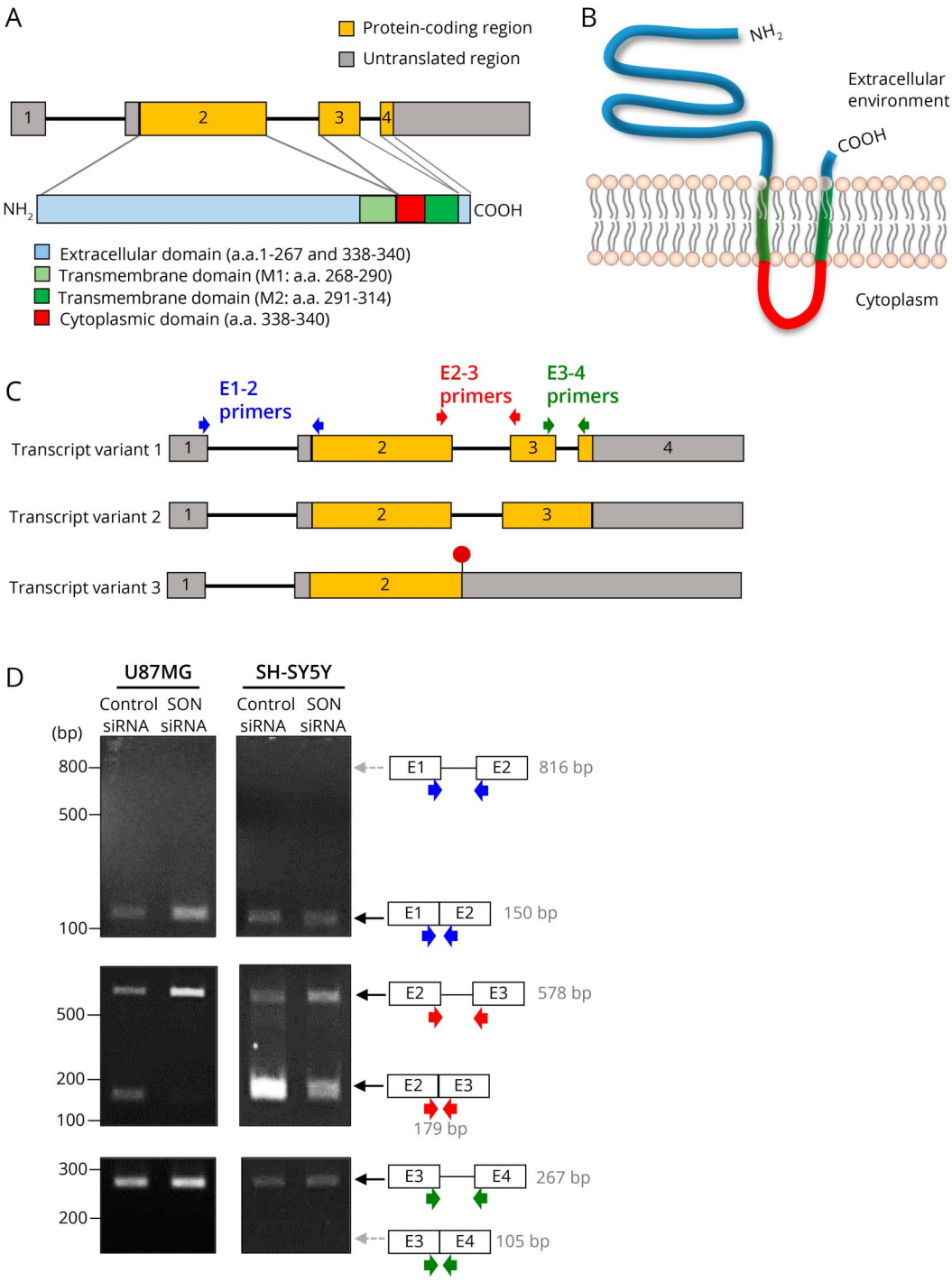
(A) qPCR determination of relative mRNA expression of *SON* and the genes known to be associated with hemiplegic migraines, *PRRT2*, *CACNA1A*, *ATP1A2*, and *SCN1A*, 48 hours after *SON* siRNA transfection into U87MG cells. Decreased *PRRT2* mRNA expression was observed on *SON* knockdown (B) and downregulation of PRRT2 in SH-SY5Y cells on siRNA-mediated *SON* knockdown. Bar graphs represent average ±SD, n = 3–6. **p* < 0.01 and ***p* < 0.001.

patient.[14] Therefore, it is conceivable that these episodic phenomena consisting of seizure-like events followed by developmental regression could in fact represent episodes of acute hemiplegic migraine rather than epilepsy. Finally, a recent report describes a single adult patient (currently 39 years of age) with severe intellectual disability because of a previously described frameshift variant in *SON* who experienced recurrent episodes possibly consistent with hemiplegic migraine beginning at age 9 years.[17] These considerations in light of our patient's case suggest that seizure-like events occurring in individuals with ZTTK syndrome warrant further characterization to better distinguish between epileptic events and hemiplegic migraines; it is possible that hemiplegic migraine could be a more prominent feature of *SON*-related disorders than previously recognized.

Although almost all patients with ZTTK syndrome experience speech delay and motor delay, the occurrence and severity of other phenotypic manifestations, including hemiplegic migraine, vary among patients. We speculate that this is due to (1) different time points at which SON mutation occurs during embryogenesis (note that these are de novo mutations), (2) variable expression of other splicing factors that may partially compensate for SON haploinsufficiency, (3) functional interaction of SON haploinsufficiency with other gene variants, and (4) sensitivity of target RNA splicing to a threshold level of SON expression. A previous study of *SON* pathogenic variants in ZTTK syndrome attempted to assess for possible genotype–phenotype correlations by analyzing 52 individuals with *SON* variants.[15] No significant genotype–phenotype correlations were identified, leading to the conclusion that it is unlikely that the position of the variants alone determines phenotype. Consequently, it is reasonable to speculate that the stage at which de novo mutation occurs during embryogenesis and genetic background, including variants in other genes, affects phenotypic expression.

SON is a large protein that has been shown to play an important role in RNA splicing and transcriptional control.[9] It has been shown that *SON* haploinsufficiency in human patients results in impaired RNA splicing of multiple genes and,

Figure 5 *SON* Knockdown Causes Intron 2 Retention in the *PRRT2* Pre-mRNA



(A) Structures of the *PRRT2* gene and the PRRT2 protein. The protein-coding region within exons and the corresponding protein domains are indicated. Each domain of the PRRT2 protein is color coded, and the amino acid numbers spanning each domain are indicated in the legend. (B) Membrane topology of PRRT2. (C) *PRRT2* transcript variants and the targeting locations of the primer sets used for splicing analysis. The red circle on transcript variant 3 represents a premature termination codon. (D) PCR analysis demonstrating increased *PRRT2* intron 2 retention on *SON* knockdown. U87MG and SH-SY5Y cells were transfected with control or *SON* siRNA and harvested after 48 hours for RNA purification and cDNA preparation. Spliced and unspliced status of indicated areas within the *PRRT2* transcripts were examined by reverse transcription, followed by PCR using the specific primer sets (color-coded arrows). Predicted size of PCR amplicons with or without intron retention is indicated next to the schematics depicting intron-retained or intron-removed forms.

in particular, many genes associated with brain development.[11,18] Herein, we demonstrate that SON dysfunction leads to altered splicing of the *PRRT2* transcript. *PRRT2* is highly expressed throughout the CNS.[2,5,7] Through its interactions with t-SNARE proteins, it serves as a modulator for presynaptic neurotransmitter release and additionally plays an important role in the development of cortical connectivity.[5,7] Dysfunction in PRRT2 is hypothesized to

decrease the inhibition of excitatory neurotransmitter release and thereby result in a hyperexcitable state.[4,5] This neurobiological consequence of *PRRT2* alteration is similar to other genes known to cause clinical manifestations of familial and sporadic hemiplegic migraine.[1,2,4-7] Our data demonstrated that reduction of SON causes failure in removal of intron 2 from the *PRRT2* transcript. Although the intron 2–retained form has been documented in NCBI and ENSEMBL databases as a transcript variant encoding a truncated PRRT2 (NCBI_Gene:112476; ENSEMBL:ENSG00000167371), it is likely that this transcript is partially degraded by nonsense-mediated mRNA decay because of the physical distance between the PTC harbored within intron 2 and the poly (A) tail of the transcript. This could contribute to the reduction of total *PRRT2* mRNA on SON knockdown. The potentially translated protein from the intron 2–retained transcript would be lacking 41 amino acids at the C terminus covering the cytoplasmic domain, transmembrane domain, and extracellular domain. It has been reported that *PRRT2* frameshift variants causing C-terminal deletion, as well as missense variants present at the C terminus of *PRRT2*, cause PRRT2 protein dysfunction.[30] Therefore, it is clear that intron 2 retention has detrimental effects on normal functions of PRRT2. Identification of SON as a splicing regulator of *PRRT2* provides a mechanism that links *SON* and hemiplegic migraines.

Of note, we did observe SCN1A upregulation on SON knockdown in the U87MG cell line. Pathogenic variants in *SCN1A*, a gene encoding the α-subunit of a neuronal voltage-gated sodium channel, cause various types of epilepsy, including Dravet syndrome, a form of early-onset epileptic encephalopathy.[31,32] Pathogenic variants in SCN1A have also been associated with familial hemiplegic migraine.[33-35] Although most of the variants identified in the patients result in putative loss of function of SCN1A,[31] at least some of these appear to result in gain of function.[34,36] However, it is not clear whether overexpression of SCN1A and gain-of-function mutations are functionally similar. Thus, although it is conceivable that SCN1A upregulation could also have contributed to the patient's symptoms, this remains uncertain. Finally, it is also possible that other as-yet unidentified genetic factors predisposing to more standard migraines reported by our patient's relatives may be contributing to his risk for hemiplegic migraine.

In summary, ZTTK syndrome or *SON*-related disorders affect the nervous system in complex ways, typically causing developmental malformation, neurodevelopmental impairment, and intellectual disability. We propose a phenotypic expansion of ZTTK syndrome or *SON*-related disorders to include hemiplegic migraine, as seen in our patient. Our case suggests that more detailed characterization of paroxysmal events of alteration of consciousness in patients with ZTTK syndrome may be warranted to better distinguish between epilepsy and hemiplegic migraine. Although the pathogenic mechanisms whereby *SON* loss of function predisposes to hemiplegic migraine remain undetermined, our data suggest that resultant defective splicing of *PRRT2* may contribute.

Acknowledgment

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Disclosure

J. Langford, L. Vukadin, J. C. Carey, L. Botto, M. Velinder, R. Mao, C. E. Miller, F.M. Filloux, and E.-Y. Erin Ahn report no disclosures relevant to the manuscript. Full disclosure form information provided by the authors is available with the full text of this article at Neurology.org/NG.

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Continued

Appendix *(continued)*

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