Brain & Development 38 (2016) 750–754

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

www.elsevier.com/locate/braindev

Case Report

# A novel *PIGA* mutation in a family with X-linked, early-onset epileptic encephalopathy

Young Ok Kim [a,*], Jae Hyuk Yang [a], Chungoo Park [b], Seul Kee Kim [c], Myeong-Kyu Kim [d], Myung-Geun Shin [e], Young Jong Woo [a]

[a] *Department of Pediatrics, Chonnam National University Medical School, Gwangju, Republic of Korea*
[b] *School of Biological Sciences and Technology, Chonnam National University, Gwangju, Republic of Korea*
[c] *Department of Radiology, Chonnam National University Medical School, Gwangju, Republic of Korea*
[d] *Department of Neurology, Chonnam National University Medical School, Gwangju, Republic of Korea*
[e] *Department of Laboratory Medicine, Chonnam National University Medical School, Gwangju, Republic of Korea*

Received 15 November 2015; received in revised form 10 February 2016; accepted 11 February 2016

**Abstract**

Early-onset epileptic encephalopathies (EOEEs) are severe and intractable infantile-onset epilepsies with progressive intellectual disability and other associated neurologic comorbidities. Whole-exome sequencing (WES) was recently used to determine the causative gene mutations in individuals with unclassified EOEEs. The present study used WES to determine the causative variant in a family with X-linked, EOEE. One potential variant (c. 427A>G, NM_002641.3; p.Lys143Glu, NP_002632.1) of the gene encoding phosphatidylinositol glycan biosynthesis class A protein (PIGA; *PIGA*) was found, which was verified by Sanger sequencing. The functional effect of this *PIGA* mutation was assessed by the surface expression levels of glycosylphosphatidylinositol-anchored proteins on blood cells: CD16 on red blood cells was significantly decreased in the proband (by 11.0%) and his mother (by 15.6%). This is the second report of a less-severe form of PIGA deficiency.


*Keywords:* *PIGA*; Epileptic encephalopathy; Focal seizure; Genetics; Infant

## 1. Introduction

Phosphatidylinositol glycan biosynthesis class A protein (PIGA) is a protein involved in the first biosynthesis step of glycosylphosphatidylinositol (GPI) anchors in plasma membrane [1–7]. GPI anchors play an important role in the attachment of proteins to the cell membrane; these GPI-anchored proteins have diverse functions and are thought to be involved in neurogenesis, complement regulation, and embryogenesis [1,3,7]. It has been proposed that germline *PIGA* mutations are lethal, whereas somatic mutations of the PIGA gene (*PIGA*) have been reported in patients with paroxysmal nocturnal hemoglobinuria [1]. Johnston et al. were the first to report a germline *PIGA* mutation in a family with neonatal seizures, brain malformation, facial dysmorphism, and other multiple anomalies [1]. Kato et al. recently reported a less-severe form of *PIGA* encephalopathy in two intellectually disabled male siblings without facial dysmorphism [2]. These patients had treatable seizures, although the previous patients

* Corresponding author at: Department of Pediatrics, Chonnam National University Medical School, 42, Jebongro, Dong-gu, Gwangju 61469, Republic of Korea. Tel.: +82 62 2206646; fax: +82 62 2226103.
*E-mail address:* ik052@jnu.ac.kr (Y.O. Kim).

http://dx.doi.org/10.1016/j.braindev.2016.02.008
0387-7604/

with a severe form of PIGA deficiency had refractory neonatal or infantile seizures and severely abnormal findings in electroencephalography (EEG), in addition to a dysmorphic face and multiple anomalies [2].

We report herein a novel missense mutation of *PIGA*, resulting in X-linked and unclassified early-onset epileptic encephalopathy (EOEE). This is only the second report of milder *PIGA* encephalopathy without facial dysmorphism, anomalies, and severe EEG abnormalities during early infancy.

## 2. Case report

A boy first presented with right hemiclonic seizures at 5 months and then exhibited multiple types of focal seizures provoked by fever or viral illness. The seizures frequently occurred in status below 2 years of age and were resistant to multiple antiepileptic drugs and a ketogenic diet. However, the seizure frequency gradually decreased, and they were transiently absent between 6 and 7 years of age. The patient developed normally before recurrent seizures, but ultimately developed a severe intellectual disability. His growth was normal up to 2 years, but his head growth slowed thereafter. He looked normal, without facial or body dysmorphism. His gait progressively deteriorated to a crouch gait by the age of 11 years. Serial EEG recordings revealed focal epileptiform discharges from multiple different parts of the brain. Brain magnetic resonance imaging (MRI) was normal at 1 year, but bilateral hippocampal sclerosis was present at 7 years (Table 1 and Fig. S1). Chromosome analysis, chromosomal microarray analysis, and extensive metabolic studies performed in this patient all produced normal results.

The proband with multifocal infantile epileptic encephalopathy was born at full term, weighing 3.2 kg, to unrelated healthy parents. Although his own birth history was uneventful, he had a brother and maternal uncles who had died at a young age. His younger brother, who also presented with seizures at 5 months, was diagnosed with Dravet syndrome at another hospital; he had no *SCN1A* mutations and died at 15 months due to pneumonia. His two uncles, who had similar phenotypes of EOEE, also died during childhood (Fig. 1).

Whole-exome sequencing (WES) was performed in trio samples composed of the proband and his parents, using the Ion AmpliSeq Exome Kit (LifeTechnologies, Carlsbad, CA, USA) on an Ion Torrent platform. The variants were prioritized using our bioinformatics workflow (Fig. S2). A novel missense mutation of *PIGA* (c.427A>G, NM_002641.3; p.Lys143Glu, NP_002632.1) was unique under the X-linked model (Table S1), which was not found in any SNP database, including the Korean Single Nucleotide Polymorphism Database (involving 400 normal Korean controls; <http://nih.go.kr/NIH_NEW/main.jsp>, in Korean). Sanger sequencing in trio samples re-verified the variant in the proband and his mother (Fig. S3).

To assess the functional effect of this *PIGA* mutation, the surface expression levels of GPI-anchored proteins were analyzed on blood cells from the proband and his mother: CD16 and CD59 on red blood cells (RBCs) and CD16 and fluorescent aerolysin on white blood cells (WBCs). The cells were stained with appropriate antibodies and analyzed by flow cytometry (FC500, Beckman Coulter, Fullerton, CA, USA). The surface expression of RBC CD16 was significantly decreased in the proband (by 11.0%) and his mother (by 15.6%), relative to the normal levels in healthy controls. However, the expression levels of other GPI-anchored proteins on RBCs and WBCs were all equivalent to the normal levels (Fig. S4).

This study was approved by the Human Research Ethics Committee of the Chonnam National University Hospital. The biospecimens provided by the Chonnam National University Hospital Biomedical Research Institute Biobank were obtained with informed consent in accordance with protocols approved by the institutional review board. Informed consent was obtained from the parents of the proband.

## 3. Discussion

Nine of the total 12 patients with germline *PIGA* mutations reported until now (including our case) exhibited a severe form of *PIGA* encephalopathy [1–7]. Eight of those nine patients exhibited myoclonic seizures, and six patients presented their first seizures at younger than 3 months. EEG revealed a suppression-burst or hypsarrhythmic pattern in most of them. Brain MRI revealed a thin corpus callosum, delayed myelination, a small cerebellum, cortical atrophy, and restricted water diffusion in the brainstem, basal ganglia, and cerebellum. Most patients were hypotonic and had facial dysmorphism. Some had anomalies of the heart, liver, and kidneys, as well as deafness and visual impairment. Serum alkaline phosphatase was elevated in five of the patients (Table 1) [1–7].

Only three patients (including our case) had a less-severe form of *PIGA* encephalopathy to date [2]. The electro-clinical characteristics in our patient were quite similar to those previously reported in two male siblings. Seizures in these three patients started at between 5 and 7 months of age. No myoclonic seizures were reported in any of the patients. Tonic or tonic–clonic seizures were noted, which in the present patient were focal seizures provoked by fever or viral illness. The seizure frequency decreased after 15 and 24 months. All had developmental slowing and moderate-to-severe intellectual disability. EEG revealed normal or focal discharges in infancy and multifocal epileptiform discharges during childhood. No generalized epileptiform discharges or