DATA REPORT

A novel *PCCB* mutation in a Thai patient with propionic acidemia identified by exome sequencingThantrira Porntaveetus¹, Chalurmpon Srichomthong^{2,3}, Kanya Suphapeetiporn^{2,3} and Vorasuk Shotelersuk^{2,3}

Propionic acidemia (PA) is an inborn error of metabolism, caused by mutations in either the *PCCA* or *PCCB* gene, leading to mitochondrial accumulation of propionyl-CoA and its by-products. Here we report a 6-year-old Thai boy with PA who was born to consanguineous parents. Exome sequencing identified a novel homozygous frameshift insertion (c.379_380insA; p.T127NfsX160) in the *PCCB* gene, expanding its mutational spectrum.

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Propionic acidemia (PA) is an autosomal recessive metabolic disorder caused by defects in the propionyl-CoA carboxylase (PCC) enzyme. PCC is involved in catabolism of branched-chain amino acids, odd chain fatty acids and cholesterol.^{1,2} Disrupted function of PCC leads to mitochondrial accumulation of propionyl-CoA and its by-products. A characteristic urine organic acid profile shows 3-hydroxypropionate, methylcitrate, tiglylglycine and propionylglycine. The clinical signs and symptoms include acute deterioration, hypotonia, metabolic acidosis, ketonuria, hypoglycemia and hyperammonemia, which usually present during infancy and early childhood.³ Without treatment, these can progress to coma and death. Patients who survive the neonatal period mostly have episodes of metabolic acidosis and hyperammonemia.⁴ It has been shown that mutations in either the *PCCA* (chromosome 13q32) or *PCCB* (chromosome 3q21-q22) genes encoding alpha or beta subunits of the PCC enzyme, respectively, can cause propionic acidemia.^{5,6} Mutations in *PCCA* are heterogeneous without a predominant mutation in the population studied, whereas *PCCB* has a limited number of mutations in various ethnic groups.^{5,7} Missense mutations are the most common defects identified in the *PCCB* gene.^{7,8} The incidence of PA ranges from 1:50,000 to 1:150,000.^{9–11} A high frequency of PA has been reported in some populations, for example, 1 in 1,000 among the Greenlandic Inuit population due to a founder effect¹² and 1:2,000–5,000 live births in Saudi Arabia partly owing to a relatively high rate of consanguinity.¹³ To date, at least 81 mutations and 17 polymorphisms have been identified in the *PCCA* gene, and 86 mutations and 7 polymorphisms in the *PCCB* gene (<http://cbs.lf1.cuni.cz/pcc/pccmain.htm>, accessed on January 2015). *PCCA* mutations have been identified in a small number of Thai patients whereas *PCCB* mutations have never been reported in Thailand.¹⁴

Here we describe a 6-year-old Thai boy with PA who was born to consanguineous parents. The first three children of this family died in infancy owing to metabolic acidosis, but without a definite diagnosis. The fourth one was healthy. The proband was the fifth child of the family. His birth weight was 3.5 kg. Newborn screening using tandem mass spectrometry (MS/MS) showed elevated

three-carbon acylcarnitine (C3). Urine organic acid analysis using gas chromatography and mass spectrometry revealed increased urinary excretion of glycine, 3-hydroxypropionate, propionylglycine, tiglylglycine and methylcitrate, a characteristic profile of PA.¹⁵ Special formula, L-carnitine and biotin were started since the first week of life. His weight and height continued to be within normal ranges, up to his last visit at the age of 6 years. However, he had delayed development; he started to walk and talk at the age of 4 years.

In this study, we aim to identify the disease-causing mutation of PA in this proband using whole-exome sequencing (WES). Blood samples were obtained. After informed consent, the patient's genomic DNA was extracted from peripheral blood leukocytes and sent to Macrogen (Seoul, Korea). Four micrograms of DNA sample was enriched by TruSeq Exome Enrichment kit and was sequenced onto HiSeq 2000 (Illumina, San Diego, CA, USA). The raw data per exome were mapped to the human reference genome hg19 using CASAVA v1.7 (Illumina). Variant calling was performed using SAM tools. Total yield of 10,475,843,220 bp was achieved. The capture efficiency varied across the target with 90.5% more than 10X and the mean read depth of target regions was 49×. The sequencing data were mapped to NCBI37 reference human genome (the version used for 1000 Genomes project), NHLBI Exome Variant Server, and in House database. WES of the proband revealed a novel homozygous frameshift insertion (NM_000532: c.379_380insA; p.T127NfsX160) in exon 4 of the *PCCB* gene.

The variant of interest was validated with Sanger sequencing. PCCBex4-F: TCT ATT TCT TCC TCC CAC TG and PCCBex4-R: GTA CCT AGC TCA GTG TCT TG were used. PCR products were treated with ExoSAP-IT (USP Corporation, Cleveland, OH, USA) and sent for direct sequencing at Macrogen. Sequence data were analyzed using Sequencher (V.5.0; Gene Codes Corporation, Ann Arbor, MI, USA). Sanger sequencing was used to confirm the presence of the identified variant in the patient and his parents. Both parents were heterozygous for the insertion (Figure 1).

Using WES, we successfully identified a mutation in the *PCCB* gene which has never previously been reported in a patient with PA. WES, next-generation sequencing (NGS), starts with breaking

¹Department of Physiology, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand; ²Center of Excellence for Medical Genetics, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand and ³Excellence Center for Medical Genetics, King Chulalongkorn Memorial Hospital, the Thai Red Cross Society, Bangkok, Thailand.

Correspondence: K Suphapeetiporn (kanya.su@chula.ac.th)

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Figure 1. Electropherograms showing (a) a homozygous mutation, c.379_380insA (p.T127NfsX160) (an arrow) in the proband. (b,c) a heterozygous mutation in both parents. (d) a homozygous wild-type in an unaffected control.

DNA into millions of short fragments and is followed by massively parallel sequencing of all exons in all genes, giving an output of > 10 GB in one run. It is suitable for detection of single-nucleotide substitutions, insertions or deletions smaller than 8–10 nucleotides.¹⁶ Compared with a conventional mutation detection technique involving PCR–Sanger sequencing, WES is both less time consuming and less expensive to identify mutations in these two genes causing PA. Moreover, it provides an opportunity to find new causative genes and actionable pathogenic variants.¹⁷

PCCA has 24 coding exons ranging from 37 to 335 bp in length, whereas PCCB have 15 exons of 57–183 bp.¹⁸ The approximate cost of PCR–Sanger sequencing is USD 45 for one exon. Setting up a molecular diagnostic test for PA using conventional sequencing of all these 39 exons would cost almost USD 2,000 in Thailand. The cost of whole human genome sequencing markedly decreases since 2007 from hundreds of millions of dollars to less than ten thousands by NGS.¹⁹ For this patient, the cost of WES was less than USD 1,000 and validation of WES with Sanger sequencing was about USD 150. Regarding turnaround time, PCR–Sanger sequencing of 39 exons would take several months, while WES takes only a few weeks.

WES reveals a homozygous frameshift mutation in PCCB expected to result in unstable mRNA, which might be degraded by nonsense-mediated mRNA decay. It has never been previously described. His parents were heterozygous for the mutation. It is absent in 100 exome Thai controls.

In conclusion, we identified a novel homozygous frameshift insertion, c.379_380insA; p.T127NfsX160, in the PCCB gene associated with PA in a Thai family.

HGV DATABASE

The relevant data from this data report are hosted at the Human Genome Variation Database at <http://dx.doi.org/10.6084/m9.figshare.hgv.693>.

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COMPETING INTERESTS

The authors declare no conflict of interest.

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