CLINICAL/SCIENTIFIC NOTE OPEN ACCESS

The First Korean Siblings With Adult-Onset 4H Leukodystrophy Related to Nonsynonymous *POLR3B* Mutations

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Neurol Genet 2022;8:e667. doi:10.1212/NXG.0000000000000667

Abstract

Objectives

4H leukodystrophy is a rare autosomal recessive hypomyelinating disorder characterized by several combinations of motor dysfunction, abnormal dentition, and ophthalmic and endocrine abnormalities. To date, only a single Korean case report of pediatric leukodystrophy caused by the *POLR1C* sequence variation has been published, while there are no reports on the *POLR3B*, *POLR3A*, or *POLR3K* variants.

Methods

Genetic tests of Korean sibling pairs with primary amenorrhea due to normosmic isolated hypogonadotropic hypogonadism and cognitive or behavioral symptoms were performed by whole-exome sequencing (WES). The WES results were validated by direct Sanger sequencing.

Results

We identified biallelic variations in the *POLR3B* gene of p.Tyr685* and p.Tyr746Cys, which have not been associated with 4H leukodystrophy. Both sequence variants lie in the hybrid-binding domain of the protein RPC2. The protein structure analysis predicted that cysteine substitution of the phylogenetically conserved amino acid tyrosine can cause destabilization.

Discussion

The siblings reported are the first *POLR3B*-related hypomyelinating leukodystrophy cases in Korea. Our report expands the mutational spectrum of 4H leukodystrophy and suggests that it is mandatory to consider its diagnostic possibility in adult patients presenting with primary amenorrhea and mild cognitive or behavioral symptoms.

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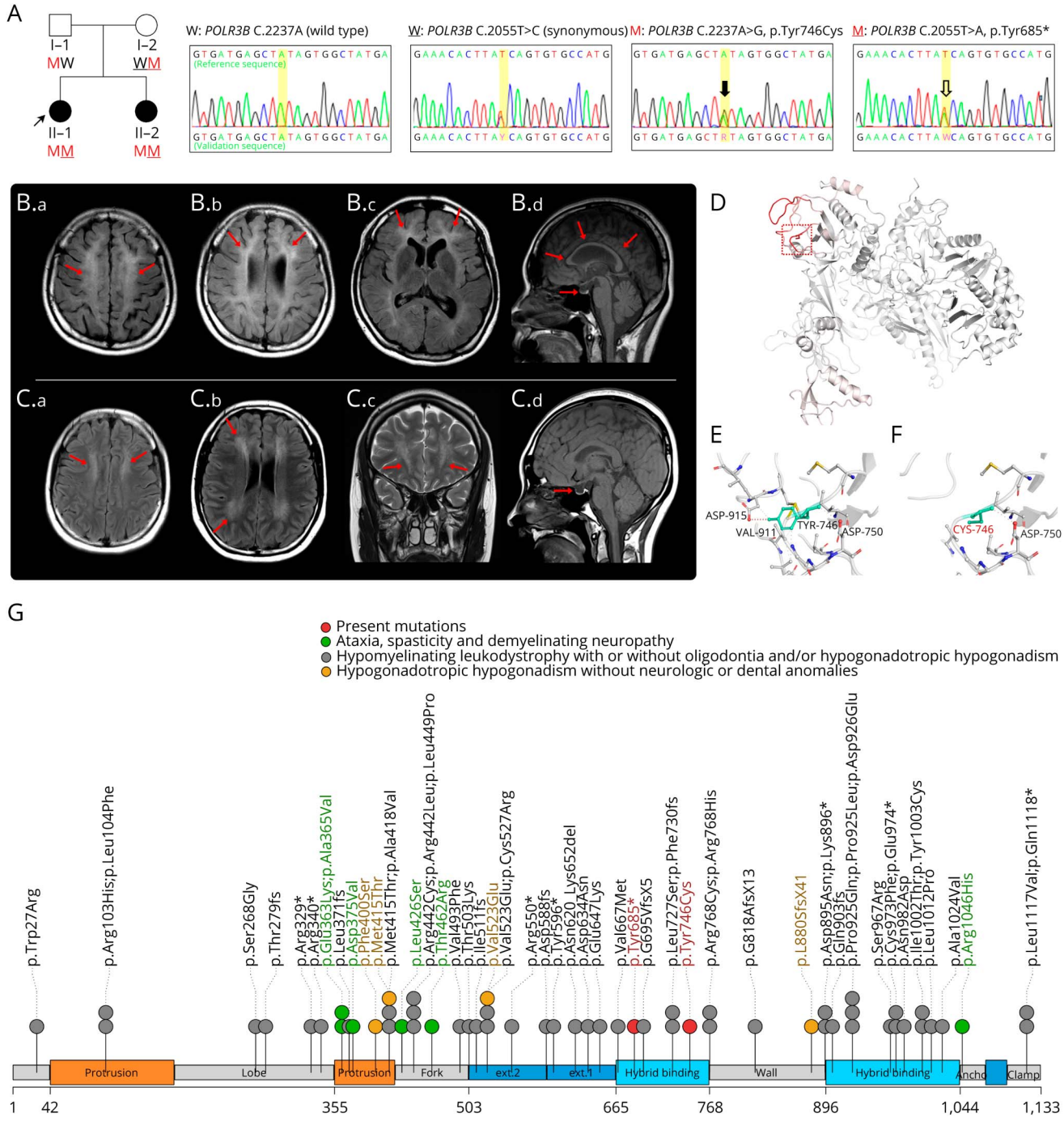
Go to Neurology.org/NG for full disclosures. Funding information is provided at the end of the article.

The Article Processing Charge was funded by the authors.

Human RNA polymerase III (Pol III) defects reportedly cause autosomal recessive hypomyelinating leukodystrophy combined with hypodontia and hypogonadotropic hypogonadism and are collectively termed as 4H leukodystrophy.[1,2] The most common genetic cause of 4H leukodystrophy is the *POLR3B* gene (MIM #614381) encoding RPC2, which is the second largest subunit of Pol III, followed by *POLR3A*, *POLR1C*, and *POLR3K* variations.[1-4] To date, only 1 pediatric leukodystrophy

Figure *POLR3B* Sequence Variation Analysis and Cerebral MRI Findings in a Korean Family With 4H Leukodystrophy



(A) Pedigree of the study family showing segregation of *POLR3B* variants; the proband is indicated by the arrow. A novel nonsense mutation (*POLR3B* c.2055T>A, p.Tyr685*; indicated by blank arrows) was found in the 2 siblings and their mother, while a missense mutation (*POLR3B* c.2237A>G, p.Tyr746Cys; indicated by solid arrows) was present in both patients and their father. (B.a–d) Brain MRI of patient 1. Fluid-attenuated inversion recovery (FLAIR) axial images show nonenhancing diffuse high-intensity areas involving the white matter of both cerebral hemispheres. T1-weighted sagittal image shows thinning of the corpus callosum and a small pituitary structure. (C.a–d) Brain MRI of patient 2. The axial FLAIR, T2-weighted coronal and T1-weighted sagittal images reveal the confluent cerebral white matter abnormalities predominantly involving the periventricular areas and descending tract with the small pituitary gland. (D) Ribbon diagram of human RNA polymerase III (PDB ID: 7D59) and the mapping of the missense mutation (p.Tyr746Cys) in the boxed area. (E and F) Close-up view of the in silico predicted structural models. The wild-type (TYR-746) and missense mutation (CYS-746) are presented in green color (please refer to Table 2). (G) *POLR3B* mutation lollipop plot. Novel mutations found in our siblings (red) and published pathologic variants mapped onto the structural domains of the *POLR3B* gene.[1,2,4,6]

Table 1 Clinical Data and Laboratory Results of Study Patients

Characteristic	Patient 1	Patient 2
Current age, y/sex	34/F	32/F
Ethnicity	Korean	Korean
Initial neurologic symptoms/onset age, y	Cognitive decline with gait disturbance/31	Mild forgetfulness/30
Cerebellar features	—	—
Muscle strength	Normal	Normal
Deep tendon reflexes	Mild hyperreflexia	Normoreflexia
Epilepsy	—	—
Abnormal dentition	—	—
Ocular abnormality	—	—
Endocrine abnormality	+	+
FSH, mIU/mL	0.40	4.67
LH, mIU/mL	<0.07	0.99
Estradiol, pg/mL	20.25	<9.0
Very long-chain fatty acid	Normal	Normal
Arylsulfatase A	Normal	Normal
Hexosaminidase A and B	Normal	NI
Beta-galactosidase	Normal	NI
Plasma lactate/pyruvate	Normal/normal	Normal/normal
Olfactory function	Normal	Normal
MMSE total score	27/30	30/30
MoCA total score	24/30	29/30

Abbreviations: CADD = combined annotation dependent depletion; CONDEL = CONsensus DELeteriousness score; FSH = follicle-stimulating hormone; INPS = impact of non-synonymous mutations on protein stability; LH = luteinizing hormone; MMSE = Mini-Mental State Examination; MoCA = Montreal Cognitive Assessment; NI = not investigated; PROVEAN = protein variation effect analyzer; SIFT = Sorting Intolerant From Tolerant program.

Table 2 In Silico Predictions of Pathogenicity and Protein Stability of *POLR3B* Mutations

In silico prediction	p.Tyr746Cys	p.Tyr685*
CADD[a] (score)	Deleterious (28.5)	
SIFT[b] (score)	Damaging (<0.00)	—
Polyphen-2 HumDiv[c] (score)	Probably damaging (1.000)	—
Polyphen-2 HumVar[c] (score)	Probably damaging (0.999)	
PROVEAN[d] (score)	Deleterious (−8.215)	
Mutation Taster[e]	Disease causing	Disease causing
DUET[f] (ΔΔ*G*)	Destabilizing (−1.218 kcal/mol)	—
CONDEL[g] (score)	Decrease the stability of protein structure (0.873)	
INPS3D[h] (ΔΔ*G*)	Reduced stability (−1.901 kcal/mol)	
DynaMut[i] (ΔΔ*G*)	Destabilizing (−0.749 kcal/mol)	
I-Mutant 3.0[j] (I-MutantΔΔ*G*)	Destabilizing (−1.07 kcal/mol)	—

[a] cadd.gs.washington.edu; if the CADD score is >20, the sequence variation is predicted to be among the top 1% of deleterious variants in the human genome.
[b] sift.bii.a-star.edu.sg; an SIFT score ranges from 0 to 1. If the SIFT score is <0.05, the mutation is predicted to be damaging.
[c] genetics.bwh.harvard.edu/pph2; HumVar and HumDiv prediction values range from 0 (benign) to 1.00 (probably damaging).
[d] provean.jcvi.org/seq_submit.php; if a PROVEAN score is <−2.5, the mutation is predicted to have a deleterious effect.
[e] mutationtaster.org.
[f] biosig.unimelb.edu.au/duet; predicted stability change (ΔΔ*G*) expressed in kcal/mol; ΔΔ*G* ≥0 as stabilizing and ΔΔG <0 as destabilizing.
[g] bg.upf.edu/fannsdb; the CONDEL scores range from 0.0 (neutral) to 1.00 (deleterious).
[h] inpsmd.biocomp.unibo.it/inpsSuite/default/index3D; the negative and positive protein stability change (ΔΔ*G*) values on single-point variation are predicted to be destabilizing and stabilizing, respectively.
[i] biosig.unimelb.edu.au/dynamut; ΔΔ*G* ≥0 as stabilizing and ΔΔG <0 as destabilizing.
[j] gpcr2.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I-Mutant3.0.cgi; the I-MutantΔΔ*G* allows to predict whether a mutation can destabilize (the I-MutantΔΔ*G* <−0.5 kcal/mol) or largely stabilize (the I-MutantΔΔ*G* >0.5 kcal/mol) the protein.

case report with *POLR1C* variants has been published in Korea.[3] In this study, we present the first Korean siblings with adult-onset hypomyelinating leukodystrophy and hypogonadotropic hypogonadism associated with biallelic *POLR3B* variations, p.Tyr685* and p.Tyr746Cys. These variants have not yet been reported as a part of this syndrome.

Case Presentation

Patient 1

The proband (II-1, Figure, A) is a woman who first visited our neurology clinic at age 33 years with a 2-year history of progressive memory disturbance. She developed mild gait difficulty and eventually resigned from her job as a nurse 8 months ago. She had a medical history of primary amenorrhea, which was gynecologically diagnosed as normosmic isolated hypogonadotropic hypogonadism at age 20 years. Neurologic examinations revealed a stooped posture with mild bilateral postural hand tremor and spastic gait. Her Montreal Cognitive Assessment score was 24, and she failed to complete the clock showing "10 minutes past 11 o'clock" in the clock drawing test. Brain MRI findings revealed diffuse hypomyelination with a small pituitary gland and thin corpus callosum (Figure, B).

Patient 2

The first clinical manifestation of patient 2 (II-2, Figure, A), the proband's younger sister, was primary amenorrhea due to normosmic isolated hypogonadotropic hypogonadism, which was diagnosed at age 19 years. At age 30 years, a neurologic examination revealed mild right hand dystonic posturing; however, no other movement disorder or gait disturbance was observed. She complained of forgetfulness and anxiety, while neuropsychological tests did not reveal any cognitive

impairment. Confluent fluid–attenuated inversion recovery and T2 high-intensity areas in the periventricular white matter and a small pituitary structure were observed on her brain MRI finding (Figure, C).

Clinical features and laboratory findings are summarized in Table 1. No hirsutism, virilization, abnormal dentition, or ophthalmic abnormalities were reported in our cases. The extended family history was unremarkable. After obtaining written informed consents, genomic DNA was extracted from the blood samples of patients 1, 2, and their parents. Whole-exome sequencing revealed compound heterozygosity for a novel nonsense variation c.2055T>A (p.Tyr685*) and a missense variation c.2237A>G (p.Tyr746Cys) in the *POLR3B* gene, which were confirmed by direct Sanger sequencing (Figure, A). The clinically unaffected parents were heterozygous carriers. There were no mutations in the *POLR3A*, *POLR1C*, or *POLR3K* genes.[2,3] The nonsense c.2055T>A mutations were not found in the Korean Variant Archive,[5] the 1000 Genomes Project Database, ExAC, dbSNP, or the Korean Reference Genome Database (KRGDB).[5] The missense c.2237A>G was present only in the heterozygous state in the KRGDB at a very low frequency (rs1228870515, minor allele frequency = 0.000582). In silico analysis using combined annotation dependent depletion (CADD) SIFT, and Polyphen-2 predicted these alterations to be pathogenic (Table 2). Protein stability analysis of the missense c.2237A>G (p.Tyr746Cys) determined that the cysteine substitution of the phylogenetically conserved tyrosine can cause destabilization (Table 2 and Figure, D–F).[5,6] The present variations are located in the RPC2 hybrid-binding domain, which binds the short Pol III DNA–RNA hybrids.[6] Several reported 4H leukodystrophy cases carry variants throughout this domain (Figure, G).

Discussion

Our sibling pairs presented with primary amenorrhea in early adulthood and therefore were initially evaluated in gynecology and endocrinology clinics. Neurologic assessments were delayed till several years later. The initial presentation of the isolated hypogonadotropic hypogonadism and absence of abnormal dentition or cerebellar ataxia, which are the clinical hallmarks of 4H leukodystrophy, is noteworthy and can be clinically misleading. Moreover, an MRI examination of patient 2 showed confluent involvement confined to the periventricular white matter, which is consistent with the previous studies revealing that diffuse hypomyelination was not obligatory for 4H leukodystrophy.[7] Of interest adult-onset hypogonadotropic hypogonadism without or with mild neurologic or dental anomalies has been previously reported only in association with the biallelic variations of the *POLR3B* gene.[2,4] A phenotypic variant related to several specific *POLR3B* mutations can be considered in which the dental and cerebellar symptoms manifest at a later course of the disease.[2,4]

In conclusion, the siblings reported are the first *POLR3B*-related hypomyelinating leukodystrophy cases in Korea. Our report expands the mutational spectrum of 4H leukodystrophy and suggests that it is mandatory to consider its diagnostic possibility in adult patients presenting with primary amenorrhea and mild cognitive or behavioral symptoms.

Study Funding

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2021R1F1A1062478).

Disclosure

The authors report no disclosures. Go to Neurology.org/NG for full disclosures.

Publication History

Received by *Neurology: Genetics* November 16, 2021. Accepted in final form February 7, 2022. Submitted and externally peer reviewed. The handling editor was Raymond P. Roos, MD, FAAN.

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