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

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Received: 16th February 2019, Revised: 1st April 2019, Accepted: 3rd April 2019

SUMMARY: Kör D, Şeker-Yılmaz B, Bulut FD, Kılavuz S, Öktem M, Ceylaner S, Yıldızdaş D, Önenli-Mungan N. Clinical features of 27 Turkish Propionic acidemia patients with 12 novel mutations. Turk J Pediatr 2019; 61: 330-336.

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 sequelae. 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.
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 Çukurova 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.
| 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, panstopenia | 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 | Feeding refusal, cyclic vomiting related to infections | Mild |
| 7 | 35.6 | F | Y | Y | 1m/6y | Cyclic vomiting related to infections, feeding difficulties, after acute viral hepatitis | Mild |
| 8 | 3.3 | F | N | N | 4m/1y | Hypotonia, feeding 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, achyptnea, 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 | 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, severe 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, Severe 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
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 admission9,12-13, most

| 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             | Yes       | 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      |
of authors reported neurological problems slightly more frequent as 77%, 83% and 91%. 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.

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. 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. 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%. In another study, mortality rate was reported as 90% up to 6 years. Whereas, in a study, involving patients diagnosed with NSP, mortality rate was reported as 8%. 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 \textit{PCCB} gene and 8 different mutations in the \textit{PCCA} gene. Further, we identified seven mutations in \textit{PCCB} gene and five mutations in \textit{PCCA} gene, that had not previously been published. Our study shows the predominance of \textit{PCCB} gene mutations, in similarity with other studies, although we have a relatively small patient cohort. On the contrary, some other studies showed a higher percentage of \textit{PCCA} gene mutations. 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 \textit{PCCA} and \textit{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 \textit{PCCB} gene and five novel mutations in the \textit{PCCA} gene were identified, and therefore expand the mutational spectrum of these \textit{PCCA-PCCB} genes.

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