POLR3A variants in striatal involvement without diffuse hypomyelination

Case Report

POLR3A variants in striatal involvement without diffuse hypomyelination

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Abstract

Background: Biallelic variants in *POLR3A* encoding the largest subunit of RNA polymerase III cause POLR3-related (or 4H) leukodystrophy characterized by neurologic dysfunction, abnormal dentition, endocrine abnormalities and ocular abnormality. Recently, whole-exome sequencing enabled the discovery of *POLR3A* variants in cases lacking diffuse hypomyelination, the principal MRI phenotype of POLR3-related leukodystrophy. Homozygous c.1771-6C>G variants in *POLR3A* were recently suggested to cause striatal and red nucleus involvement without white matter involvement.

Case report: Here, we report three cases in two families with biallelic *POLR3A* variants. We identified two sets of compound heterozygous variants in *POLR3A*, c.1771-6C>G and c.791C>T, p.(Pro264Leu) for family 1 and c.1771-6C>G and c.2671C>T, p.(Arg891*) for family 2. Both families had the c.1771-6C>G variant, which led to aberrant mRNA splicing. Neuropsychiatric regression and severe intellectual disability were identified in three patients. Two cases showed dystonia and oligodontia. Notably, characteristic bilateral symmetric atrophy and abnormal signal of the striatum without diffuse white matter signal change were observed in brain MRI of all three individuals.

Conclusions: Striatum abnormalities may be another distinctive MRI finding associated with *POLR3A* variants, especially in cases including c.1771-6C>G variants and our cases can expand the phenotypic spectrum of *POLR3A*-related disorders.

Keywords: *POLR3A*, POLR3-related leukodystrophy, 4H leukodystrophy, striatal involvement, whole-exome sequencing

Introduction

POLR3A (RNA polymerase III subunit A) encodes the catalytic component of RNA polymerase III (Pol III), which synthesizes small RNAs such as nuclear-encoded transfer RNAs (tRNA) and various other small housekeeping noncoding RNAs (ncRNA) [1]. Biallelic *POLR3A* variants cause POLR3-related (or 4H) leukodystrophy (OMIM # 607694), which is characterized by neurologic dysfunction, abnormal dentition, endocrine abnormalities and ocular abnormality [2]. POLR3-related leukodystrophy showed characteristic magnetic resonance imaging (MRI) findings including high-intensity areas in the white matter on the T2-weighted images, cerebellar atrophy and thinning of the corpus callosum [3].

Recent studies described several cases with *POLR3A* variants who did not show diffuse hypomyelination [4-7]. Azmanov et al. indicated that striatal and red nucleus involvement without white matter involvement was observed in individuals with a homozygous splice site variant (c.1771-6C>G) in *POLR3A* [5].

Here, we report three individuals with biallelic *POLR3A* variants including c.1771-6C>G, who show characteristic brain images of striatal lesions without abnormal signal changes of white matter. We reviewed the literature and discussed the phenotypic features of *POLR3A*-related disorders.

Genetic analyses

This study was approved by the Institutional Review Board Committee at Hamamatsu University School of Medicine. After receiving written informed consent, genomic DNAs extracted from peripheral blood samples from the probands and their parents were analyzed using whole-exome sequencing (WES). Data processing, variant calling,

annotation and filtering were performed as described previously [8]. Using the trio-WES data, we found compound heterozygous variants in *POLR3A* (NM_007055.4), c.1771-6C>G and c.791C>T, p.(Pro264Leu) in patient 1 and c.1771-6C>G and c.2671C>T, p.(Arg891*) in patient 2 (Figure 1A). These variants were validated using Sanger sequencing of the two patients and patient 3, an affected younger sister of patient 2 (Figure 1A). A previous study revealed that the c.1771-6C>G variant caused skipping of exon 14 in the mutant transcript, which introduced the premature termination codon (p.(Pro591Metfs*9)) [5]. The novel c.791C>T and the c.2671C>T variants are absent in the public databases and predicted to be deleterious by *in silico* pathogenicity prediction tools (Supplemental Table S1). These findings suggested that these *POLR3A* variants were likely to be pathogenic in our cases.

Case Presentation

Patient 1

After 37 weeks of gestation without asphyxia, a Japanese boy was born to nonconsanguineous healthy parents as their first child (Figure 1A). There was no family history of neurodevelopmental disorders. He had thick lips and thick eyebrows (Figure 2A), but no dental abnormalities were found (Figure 2B). His developmental milestones were delayed: head control at 6 months and walking independently at 1 year and 5 months. He was diagnosed with autism spectrum disorder at 3 years. He began speaking meaningful words at 4 years, but could not combine two words. Dysarthria developed at 8 years and progressed. His gait was unstable whenever he started walking, but he was able to go up and down the stairs at 6 years old. However, his motor dysfunction

gradually regressed and he could not walk unsupported because of progressive gait ataxia and muscle hypotonia at 11 years old. Brain MRI at 12 years old revealed bilateral symmetric atrophy and increased signal of the caudate nucleus and the putamen (Figure 2D, H). The white matter and red nucleus showed no abnormal signal changes, and cerebellum was normal (Figure 2E, I). Upon final examination at 18 years old, he could not maintain a sitting position without support. He showed tremor but no dystonia or rigidity. He was unable to speak any words, but he could communicate with others using a letter board.

Patient 2

A Japanese girl was born to nonconsanguineous healthy parents without asphyxia as a second child after an uneventful 40-week pregnancy. The first child was stillborn after 7 months of gestation. Her developmental milestones were normal until 1 year: head control at 4 months and pulling herself up at 10 months. Thereafter, her development regressed: she could not pull herself to standing at 1 year and 6 months and she could not sit unassisted at 2 years old. She thrashed her extremities randomly since the age of 1 year and 6 months; these were considered progressive hyperkinetic movements. Progressive muscle hypotonia, dysarthria, ophthalmoparesis and dystonia were noted. Biochemical analyses of blood and metabolic screenings including amino acids, lactic acid and pyruvic acid were unremarkable. MRI at 8 years showed bilateral symmetric atrophy and increased signal of the striatum and cerebral atrophy (Figure 2F, J). The red nucleus and cerebellum were normal. At 23 years old, she was bedridden and received

tube feeding from the gastrostoma. Severe dystonia, rigidity and congenital hypodontia was observed (Figure 2C).

Patient 3

A Japanese girl was born without asphyxia as a third child after 38 weeks of gestation. Her older sister was patient 2. Her development was normal until 1 year of age. She could hold her head up at 3 months and could pull herself up at 11 months. However, she stopped laughing at 1 year and she could not stand up even with support at 1 year and 9 months. Myoclonus was recognized at 1 year and 5 months, and dystonia was seen at 1 year and 11 months. Tube feeding started because of dysphagia at 3 years and gastrostomy was performed at 8 years. Home ventilator therapy was initiated at 9 years. The biochemical analyses of blood and metabolic screenings including amino acids, lactic acid and pyruvic acid showed normal values. Brain MRI at 10 years old showed bilateral-atrophy-associated increased signal in the striatum and frontal predominant cerebral atrophy (Figure 2G, K). Neither signal changes in red nucleus nor cerebellar atrophy were recognized. She died at 15 because of respiratory disturbance due to increased tracheal granulation.

Discussion

In this study, we described three individuals with compound heterozygous *POLR3A* variants. We summarized the clinical manifestations of eight patients who shared c.1771-6C>G variant including our cases in Table 1 [4-6]. Dystonia (6/8), ataxia (6/8), dysphagia (5/8), muscle weakness (4/8) and Babinski sign (4/8) were frequently

observed. Cerebellar features like ataxia, dysphagia and mild pyramidal features overlapped with those of POLR3-related leukodystrophy [2]. Other common features of POLR3-related leukodystrophy, including dental abnormalities (3/8), hypogonadism (0/6) and developmental delay (2/7) [3], were found in a minority of patients. Extrapyramidal signs are not commonly seen in patients with POLR3-related leukodystrophy [3]; however, most individuals had dystonia. Considering these findings, extrapyramidal signs may be characteristic physical features for patients with c.1771-6C>G variants.

POLR3A variants have been identified in individuals with neurological disorders such as hereditary ataxia and spastic paraparesis (Figure 1B) [6,7]. Brain images of these cases showed various brain abnormalities; abnormal signal of the posterior limb of the internal capsule, involvement of superior cerebellar peduncles, thin cervical spinal cord, cerebellar atrophy and thin corpus callosum [6,7]. Furthermore, atypical brain abnormalities without diffuse hypomyelination, including a selective involvement of the corticospinal tracts or moderate to severe cerebellar atrophy, were reported in cases of POLR3-related leukodystrophy [4]. MRI findings of eight cases with c.1771-6C>G variant also showed no signal changes of white matter, but the caudate nucleus and putamen involvement were observed in six of the eight individuals. Interestingly, striatal involvement was recognized only in individuals with c.1771-6C>G variants. Therefore, our study expands MRI phenotypes of brain abnormalities associated with *POLR3A* variants.

Although the underlying mechanism of *POLR3A*-related disorders is unsolved, dysregulation of tRNAs expression and RNA polymerase III transcribed ncRNAs may

impair the myelin development and maintenance [9]. Homozygous c.1771-6C>G variants in *POLR3A* reduced Pol III-transcribed tRNA expression and affected the balance of tRNAs and ncRNAs [5]. Recent studies suggested that tRNA hypomodification and tRNA-modifying-enzyme deregulation affected the efficiency and fidelity of protein translation and were associated with developments of several neurodegenerative diseases [10]. Thus, in patients with c.1771-6C>G variant, it is possible that disturbance of Pol III transcription involving tRNAs could affect proper translation and impair cellular processes, especially in the striatum.

In conclusion, we identified three cases with neuropsychiatric regression with characteristic striatal involvements without diffuse hypomyelination related to the c.1771-6C>G splicing variant in *POLR3A*. Our cases expand the phenotypic spectrum of POLR3-related leukodystrophy.

Acknowledgments

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Figure legends

Figure 1. Pedigrees and schematic structure of *POLR3A*

(A) Familial pedigrees and Sanger sequencing confirmation of Family 1 and Family 2. *POLR3A* (NM_007055.4) variants are shown below each individual. Arrows, probands; SB, stillbirth; WT, wild-type allele of *POLR3A*. (B) Schematic presentation of RPC1 protein encoded by *POLR3A* and location of altered residues. Previously-reported *POLR3A* variants, which showed no diffuse hypomyelination, are depicted above (previously reported in spastic ataxia [6,7], blue; previously reported in the cases with selective involvement of the corticospinal tracts or cerebellar atrophy [4], purple). The *POLR3A* variants identified in our case are shown below. Multiple amino acid sequences of RPC1 were aligned using the ClustalW tool (see http://www.genome.jp/tools/clustalw). The c.791C>T variant was highly evolutionarily conserved.

Figure 2. Clinical features of patients with *POLR3A* variants

(A, B) Photographs of patient 1 at 18 years old. Thick lips and thick eyebrows are observed (A), but no dental abnormalities are recognized (B). (C) A dental cavity photograph of patient 2 taken at 23 years old shows hypodontia. Eight upper and lower anterior teeth and molars were lacking. (D-K) MRI of three patients: patient 1 at 12 years old (D, E, H, I), patient 2 at 8 years old (F, J), patient 3 at 10 years old (G, K). Axial (D-G) and a coronal (H) T2-weighted image and a coronal T2–fluid-attenuated inversion recovery image (J) all show elevated signal of the caudate nucleus and the putamen and bilateral symmetric atrophy. Frontal predominant brain atrophies are

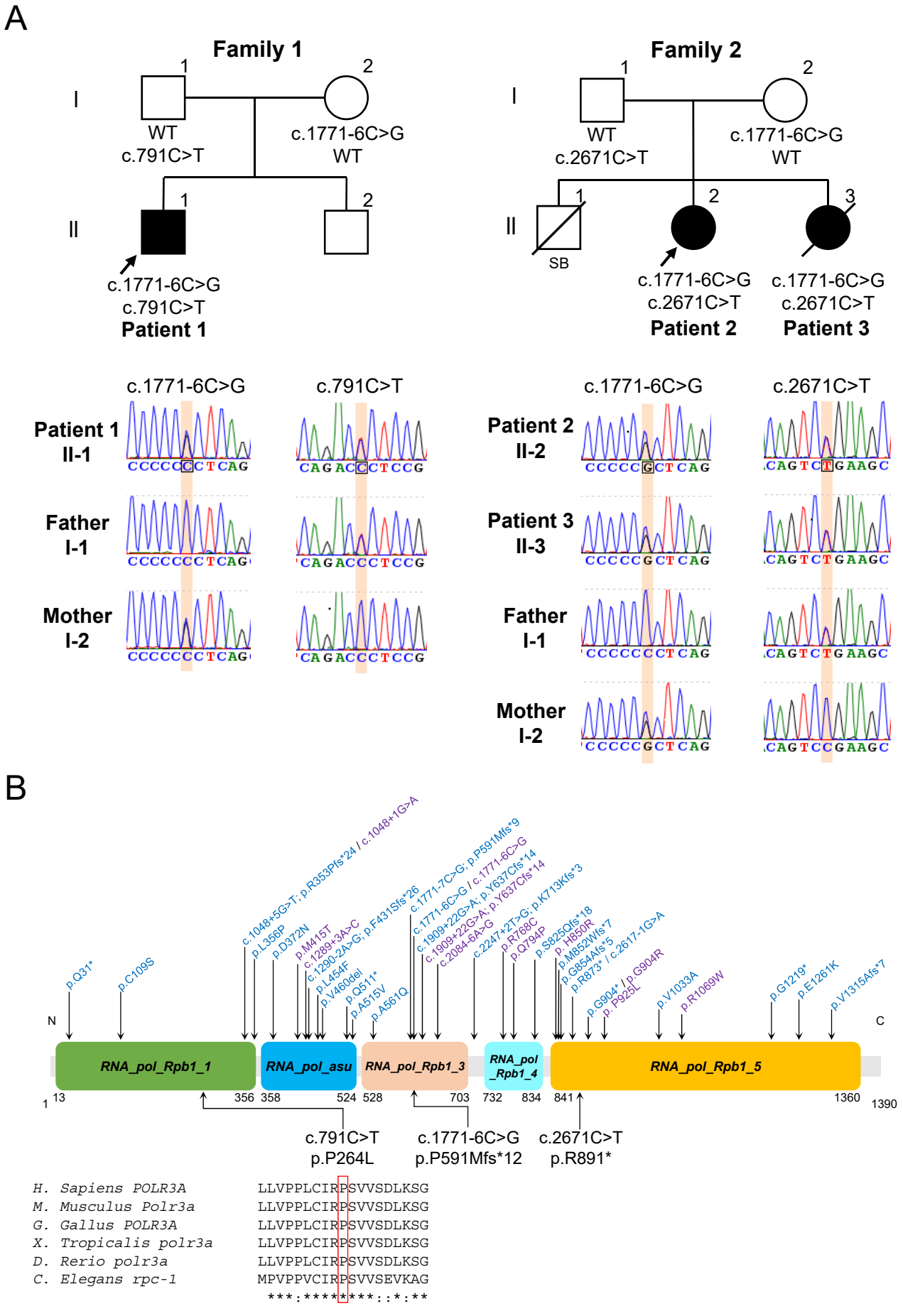
recognized in patients 2 and 3 (F, G). No signal change in red nucleus is seen in an axial T2-weighted image (E). A sagittal T1-weighted image shows no cerebellar atrophy (I). An axial T1-weighted image reveals hypointensity of putamen (K). We obtained written informed consents to post these photographs and images from their parents.

Table 1. Clinical findings of individuals with c.1771-6C>G variant in *POLR3A* variants [4-6].

Individuals	This study			Azmanov et al.			Rydning et al.	La Piana et al.
	Patient 1	Patient 2	Patient 3	ShII-1	IzII-1	IzII-2		
Variant 1	c.1771-6C>G	c.1771-6C>G	c.1771-6C>G	c.1771-6C>G	c.1771-6C>G	c.1771-6C>G	c.1771-6C>G	c.1771-6C>G
	p.(Pro591Metfs*9)	p.(Pro591Metfs*10)	p.(Pro591Metfs*11)	p.(Pro591Metfs*12)	p.(Pro591Metfs*13)	p.(Pro591Metfs*14)	p.(Pro591Metfs*16)	p.(Pro591Metfs*15)
Variant 2	c.791C>T	c.2671C>T	c.2671C>T					c.3205C>T
	p.(Pro264Leu)	p.(Arg891*)	p.(Arg891*)					p.(Arg1069Trp)
Status	C Het	C Het	C Het	Homo	Homo	Homo	Homo	C Het
Sex, age	M, 18 years	F, 23years	F, 15 years (died)	M, 52 years	M, 27 years	M, 23 years	M, 41 years	NA
Age at onset	6 months	1 year and 6 months	1 year	8 years	7 years	8 years	4 years	NA
Upper motor neuron signs								
Muscle weakness	+	+	+	–	–	–	+	NA
Spasticity	–	–	NA	–	–	–	+	+
Brisk tendon reflexes	–	+	NA	+	–	–	–	NA
Babinski sign	–	–	NA	+	+	+	+	NA
Extrapyramidal signs								
Rigidity	–	+	NA	+	+	–	NA	NA
Dystonia	–	+	+	+	+	+	+	NA
Tremor	+	–	NA	–	–	–	+	NA
Cerebellar features								
Ataxia	+	–	NA	+	+	+	+	+
Gaze-evoked nystagmus	–	+	+	+	–	–	–	NA
Abnormal smooth pursuits	–	+	NA	+	–	–	–	NA
Dysphagia	+	+	+	–	+	–	+	NA
Developmental delay	+	–	–	–	–	–	NA	+
Intellectual disability	+	+	+	–	–	–	NA	NA
Neuropsychiatric regression	+	+	+	–	–	–	NA	NA
Neuroimaging								
Signal changes in diffuse white matter	–	–	–	–	–	–	–	–
Atrophy and signal chenges in striatum	+	+	+	+	+	+	NA	NA
Signal changes in red nucleus	–	–	–	+	+	+	NA	NA
PLIC	–	–	–	–	–	–	+	+
SCP hyperintensity	–	–	–	NA	NA	NA	+	NA
Thin cervical spinal	–	–	–	NA	NA	NA	+	NA

cord								
Cerebellar atrophy	–	–	–	–	–	–	+	–
Limb deformities	–	–	–	–	Bilateral pes cavus	Bilateral pes cavus	–	NA
Dental abnormalities	–	+	+	–	–	–	–	+
Hypogonadism	–	–	NA	–	–	–	–	NA
Others							LD, HSP	

C Het, compound heterozygous; HSP, hereditary spastic paraparesis; LD, learning difficulties; NA not assessed or not available; PLIC, Abnormal signal of the posterior limb of the internal capsule; SCP, superior cerebellar peduncles


A
Family 1
I
1
2
WT
c.791C>T
c.1771-6C>G
WT
II
1
2
c.1771-6C>G
c.791C>T
Patient 1
Family 2
I
1
2
WT
c.2671C>T
c.1771-6C>G
WT
II
1
2
3
SB
c.1771-6C>G
c.2671C>T
Patient 2
c.1771-6C>G
c.2671C>T
Patient 3
c.1771-6C>G
c.791C>T
Patient 1
II-1
Father
I-1
Mother
I-2
c.1771-6C>G
c.2671C>T
Patient 2
II-2
Patient 3
II-3
Father
I-1
Mother
I-2
B
N
C
p.Q31*
p.C109S
c.1048+5G>T; p.R353Pfs*24 / c.1048+1G>A
p.L356P
p.D372N
p.M415T
c.1289+3A>C
c.1290-2A>G; p.F431Sfs*26
p.L454F
p.V460del
p.Q511*
p.A515V
p.A561Q
c.1771-7C>G; p.P591Mfs*9
c.1771-6C>G / c.1771-6C>G
c.1909+22G>A; p.Y637Cfs*14
c.1909+22G>A; p.Y637Cfs*14
c.2084-6A>G
c.2247+2T>G; p.K713Kfs*3
p.R768C
p.Q794P
p.S825Qfs*18
p.H850R
p.M852Wfs*7
p.G854Afs*5
p.R873* / c.2617-1G>A
p.G904* / p.G904R
p.P925L
p.V1033A
p.R1069W
p.G1219*
p.E1261K
p.V1315Afs*7
RNA_pol_Rpb1_1
RNA_pol_asu
RNA_pol_Rpb1_3
RNA_pol_Rpb1_4
RNA_pol_Rpb1_5
1
13
356
358
524
528
703
732
834
841
1360
1390
c.791C>T
p.P264L
c.1771-6C>G
p.P591Mfs*12
c.2671C>T
p.R891*
H. Sapiens POLR3A LLVPPLCIRPSVVSDLKSG
M. Musculus Polr3a LLVPPLCIRPSVVSDLKSG
G. Gallus POLR3A LLVPPLCIRPSVVSDLKSG
X. Tropicalis polr3a LLVPPLCIRPSVVSDLKSG
D. Rerio polr3a LLVPPLCIRPSVVSDLKSG
C. Elegans rpc-1 MPVPPVCIRPSVVSEVKAG
:*:****::*:**

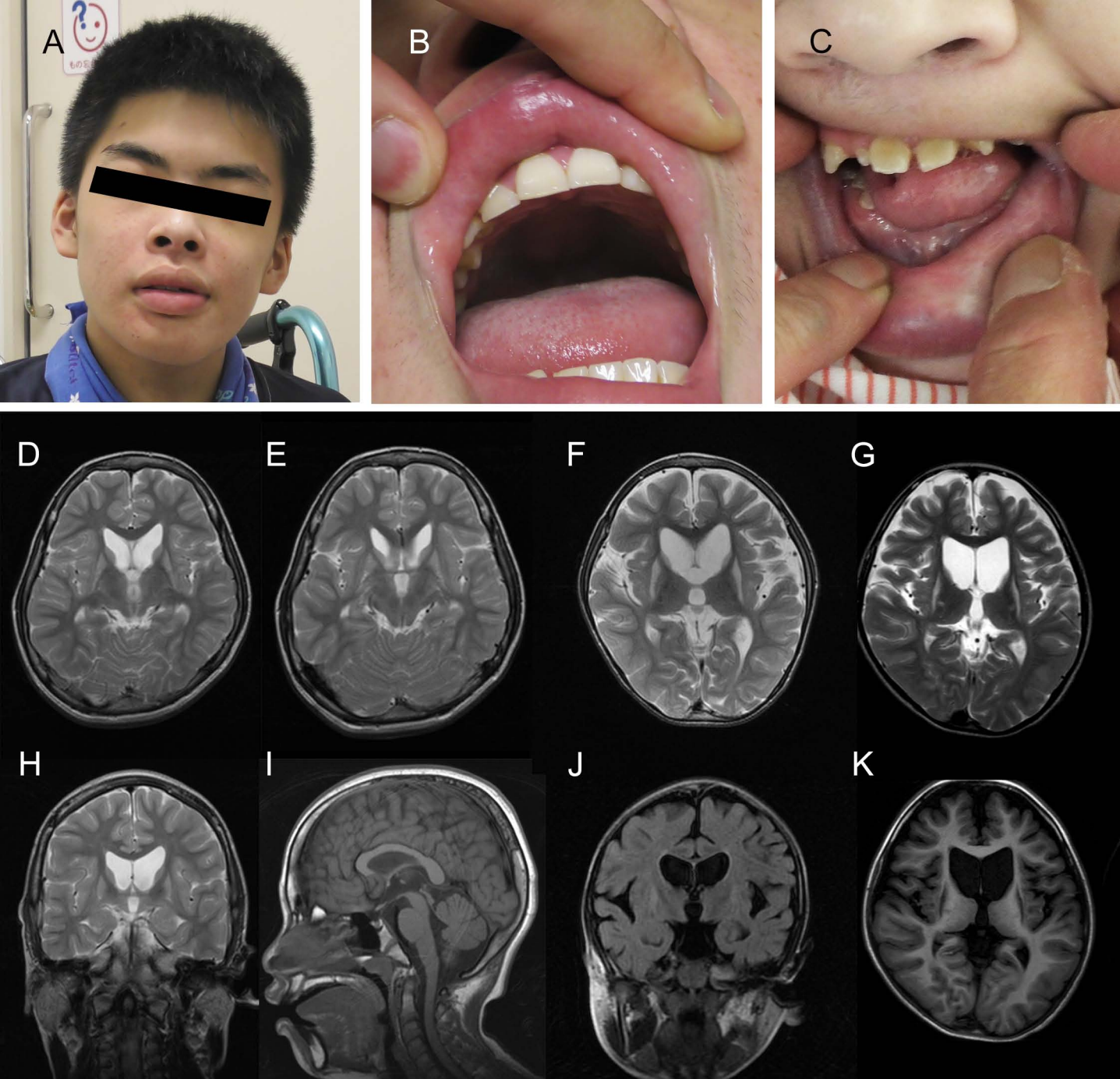

A
B
C
D
E
F
G
H
I
J
K

Table S1. Allele frequency and *in silico* prediction of *POLR3A* variants (NM_007055.4)

Family	Variant	3.5KJPN	gnomAD	SIFT	PP2 HVAR	CADD phred	M-CAP	Mutation Taster
I, II	c.1771-6C>G, p.(Pro591Metfs*9)	0.0004	0.00006024			11.52		
I	c.791C>T, p.(Pro264Leu)	–	–	0.001	0.977	28.6	0.751	1
II	c.2671C>T, p.(Arg891*)	–	–			39		

3.5KJPN, https://jmorp.megabank.tohoku.ac.jp/; gnomAD (the Genome Aggregation Database), http://gnomad.broadinstitute.org/; SIFT (Sorting Intolerant From Tolerant), http://sift.jcvi.org/; Polyphen-2 Hum Var, http://genetics.bwh.harvard.edu/pph2/; CADD (Combined Annotation–Dependent Depletion), http://cadd.gs.washington.edu/score; M-CAP (Mendelian Clinically Applicable Pathogenicity), http://bejerano.stanford.edu/mcap/index.html; MutationTaster, http://www.mutationtaster.org/.