Case Report

DOI: 10.5582/irdr.2017.01020

A novel *PGK1* mutation associated with neurological dysfunction and the absence of episodes of hemolytic anemia or myoglobinuria

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Summary **Phosphoglycerate kinase (PGK) deficiency affects three different organs: red blood cells (RBC), the central nervous system, and muscles. Next-generation sequencing identified a hemizygous *PGK1* mutation (p.V217I) in a 16-year-old Japanese male patient presenting with intellectual disability and episodes of muscle weakness of unknown etiology. Enzymatic analysis demonstrated slightly lower RBC-PGK activity and compensatory increases of other glycolysis enzymes. This is the first *PGK1* mutation found through next-generation sequencing.**

Keywords: Phosphoglycerate kinase 1 gene (*PGK1*), novel mutation, PGK deficiency, intellectual disability, muscle involvement

1. Introduction

Phosphoglycerate kinase (PGK) deficiency is a rare cause of congenital hemolytic anemia (*1*). Mutations in the phosphoglycerate kinase 1 gene (*PGK1*) result in an X-linked recessive disorder (MIM #311800) involving three tissues: red blood cells (RBC), the central nervous system (CNS), and muscles (*2*). Variable symptoms have been observed, for example, chronic anemia, exercise-intolerant myopathy, muscle weakness, cramping, myalgia, myoglobinuria, and intellectual disability (*3*).

Here, we report a rare patient with a *PGK1* mutation associated with only CNS and muscular symptoms.

2. Case Report

A 16-year-old Japanese male patient with no family history of neuromuscular or blood-cell disorders was born uneventfully at 41 weeks of gestation, weighing 3,460 g. Since early infancy, his development was delayed: head control at 7 months, sitting at 13 months, crawling and standing with support at 17 months, and walking independently at 29 months of age. Hence, he was referred to our hospital. Neurological examination showed no finding of muscular involvement, and routine laboratory examinations revealed no abnormalities.

He suffered epileptic seizures starting at 20 months of age. By 35 months, he exhibited recurrent attacks, 2-3 times per month, of transient hemiplegia, with or without tonic stiffness of the unilateral extremity, and nystagmus occurring during sleep. These attacks alternated between the two sides of the extremities. Interictal electroencephalogram (EEG) showed only mild, diffuse, background abnormality. Combinatorial use of antiepileptic drugs has controlled the attacks since 6 years of age.

At 7 years, the patient was examined by the modified Binet Intelligence Scales test, revealing an intelligence quotient (IQ) of 30. Brain magnetic resonance imaging showed nonspecific, mild, cerebral and cerebellar atrophy.

At 16 years, the patient developed recurrent peculiar episodes, characterized by sudden, early morning onset of muscle weakness lasting 1-2 hours. There were no trigger events, such as exercise, before the episodes. During the episodes, he was unable to sit or stand and

Released online in J-STAGE as advance publication May 24, 2017.

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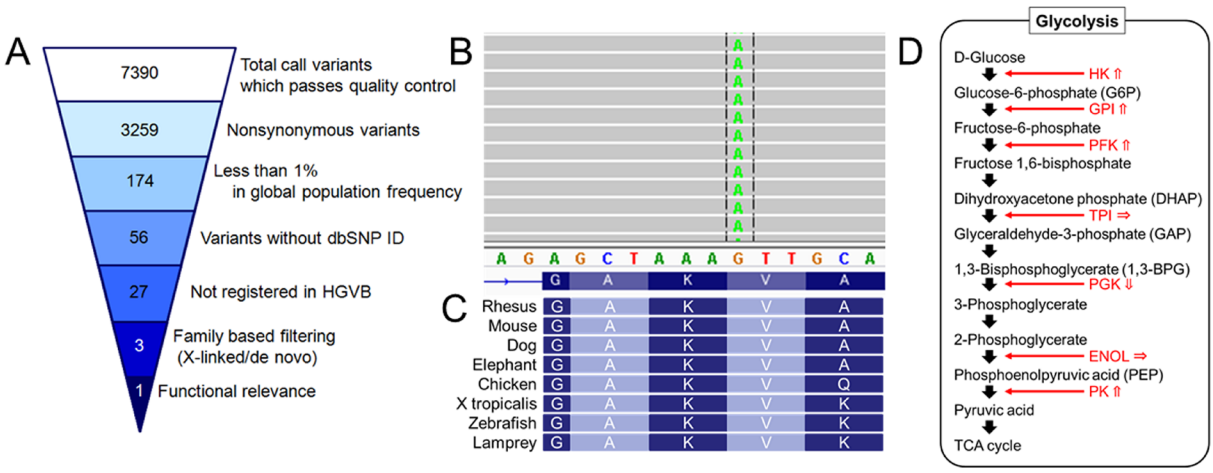

Figure 1. Results of next-generation sequencing. (A) Filtering steps and the number of filtered variants are shown in inverted pyramids. **(B)** The Integrative Genomics Viewer (IGV) shows the identified *PGK1* variant in 100% of reads. **(C)** The affected codon is conserved among species. **(D)** A schematic representation of glycolysis and the results of the enzymatic activities in this patient. Many enzymes other than PGK show increased levels.

Table 1. Summary of the previously reported mutations and its organ involvements

Items	Nucleotide change	Protein change	Anemia	CNS symptoms	Muscle involvement	RBC residual activity
Noel *et al*. (2006)	c.140T>A	p.I47N	+	+	-	5.9-10.4%
Ramirez-Bajo *et al*. (2011)	c.140T>A	p.I47N	+	+	-	20%
Maeda and Yoshida (1991)	c.266T>C	p.L89P	+	+	-	
Tsujino *et al*. (1994)	c.417+1G>T	splicing	-	+	+	
Fujii *et al*. (1992)	c.473G>T	p.G158V	+	-	+	0.7%
Cohen-Sola *et al*. (1994)	c.491A>T	p.D164V	+	+	-	
Turner *et al*. (1995)	c.491A>T	p.D164V	+	-	-	
Flanagan *et al*. (2006)	c.491A>T	p.D164V	+	+	-	<5%
Yoshida *et al*. (1995)	c.571_573del	p.K191del	+	-	-	4%
Fujii and Yoshida (1980)	c.617G>C	p.R206P	+	+	-	20-30%
Svaasandet *et al*. (2007)	c.639C>T	p.G213G (splicing?)	-	-	+	2-3%
Present patient	**c.649G>A**	**p.V217I**	**-**	**+**	**+**	**78-91%**
Hamano *et al*. (2000)	c706_709del	frameshift	-	-	+	2.9%
Ookawara *et al*. (1996)	c.755A>C	p.E252A	-	-	+	8%
Coppens *et al*. (2016)	c.756+3A>G	splicing	-	+	+	11-18%
Shirakawa *et al*. (2006)	c.756+5G>A	splicing	-	+	+	8.9%
Sugie *et al*. (1989)	c.758T>C	p.I253T	-	+	+	44%
Fujii *et al*. (1981)	c.796G>A	p.V266I*	+	+	-	16%
Fujii *et al*. (1980)	c.802G>A	p.D268N	-	-	-	20%
Valentin *et al*. (1998)	c.854A>T	p.D285V	+	-	-	49%
Rosa *et al*. (1982)	c.943G>A	p.D315N	-	-	+	21-37%
Cohen-Sola *et al*. (1994)	c.943G>A	p.D315N	-	-	+	
Maeda *et al*. (1992)	c.946T>C	p.C316R	+	+	-	5%
Noel *et al*. (2006)	c.959G>A	p.S320N	+	+	-	28-49%
Yoshida *et al*. (1972)	c.1055C>A	p.T352N	-	-	-	
Morimoto *et al*. (2003)	c.1060G>C	p.A354P	+	+	+	4.9-6.3%
Fermo *et al*. (2012)	c.1112T>A	p.I371K	+	+	+	
Sotiriou *et al*. (2010)	c.1132A>C	p.T378P	-	+	+	1.1%
Spiegel *et al*. (2009)	c.1132A>C	p.T378P	-	-	+	1.6%
Tamai *et al*. (2014)	c.1180A>G	p.T394A	+	-	-	11.2-13.9%

*; It may be a misdescription in original manuscript, suggested by Beutler (2006).

he had difficulty responding to our verbal commands. He had a dull facial appearance with ptosis. EEG taken during the episodes indicated a slight slowing of background activity, similar to that in periodic paralysis; however, levels of creatine kinase and electrolyte were unremarkable.

This study was performed in accordance with the declaration of Helsinki and was approved by the ethics committee of Tokyo Women's Medical University. After receiving informed consent, we obtained blood samples from the patient and his parents and extracted genomic DNA for sequence analysis. Next-generation

sequencing (NGS) was performed using the TruSight One v1.0 sequencing panel (Illumina, San Diego, CA) (*4*). After annotation using GATK, 7390 variants were obtained. Those variants were filtered by Variant Studio software (Illumina) (Figure 1A). Next, synonymous variants, variants more than 1% in global population frequency, variants registered in the dsSNP database, and variants registered in the Human Genetic Variation Database (HGVD) (*http://www.genome.med.kyoto-u.ac.jp/SnpDB*), which is the database provided from Kyoto University in Japan (*5*), were removed. Finally, variants with *de novo* origin or inherited in accordance with a Mendelian inheritance trait were selected. As a result, a hemizygous single nucleotide variant, NM_000291.3(PGK1):c.649G>A [NP_000282.1:p.Val217Ile] on the X-chromosome, was retained in association with X-linked recessive inheritance. This variant has never been reported previously (Table 1). The predicted functional importance scores were calculated using wANNOVAR (*http://wannovar.usc.edu/*). As shown in Table 2, many scores indicated that this variant would be damaging to the encoded protein. The identified variant was checked visually by Integrative Genomics Viewer (IGV; *http://www.broadinstitute.org/igv/*) (Figure 1B) and was verified by Sanger sequencing (data not shown). The mother of the patient was confirmed as an obligate carrier. The affected codon was conserved among species (Figure 1C).

Table 2. Results of prediction scores

SIFT_score	0.08
SIFT_pred	T
Polyphen2_HDIV_score	0.61
Polyphen2_HDIV_pred	P *
Polyphen2_HVAR_score	0.347
Polyphen2_HVAR_pred	B
LRT_score	0
LRT_pred	D *
MutationTaster_score	1
MutationTaster_pred	D *
MutationAssessor_score	1.67
MutationAssessor_pred	L
FATHMM_score	-3.28
FATHMM_pred	D *
RadialSVM_score	0.462
RadialSVM_pred	D *
LR_score	0.743
LR_pred	D *
VEST3_score	0.571
CADD_raw	3.712
CADD_phred	18.85 *
GERP++_RS	4.32
phyloP46way_placental	1.064
phyloP100way_vertebrate	9.756
SiPhy_29way_logOdds	13.55

T, tolerate; P, possibly damaging; B, benign; L, low; *, damaging is suggested.

Routine laboratory test values from the proband did not indicate hemolysis: hemoglobin, 16.7 g/dL; reticulocyte, 0.7%; total bilirubin, 0.2 mg/dL; haptoglobin, 180 mg/dL. However, the screening test for hemolytic anemia revealed RBC-PGK activity as 194 IU/gHb (normal range: 214-249 IU/gHb [mean ± SD]), suggesting slightly lower activity in this patient. In comparison with previously reported patients with PGK deficiency, the decreased level of PGK activity in this patient was not so severe (Table 1). Unexpectedly, other enzymatic activities related to glycolysis were mildly increased (Table 3). For clarity, these findings are depicted in a schematic representation of glycolysis (Figure 1D). From these results, we considered that mildly elevated activities of glycolytic enzymes other than PGK might suggest compensation for the decreased PGK activity in this patient.

3. Discussion

Generally, patients with PGK deficiency show clinical symptoms in any of three organs including RBC, muscles, and the CNS. Major symptoms are chronic

Table 3. Results of the enzymatic activities

Items	Standard (mean ± SD)	Reference	Patient	Evaluation
Hexokinase (HK)	1.08~1.46	1.44	2.08	↑
Glucose phosphate isomerase (GPI)	57.2~70.3	65.1	70.7	↑
Phosphofructokinase (PFK)	14.1~20.0	20.0	22.9	↑
Aldolase (ALD)	2.62~6.30	3.08	3.89	
Triosephosphate isomerase (TPI)	1,052~1,567	1,353	1,238	
Phosphoglycerate kinase (PGK)	214~249	246	194	↓
Enolase (ENOL)	3.89~6.30	5.62	6.01	
Pyruvate kinase (PK)	13.0~19.8	14.4	24	↑
Glucose-6-phosphate dehydrogenase (G6PD)	7.61~9.81	7.00	8.88	
6-Phosphogluconate dehydrogenase (6PGD)	9.00~10.70	8.92	9.78	
Glutathione peroxidase (GSH-Px)	37.2~51.4	40.3	42.1	
Adenylate kinase (AK)	165~307	285	319	↑
Adenosine deaminase (ADA)	0.87~1.59	0.59	0.94	
Acetylcholineesterase (Ach-E)	28.6~42.7	33.7	36.1	
Pyrimidine 5'-nucleotidase (P5N) (CMPase)*	6.90~10.8	11.9	11.7	↑
Pyrimidine 5'-nucleotidase (P5N) (UMPase)*	9.75~15.5	15.4	15.0	

units = IU/gHb (*, μmole Pi liberated/hr/gHb)

anemia (followed by recurrent hemoglobinuria caused by rhabdomyolysis), intellectual disability, and seizures (*1*). In Table 1, previously reported *PGK1* mutations are summarized (*1-3,6-27*). Some patients exhibited symptoms only in the CNS and the muscles, but not in the RBC (*24*). In particular, a few patients showed neurological symptoms similar to those in the present patient, such as hemiplegic migraines (*20,27,28*), although the details of the clinical manifestations appear to be different. Dysfunction of some glycolytic enzymes other than PGK impairs not only for RBC but also for the CNS (*29,30*). The CNS may not tolerate minor glycolysis dysfunction better than the RBC, because the CNS requires substantial energy compared to RBC.

Usually, patients with anemia or myoglobinuria are suspected to exhibit PGK deficiency, and are referred for examination of PGK activities and *PGK1* mutations. However, this patient had no clinical symptoms to suggest PGK deficiency; instead, he presented with neurological symptoms, mimicking alternating hemiplegia and later periodic paralysis. Episodes of dullness were considered the consequence of muscular involvement. Hence, this patient was referred for exome sequencing based on neurological impairments rather than for hemolytic anemia, one of the key symptoms of PGK deficiency. Therefore, similar patients with mildly impaired PGK activities, who show no sign of hemolysis and presenting only CNS and muscle symptoms, might be underdiagnosed.

Recently, a PGK heterozygous carrier mother was reported to show parkinsonism, although she showed normal PKG activity (*31*). In the future, the disease characteristics associated with *PGK1* mutations may expand as more patients with similar CNS symptoms are identified. On the other hand, this patient may still possess an unidentified etiology for his pathology. To confirm the association between mild reduction of PGK activity and neurological impairment, more patients need to be identified.

In this study, a new *PGK1* variant was identified. This is the first case of a *PGK1* variant discovery through next-generation sequencing.

Acknowledgements

We would like to express our gratitude to the patients and their families for their cooperation. We are grateful to the technicians from our laboratories, including Ms. Yumiko Ondo, Ms. Takako Aoki, and Mr. Takuya Iwasaki, for their skillful help.

This research was supported by the Practical Research Project for Rare/Intractable Diseases from the Japan Agency for Medical Research and Development (AMED) and the Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Number 15K09631 (TY). This work was supported in part by JSPS KAKENHI Grant Number 16K10041 (HK).

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(Received April 1, 2017; Revised May 10, 2017; Accepted May 18, 2017)