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Leaky splicing variant in sepiapterin reductase deficiency

Are milder cases escaping diagnosis?

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Sepiapterin reductase deficiency (SRD), an extremely rare but treatable neurotransmitter disease, is an enzyme defect in the final step of tetrahydrobiopterin (BH_4) synthesis.[1] Unlike other forms of BH_4-deficient dopa-responsive dystonia, SRD uniquely does not manifest hyperphenylalaninemia and thus slips through detection by newborn screening. Owing to its variable presenting features and need for a sensitive method of CSF analysis, diagnosis of SRD may be compromised in mild phenotypes.[2]

We describe a novel splice site variant leading to leaky splicing control of the *SPR* gene. Our observation adds evidence to the notion that leaky splicing may take part in SRD heterogeneity and evokes the image of an iceberg beneath the water: patients at the milder end of the spectrum escaping recognition.

Case report

An 8-month-old girl presented with postural limb dystonia that worsened in the evening. Brain imaging, EEG, routine blood, urine, and CSF testing were nondiagnostic. Recognition of her episodic oculogyric crises and convergence spasms prompted us to analyze her CSF for pterins and biogenic amines. CSF homovanillic acid (132 nmol/L) and 5-hydroxyindoleacetic acid (11.5 nmol/L) were decreased (normal range: 295–932 nmol/L and 114–336 nmol/L, respectively). The CSF BH_4 level, analyzed by the method described by Fukushima and Nixon,[3] was below the detection limit, whereas total biopterin (27.06 nmol/L) and neopterin (22.06 nmol/L) levels were within the normal range, suggesting that most of the patient's total biopterin was a sum of biopterin and dihydrobiopterin. Findings were suggestive of monoamine neurotransmitter disease due to BH_4 deficiency. L-dopa/carbidopa therapy completely suppressed her dystonia and resulted in near-normal psychomotor development.

Genetic analysis established the diagnosis of SRD by identifying compound heterozygous variants in the *SPR* gene (NM_003124.4): c.512G>A and c.304+1_+12del. The former is a novel missense variant, absent in the Exome Aggregation Consortium (ExAC) and gnomAD databases, estimated to substitute a well-conserved cysteine for tyrosine, and predicted as damaging according to in silico analyses. The latter, also absent in the ExAC and gnomAD databases, destroys the 5′ splice donor site in intron 1, rendering the gene prone to aberrant splicing (figure, A).

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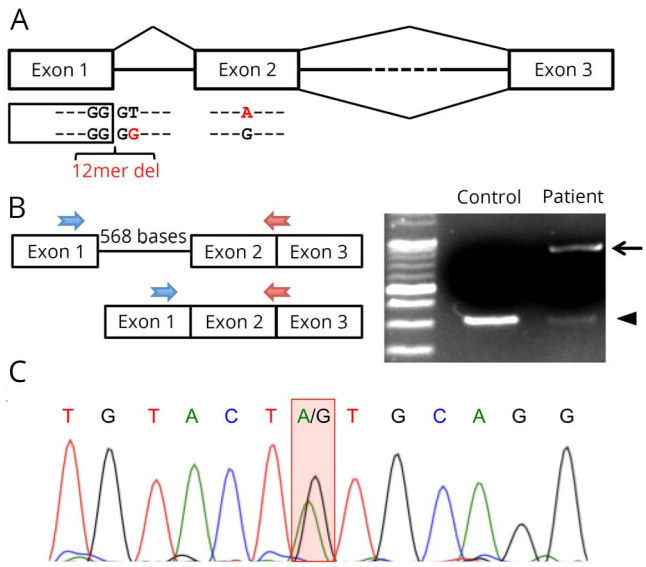
Figure Mutational and splicing analyses



(A) The patient was compound heterozygous for an exonic c.512G>A and an intronic c.304+1_+12del (*SPR*, NM_003124.4) (B and C) The destroyed splice site and retention of intron 1 resulted in a larger size 899-bp band (B, arrow) specific to the patient. Because allelic origin was identifiable based on the c.512G>A variant, sequencing the normally spliced 319-bp product (B, arrowhead) showed significant wild-type splicing from the allele carrying c.304+1_+12del (C). Primers were designed as depicted, and sequences are available upon request.

Next, splicing analysis was performed, using blood cell transcripts extracted from the patient and a healthy control. Primers were designed to flank intron 1 and exon 2 of the *SPR* gene and to specifically amplify the RNA sequences (figure B). Reverse transcription-PCR–based splicing analysis not only confirmed aberrant splicing causing intron retention (figure B, arrow) but also discovered evidence for leaky splicing control related to c.304+1_+12del. Because the allelic origin was identifiable based on the presence or absence of c.512G>A, directly sequencing the shorter 319-bp amplicon (figure B, arrowhead) showed significant wild-type splicing from the allele carrying c.304+1_+12del (figure C).

Discussion

Leaky splicing control contributes to phenotypic variation by affecting disease onset and/or severity. The extent of leaky wild-type transcription determines, for example, residual acid alpha-glucosidase activity in Pompe disease and relates to a specific-form of adult-onset disease.[4] As for SRD, others have reported the possibility of leaky splicing causing intrafamilial heterogeneity.[5] In the report, however, splicing was assessed indirectly using the minigene system. Our report proves by directly analyzing patient RNA that leaky splice site variants indeed underlie SRD. Phenotypic variability owing to such leaky splicing control may further expand the SRD spectrum.

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Appendix Author contributions

Name	Location	Role	Contribution
Yu Nakagama, MD	The University of Tokyo, Tokyo	Author	Interpreted clinical data, performed splicing analysis, and drafted the manuscript.
Masakazu Mimaki, MD, PhD	Teikyo University, Tokyo	Author	Interpreted clinical data and revised the manuscript.
Haruo Shintaku, MD, PhD	Osaka City University, Osaka	Author	Performed CSF analysis for pterins and amines.
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Appendix *(continued)*

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Ryo Inuzuka, MD, PhD	The University of Tokyo, Tokyo	Author	Critically revised the manuscript.
Akira Oka, MD, PhD	The University of Tokyo, Tokyo	Author	Critically revised the manuscript.

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