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Novel mutations of PCCA and PCCB genes found by whole-exome sequencing related to propionic acidemia patients

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ABSTRACT: Propionic acidemia (PROP) is an autosomal recessive inherited metabolic deficiency caused by multimeric mitochondrial enzyme propionyl-coenzyme A (CoA) carboxylase (PCC). PCC enzyme contains a and b subunits, encoded by the *PCCA* and *PCCB* genes that mutations in both subunits are related to propionic acidemia. About 50% of disease-causing variants have been found in *PCCA* and most mutations related to propionic acidemia are missense mutations. The present study involves three families that are suspicious to hereditary propionic acidemia syndrome. The first family has four, the second family has one, and the third family has two passed-away children. All these families were diagnosed with the same clinical conditions such as poor feeding, vomiting, hypotonia, and lethargy. In the process of finding and confirming the mutation, pathological tests and whole-exome sequencing and sanger sequencing were done. In order to pathological tests and whole-exome sequencing, this is the first report of three novel variants related to propionic acidemia: 1. Novel pathogenic homozygous NM_000532.5: c.503_505del: p.Glu168del mutation of the *PCCB* exon5 gene, 2. Novel pathogenic homozygous splicing NM_000282:c.1900- 1G>A mutation of *PCCA* exon22 and exon21, 3. Novel compound heterozygous pathogenic NM_000532.5: c.503_505del: p.Glu168del and likely pathogenic NM_000532.5:c.539T>C: P.F180S mutation of the *PCCB* exon5 gene. The study shows that *PCCA* and *PCCB* have a great role in hereditary propionic acidemia and the results of the present study may be of importance in genetic counseling and finding the best treatment of this syndrome.

Keywords: Biochemistry; Children; Clinical molecular diagnostics; Molecular diagnostics.

1. INTRODUCTION

Metabolic disorders as a worldwide problem include various medical conditions as results of genetic defects that in most cases, they are inherited from both father and mother to offspring. Metabolic disorders as it is obvious from their name, change the body's metabolism and occur as a hormone or enzyme deficiency. There are different types of inherited metabolic disorders from an extra amount of a special molecule to its missing or low amount and several conditions, as possible [1].

Propionic acidemia (PROP) (OMIM 606054) is an autosomal recessive inherited metabolic deficiency that is caused by multimeric mitochondrial enzyme propionyl-coenzyme A (CoA) carboxylase (PCC) (EC 6.4.1.3) mutations [2, 3] and its spectrum ranges from neonatal-onset to late-onset disease [2]. PROP rate is about 1 in 100 000 in all nations except the middle east and north Africa. The two named nations have higher rates of metabolic disorders [3]. This enzyme manages the biotin-dependent conversion of propionyl-CoA to D-methylmalonyl-CoA. Now, let's find more about the importance of this interaction [3]. Catabolism of isoleucine (ILE) and valine (VAL), as well as threonine (THR), methionine (MET), odd-chain fatty acids, and cholesterol is mediated by Propionyl CoA as an intermediate [3, 4].

Finally, in order to energy production and making the precursor of the citric acid (Krebs) cycle, propionyl CoA turns into succinyl CoA [4]. Propionyl-coenzyme A (CoA) carboxylase dysfunction will cause the presence of intermediates of methyl citrate, 3-hydroxypropionate and propionyl carnitine in urine and plasma [2]. Defective propionyl CoA carboxylase will cause in the gathering of propionyl CoA and also mixing it with oxaloacetate. In the last part, methylcitric acid is made, which is the diagnostic molecule of propionic acidemia [4].

PCC (Propionyl-CoA carboxylase) enzyme contains a and b subunits, encoded by the *PCCA* and *PCCB* genes [5] that mutations in both subunits are related to propionic acidemia [6]. They are located on 13q32.3 and 3q22.3 [7], respectively and about 50% of disease-causing variants have been found in *PCCA*. Notably, most propionic acidemia mutations are missense mutations, and *PCCA* null mutations result in the most severe types of propionic acidemia [6].

Propionic acidemia occurs in different stages of life and contains various medical conditions. The most common and severe form of propionic acidemia is neonatal-onset and its symptoms will appear in the first few days of infant life. In this form of propionic acidemia, individuals suffer from medical conditions ranging from poor feeding, vomiting, hypotonia, progressive encephalopathy to lethargy, seizures, or coma that would cause death. Metabolically unstable individuals even have more suffering situations. Also, sometimes there are metabolic decompensations found in neonatal-onset propionic acidemia such as lactic acidosis, ketonuria, hypoglycemia, hyperammonemia, and cytopenia [2, 8].

Late-onset and chronic propionic acidemia will start asymptomatic most of the times. It can start with metabolic stresses such as illness, surgery or fasting and may have some other manifestations including vomiting, protein intolerance, hypotonia, skeletal myopathies, developmental delays, movement disorders such as dystonia and choreoathetosis [2, 8]. Some conditions are reported in both neonatal-onset and late-onset propionic acidemia over time. Growth impairment, intellectual disability, seizures, basal ganglia lesions, pancreatitis, and cardiomyopathy are more common and rare conditions include optic atrophy, hearing loss, premature ovarian insufficiency, and chronic renal failure [8]. It is notable that dysfunction of the immune system has been reported in 30-65% of propionic acidemia sufferers [2].

One of the most well-studied medical conditions of acidurias is neurocognitive complications. Some behavioral signs such as self-damaging or self-regulating issues, anxious and avoidant behaviors have been reported with a higher prevalence through aciduria affected individuals. Additionally, propionic acidemia is the most common disorder through aciduria spectrum [9].

Here, for the first time we report three cases, the first one which contains novel likely pathogenic homozygous NM_000532.5: c.503_505del: p.Glu168del mutation of the *PCCB* exon5 gene that was identified by whole exome-sequencing and confirmed by Sanger sequencing. Our second proband contains novel pathogenic homozygous splicing NM_000282:c.1900- 1G>A mutation of *PCCA* exon22 and exon21 that was

identified by whole exome-sequencing and confirmed by Sanger sequencing. Our third proband contains novel compound heterozygous likely pathogenic NM_000532.5: c.503_505del: p.Glu168del and likely pathogenic NM_000532.5:c.539T>C: P.F180S mutation of the *PCCB* exon5 gene that was identified by whole exome-sequencing and confirmed by Sanger sequencing.

Our aim with this study was to propose that novel variants including likely pathogenic NM_000532.5: c.503_505del: p.Glu168del, pathogenic NM_000282: c.1900- 1G>A, likely pathogenic NM_000532.5: c.503_505del: p.Glu168del and likely pathogenic NM_000532.5:c.539T>C: P.F180S can cause propionic acidemia.

2. MATERIALS AND METHODS

2.1. Patients

2.1.1. First Patient

Our first proband involves a family with four dead children, as a result of a consanguineous marriage. Our proband passed away after twenty days and other three children of this family passed away after two days, two days and twelve weeks, respectively. The proband was normal in all new born screening tests and there was no abnormal appearance in the proband, her mother and father. Even the pregnancy screening tests were reported normal. It is notable that one brother and one sister of proband's mother passed away in their childhood (Figure 1-A). To find out the cause of consecutive children deaths in this family, pathological tests, metabolic tests and WES were requested for proband and her parents. Also, sanger sequencing was done for confirming the mutation.

2.1.2. Second patient

Our second proband involves a family with two children, as a result of a consanguineous marriage. They have one healthy six-year-old child and their second child as our proband, who passed away after nine days. Newborn screening tests were done for proband. The proband has been diagnosed by cyanosis and rapid breathing. To find out the cause of proband's death, in this family, pathological tests, metabolic tests and WES were requested for proband and his parents. Also, sanger sequencing was done for confirming the mutation (Figure 1-B).

2.1.3. Third patient

Our third proband involves a family with two dead children. Our proband passed away after 13 months as a result of a heart attack. Days and other three children of this family passed away after two years. The proband was normal in all newborn screening tests and there was no abnormal appearance in the proband, her mother and father. To find out the cause of proband's death, in this family, pathological tests, metabolic tests and WES were requested for proband and his parents. Also, sanger sequencing was done for confirming the mutation (Figure 1). To find out the cause of proband's death, in this family, pathological tests, metabolic tests and WES were requested for proband and his parents. Also, sanger sequencing was done for confirming the mutation (Figure 1-C).

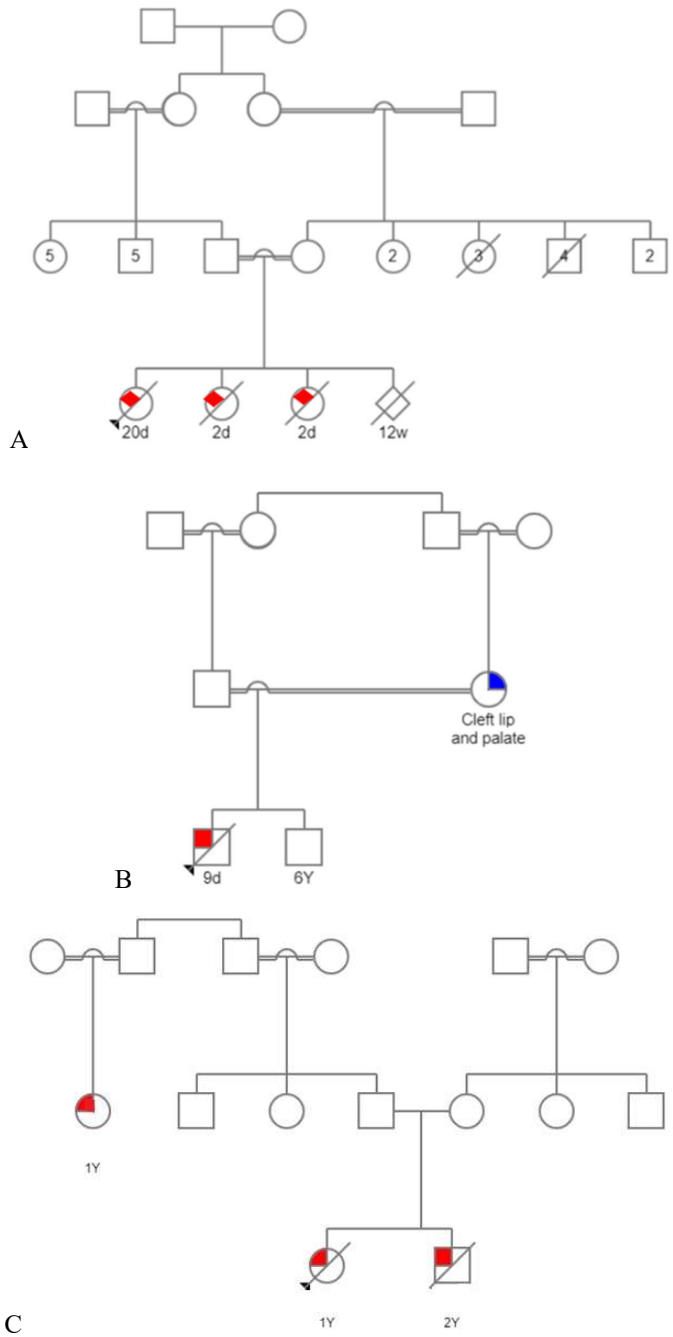


Figure 1. A) Pedigree of first proband, B) Pedigree of second proband and C) Pedigree of third proband.

Informed consent was obtained from all human adult participants and from the parents or legal guardians of minors in Genome laboratory of Isfahan. In this study, internal consent has been prepared, adjusted and available in Genome Laboratory of Isfahan. Local ethics committees received informed consent from the subjected families by IRCT Ethics Committee Agreement number: 52793.

2.2. Mutation analysis

Genomic deoxyribonucleic acid (gDNA) is isolated from the patient's specimen using a filter-based methodology and quantified. A total amount of 1.0 µg genomic DNA per sample was used as input material for the DNA sample preparation. Sequencing libraries were generated using Agilent SureSelect Human All ExonV7 kit (Agilent Technologies, CA, USA) following manufacturer's recommendations and x index codes were added

to attribute sequences to sample. Briefly, fragmentation was carried out by hydrodynamic shearing system (Covaris, Massachusetts, USA) to generate 180-280 bp fragments. Remaining overhangs were converted into blunt ends via exonuclease/polymerase activities and enzymes were removed. After adenylation of 3' ends of DNA fragments, adapter oligonucleotides were ligated. DNA fragments with ligated adapter molecules on both ends were selectively enriched in a PCR reaction. Captured libraries were enriched in a PCR reaction to add index tags to prepare for hybridization. Products were purified using AMPure XP system (Beckman Coulter, Beverly, USA) and quantified using the Agilent high sensitivity DNA assay on the Agilent Bioanalyzer 2100 system. The qualified libraries are fed into NovaSeq 6000 Illumina sequencers. Then data quality control, analysis and interpretation were run on G9 generation of HP server using unix based operating system.

Sanger sequencing was performed by ABI prism 3730 sequencer (Applied Biosystems, Waltham, MA,USA) to validate the likely pathogenic mutation and segregation the mutation in this family. Mutation Surveyor program version 4.0.9 was used to analyze the sequences (SoftGenetics, State College, PA).

We employed the 48-well thermocycler device (BioRad) in this reaction. The materials utilized in PCR as well as their concentration and amount were as follows: 6 µL of master (1X), 2 µL of template DNA, 0.5 µL of (10 pmol) forward primer, 0.5 µL of (10 pmol) reverse primer, and 3.5 L of sterile distilled water (total: 12.5 µL). To prepare the PCR solution, we used 0.2 mL microtubes. We poured the mentioned materials in the tubes and stirred them by pipetting. We amplified the noted gene segment by primers.

For the first patient as a 20 days old passed chid, to amplify the 634-base pair segment related to NM_000532: c.500-502del: P.E168del, the sequence of forward and reverse primers was CCTCAGATTAACAGATGGGTCAACA and GACACAATGCGGCAGAGAACAA, respectively. The utilized primers were manufactured by Tag Copenhagen Co. The 634-base pair segment was amplified in the thermocycler as follows: cycle 1 for the initial denaturation: once for 5 min at 94°C; cycle 2 including three steps: denaturation, binding the primer to the template strand, and polymerase expansion: 35 times, each for 30 sec at 94°C, F: 60.57/R: 61.95, and 72°C, respectively; cycle 3 for the final expansion: once for 10 min at 72°C; cycle 4 for maintaining the products: once at 4°C.

For the second patient as a 9 days old passed chid, to amplify the 585-base pair segment related to NM_000282: c.1900-1G>A, the sequence of forward and reverse primers was GATAGGGATAAGTTTGTAGGTGGTG and AAGTAAGAACTGCAAAGAGCCGA, respectively. The utilized primers were manufactured by Tag Copenhagen Co. The 585-base pair segment was amplified in the thermocycler as follows: cycle 1 for the initial denaturation: once for 5 min at 94°C; cycle 2 including three steps: denaturation, binding the primer to the template strand, and polymerase expansion: 35 times, each for 30 sec at 94°C, F: 58.71/R: 60.49, and 72°C, respectively; cycle 3 for the final expansion: once for 10 min at 72°C; cycle 4 for maintaining the products: once at 4°C.

For the third patient as a 13 months old passed child, to amplify the 387-base pair segment, the sequence of forward and reverse primers was CCATGAAGGTACCCAATCGTG and ACACAATGCGGCAGAGAAC, respectively. The utilized primers were manufactured by Tag Copenhagen Co. The 387-base pair segment was amplified in the thermocycler as follows: cycle 1 for the initial denaturation: once for 5 min at 94°C; cycle 2 including three steps: denaturation, binding the primer to the template strand, and polymerase expansion: 35 times, each for 30 sec at 94°C, F: 58.71/R: 58.75, and 72°C, respectively; cycle 3 for the final expansion: once for 10 min at 72°C; cycle 4 for maintaining the products: once at 4°C. Also for the third patient as a 13 months old passed child, to amplify the 634-base pair segment related to NM_000532: c.500-502del: P.E168del, the sequence of forward and reverse primers was CCTCAGATTAACAGATGGGTCAACA and

GACACAATGCGGCAGAGAACAA, respectively. The utilized primers were manufactured by Tag Copenhagen Co. The 634-base pair segment was amplified in the thermocycler as follows: cycle 1 for the initial denaturation: once for 5 min at 94°C; cycle 2 including three steps: denaturation, binding the primer to the template strand, and polymerase expansion: 35 times, each for 30 sec at 94°C, F: 60.57/R: 61.95, and 72°C, respectively; cycle 3 for the final expansion: once for 10 min at 72°C; cycle 4 for maintaining the products: once at 4°C.

3. RESULTS

3.1. Proband 1

3.1.1. Pathological results

Abnormal pathological test results of the fetus are written in Table 1. The results are summarized from metabolic panel tests and disorders of amino acids metabolism tests. All other items were reported in normal range.

Table 1. Pathological abnormal test results (μmol/l).

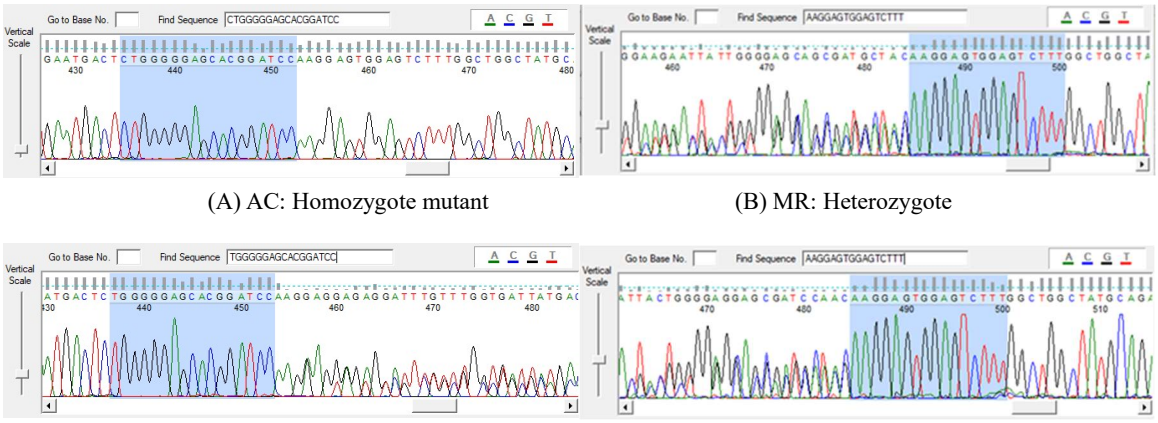
Items	Results	Reference range	Method
Lactate	63	4.5-20	HPLC
Ammoniac	0.340	0.017-0.080	HPLC
Pyrovate	0.85	0.3-0.7	HPLC
Glycine	478.8	111-426	HPLC
Alanine	130.2	139-474	HPLC
Citrulline	3.1	9-42	HPLC
Propionylcarnitine	3.28	<0.62	HPLC
Free carnitine	1.12	7.6-32	HPLC
Valine	70.7	83-312	HPLC
Leucine	43.1	48-205	HPLC
Isoleucine	20.8	24-105	HPLC

3.1.2. Sequencing results

Performing WES on proband 1, identified novel likely pathogenic homozygous NM_000532.5: c.503_505del: p.Glu168del mutation of the *PCCB* exon5 gene. Sanger sequencing confirmed homozygousity of NM_000532.5: c.503_505del: p.Glu168del mutation in the proband, suggesting it as the putative disease-causing mutation, and autosomal recessive inheritance pattern in propionic acidemia syndrome (Figure 2).

It should be noted that basic clinical information and relationship for each analyzed family member is needed for a comprehensive evaluation of the data. On the basis of these findings, additional genetic testing to confirm results, clinical screening tests, or preventive care may be recommended.

There is no report of the NM_000532.5: c.503_505del: p.Glu168del mutation in *PCCB* gene in ExAC, 1000G and other control datasets. This variant does not have a gnomAD exomes entry, and also it has not a Clinvar entry [10]. Also it is notable that NM_000532.5: c.503_505del: p.Glu168del mutation was found as a pathogenic mutation in Franklin predictor [11] as we know in 135980867 position [10].



(A) AC: Homozygote mutant (B) MR: Heterozygote

(C) PF: Heterozygote (D) PR: Heterozygote

Figure 2. Confirmation sequences of the proband (A), her mother (B) and father (C) and (D). AC: affected child, MR: mother reverse strand and PF: father forward strand.

3.2. Proband 2

3.2.1. Pathological results

Abnormal pathological test results of the fetus are written in Table 2. The results are summarized from metabolic panel tests and disorders of amino acids metabolism tests. All other items were reported in normal range.

Table 2. Pathological abnormal test results (μmol/l).

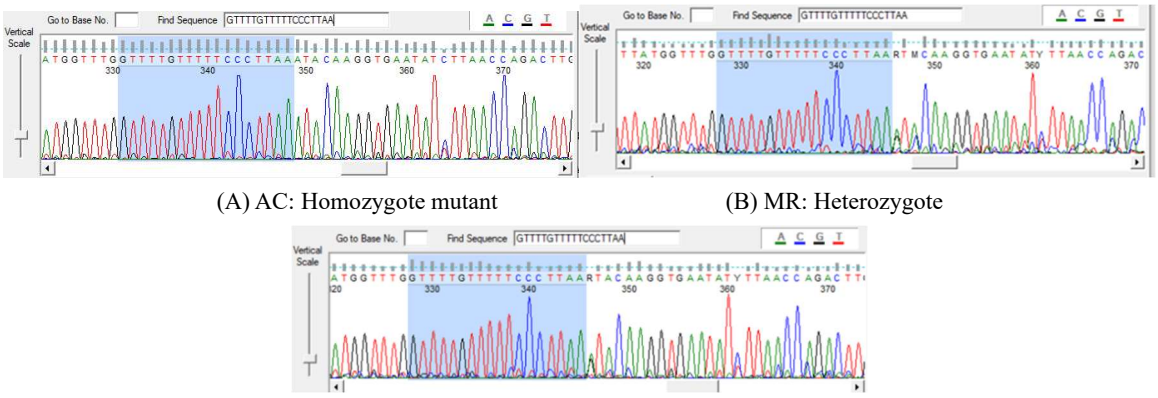
Items	Results	Reference range	Method
Glycine	654.5	111-426	HPLC
Valine	62	83-312	HPLC
Acetylcarnitine	0.9	1.5-12	HPLC
Propionylcarnitine	3.1	<0.62	HPLC
Lactate	55	4.5-20	HPLC
Free carnitine	1.74	7.6-32	HPLC

3.2.2. Sequencing results

Performing WES on proband 2, identified a novel pathogenic homozygous splicing NM_000282:c.1900-1G>A mutation of *PCCA* exon22 and exon21. Sanger sequencing confirmed homozygosity of NM_000282:c.1900-1G>A splicing mutation of *PCCA* exon22 and exon21 mutation in the proband, suggesting it as the pathogen disease-causing mutation, and autosomal recessive inheritance pattern in propionic acidemia disease (Figure 3).

Basic clinical information and relationship for each analyzed family member is needed for a comprehensive evaluation of the data. In order to figure 3, by sanger confirming, It should be noted that basic clinical information and relationship for each analyzed family member is needed for a comprehensive evaluation of the data. On the basis of these findings, additional genetic testing to confirm results, clinical screening tests, or preventive care may be recommended.

There is no report of the NM_000282:c.1900-1G>A splicing mutation of *PCCA* exon22 and exon21 in ExAC, 1000G and other control datasets. This variant does not have a gnomAD exomes entry, but its locus is covered in gnomAD exomes as follows c.1900-1G>A splicing mutation [10] DANN score [12] was 0.9906 and was found as pathogenic mutation in EIGEN predictor [13]. This variant was found as a damaging variant in FATHMM-MKL [14], BayesDel noAF and BayesDel addAF meta-predictors [15] through searching dbNSFP v4 [16]. Also it is notable that NM_000282: c.1900-1G>A splicing mutation of *PCCA* gene was found as a disease causing mutation in mutation taster predictor [17] as we know in 101167680 position [10].



(A) AC: Homozygote mutant (B) MR: Heterozygote

(C) PR: Heterozygote

Figure 3. Confirmation sequences of the proband (A), his mother (B) and father (C). AC: affected child, MR: mother reverse strand and PR: father reverse strand.

3.3. Proband 3

3.3.1. Pathological results

Abnormal pathological test results of the fetus are written in Table 3. The results are summarized from metabolic panel tests and disorders of amino acids metabolism tests. All other items were reported in normal range.

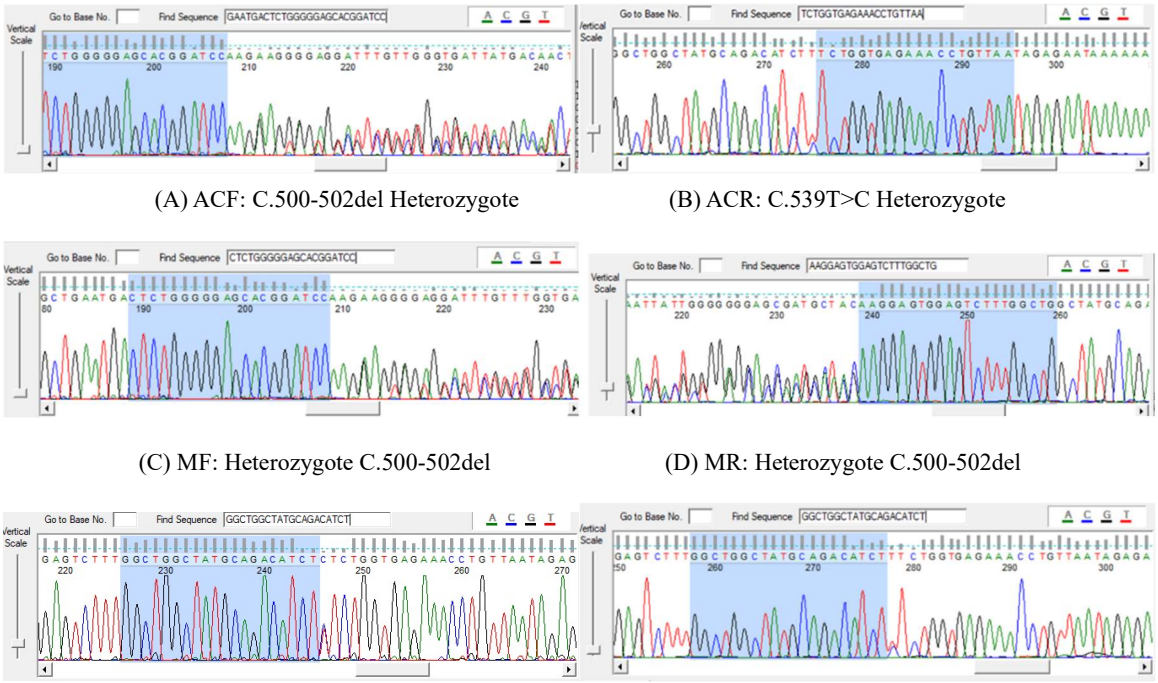
Table 3. Pathological abnormal test results (µmol/l).

Items	Results	Reference range	Method
Glycine	866.1	111-426	HPLC
Acetylcarnitine	1.3	1.5-12	HPLC
Propionylcarnitine	3.57	<0.62	HPLC
Free carnitine	1.92	7.6-32	HPLC

3.3.2. Sequencing results

Performing WES on proband 3, identified novel likely pathogenic compound heterozygous pathogenic NM_000532.5: c.503_505del: p.Glu168del and likely pathogenic NM_000532.5:c.539T>C: P.F180S mutation of the *PCCB* exon5 gene. Sanger sequencing confirmed compound heterozygosity of pathogenic NM_000532:c.500-502del: P.E168del and likely pathogenic NM_000532.5:c.539T>C: P.F180S mutation of the

PCCB exon5 gene in the proband, suggesting it as the pathogen disease-causing mutation, and autosomal recessive inheritance pattern in propionic acidemia disease (Figure 4).



(A) ACF: C.500-502del Heterozygote (B) ACR: C.539T>C Heterozygote

(C) MF: Heterozygote C.500-502del (D) MR: Heterozygote C.500-502del

(E) PF: Heterozygote c.539T>C (F) PR: Heterozygote c.539T>C

Figure 4. Confirmation sequences of the proband (A) and (B), her mother (C) and (D) and her father (E) and (F). ACF: affected child forward strand, ACR: affected child reverse strand, MF: mother forwad strand, MR: mother reverse strand, PF: father forward strand and PR: father reverse strand.

Basic clinical information and relationship for each analyzed family member is needed for a comprehensive evaluation of the data. In order to figure 4, by sanger confirming, It should be noted that basic clinical information and relationship for each analyzed family member is needed for a comprehensive evaluation of the data. On the basis of these findings, additional genetic testing to confirm results, clinical screening tests, or preventive care may be recommended.

There is no report of the NM_000532.5:c.539T>C: P.F180S mutation in *PCCB* gene in ExAC, 1000G and other control datasets. This variant does not have a gnomAD exomes entry, but its locus is covered in gnomAD exomes as follows. c.539T>C: P.F180S [10] DANN score [12] was 0.9987 and was found as likely pathogenic mutation in EIGEN predictor [13]. This variant was found as a damaging variant in FATHMM-MKL [14], BayesDel noAF [15], SIFT, SIFT4G [18] and BayesDel addAF meta-predictors [15] through searching dbNSFP v4 [16]. Also it is notable that NM_000532.5:c.539T>C: P.F180S mutation was found as a disease causing mutation in mutation taster predictor [17] as we know in 135980903 position [10].

There is no report of the NM_000532.5: c.503_505del: p.Glu168del mutation in *PCCB* gene in ExAC, 1000G and other control datasets. This variant does not have a gnomAD exomes entry, and also it has not a Clinvar entry [10]. Also it is notable that NM_000532.5: c.503_505del: p.Glu168del mutation was found as a pathogenic mutation in Franklin predictor [11] as we know in 135980867 position [10].

3.4. Protein models

Different changes of gene sequences such as deletion and missense mutations will result in different 3D structure of RNA and proteins, and can be the real cause of different diseases. In this part we want to take a quick glance at the wild type of PCC protein that was made by X-RAY DIFFRACTION method and 3.20 Å resolution as well as cryo-electron microscopy (cryo-EM) reconstruction at 15-A resolution demonstrating a similar structure for human PCC from protein data bank server [19, 20] (Figure 5).

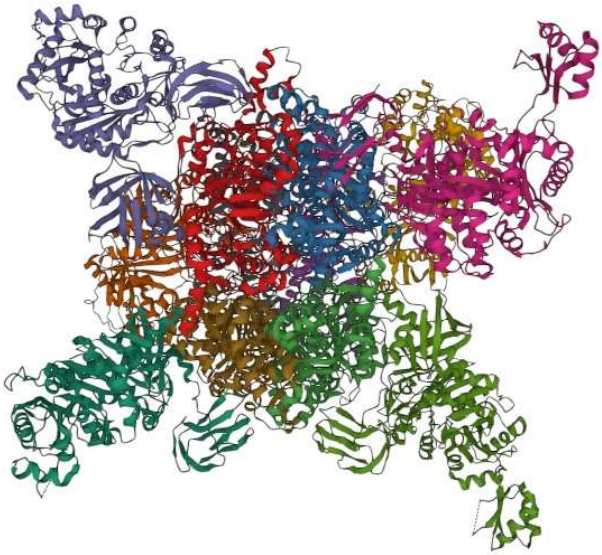


Figure 5. Wild type of PCC protein made by X-RAY DIFFRACTION method and 3.20 Å resolution [19, 20].

4. DISCUSSION

Many people suffer from metabolic diseases through the world and after a lot of studies now, we know that genes are responsible for most of them but they are not completely understood, yet. In these days, genome-wide association studies really help us in order to find the underlying genetic factors of metabolic diseases in different nations and genders [21].

Based on the results, our first proband that is homozygous for NM_000532.5: c.503_505del: p.Glu168del mutation of the PCCB exon5 gene, as a novel mutation, is a pathogenic variant that has a great risk for propionic acidemia. In order to WES results, our proband's father and mother were heterozygous for the above variant and it is confirmed that NM_000532.5: c.503_505del: p.Glu168del is an autosomal recessive inherited.

Our second proband that is homozygous for pathogenic splicing NM_000282:c.1900-1G>A mutation of *PCCA* exon22 and exon21, as a novel mutation, is a pathogenic variant that has a great risk for propionic acidemia. In order to WES results, our proband's father and mother were heterozygous for the above variant and it is confirmed that NM_000282:c.1900- 1G>A is an autosomal recessive hereditary propionic acidemia cause.

Our third proband contains novel compound heterozygous NM_000532.5: c.503_505del: p.Glu168del and likely pathogenic NM_000532.5:c.539T>C: P.F180S mutations of the *PCCB* exon5 gene. As a novel mutation, the named variant has a great risk for propionic acidemia. In order to WES results, our proband's father and mother were heterozygous for the above variant, father was heterozygous for

NM_000532.5:c.539T>C: P.F180S and mother was heterozygous for NM_000532.5: c.503_505del: p.Glu168del. It is confirmed that NM_000532.5: c.503_505del: p.Glu168del and NM_000532.5:c.539T>C: P.F180S are a compound heterozygous autosomal recessive hereditary propionic acidemia cause.

There are a lot of complications about studying the mechanism and diagnosis the probable affected individuals of metabolic diseases. Early diagnosis specially in newborns is significant to reduce their severe symptoms and increase their life span quality. It is possible to diagnose several metabolic disorders by birth routine screening tests, so we should have programs about newborn screening [4].

Prenatal diagnosis is another method to screen and find propionic acidemia. In amniotic fluid, elevated quantity of the metabolite methylcitrate is a real sign of this metabolic syndrome. Also deficient activity of propionyl-CoA carboxylase in amniocytes is another experiment to find propionic acidemia affected fetus. In prenatal diagnosis, direct enzyme assay will help us too. Assay in uncultured chorionic villi in the first three months of pregnancy navigates us to find PCC deficiency [22, 23].

Propionyl-CoA carboxylase deficiency will cause increased propionic acid and propionyl-CoA related metabolites. By biochemical analysis of urine and plasma, propionic acidemia would be confirmed. Generally biochemical findings in this syndrome include: plasma elevated propionylcarnitine (C3), plasma elevated glycine, urine elevated 3-hydroxypropionate, urine presence of methylcitrate, tiglylglycine, propionylglycine and lactic acid. Also during different tests, these medical conditions may appear: high-anion gap metabolic acidosis, lactic acidosis, elevated plasma and urinary ketones, low to normal blood glucose concentration, hyperammonemia, neutropenia, anemia, and thrombocytopenia [24].

It has not been an exact treatment approach found yet for propionic acidemia as the most common condition through aciduria spectrum; but the treatment and using different drugs and dietary restrictions to reduce the symptoms such as infection, dehydration and vomiting is directly related to the type and also severity of the disorder. Additionally, because of autosomal recessive inheritance pattern of propionic acidemia, genetic counseling would be useful specially if pathogenic variants have been reported in the family [1].

To our knowledge, this is the first report of three novel variants: 1. Novel pathogenic homozygous NM_000532.5: c.503_505del: p.Glu168del mutation of the *PCCB* exon5 gene, 2. Novel pathogenic homozygous splicing NM_000282:c.1900- 1G>A mutation of *PCCA* exon22 and exon21, 3. Novel compound heterozygous pathogenic NM_000532.5: c.503_505del: p.Glu168del and likely pathogenic NM_000532.5:c.539T>C: P.F180S mutation of the *PCCB* exon5 gene. Consequently, the results of the present study may be of importance in genetic counseling.

Authors' Contributions: Conceptualization: MS, SRK; Data curation: SRK, SRK; Formal analysis: SRK, ZS; Funding acquisition: MS; Methodology: SRK, ZS; Project administration: DMKT, MS; Writing - original draft: ZS; Writing - review & editing: SRK, ZS. All authors read and approved the final version of the manuscript.

Conflict of Interest: The authors declare no conflict of interest.

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