Clinical characteristics and mutation analysis of propionic acidemia in Thailand

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Original article

Background: **Propionic acidemia (PA) is caused by a deficiency of propionyl CoA carboxylase. A characteristic urine organic acid profile includes 3-hydroxypropionate, methylcitrate, tiglylglycine, and propionylglycine. The diagnosis of PA is confirmed by detection of mutations in the *PCCA* or *PCCB* genes. We herein report the clinical and molecular findings of four Thai patients with PA.**

Methods: **Clinical findings of four Thai patients with PA were retrospectively reviewed. Urine organic acids were analyzed by gas chromatography-mass spectrometry. PCR-sequencing analyses of encoding exons and intron/exon boundaries of the *PCCA* and *PCCB* genes were performed.**

Results: **All patients had neonatal onset of PA. One patient died of cardiomyopathy, and another one of pneumonia and metabolic decompensation. The remainder experienced significant neurocognitive impairment. Mutation analysis of the *PCCA* gene identified homozygous c.1284+1G>A in patient 1, c.230G>A (p.R77Q) and c.1855C>T (p.R619X) in patient 2, homozygous c.2125T>C (p.S709P) in patient 3, and only one mutant allele, c.231+1G>T in patient 4. No *PCCB* mutation was identified. Four mutations including c.230G>A, c.231+1G>T, c.1855C>T, and c.2125T>C have not been reported previously.**

Conclusions: **The clinical and molecular study of these Thai patients provided additional knowledge of the genotype and phenotype characteristics of PA. The results of the study suggested that *PCCA* mutations in Asian populations were distinct from those of other populations.**

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Key words: **mutations; propionic acidemia; Thailand**

Introduction

Propionic acidemia (PA; MIM 606054) is an autosomal-recessive disorder caused by deficiency of propionyl CoA carboxylase (PCC; EC 6.4.1.3), a mitochondrial biotin-dependent enzyme in the catabolic pathway of amino acids, including valine, threonine, isoleucine, cholesterol side chains, and odd-chain fatty acids.[1] PCC catalyzes the carboxylation of propionyl CoA to yield D-methylmalonyl-CoA. PCC comprises a α6β6 structure.[2] The α and β subunits are encoded by the *PCCA* and *PCCB* genes localized on chromosomes 13q32 and 3q21-q22, respectively.[3,4] To date, 81 mutations and 17 polymorphisms, and 86 mutations and 7 polymorphisms have been identified in the *PCCA* and *PCCB* genes, respectively.[5] Missense mutations are predominant (-40%), followed by small insertions/deletions and splicing mutations and, in the case of the *PCCA* gene, large genomic deletions.[6]

The clinical picture of PA is heterogeneous. Affected patients most commonly present within the neonatal period with nonspecific clinical signs, such as vomiting, refusal to feed, lethargy, hypotonia, seizures, metabolic acidosis and/or hyperammonemia. Patients can deteriorate quickly and lapse into coma, then death if the disorder is not recognized or if patients are already too severely compromised to respond to therapy.[7] Late-onset PA may be characterized by failure to thrive, developmental delay, and various neurological symptoms.[8] Brief clinical summaries and the urine organic acid profiles of only two Thai PA patients have been described,[9] but their causative mutations have not

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been reported. Here we report the clinical course and mutation spectrum of four Thai patients with PA.

Methods

Patients

We retrospectively reviewed the cases of four unrelated Thai patients diagnosed with PA at Siriraj and Phramongkutklao Hospitals between 2000 and 2010. All patients were diagnosed via urine organic acid analysis using urease treatment extraction with gas chromatography-mass spectrometry (GC-MS)[10] after presenting clinical symptoms, and not through neonatal screening. Informed consent was obtained from the families of the patients. The study was approved by the Institutional Ethical Review Board of Faculties of Medicine of Siriraj Hospital.

Mutation analysis of the *PCCA* and *PCCB* genes

Genomic DNA was extracted from peripheral blood. All coding exons as well as flanking introns in the *PCCA* and *PCCB* genes were PCR-amplified and directly sequenced as described previously.[11,12]

Results

Case presentations

Case 1 was a boy of first-degree-cousin parents. He was born full term with birth weight of 3 kg. He had a tachypnea at 10 hours, then developed vomiting and coma. Basic blood chemistries showed metabolic acidosis and hyperammonemia. A metabolic work-up at 2 months of age yielded the following: urine organic acids showed increased excretions of methylcitrate, propionylglycine, and 3-hydroxypropionate; plasma amino acids showed increased glycine (3405 μmol/L, reference range: 94-463 μmol/L); and acylcarnitine profile revealed increased C3-acylcarnitine (22.7 μmol/L, reference: <8.17 μmol/L), and C3/C2 acylcarnitine ratio (8.54, reference: <1.8). He was initially treated with intravenous fluid, low-isoleucine, -methionine, -threonine, and -valine special formulas, oral carnitine, and biotin. He developed seizures at 2 years of age. Thereafter, he had several episodes of infections, diarrhea, metabolic acidosis and epilepsy. Gastrostomy tube placement with fundoplication was performed at the age of 8 years which improved caloric intake, and increased his weight from the 3rd percentile to 25th percentile. However, this did not decrease the frequency of his hospital admissions due to infections and metabolic decompensation. He is now 12 years old with profound mental retardation. His most recent cardiac evaluation did not show long QT or cardiomyopathy.

Case 2 was a girl of non-consanguineous parents. She was diagnosed with neonatal sepsis on 2 days of life. At 25 days, she developed skin abscesses and seizures. Laboratory revealed pancytopenia [hemoglobin (Hb), 11 g/dL; white blood cells (WBC), 2200/mm^3; absolute neutrophil count (ANC), 576/mm^3; platelets 83 000/mm^3]. Blood chemistries did not show acidosis (bicarbonate, 24 mmol/L) but urine analysis revealed ketonuria. Blood ammonia was not obtained at that time. She was admitted in the hospital several times due to recurrent urinary and respiratory tract infections, and neutropenia. Bone marrow aspiration indicated maturation arrest of myeloid series. This led to a diagnosis of cyclic neutropenia. She had mild developmental delay with normal growth parameters. During an admission at 10 months of age, blood chemistry revealed mild metabolic acidosis (bicarbonate, 18 mmol/L) and slight hyperammonemia (ammonia, 127 μmol/L, reference: 11.2-48.2 μmol/L). This prompted a metabolic work-up. Urine organic acids revealed increased excretions of propionylglycine, 3-hydroxypropionate, and methylcitrate, which indicated propionic acidemia. Plasma amino acids showed increased glycine (707 μmol/L, reference: 90-221 μmol/L). The patient was treated with low-isoleucine, -methionine, -threonine, and -valine diet, L-carnitine (100 mg/kg per day), and biotin (10 mg/day). Thereafter, she experienced only a few metabolic crises and mild developmental delay. She died at 6 years of age from sudden cardiac arrest probably secondary to cardiomyopathy. Free carnitine level was not measured before the event. The parents refused postmortem autopsy.

Case 3 was a boy of a consanguineous marriage. He was born at term after an uneventful delivery with a birth weight of 2.76 kg. He developed tachypnea at day 11, and was diagnosed with presumed sepsis. At one month of age, he had generalized tonic clonic seizures, poor weight gain, and metabolic acidosis (bicarbonate, 16 mmol/L). Blood ammonia was mildly elevated (84 μmol/L). Complete blood count (CBC) showed leukopenia (WBC, 3350/mm^3). Urine organic acids at 2 months of age demonstrated increased excretions of 3-hydroxypropionate, methylcitrate, tiglylglycine, and propionylglycine. He was treated with low-isoleucine, -methionine, -threonine, and -valine special formulas, L-carnitine, biotin, and metronidazole. The patient could not achieve good metabolic control due in part to inadequate caloric intake. The patient's weight and height remained below the third percentile since one month of age. The parents refused a gastrostomy tube placement. The patient's cognitive development was severely delayed at 2.5 years of age. The patient's most recent echocardiogram revealed no cardiomyopathy. He died at 3 years of age from pneumonia and metabolic decompensation.

Case 4 was a girl of non-consanguineous parents. She was born at term with a birth weight of 3325 g. She developed lethargy at 7 days of life. Physical examination showed nothing remarkable except for hypotonia. Initial investigations showed metabolic acidosis with a wide anion gap, and severe hyperammonemia (ammonia > 700 μmol/L). Urine organic acids at that time revealed increased excretion of only 3-hydroxypropionate and lactate. She was treated since 2 weeks of age. When she developed metabolic decompensation again at 10 months, urine organic acids showed the typical profile of PA including increased levels of 3-hydroxy propionate, propionylglycine, tiglylglycine, and methylcitrate. Her cognitive development was moderately delayed at age of 2.5 years.

Mutation analysis

Five different *PCCA* mutations and no *PCCB* mutations were identified in this study. Two mutant alleles were identified in three patients (case 1-3), which confirmed a clinical diagnosis of PA. For case 1, homozygous c.1284+1G>A mutation of the *PCCA* gene was identified, and the heterozygous mutation was identified in both parents. Compound heterozygosity of c.230G>A (p.R77Q) and c.1855C>T (p.R619X) mutations of the *PCCA* gene were identified in case 2. The heterozygous c.230G>A and c.1855C>T mutations were identified in the father and mother, respectively. Homozygous c.2125T>C (p.S709P) mutation of the *PCCA* gene was identified in case 3, and the heterozygous mutation was identified in both parents. Only one mutant allele, heterozygous c.231+1G>T, was identified in case 4 but the clinical and urine organic acid findings strongly suggested PA. The heterozygous c.231+1G>T mutation was also identified in the father, and no *PCCA* mutation was identified in the mother. Clinical findings and genotypes are summarized in the Table and Fig.

Table. Summary of the clinical features and genotypes of the patients with propionic acidemia

Case *no.*	Consanguinity	Onset	Diagnosis	Current age	Outcome	Mutations identified					
						Allele 1	Predicted protein change	Ex./ In.	Allele 2	Predicted protein change	Ex./In.
1	+	10 h	2 mon	12 y	Profound MR, epilepsy	c.1284+1G>A	Ex. 13-14 skipping	In. 14	c.1284+1G>A	Ex. 13-14 skipping	In. 14
2	-	2 d	11 mon	Died at 6 y	Mild DD, possible cardiomyopathy	c.230G>A	p.R77Q	Ex. 3	c.1855C>T	p.R619X	Ex. 21
3	+	11 d	1 mon	Died at 3 y	Severe DD, growth failure, epilepsy	c.2125T>C	p.S709P	Ex. 24	c.2125T>C	p.S709P	Ex. 24
4	-	2 d	11 d	2 y 11 mon	Moderate DD, normal growth	c.231+1G>T	Ex. 3-4 skipping	In. 3	ND	-	-

MR: mental retardation; DD: developmental delay; ND: not detected; In.: intron; Ex.: exon.

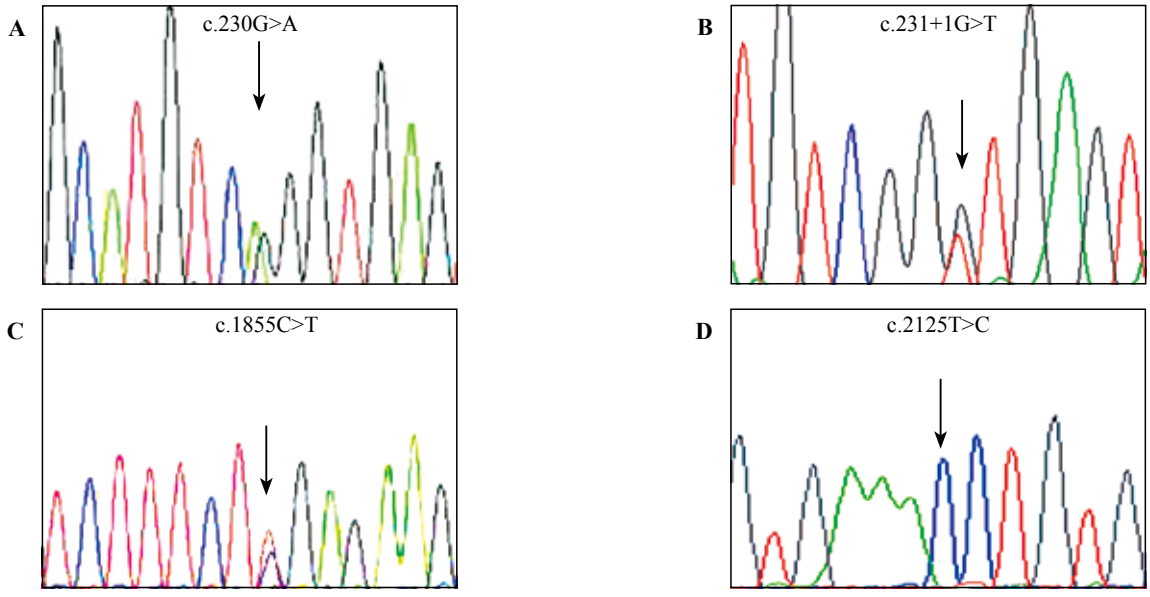

Fig. Novel *PCCA* mutations. Chromatograms demonstrating the nucleotide changes detected. Black arrows indiate the c.230G>A (**A**), c.231+1G>T (**B**), c.1855C>T (**C**), and c.2125T>C (**D**).

Discussion

In Thailand, as in other developing countries, there has been a gradual development of technologies to diagnose inborn errors of metabolism over the last decade.[13] In this study, all PA cases presented in an acute neonatal form. Patient 2 initially presented with severe neutropenia without metabolic acidosis but her blood ammonia level was not measured. This resulted in a delay until an accurate diagnosis, during which the patient had recurrent infections and metabolic acidosis appeared. Neutropenia/anemia/pancytopenia in PA and other organic acidemias might be a consequence of bone marrow suppression. This is probably due to accumulation of CoA esters which inhibits the maturation of bone marrow precursors.[14] Metabolic acidosis was documented in only two thirds of PA patients whereas hyperammonemia was found in >90% of patients.[15] Therefore, blood ammonia could have been elevated in patient 2, and this should be a better screening test for PA rather than serum electrolytes alone. For the final outcomes, two patients died and the survivors had moderate to profound developmental delay/mental retardation. The unfavorable outcomes of our patients are partly related to the delayed diagnosis (three of the four patients were diagnosed at or after 1 month of age). According to a study by Grünert et al,[15] the mortality rate was reduced in patients identified by newborn screening; however, the clinical course or neurocognitive outcome was not improved. Cardiomyopathy, which is a common long-term complication in PA, was suspected in case 2 and was probably a cause of death.[16]

To our knowledge, our study is the first mutation study of PA in Southeast Asia. Among Asian countries, common mutations in the *PCCA* and *PCCB* genes have been identified in Japanese and Korean patients.[17,18] In our study, we identified five different mutations in the *PCCA* gene, whereas no mutation in the *PCCB* gene was identified. The same mutations were identified only as homozygosity in patients with parental consanguinity. In case 4, only one mutant allele of the *PCCA* gene was identified. However, the clinical and organic acid findings were typical for PA. An unidentified mutation in another allele of patient 4 could be due to many possibilities: for example, a mutation is located in unexplored regions, such as regulatory element, promoter, or intronic regions; or a mutation is a large genomic deletion, rendering it undetectable by our methods. However, the unidentified mutation is most likely to be a large deletion, which contributes to the high percentage (-20%) of alleles in *PCCA*-deficient patients.[6] Therefore, without using multiple ligation probe amplification (MLPA) and/or Southern blot analysis to detect large genomic copy variations, a substantial number of *PCCA* mutations would be missed. The c.1284+1G>A mutation has been previously reported[11] that is located at the splice donor site of intron 14, and causes exons 13-14 skipping which affects the biotin carboxylase domain.[11] The c.230G>A, c.231+1G>T, c.1855C>T, and c.2125T>C mutations are novel. Effects of the novel missense mutations on protein are predicted by in silico prediction programs [PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/)]. For the p.R77Q mutation, it is predicted by PolyPhen-2 to be probably damaging with a score of 1.00, and by SIFT to be damaging with a score of 0. For the p.S709P mutation, it is predicted by PolyPhen-2 to be possibly damaging with a score of 0.555, and by SIFT to be damaging with a score of 0.03. Both amino acid residues are located at the important functional domains, namely biotin carboxylase and biotinylation domains, respectively. Therefore, p.R77Q and p.S709P are likely to be pathogenic mutations. Another *PCCA* mutation at codon 77, namely c.229C>T (p.R77W), has been reported in several populations.[17,19,20] The recurrent mutations at the same codon 77 could be explained by a mutation hot spot (CpG sequence). The c.231+1G>T mutation is located at the splice donor site of intron 3, and predicted to cause splicing aberration. The previously reported mutation at the same site, namely c.231+1G>C, results in exons 3-4 skipping which also affects the biotin carboxylase domain.[21] The p.R619X mutation is located in exon 21, and predicted to cause premature termination codon. Therefore, both are likely to be pathogenic mutations.

In summary, the clinical and molecular study of these four Thai patients provides additional knowledge of the genotype and phenotype of PA. Our data suggest that *PCCA* mutations in Asian populations are distinct from other populations. In addition, four novel mutations, c.230G>A, c.231+1G>T, c.1855C>T, and c.2125T>C in the *PCCA* gene, were identified, expanding the mutation spectrum of this gene.

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