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Novel Biallelic Synonymous Exonic Variant in *VPS13A* Affecting mRNA Splicing

Case Report

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Abstract

Objectives

Chorea-acanthocytosis is an autosomal recessively inherited condition caused by loss-of-function pathogenic variants in *VPS13A*. We identified a novel synonymous exonic variant leading to abnormal mRNA splicing in a patient with chorea-acanthocytosis.

Methods

A patient with focal epilepsy developed generalized chorea with orolingual dystonia, cognitive decline, and peripheral neuropathy, consistent with chorea-acanthocytosis. Her parents were first cousins, but there was otherwise no family history. Targeted gene sequencing for variants in *VPS13A*, mRNA splicing analysis, and Western blot for chorein were performed.

Results

A homozygous synonymous variant in exon 41 of *VPS13A* (NM_033305.3): c.5157C>T; p.Gly1719 = was identified; this was previously classified as a variant of uncertain significance. SpliceAI predicted a splice donor gain with a score of 0.75 2 base pairs upstream of the reported variant. RNA splicing analysis revealed the creation of a type III splice variant, resulting in a frameshift and a premature termination codon. Western blot showed absent chorein/VPS13A protein.

Discussion

The variant is reclassified as likely pathogenic based on the American College of Medical Genetics criteria. This is the first reported case of ChAc caused by a synonymous variant in *VPS13A* proven to affect splicing. Our report further expands the spectrum of variants known to cause ChAc.

Introduction

Chorea-acanthocytosis (ChAc) or *VPS13A* (vacuolar protein sorting 13 homolog A) disease is an autosomal recessively inherited disorder.[1] Diagnosis is established when a patient with suggestive clinical features is found to have biallelic pathogenic variants in *VPS13A* on chromosome 9 or absent or significantly reduced chorein/VPS13A expression.[2] Chorein/VPS13A is a bridge-like lipid transfer protein located at membrane contact sites enabling direct bulk lipid

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transfer between organelle membranes.[3] We report a novel synonymous variant in *VPS13A*, proven to be pathogenic through in silico and in vitro approaches, in a patient with ChAc.

Case Summary

Our patient presented at age 35 with focal epilepsy. Brain MRI was normal while EEG showed an epileptogenic focus in the right hemisphere. Eleven years after diagnosis of epilepsy, she developed repeated mouth and tongue ulcers from accidental bites while eating and mild dysphagia and dysarthria. Three years later, involuntary cranial and bilateral limb movements appeared, with smacking of the lips, involuntary vocalizations, twisting movements of the neck, shoulder shrugging, and writhing movements of the hands and legs, consistent with generalized chorea (Video 1). She also had mild cognitive impairment, without psychiatric or behavioral problems. Examination showed decreased tendon reflexes with distal weakness and wasting. Her gait was lurching and unsteady. Tetrabenazine provided symptomatic relief of chorea. Her late parents were first cousins, but there was no family history of neurologic disorders (Figure 1). Laboratory tests showed raised creatine kinase (434 U/L, range 30–250 U/L), and peripheral blood film demonstrated acanthocytosis of 7.5% (normal value using EDTA/dry smear <1.2[4]) (eFigure 1). Evaluation for alternative etiologies including serum ceruloplasmin, antibodies against cell-surface antigens, and screen for infection was unyielding. Genetic tests for *HTT* and *SCA17* were negative. Repeat brain MRI showed bilateral caudate nucleus atrophy. The patient's phenotype was consistent with ChAc.

Targeted genetic testing of *VPS13A*, mRNA splicing analysis, and Western blot for chorein/VPS13A protein were performed.

Methods

Patient Recruitment and Ethical Consideration

Written informed consent for publication of case details and videos was obtained. Genetic counseling was performed in accordance with local guidelines. The research was approved by the SingHealth Institutional Review Board (ID 2019/2330).

VPS13A Gene Sequencing

Gene sequencing and deletion/duplication analysis of *VPS13A* were performed on genomic DNA obtained from the patient's saliva through a commercial test using the Illumina sequencing technology.

mRNA Splicing Analysis

RNA Extraction

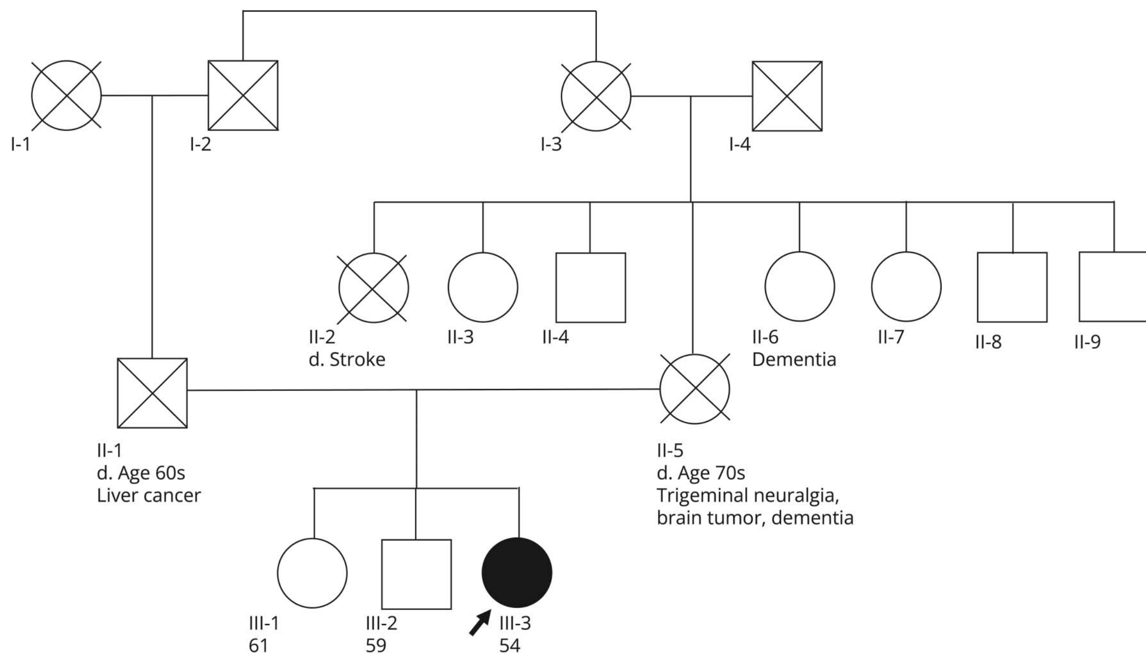
RNA was extracted from the patient's blood with trizol and miRNaeasy Mini Kit (Qiagen). A high-fidelity reverse-transcription kit (Applied Biosystems) was used for cDNA transcription.

cDNA Analysis for Evaluation of VPS13A mRNA Splicing

Using the patient's cDNA template, PCR was performed using reverse transcription-PCR primers designed to cover VPS13A mRNA sequences from exon 39 to exon 44 (VPS13A-F-5′-GATCTCCAAGTGAGAGCCTGC-3′ and VPS13A-R2-5′-GCTGCTTAGGCTGGTCATTGC-3′).

Electrophoresis with 2.0% agarose gel was used to analyze the PCR products. Thereafter, PCR product purification was performed, followed by Big Dye Sanger sequencing using the 3500 Series Genetics Analyzer (Applied Biosystems, USA). Comparison was made with 2 normal control specimens.

Figure 1 Pedigree



Western Blot

Western blot for detection of chorein/VPS13A protein was performed on erythrocyte membrane preparations from EDTA blood using 2 different specific anti-VPS13A antibodies (Anti-VPS13A, rabbit, Sigma-Aldrich; Cat#HPA021662; Anti-VPS13A, rabbit, Invitrogen, Cat#PA5-54483) in 2 independent experiments in accordance with previous reports.[2]

Data Availability

Unpublished anonymized data will be made available on request from any qualified investigator.

Results

Commercial sequencing identified a homozygous synonymous variant in *VPS13A* (NM_033305.3): c.5157C>T; p.Gly1719=. This variant had previously been classified in ClinVar as a variant of unknown significance, is found in gnomAD exomes with an allelic frequency of 0.000137, and has a PhyloP100 conservation score of 0.455. In silico analysis with SpliceAI[5] predicted a donor gain score of 0.75 2 base pairs (bp) upstream of the variant and a donor loss score of 0.84 156 bp downstream of the variant.

On mRNA splicing analysis, agarose gel electrophoresis revealed a single shorter *VPS13A* cDNA PCR amplification product (847 bp) of the patient compared with healthy controls (1,005bp) (Figure 2A). Sanger sequencing of the cDNA PCR product revealed a 158 mRNA base pair deletion that corresponded with a deletion between c.5156 on exon 41 and c.5313 on exon 42. This deletion results in a frameshift, in turn leading to the creation of a stop codon (TAA) 12 bp downstream from the start of exon 42 (Figure 2D). Western blot for chorein/VPS13A protein revealed absence of detectable chorein/VPS13A (Figure 3). The variant is reclassified as likely pathogenic based on American College of Medical Genetics criteria.

Figure 2 Results of mRNA Splicing Analysis

(A) Agarose gel electrophoresis of PCR products from controls and from the patient. (B) Reference cDNA sequence (transcript ID: ENST00000360280.8) with black box showing the site of the c.5157C>T variant, strikethrough indicating the 158 missing RNA resulting from the introduction of an exonic splice site. Blue highlight represents exon 40, grey represents exon 41, and yellow represents exon 42. cDNA sequence from the normal control (C) and the patient (D). (D) The dotted line indicating the site of frameshift while the dotted box indicating the site of premature stop codon.

Discussion

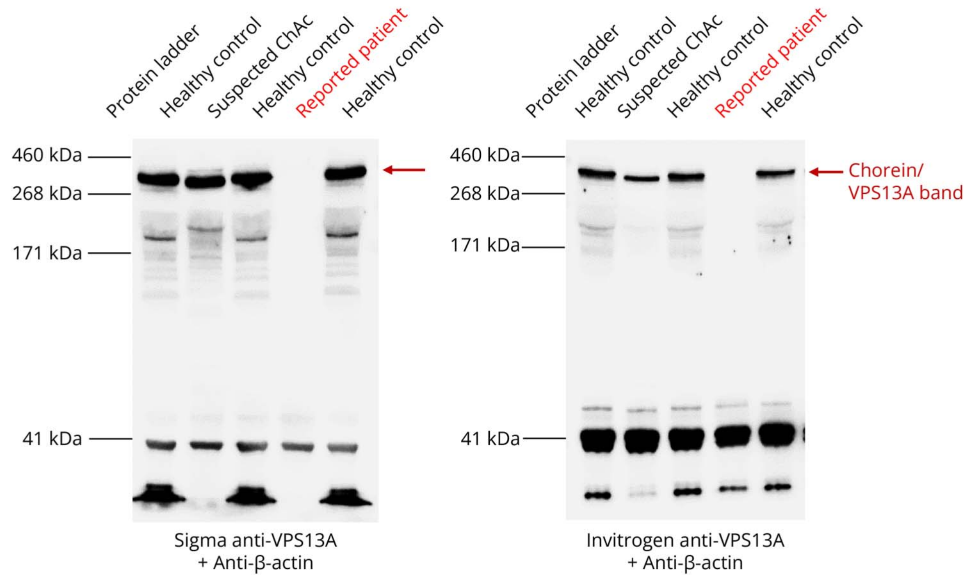
We report a novel synonymous exonic variant in *VPS13A* (*VPS13A*: c.5157C>T; p.Gly1719=), proven to affect splicing, in a patient with a clinical phenotype consistent with ChAc.

We performed further studies because (1) the patient's phenotype and negative chorein Western blot were fully consistent with ChAc and (2) in silico analysis using SpliceAI[5] predicted a moderate-to-high probability of a splicing effect secondary to the variant. The variant likely unveiled a cryptic donor splice site (type III splice variant[6]) at c.5155. This splice donor gain is in keeping with in silico analysis by SpliceAI. The creation of an exonic splice site, causing a partial exonic deletion, resulted in a frameshift and development of a premature termination codon (PTC). Because there are 73 exons in wild-type *VPS13A*, the PTC would prematurely terminate the translation of VPS13A mRNA at exon 42. The truncation of >10% of VPS13A mRNA supports the development of nonsense-mediated mRNA decay (NMD),[7,8] leading to absence of chorein/VPS13A in EDTA blood. Gel electrophoresis only identified 1 cDNA transcript, suggesting that the unveiled cryptic splice site is a strong splice site that did not allow for alternative splicing, unlike transcript mosaicism observed in other type III splicing variants.[9]

A limitation of our analysis is that, because of infection control protocols, the patient's peripheral blood film was obtained using dry smears of EDTA blood. An unfixed wet blood smear achieves the highest sensitivities for quantifying acanthocyte levels in suspected neuroacanthocytosis, although the presence of >1.2% acanthocytes using our method remains highly specific.[4] In addition, we were unable to perform genetic evaluation of the patient's family members.

Of the small proportion of synonymous variants that have a pathogenic effect, most seem to affect splicing. Rarely, these synonymous variants may also affect micro-RNA binding, mRNA structure, and protein translation because of changing codon usage.[10] Techniques to analyze the effect of synonymous variants include RNA structure prediction using computational and experimental approaches.[11,12] Reverse transcription-PCR, as used in our patient, can evaluate the perturbations to pre-mRNA splicing from synonymous variants.[13] A limited number

Figure 3 Western Blot Showing Absence of Chorein/VPS13A Band in Our Patient (4th Column From the Left) Using 2 Different Specific Anti-VPS13A Antibodies (Anti-VPS13A, Rabbit, Sigma-Aldrich; Cat#HPA021662; Anti-VPS13A, Rabbit, Invitrogen, Cat#PA5-54483) in 2 Independent Experiments



of in silico computational tools are available to evaluate the functional effects of synonymous variants, although most, like SpliceAI, were designed to evaluate splicing effects. Further development is required to comprehensively evaluate the functional effects of synonymous variants.

A highly suspected clinical diagnosis can support the interrogation of the causative gene with new analytic techniques.[14] Direct Sanger sequencing and next-generation sequencing are the usual methods of identifying genetic variants and allow easy identification of variants altering protein coding but may overlook single nucleotide variants affecting noncoding regions or disrupting gene function through more complex pathways. Patients without genetic diagnosis are often without diagnostic closure, limiting their accessibility to prenatal diagnosis, certain clinical treatments, and accurate prognostication. An awareness of the limitations of the molecular approach chosen is key to interpret the genetic diagnosis and reinforce utilization of alternative techniques that may lead to identification of missing variants.

In conclusion, we report a case of ChAc caused by a synonymous variant in *VPS13A* and prove that this variant affects splicing. Our report further expands the spectrum of variants known to cause ChAc.

Study Funding

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Disclosure

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Publication History

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Helen Lisa Ong, MSc	Department of Clinical Translational Research, Singapore General Hospital, Singapore	Major role in the acquisition of data; analysis or interpretation of data
Karine Su Shan Tay, BSc	Department of Neurology, National Neuroscience Institute (Tan Tock Seng Hospital Campus), Singapore	Major role in the acquisition of data
Nigel Choon Kiat Tan, MBBS, FAMS, FRCP (Edinburgh), MHPEd	Department of Neurology, National Neuroscience Institute (Tan Tock Seng Hospital Campus), Singapore	Major role in the acquisition of data

Continued

Appendix (continued)

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