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CLINICAL REPORT

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Expanding the genotypic spectrum of *PYCR2* and a common ancestry in Thai patients with hypomyelinating leukodystrophy 10

Chawan Manaspon[1,2] | Ponghatai Boonsimma[3] | Chureerat Phokaew[4,5] | Thanakorn Theerapanon[2] | Kanokwan Sriwattanapong[2] | Thantrira Porntaveetus[2] | Vorasuk Shotelersuk[4,5]

[1]Biomedical Engineering Institute, Chiang Mai University, Chiang Mai, Thailand

[2]Genomics and Precision Dentistry Research Unit, Department of Physiology, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand

[3]Division of Medical Genetics and Metabolism, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

[4]Center of Excellence for Medical Genomics, Medical Genomics Cluster, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

[5]Excellence Center for Genomics and Precision Medicine, King Chulalongkorn Memorial Hospital, The Thai Red Cross Society, Bangkok, Thailand

Correspondence
Thantrira Porntaveetus, Genomics and Precision Dentistry Research Unit, Department of Physiology, Faculty of Dentistry, Chulalongkorn University, Bangkok 10330, Thailand.
Email: thantrira.p@chula.ac.th

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Abstract

PYCR2 pathogenic variants lead to an autosomal recessive hypomyelinating leukodystrophy 10 (HLD10), characterized by global developmental delay, microcephaly, facial dysmorphism, movement disorder, and hypomyelination. This study identified the first two unrelated Thai patients with HLD10. Patient 1 harbored the novel compound heterozygous variants, c.257T>G (p.Val86Gly) and c.400G>A (p.Val134Met), whereas patient 2 possessed the homozygous variant, c.400G>A (p.Val134Met), in *PYCR2*. Haplotype analysis revealed that the two families' members shared a 2.3 Mb region covering the c.400G>A variant, indicating a common ancestry. The variant was estimated to age 1450 years ago. Since the c.400G>A was detected in three out of four mutant alleles and with a common ancestry, this variant might be common in Thai patients. We also reviewed the phenotype and genotype of all 35 previously reported *PYCR2* patients and found that majorities of cases were homozygous with a consanguineous family history, except patient 1 and another reported case who were compound heterozygous. All patients had microcephaly and developmental delay. Hypotonia and peripheral spasticity were common. Hypomyelination or delayed myelination was a typical radiographic feature. Here, we report the first two Thai patients with HLD10 with the novel *PYCR2* variants expanding the genotypic spectrum and suggest that the c.400G>A might be a common mutation in Thai patients.

KEYWORDS
dysmorphism, global developmental delay, hypomyelination, hypotonia, microcephaly

1 | INTRODUCTION

Hypomyelinating leukodystrophy 10 or HLD10 (OMIM #616420) is an autosomal recessive neurological disorder characterized by global developmental delay, postnatal progressive microcephaly, movement disorder, and hypomyelination (Meng et al., 2017; Nakayama et al., 2015; Spagnoli et al., 2019; Zaki et al., 2016). The disorder is caused by alterations in the *PYCR2* gene (OMIM *616406) which encodes the mitochondrial enzyme, pyrroline-5-carboxylate reductase 2, that converts pyrroline-5-carboxylate to proline (Nakayama et al., 2015; Pérez-Arellano et al., 2010). Proline is an important amino acid for the function of central nervous system and connective

tissues. The pyrroline-5-carboxylate reductase 2 also plays roles in regulating $NADP^+$/NADPH balance, apoptosis, and intracellular redox potential (Liang et al., 2013; Wu et al., 2011).

To date, there have been nine studies reporting 35 patients with the *PYCR2* variants and HLD10 features (Afroze & Mercimek-Andrews, 2020; Al-Shamsi et al., 2016; Bick et al., 2017; Escande-Beillard et al., 2020; Meng et al., 2017; Nakayama et al., 2015; Spagnoli et al., 2019; Theunissen et al., 2018; Zaki et al., 2016). This study reported the first two unrelated Thai patients affected with HLD10 and two novel variants in *PYCR2*. We observed that both patients shared the c.400G>A (p.Val134Met) variant which was originated from a common ancestor and estimated to arise 1450 years ago. Additionally, we summarized the phenotype and genotype of all patients with *PYCR2* variants published in the literature.

2 | CASE REPORT

Patient 1 was a 13-year-old Thai girl. She was born at 38 weeks. The pregnancy was unremarkable. The patient started to have occasional twitching on her right cheek and a developmental delay was noticed at the 6 months of age. Brain MRI at age 9 months was unremarkable. Electroencephalogram at age 2 years showed no epileptiform discharge. Vision screening was normal. The patient was lost to follow-up and presented at age 7 years with occasional muscle spasticity for 2–3 times a week. Each episode started from rapid eye blinking followed by persistent whole-body twitching for 2–3 min. Physical examinations showed severe muscle wasting, spastic tone, clonus, hyperreflexia, deep tendon reflexes (DTR) 3+, and motor scoring scale III. Seizure and facial palsy were not observed. Plasma amino acid and urine organic acid profiles were within normal limits. Brain MRI at age 7 years showed incomplete myelination, pronounced brain volume loss along deep cerebral white matter, small corpus callosum, and prominent size of posterior body-trigone of the lateral ventricles. The EEG revealed focal spikes in the left temporo-parietal area with moderate encephalopathy, suggesting multifocal epileptic disorder. Valproic acid was prescribed at age 8 years. Clonazepam and ferrous sulfate supplement were added at age 10 years, followed by a muscle relaxer, Baclofen, at age 11 years. With medications, the duration and frequency of spastic tones were subsided. Swollen legs and feet were noticed at age 11 years.

At age 13 years, the patient showed global developmental delay. She could neither walk, sit, nor say a word. Physical examination revealed microcephaly (OFC 44.5 cm, <3rd percentile), truncal hypotonia, pectus excavatum, appendicular hypertonia, hyperreflexia, and delayed puberty. Her breast development was at Tanner stage II. She had facial dysmorphism including narrow forehead, low frontal hairline, thick hairs, deep set eyes, bulbous nose, full lips, short columella, large pinnae, overfolded helix, and high-arched palate (Figure 1(a–h)).

Patient 2 was a 2-year-old Thai girl. She was born full term with a birth weight of 3332 g. The pregnancy was unremarkable. No perinatal complication was noted. At age 1 year, the patient had feeding difficulty and showed developmental delay. Physical examination showed microcephaly (OFC 42 cm, <3rd percentile), strabismus, upturned bulbous nose, full lips, prominent ears, medial strabismus without nystagmus, hyperreflexia, poor coordination, and spasticity. Brain MRI demonstrated hypomyelination with thin corpus callosum and brainstem (Figure 1(i–k)). The videofluoroscopic swallowing study with barium sulfate solution showed normal structure and function of esophagus, esophagogastric junction, stomach, duodenum, and duodenojejunal junction. The parents of both patient 1 and patient 2 were nonconsanguineous (Figure S1, Supporting Information). A history of brain anomalies in both families was not noted.

Whole exome sequencing and Sanger sequencing were performed as previously described (Intarak et al., 2019; Porntaveetus et al., 2017) (Table S1). We identified that patient 1 harbored the novel compound heterozygous variants in *PYCR2* (NM_013328.3). A novel missense variant c.400G>A, p.Val134Met (ClinVar accession VCV000872905.1) was detected on the maternal allele and a novel missense variant, c.257T>G, p.Val86Gly (ClinVar accession VCV000872904.1) on another allele. Patient 1's father was not available for genetic test. Patient 2 was homozygous for the missense variant, c.400G>A, p.Val134Met in *PYCR2*. Both the c.257T>G and c.400G>A variants were not observed in gnomAD exome v2.1.1, dbSNP, and in-house exome database of 3206 Thai individuals consisting of patients with rare diseases and their healthy family members. The variants were located in the NAD(P) binding domain of the PYCR2 protein. They were highly conserved among species (Figure S1) and predicted to be deleterious (SIFT), probably damaging (Polyphen), possibly pathogenic (MCAP), and uncertain significance (PM2, PP3) according to the ACMG guideline (Richards et al., 2015). Based on the phenotype and genotype of both patients, the diagnosis of hypomyelinating leukodystrophy 10 (HLD10) is highly likely.

We speculated that the two patients might have a common ancestry as they shared the c.400G>A variant. Microarray genotyping was performed using the Infinium OmniZhongHua-8 Kit (Illumina, Seoul, South Korea). Three members of family 1 (patient 1, brother, and mother) and three members of family 2 (patient 2, father, and mother) were included. Using microarray and homozygosity haplotype analysis of patient 2's family members, we detected the homozygous segment of 3.2 Mb between coordinates 223632876 and 226846391 (1312 SNPs) on chromosome 1q42.13 that covered the c.400G>A variant. These coordinates were then applied to determine the haplotype of family 1. We then observed that both families shared a 2.3 Mb segment between coordinates 224440468 and 226846391 (Figure S2). These coordinates were input into the Genetic Mutation Age Estimator, which is the application that estimates the age of genetic variants based on the length of ancestral haplotypes common to individuals who share the variant (https://shiny.wehi.edu.au/rafehi.h/mutation-dating/) (Figure S3) (Gandolfo et al., 2014; Yeetong et al., 2019). The c.400G>A variant was estimated to age 58 generations or 1450 years (assuming one generation = 25 years) with a 95% confidence interval (CI) between 17.4 (425 years) and 218 (5450 years) generations. This indicates that the c.400G>A variant is identity by descent and the two families share a common ancestry.

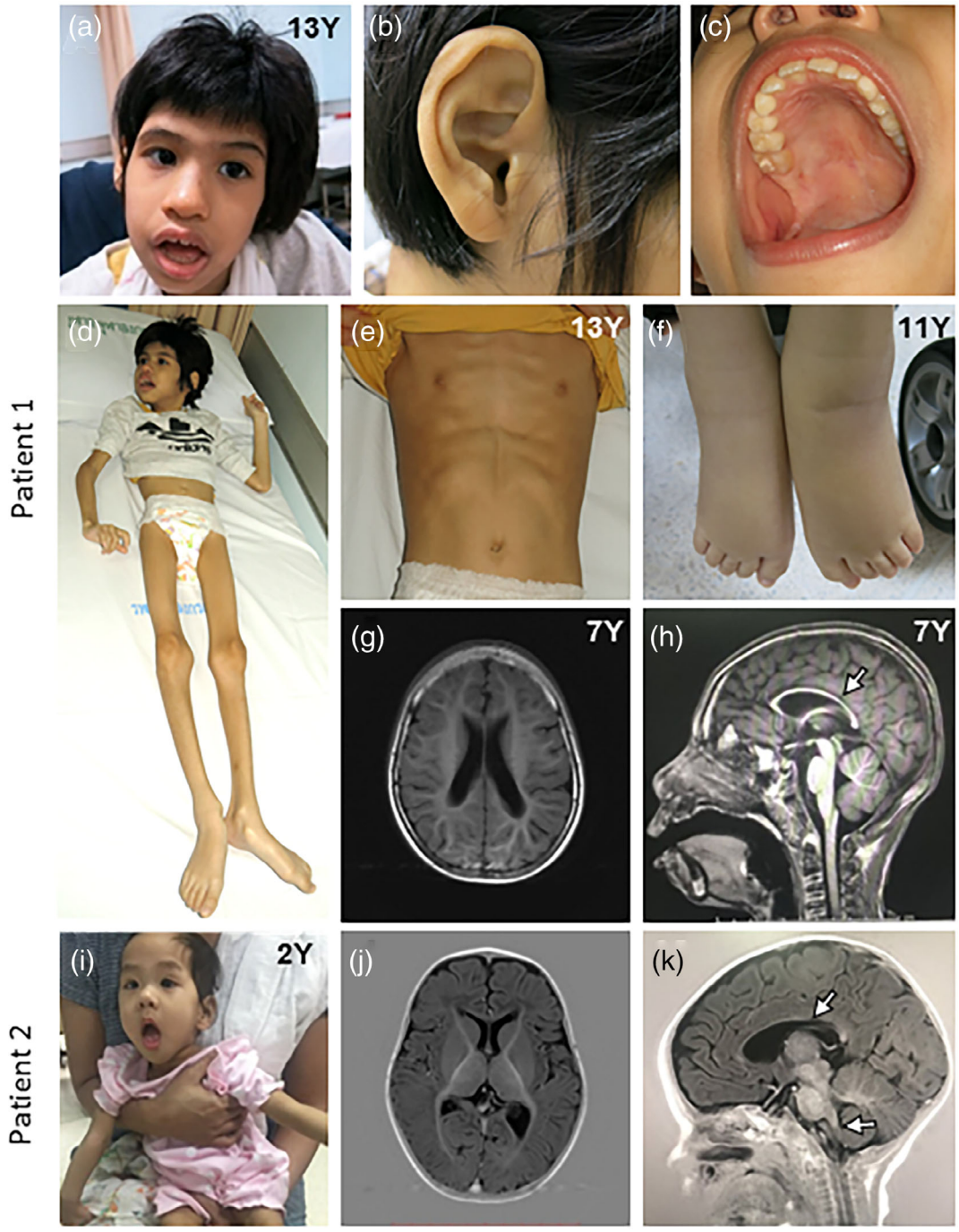

FIGURE 1 Phenotypic features of the patients. Patient 1 at age 13 years showed facial dysmorphism including oval face, narrow forehead, low frontal hairline, thick hairs, deep set eyes, bulbous nose, full lips, short columella, large pinnae, overfolded helix, and high-arched palate (a–c). She had severe muscle wasting and pectus excavatum (d, e). Swollen legs and feet were noticed at age 11 years (f). T2 weighted images (T2WI) of brain MRI showed incomplete myelination, brain volume loss, and small corpus callosum (arrow) (g, h). Facial features of patient 2 at age 2 years showed microcephaly, upturned bulbous nose, full lips, and prominent ears (i). Brain MRI showed hypomyelination, and thin corpus callosum and brainstem (arrows) (j, k)

Additionally, we reviewed and summarized the phenotypic and genotypic features of all 37 patients (including patients 1 and 2 in this study) reported with *PYCR2* variants as shown in Figure 2 and Tables S2 and S3.

3 | DISCUSSION

This study reported the first two Thai patients having HLD10 and two novel *PYCR2* variants. Both patients showed global development

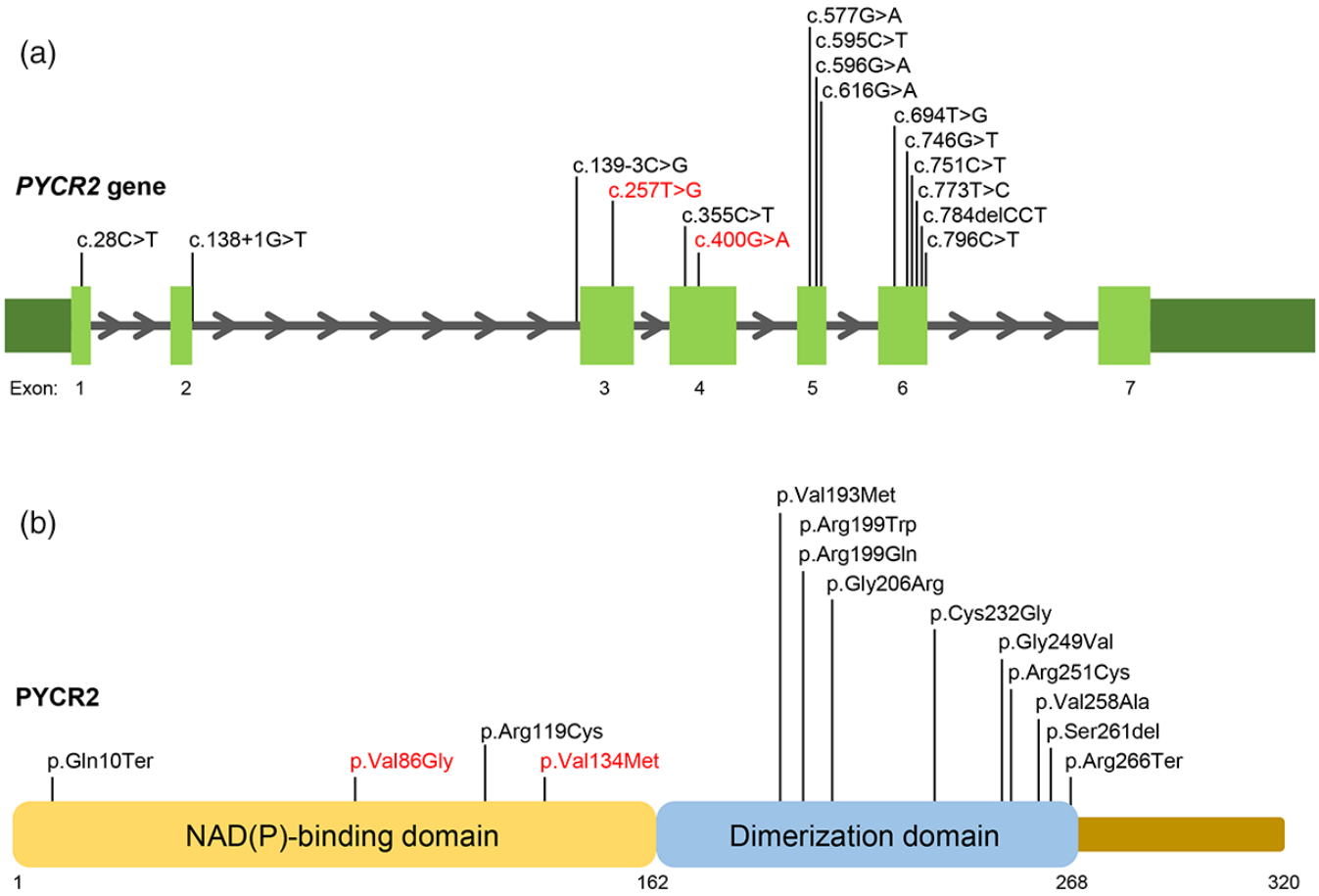

FIGURE 2 Schematic diagrams PYCR2 gene and protein with mutations. The variants identified in this study, c.257T>G (p.Val86Gly) and c.400G>A (p.Val134Met), are shown in red

delay, failure to thrive, microcephaly, facial dysmorphism, muscle wasting, spastic tone, and hyperreflexia. According to our review (Tables S2 and S3), we observed that all *PYCR2* patients had microcephaly, failure to thrive, and global developmental delay. Abnormal muscle tone and coordination including muscle wasting, central hypotonia, and peripheral spasticity were common. Only 2 out of 30 patients were able to walk and 4 out of 12 patients were able to sit with support. Seizure was present in 17 out of 31 patients (54.8%) and feeding difficulty in 10 out of 21 patients (47.6%). The consistent radiographic features were hypomyelination or delayed myelination, diminished while-matter volume, and small/thin corpus callosum. Neurodevelopmental delay was usually detected in a brain MRI within the first 5 years of life. Only 1 out of 22 patients having brain MRI image did not show a thin brainstem. However, two MRIs at 6 months intervals are required to differentiate between hypomyelination and delayed myelination, and a finding of increased myelin content indicates delayed myelination (Afroze & Mercimek-Andrews, 2020; Schiffmann & van der Knaap, 2009). We noticed that most reported cases with hypomyelination were based on a single MRI. It is therefore uncertain to determine delayed myelination or hypomyelination in the published cases. None of the reported cases had abnormal plasma and urine profiles and there was no evidence for proline depletion, suggesting that the HLD10 features are restricted to the central nervous system.

Majority of HLD10 cases were from consanguineous families (20/25 families: 80.0%). Twenty-nine out of 37 patients (78.4%) harbored the homozygous *PYCR2* variants except one previously reported patient (Bick et al., 2017) and patient 1 in this study who were compound heterozygous. In total, 16 different *PYCR2* variants were identified from 26 families, 11 variants were missense (68.75%), 2 were nonsense (12.5%), 2 were splice site (12.5%), and 1 was in-frame deletion (6.25%) (Table S4). The PYCR2 protein consists of two common domains, the NAD(P) binding and dimerization domains, which encompass 268 amino acid residues or 84% of the PYCR2 protein (320 amino acids). All variants except the splice site were observed in these two functional domains, emphasizing their functional importance (Figure 2).

Afroze and Mercimek-Andrews (2020) demonstrated that the c.796G>A (p.Arg26*) variant reported by Zaki et al. (2016) should be corrected to the c.796C>T (p.Arg266Ter) variant. We then checked all variants reported by Zaki et al. (2016) against the *PYCR2* transcript (NM_013328.4) and protein (NP_037460.2) and found the mismatches. The corrections are shown in Table S5.

The c.400G>A variant identified in both patient 1 and patient 2 has never been reported in any HLD10 cases with other ethnicities. Using haplotype analysis, it detected the shared 2.3 Mb segments covering the c.400G>A variant in the two families, indicating a common ancestry. The variant was estimated to occur around 1450 years or 58 generations ago. Regarding the c.257T>G (p.Val86Gly) variant which was identified in compound heterozygous with c.400G>A in the patient 1, the variant is conserved among several species and located in the NAD(P)-binding domain. This domain is important for the function of the pyrroline-5-carboxylate reductase (PYCR) enzyme. The PYCR uses NAD(P)H to catalyze the reduction of L-Δ1-pyrroline-5-carboxylate (L-P5C) to L-proline in the final stage of L-proline biosynthesis. The variants in the NAD(P)-binding domain have been reported in the other patients with HLD10 (Table S4). In addition, a recent study has validated the pathogenicity of the Arg119Cys variant, also in the NAD(P)-binding domain, and showed that the Arg119Cys impairs catalytic efficiency of PYCR2 up to 366 times lower than that of the wild-type enzyme, confirming its pathologic role in HLD10 (Patel et al., 2021). The above evidences suggest that the variants in the NAD(P)-binding domain could interfere the PYCR2 function, leading to HLD10 phenotype.

To conclude, we report the first two Thai patients with HLD10 and the two novel *PYCR2* variants, expanding the genotypic spectrum. We also suggest that the c.400G>A might be a common *PYCR2* variant in Thai patients with HLD10.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

ETHICS STATEMENT

This study was approved by the Institutional Review Board, Faculty of Medicine, Chulalongkorn University (IRB 264/62) and in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

AUTHOR CONTRIBUTIONS

Chawan Manaspon: Initial writing of manuscript draft; experimental design; data acquisition; data interpretation. **Ponghatai Boonsimma**: Obtained patients consent; clinical data analysis; clinical data acquisition. **Chureerat Phokaew**: Computational analysis; interpretation. **Thanakorn Theerapanon**: Edited manuscript; computational analysis; interpretation. **Kanokwan Sriwattanapong**: Assisted with writing of manuscript draft; edited manuscript. **Thantrira Porntaveetus**: Conceptualization; initial writing of manuscript draft; data interpretation; critical revision of the manuscript; submitted the manuscript. **Vorasuk Shotelersuk**: Edited manuscript; reviewed the study; conceptualization of the manuscript. All authors critically revised the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in ClinVar at https://www.ncbi.nlm.nih.gov/clinvar/, accession number VCV000872905.1 and VCV000872904.1.

ORCID

Chawan Manaspon https://orcid.org/0000-0001-8961-7901
Ponghatai Boonsimma https://orcid.org/0000-0002-2414-7010
Chureerat Phokaew https://orcid.org/0000-0002-4246-2604
Thanakorn Theerapanon https://orcid.org/0000-0001-6727-862X
Kanokwan Sriwattanapong https://orcid.org/0000-0003-1133-1983
Thantrira Porntaveetus https://orcid.org/0000-0003-0145-9801
Vorasuk Shotelersuk https://orcid.org/0000-0002-1856-0589

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