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Original

Clinical features of 27 Turkish Propionic acidemia patients with 12 novel mutations

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Propionic acidemia (PA) is an inherited metabolic disease caused by the deficiency of one of the four biotin-dependent enzymes propionyl-CoA carboxylase (PCC), and is characterized by coma and death in unrecognized patients, additionally late diagnosis leads to severe developmental delay and neurological sequels. Manifestations of PA over time can include growth impairment, intellectual disability, seizures, basal ganglia lesions, pancreatitis, and cardiomyopathy. Other rarely reported complications include optic atrophy, hearing loss, premature ovarian insufficiency, and chronic renal failure. Mutations in PCCA-PCCB genes cause the clinically heterogeneous disease of PA. In this study, we investigate the mutation spectrum of PCCA-PCCB genes and phenotypic features of 27 Turkish patients with PA from the South and Southeast parts of Turkey. We report 12 novel PA mutations, five affecting the PCCA gene and 7 affecting the PCCB gene.

Key words: Propionic acidemia, novel mutation, clinical features, PCCA, PCCB.

Propionic acidemia (PA; MIM# 232000 and 232050) is an autosomal recessive inherited metabolic disorder caused by the deficiency of the mitochondrial biotin-dependent enzyme propionyl-CoA carboxylase (PCC), which catalyzes the conversion of propionyl coenzyme A (propionyl-CoA) to methylmalonyl-coenzyme A (methylmalonyl-CoA).[1] PCC is essential for the catabolism of the branched-chain amino acids, odd-numbered fatty acids, cholesterol, and other metabolites.[2] The PCC holoenzyme is a dodecamer composed of an equal number of alpha and beta subunits.[3] The alpha subunit is encoded by the *PCCA* gene (chromosome 13q32, MIM#232000). The beta subunit is encoded by the *PCCB* gene (chromosome 3q13.3–q22, MIM#232050).[4] Mutations in either the *PCCA* or *PCCB* gene cause PCC deficiency. To date, 149 mutations in the *PCCA* gene and 131 in the *PCCB* gene have been collected in the Human Genome Mutation Database (HGMD® Professional 2018.4). The PCC deficiency results in increased amounts of diagnostic intermediates of methylcitrate, 3-hydroxypropionate and propionylcarnitine in urine and plasma.[5] The clinical features of PA are highly heterogeneous. Affected patients most commonly present in the neonatal period. Vomiting, refusal of feeding, dehydration, lethargy, hypotonia, seizures, metabolic acidosis, hyperammonemia, and hypoglycemia are the prominent signs and symptoms of the disease. The clinical picture can quickly progress to coma and death in unrecognized patients, however late diagnosis leads to severe developmental delay and neurological sequelae.[6-7] Additionally, chronic complications of PA may affect the whole body with neurological, cardiologic, hematologic, immunologic, endocrine, and gastrointestinal

manifestations.[8] Therefore, early diagnosis and proper treatment are crucial. The main principles of PA treatment consist of protein-restricted diet, carnitine supplementation, and prevention of catabolism in fasting situations.

In this study, we investigate the mutation spectrum of *PCCA-PCCB* genes and phenotypic features of 27 Turkish patients with PA from the South and Southeast parts of the country.

Material and Methods

We conducted a retrospective study of patients with PA at Çukurova University Faculty of Medicine, Department of Pediatric Metabolism and Nutrition. Twenty-seven patients with PA, who came from 21 unrelated families were included in this study. All the patients were diagnosed with the clinical features confirmed by urine organic acid analysis and gas chromatography-mass spectrometry (GC-MS) and not through a neonatal screening program (NSP). Clinical diagnosis was confirmed by *PCCA* and *PCCB* genes analysis. *PCCA* and *PCCB* genes sequence analysis was performed by using MiSeq next generation sequencing (NGS) platform (Illumina, San Diego, CA, USA). The Ethics Committee of the Cukurova University Faculty of Medicine approved this study (Approval number: 2017/85-52).

Mutation Analyses of PCCA and PCCB genes

Genomic DNA was extracted according to the manufacturer's standard procedure using the Anatolia Magnesia Blood Kit (Anatolia Geneworks, Turkey). All coding exons and their flanking splice site junctions were amplified using PCR primers, designed with PRIMER© – Primer Designer v.2.0 (Scientific & Educational Software programme) software. Next-generation sequencing was carried on MiSeq (Illumina Inc. (Illumina, San Diego, CA, USA)) Sequences were aligned to the hg19 genome within MiSeq Reporter software (Illumina Inc.). Visualization of the data was performed with IGV 2.3 (Broad Institute, Cambridge, Massachusetts) software.

Mutations were screened in the literature. *In silico* analysis of the variations was done with Varsome software. Variants were also checked in 2500 exome sequencing data of our patients. This data is of patients applied to our Intergen Genetics Center for diagnostic purposes. HiSeqControl Software, CLC Bio Genomics Workbench, SeattleSeq Annotation was used for analysis.

Results

The frequently observed initial symptoms, signs and laboratory findings that lead to PA diagnosis were feeding difficulties (48.1%), asymptomatic with family history of PA (40.7%), vomiting (37%), tachypnea (37%), hypotonia (26%), lethargy (14.8%), seizures (11.1%), pancytopenia (7.4%), hypoglycemia (3.7%), and feeding refusal (3.7%). Patients' characteristics and clinical data are presented in Table 1. In this study, most of the parents (96.3%) were consanguineous. Positive family history rate was 19/27 (70.4%). The current age of the patients ranged from 0.3-35.6 years. The age at onset of symptoms was from birth to 18 months (median:3 days, mean: 40.44±108.8), and the age of diagnosis ranged from the prenatal period to 10.5 years (median: 35.5 days, mean: 295.12±828.3 days). Nineteen patients became symptomatic within the first 10 days of life, 4 patients within 10-30 days of life, and the remaining 4 patients in the second month of life. Twenty-two patients had severe clinical phenotype, and the remaining five patients had milder phenotype. Seventeen patients (63%) were still alive, while 10 patients had died during an acute metabolic crisis. Metabolic acidosis was reported in 23 patients, and 16 out of 23 patients had hyperammonemia (Table I). Hyperammonemia was seen as high as in urea cycle defects. The highest concentration found in our study cohort was >1000 μmol/L. Pancytopenia was found in six patients and creatine kinase levels were increased in six patients (Table II). A total of 12 different *PCCB* and 8 different *PCCA* gene mutations were identified (Table III), of which 12 were novel. Only one same mutant allele was identified in two unrelated patients (patient 16-26). Different mutations were detected in all other patients. No genotype-phenotype correlations were found.

Table I. Clinic Features of Patients.

Patient no	Current age (years)	Sex	Consanguinity	Abnormality in family history	Age at onset /age at diagnosis	Onset of symptoms	Clinical phenotype
1* sib of 2	0.3	M	Y	Y	Prenatal	Prenatal	Severe
2* sib of 1	Ex	F	Y	Y	3d/55d	Hypotonia,feeding difficulties, seizures, pansitopenia	Severe
3	3.3	M	Y	N	13d/56d	Hypotonia,feeding difficulties, lethargy, tachypnea	Severe
4	13.8	M	Y	Y	6m/7m	Vomiting, seizures	Mild
5* sib of 6	1.2	M	Y	Y	2d/5m	Family scan	Mild
6* sib of 5	11.8	F	Y	Y	18m/10.5y	Feding refusal, cyclic vomiting related to infections	Mild
7	35.6	F	Y	Y	1m/6y	Cyclic vomiting related to infections, feding difficulties, after acute viral hepatitis	Mild
8	3.3	F	N	N	4m/1y	Hypotonia, feding difficulties, lethargy, metabolic acidosis	Severe
9* sib of 10	0.3	M	Y	Y	1d/3d	Tachypnea	Severe
10* sib of 9	Ex	M	Y	Y	2d/7d	Tachypnea, hypoactivity	Severe
11	5.5	F	Y	Y	2d/25d	Feeding difficulties, hypoactivity	Severe
12	6.8	F	Y	N	3d/1m	Feeding difficulties, hypoglycemia, metabolic acidosis	Severe
13	7.9	M	Y	N	1m/5m	Cyclic vomiting related to infections, tachypnea, metabolic acidosis	Severe
14	2.1	M	Y	N	3m/4m	Vomiting	Severe
15	Ex	F	Y	N	3d/25d	Feeding difficulties, metabolic acidosis, hyperammonemia	Severe
16	2.5	F	Y	Y	10d/6m	Hypotonia, achypnea, family history	Mild
17	Ex	F	Y	Y	2d/5d	Family history	Severe
18* sib of 19	12.1	M	Y	Y	3d/NA	Family history, vomiting	Severe
19* sib of 18	3.6	M	Y	Y	3d/prenatal	Prenatal, vomiting, lethargy, metabolic acidosis	Severe
20* sib of 21	Ex	M	Y	Y	3d/11d	Vomiting, feeding difficulties, metabolic acidosis, lethargy	Severe
21* sib of 20	0.8	F	Y	Y	3d/41d	Feeding difficulties, tachypnea, metabolic acidosis, hyperammonemia, family history	Severe
22* cousin of 23	Ex	M	Y	Y	9d/11d	Feeding difficulties, tachypnea, hyperammonemia, family history	Severe
23* cousin of 22	Ex	M	Y	Y	3d/14d	Feeding difficulties, vomiting, tachypnea, family history, dehydration	Severe
24	Ex	F	Y	Y	4d/10d	Feeding difficulties, hypotonia, lethargy	Severe
25	Ex	F	Y	Y	1d/10d	Feeding difficulties, vomiting, tachypnea, metabolic acidosis, pansitopenia	Severe
26	6.4	F	Y	N	20d/6m	Seizures	Severe
27	Ex	M	Y	N	2d/2m	Tachypnea	Severe

M: Male, F: Female, Y: Yes, N: No, d: day, m: month

Table II. Laboratory Findings of Patients.

Patient no	Metabolic acidosis	Hyperammonemia	Hypotonia	Pancytopenia	CK level
1* sib of 2	No	No	No	No	575
2* sib of 1	Yes	No	Yes	Yes	119
3	Yes	Yes (>500)	Yes	No	
4	Yes	Yes	No	No	
5* sib of 6	No	No	No	No	
6* sib of 5	No	No	No	No	
7	Yes	No	No	No	107
8	Yes	No	Yes	No	250
9* sib of 10	Yes	Yes	Yes	No	
10* sib of 9	Yes	Yes (>500)	Yes	No	
11	Yes	No	Yes	No	99
12	Yes	Yes (>500)	Yes	No	
13	Yes	No	Yes	Yes	101
14	Yes	No	Yes	No	
15	Yes	Yes	No	No	
16	No	No	Yes	No	75
17	Yes	Yes	Yes	No	
18* sib of 19	Yes	Yes	No	No	129
19* sib of 18	Yes	No	Yes	No	
20* sib of 21	Yes	Yes	Yes	Yes	808
21* sib of 20	Yes	Yes	Yes	No	297
22* cousin of 23	Yes	Yes (>1000)	Yes	Yes	117
23* cousin of 22	Yes	Yes (>1000)	Yes	No	613
24	Yes	Yes (>1000)	Yes	Yes	
25	Yes	Yes	Yes	Yes	88
26	Yes	Yes	Yes	No	43
27	Yes	Yes	Yes	No	744

Discussion

In this report, we presented the clinical and molecular features of 27 Turkish PA patients from South and Southeast parts of Turkey which does not entirely represent the whole genotypic spectrum of PA in Turkey.

The clinical outcomes of the patients were evaluated and the disease-causing mutations were determined. Most of our PA patients' symptoms began in neonatal period and late-onset forms are rare. Of patients included in the study, 70.4% (19/27) became symptomatic within the first ten days of life. Other studies also reported early occurrence of symptoms as 71-92%.[9-11]

The most common initial symptoms of PA patients were vomiting, feeding difficulties, feeding refusal, and failure to thrive. In our patient group also feeding problems, vomiting and feeding refusal were the most frequent manifestations (70%), especially when compared with neurological signs. Although in some series, gastroenterological problems are more prominent at admission[9,12-13], most

Table III. Mutations of The Patients.

Patient no	Gene	Exon/intron	Nucleotide change allele 1-2	Effect on codig region allele 1-2	Reference
1* sib of 2	PCCA	19	c.1746G>A	p.S582S	
2* sib of 1	PCCA	19	c.1746G>A	p.S582S	
3	PCCB	15	c.1555C>T	p.R519*	
4	PCCA	13	c.1209+3A>G	IVS13+3A>G	
5* sib of 6	PCCA	4	c.424C>A	p.T81N	This study
6* sib of 5	PCCA	4	c.424C>A	p.T81N	This study
7	PCCB	7	c.C683T	p.P228L	
8	PCCB	2	c.207G>C	p.R69S	This study
9* sib of 10	PCCA	11	c.843_843delT	p.N281Kfs*41	This study
10* sib of 9	PCCA	11	c.843_843delT	p.N281Kfs*41	This study
11	PCCA	16	c.1430-1G>A	IVS16-1G>A	This study
12	PCCA	10	c.769A>T	p.R257*	This study
13	PCCB	13	c.1373C>T	p.A458V	This study
14	PCCA	16	c.1412A>T	p.D471V	This study
15	PCCB	2	c.241G>A	p.E81K	This study
16	PCCB	3	c.337C>T	p.R113*	
17	PCCB	3	c.331C>T	p.R111X	This study
18* sib of 19	PCCB	3	c.304_304delT	p.P103Lfs*24	This study
19* sib of 18	PCCB	3	c.304_304delT	p.P103Lfs*24	This study
20* sib of 21	PCCA	22	c.2041-2A>G	IVS22-2A>G	
21* sib of 20	PCCA	22	c.2041-2A>G	IVS22-2A>G	
22* cousin of 23	PCCB	7	c.764_2A>G	IVS7-2A>G	This study
23* cousin of 22	PCCB	7	c.764_2A>G	IVS7-2A>G	This study
24	PCCB	15	c.1600C>T	p.R534*	
25	PCCB	11	c.1050insT	p.E351*	
26	PCCB	3	c.337C>T	p.R113*	
27	PCCB	1	c.183+5G>T	IVS1+5G>T	This study

of authors reported neurological problems slightly more frequent as 77%, 83% and 91%.[11,14-15] Neurological symptoms like hypoactivity, lethargy, and seizures were present in 40% of our patients. Eleven % of our patients had seizures. However, occurrence rate of seizures was as high as 43% in some reports.[14]

Although the median age at onset of symptoms was 3 days, diagnosis was made at a median age of 35.5 days (range from prenatal-10.5 year). Another study reported 12 days apart from those diagnosed with the NSP[13]. In another study, clinical manifestations were reported to occur in the first week in 84% and the first 2 weeks of life in 92% of patients.[11] In this study, median age at diagnosis is later than the reported in the literature because the time interval between the onset of symptoms and the admission to hospital is longer in our patient population.

The mortality rate was found to be 37% in our study. In a similar study 42 early-onset PA patients were included and mortality rate

was reported to be 33%.[9] In another study, mortality rate was reported as 90% up to 6 years.[16] Whereas, in a study, involving patients diagnosed with NSP, mortality rate was reported as 8%.[13] This clearly shows that early diagnosis of PA through NSP is associated with a tendency toward lower mortality rates.

In our study, we identified 12 different mutations in the *PCCB* gene and 8 different mutations in the *PCCA* gene. Further, we identified seven mutations in *PCCB* gene and five mutations in *PCCA* gene, that had not previously been published. Our study shows the predominance of *PCCB* gene mutations, in similarity with other studies, although we have a relatively small patient cohort.[12,17-19] On the contrary, some other studies showed a higher percentage of *PCCA* gene mutations.[20-22] No single common mutation was identified in both genes. A large number of novel mutations was detected in our study (60%). The variety of mutations may suggest the highly heterogeneous nature of genotype of PA in Turkey. The study included patients from the south and southeast parts of Turkey. This geographical region has the highest rates of parental consanguinity in Turkey. Patients included in this study were from different socioeconomic status and ethnicities. So, this cohort may not reflect the entire country therefore this may be the limitation of this study. Further multicentre, longitudinal studies are needed to better elucidate the clinical spectrum of the disease. Lack of NSP and delay in diagnosis seem to be major factors associated with poor outcomes and increased mortality of children in the present study.

In this study, we report the clinical and molecular characteristics of 27 Turkish patients which provides additional knowledge to the genotype and phenotype of PA. Our data suggest that *PCCA* and *PCCB* mutations in Turkish populations are distinct from other populations. Genetic heterogeneity was observed in our cohort in spite of the high rate of consanguineous marriages in Turkey, which are considered to be an important factor contributing to the higher incidences of autosomal recessive hereditary diseases. In addition, seven novel mutations in the *PCCB* gene and five novel mutations in the *PCCA* gene were identified, and therefore expand the mutational spectrum of these *PCCA-PCCB* genes.

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