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Spectrum of mutations underlying Propionic acidemia and further insight into a genotype-phenotype correlation for the common mutation in Saudi Arabia

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ABSTRACT

Propionic acidemia (PA) is an autosomal recessive metabolic disorder. PA is characterized by deficiency of the mitochondrial enzyme propionyl CoA carboxylase (PCC) that results in the accumulation of propionic acid. Alpha and beta subunits of the PCC enzyme are encoded by the *PCCA* and *PCCB* genes, respectively. Pathogenic variants in *PCCA* or *PCCB* disrupt the function of the PCC enzyme preventing the proper breakdown of certain amino acids and metabolites. To determine the frequency of pathogenic variants in PA in our population, 84 Saudi Arabian patients affected with PA were sequenced for both the *PCCA* and *PCCB* genes. We found that variants in *PCCA* accounted for 81% of our cohort (68 patients), while variants in *PCCB* only accounted for 19% (16 patients). In total, sixteen different sequence variants were detected in the study, where 7 were found in *PCCA* and 9 in *PCCB*. The pathogenic variant (c.425G > A; p.Gly142Asp) in *PCCA* is the most common cause of PA in our cohort and was found in 59 families (70.2%), followed by the frameshift variant (c.990dupT; p.E331Xfs*1) in *PCCB* that was found in 7 families (8.3%). The p.Gly142Asp missense variant is likely to be a founder pathogenic variant in patients of Saudi Arabian tribal origin and is associated with a severe phenotype. All variants were inherited in a homozygous state except for one family who was compound heterozygous. A total of 11 novel pathogenic variants were detected in this study thereby increasing the known spectrum of pathogenic variants in the *PCCA* and *PCCB* genes.

1. Introduction

Propionic acidemia (PA; OMIM 606054) is an autosomal recessive metabolic disorder caused by the deficient activity of the propionyl coenzyme A carboxylase (PCC) [1]. Most PA patients have an early onset (diagnosed < 3 months of age). Classically, this group presents in the neonatal period with lethargy, vomiting, refusal to feed, hypotonia and less frequently, seizures. Biochemical features include metabolic acidosis, hyperammonemia, hypoglycaemia, bone marrow suppression and ketonuria. The later course is dominated by recurrent metabolic decompensation characterized by the same above symptoms and biochemical abnormalities. Recurrent pancreatitis, cardiomyopathy and abnormal movements are also seen. Almost all PA patients surviving into childhood and adolescence have some degree of intellectual disability [1]. PA is a life threatening disease and has been reported in Saudi Arabia and other Arab countries [2–4] with most patients showing an early age of onset [5,6]. In previously reported Saudi PA patients, poor feeding, vomiting and lethargy were the most common clinical features sometimes in parallel with ketoacidosis and hyperammonemia [5,7].

PA is a reported to be a rare condition with incidence ranges from 1:50,000 to 1:100,000 worldwide (http://www.ncbi.nlm.nih.gov/books/NBK92946). PA is however the most frequently encountered organic aciduria in newborn screening in Saudi Arabia with an

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Table 1
Mutations in *PCCA* and *PCCB* genes identified in Saudi Arabian cases with Propionic Acidemia (PA).

Gene	Nucleotide change	Amino acid change	Number of families	Reference
PCCA	**c.111_130del20**	**Cys44Leufs*6**	3	This study
PCCA	**c.266C > G**	**Thr89Arg**	1	This study
PCCA	c.425G > A	Gly142Asp	59	[26]
PCCA	**c.955C > T**	**Gln319***	1	This study
PCCA	c.1288C > T	Arg430*	2	[29]
PCCA	**c.1375_1391dup + c.1391ins**		1	This study
PCCA	**c.2120 T > G**	**Val707Gly**	1	This study
PCCB	**c.90_108del(19N)ins(14N)**	**Ser30Argfs*25**	1	This study
PCCB	c.337C > T	Arg113*	1	[41]
PCCB	**c.518_543del26**	**Leu173***	1	This study
PCCB	**c.629 T > C**	**Leu210Pro**	1	This study
PCCB	**c.866G > C**	**Arg289Pro**	1	This study
PCCB	c.990dupT	Glu331*	7	[16]
PCCB	**c.1088C > T**	**Ser363Leu**	1	This study
PCCB	**c.1163 T > A**	**Leu388His**	2	This study
PCCB	c.1210G > A	Glu404Lys	1	[26]

Novel mutations are in bold

estimated incidence of 1 per 12,500 live births [2,8], which is much higher than the previously reported prevalence estimated to be 1 in 27,264 live births [9]. The incidence of PA is even higher in certain tribes of Saudi Arabia in the range of 1:2000 to 1:5000 [10].

PCC is a mitochondrial biotin-dependent enzyme that catalyses the conversion of propionyl-CoA to D-methylmalonyl-CoA. Deficiency of PCC leads to accumulation of propionic acid leading to PA. PCC comprises of six α-subunits that form binding sites and six β-subunits that form a central core of the enzyme, in addition to a biotin domain that located on α-subunits [11]. Alpha and beta subunits of the PCC enzyme are encoded by the *PCCA* and *PCCB* genes, respectively.

The *PCCA* gene is located on chromosome 13q32 [12], spans > 360 kb and consists of 24 exons encoding 728 amino acids. The alpha subunit encoded by *PCCA* is responsible for the formation of carboxybiotin upon hydrolysis of ATP and it contains a C-terminus biotin-binding domain and a biotin carboxylase domain [13]. In fibroblasts, the level of PCCA expression does not normally limit the activity of PCC enzyme or propionate flux [14]. Pathogenic variants in *PCCA* account approximately for 50% of molecularly diagnosed individuals with PA [15]. According to the Human Gene Mutation Database; HGMD (http://www.hgmd.org); currently there are 126 pathogenic variants were reported in *PCCA* gene implicated in causing PA including gross deletions. Most variants in *PCCA* gene cause protein instability [16]. *PCCB* gene was mapped to chromosome 3q22.3 and contains 15 exons [17] coding for 539 amino acids. Functional analysis of *PCCB* sequence variants revealed 3 effects: retention of PCC activity, null or very low activity [18]. To date, there are 112 pathogenic variants in *PCCB* gene reported to cause PA; HGMD (http://www.hgmd.org). Large deletions in *PCCB* gene are far less frequent than in the *PCCA* gene. The beta subunit of PCC enzyme contains a propionyl-CoA binding site that is responsible for transferring the carboxyl group to propionyl-CoA and most pathogenic variants in *PCCB* are predicted to alter the active site and reduce PCC activity [16].

In newborn screening programs, plasma acylcarnitine analysis using tandem mass spectrometry is the initial step in the diagnosis of PA [19] and is followed by urine organic acid analysis using gas chromatography/mass spectrometry [20]. Further confirmation can be pursued by measuring the PCC enzyme activity in leucocytes or fibroblasts and sequencing the *PCCA* and *PCCB* genes for pathogenic variants [13,21,22]. The Clavero et al. study also allows for genotype- phenotype correlation and prevention through reproductive genetic counselling. Apart from newborn screening, the diagnosis is usually established through clinical assessment and suspicion during a metabolic crisis and confirmed using the same biochemical and molecular tests described above [23].

Despite its high prevalence, many reports from Saudi Arabia describing PA and the corresponding molecular causes were in singleton cases [24–26]. In this study, we screened for sequence variants in both the *PCCA* and *PCCB* genes implicated in PA in 84 Saudi Arabian patients. To date, this is the largest cohort to be studied from this part of the world. The Saudi Arabian population has a tribal structure and the overall rate of consanguineous marriage is reported to be over 55%, with regional variations [27]. In such a population, the identification of a molecular genetic cause of PA allows both a confirmatory diagnosis, cascade carrier screening and prenatal testing for at-risk relatives.

Our study is aimed to describe the molecular basis of PA in Saudi Arabian patients and to determine possible founder variants in this population. Although there has been no significant genotype-phenotype correlation described in our population to date, this study attempts to draw a relevant relationship between the genotype and clinical phenotype in our patient cohort.

2. Materials and methods

2.1. Study cohort

The cohort consists of 84 Saudi Arabian patients from individual 84 families. PA diagnosis was based on newborn screening programme where elevation of C3-carnitine and C3-/C2-carnitine ratio was recorded in addition to clinical phenotype that include poor feeding, vomiting, lethargy, metabolic acidosis, and hyperammonemia. The study has been approved by the IRB of King Faisal Specialist Hospital, Riyadh, Saudi Arabia (RAC# 2020 011). DNA was extracted from peripheral blood cells using the Gentra Systems PUREGENE DNA Isolation kit (Qiagen, Valencia, California, USA).

2.2. Sequence variants analysis

Screening for sequence variants was undertaken of known genes implicated in PA; *PCCA* gene (RefSeq: NM_000282.3) and *PCCB* gene (RefSeq: NM_000532.4). Direct sequencing of all coding exons and exon–intron boundaries was performed for both genes. Oligonucleotide primers for PCR amplification of genomic DNA were designed using Primer3 software (http://frodo.wi.mit.edu/) and synthesized by Metabion International AG (Munich, Germany). PCR was performed in a final volume of 25 μl containing approximately 20 ng of genomic DNA and Qiagen (Manchester, UK) master mix kit (including 1 × PCR buffer, 100 mmol l^{-1} dNTP, and 1 U per reaction HotStar Taq polymerase) and 0.5 mmol l^{-1} primer. PCR products were treated with the Agencourt AMPure PCR purification system (Agencourt Bioscience Corporation,

Table 2
Novel genetic variants detected in *PCCA* and *PCCB* genes and *in silico* analysis of pathogenicity.

Gender	Date of birth	Gene	Nucleotide change	Amino-acid change	Zygosity	Mutation category	PolyPhen-2	SIFT	Provean	MutationTaster	Age of diagnosis	Clinical Features	Laboratory findings
Male	06 Jul 2014	*PCCA*	c.111_130del20	Tyr39Asnfs*5	Homozygous	frame-shift	N/A	N/A	N/A	N/A	Neonatal period	ID Hypotonia, Recurrent MC, ECEPH and pancreatitis	Recurrent; metabolic acidosis, hyperammonemia, ketonuria. Pancytopenia and increased serum lipase
Male	12 Aug 2007	*PCCA*	c.266C > G	Thr89Arg	Homozygous	Missense	Probably damaging	Damaging	Deleterious	Disease causing		ID Hypotonia, Recurrent MC, ECEPH. infrequent pancreatitis, SZ disorder	Recurrent; metabolic acidosis, hyperammonemia, ketonuria. Pancytopenia and increased serum lipase
Male	01 Oct 2013	*PCCA*	c.955C > T	Gln319*	Homozygous	Nonsense	N/A	N/A	N/A	N/A		Mild ID, rare MC	metabolic acidosis
Male	05 May 2009	*PCCA*	c.1375_1391dup + c.1391ins		Homozygous	frame-shift	N/A	N/A	N/A	N/A	Neonatal period	ID Hypotonia, Recurrent MC, ECEPH pancreatitis	Recurrent; metabolic acidosis, hyperammonemia, ketonuria. Pancytopenia and increased serum lipase
Female	01 Aug 1991	*PCCA*	c.2120 T > G	Val707Gly	Homozygous	Missense	Probably damaging	Damaging	Deleterious	Disease causing		ID, Hypotonia, Recurrent MC ECEPH	Recurrent; metabolic acidosis, hyperammonemia, ketonuria. Pancytopenia
Male	1993	*PCCB*	c.90_108delins14	Ser30Argfs*25	Homozygous	frame-shift	N/A	N/A	N/A	N/A		ID, Hypotonia, Recurrent MC, ECEPH, Pancreatitis	Recurrent; metabolic acidosis, hyperammonemia, ketonuria. Pancytopenia
Male	04 Oct 2010	*PCCB*	c.518_543del26	Leu173*	Homozygous	frame-shift	N/A	N/A	N/A	N/A	Neonatal period	ID, Hypotonia, Recurrent MC, ECEPH pancreatitis choreoathetoid movements	Recurrent; metabolic acidosis, hyperammonemia, ketonuria. Pancytopenia
Female	19 Oct 2010	*PCCB*	c.629 T > C	Leu210Pro	homozygous	Missense	Probably damaging	Damaging	Deleterious	Disease causing	Neonatal period	ID, Hypotonia, Recurrent MC, ECEPH pancreatitis choreoathetoid movements	Recurrent; metabolic acidosis, hyperammonemia, hypoglycemia ketonuria. Pancytopenia increased serum lipase
Male	22 Oct 2000	*PCCB*	c.866G > C/c.990dupT	Arg289Pro Glu331*/	Compound heterozygous	Missense	Probably damaging	Tolerated	Deleterious	Disease causing		ID, Hypotonia, Recurrent MC less with age, ECEPH infrequent pancreatitis, SZ disorder,	Recurrent; metabolic acidosis, hyperammonemia, ketonuria. Pancytopenia increased serum lipase
Male	12 Sep 2011	*PCCB*	c.1088C > T	Ser363Leu	Homozygous	Missense	Possibly damaging	Damaging	Deleterious	Disease causing	Neonatal period	ID, Hypotonia, Recurrent MC, ECEPH pancreatitis preterm.	Recurrent; metabolic acidosis, hyperammonemia, hypoglycemia ketonuria. Pancytopenia increased serum lipase
Female	15 Apr 2004	*PCCB*	c.1163 T > A	Leu388His	Homozygous	Missense	Probably damaging	Damaging	Deleterious	Disease causing		ID, Hypotonia, Recurrent MC ECEPH	Recurrent; metabolic acidosis, hyperammonemia, ketonuria. Pancytopenia

ID: Intellectual Disability, SZ: Seizure, MC: MC, ECEPH: encephalopathy

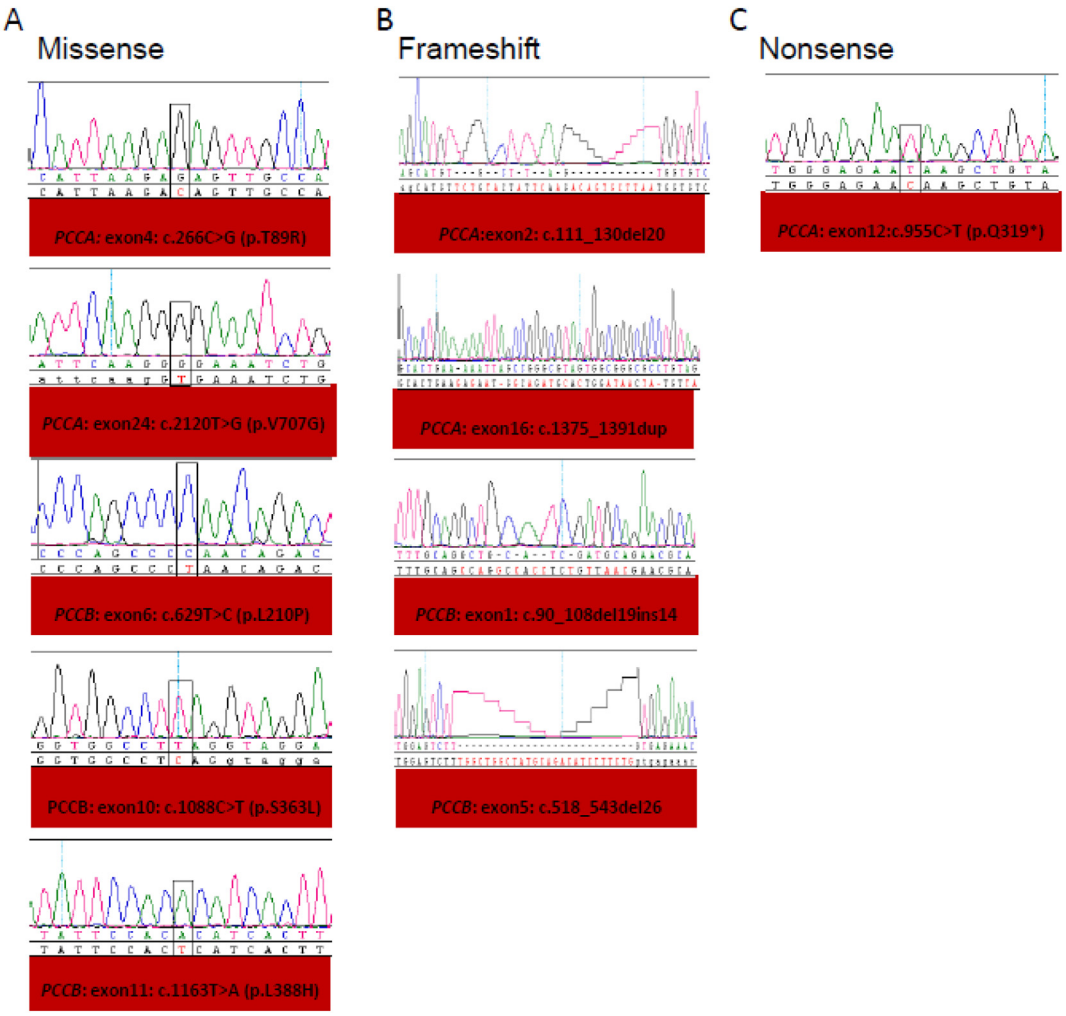

Fig. 1. Novel mutations identified in cohort with PA phenotypes in Saudi population (A) Five missense (B) Four frame shift and (C) One nonnense novel mutations (boxed) were detected homozygously in patients. Normal sequence is show alongside.

Beverly, MA, USA). PCR products were sequenced using BigDye Terminator Cycle Sequencing kit (PE Applied Biosystems, Beverly, MA, USA) as described by the manufacturer. Sequences were analysed using Mutation Surveyor software Version 3.24 (SoftGenetics LLC, State College, PA, USA) and SeqMan II software 6.1 (DNAStar, Madison, WI, USA). For predicting the damaging effect of novel missense variants, three *in silico* prediction tools were used: PolyPhen-2 (http://genetics.bwh.harvard.edu/pph/), Provean (http://provean.jcvi.org/index.php), and Mutation Taster (http://www.mutationtaster.org/). PolyPhen-2 scores range from 0 to 1, the higher the score the more damaging the amino-acid substitution. Provean scores range from 0 to 1. The amino-acid substitution is predicted damaging if the score is ≤ 0.05, and tolerated if the score is > 0.05. Allele frequency of all novel sequence variants was determined using a local database (SGP737) of 550 patients containing Arab-specific variants and the ExAC database (http://exac.broadinstitute.org/). Evolutionary conservation was determined from sequence alignments using Mutation Taster and UCSC (https://genome.ucsc.edu).

Protein folding predictions were generated with the Phyre2 application [28] (http://www.sbg.bio.ic.ac.uk/phyre2) using the 'intensive' modelling mode.

3. Results and discusion

Eighty four individual cases affected with PA representing 84 families from Saudi Arabia were screened for sequence variants in the *PCCA* and *PCCB* genes. A molecular genetic diagnosis was established in all families studied. A total of sixteen different sequence variants were detected in the study, where 7 pathogenic variants were detected in *PCCA* and 9 in *PCCB* (Table 1). Pathogenic variants in *PCCA* represent over 80% of genetically proven PA in this population. Previously published data from a world-wide cohort suggested that ~50% of PA is caused by pathogenic variants in *PCCA* gene and other 50% by variants in *PCCB* gene [15]. Although it has been reported that most *PCCA* pathogenic variants are private [15]; the missense variant (c.425G > A; p.Gly142Asp) in *PCCA* is the most common cause of PA in our cohort and was found in 59 patients (70.2%). All sequence variants detected in our cohort were in homozygous state except one family who was compound heterozygous.

Of the novel homozygous or compound heterozygous sequence variants in coding regions (Table 2 and Fig. 1), we identified 1 novel nonsense variant, 4 frameshift variants and 6 missense variants (11 novel disease causing variants). *In silico* predictions suggested that the missense changes are indeed pathogenic affecting the function of PCC (Table 2). A detailed analysis of the results for *PCCA* and *PCCB* genes is given below:

3.1. PCCA gene mutational analysis

All variants in affected individuals were found in a homozygous state consistent with known parental consanguinity. Variants detected in *PCCA* accounted for around 81% of all the variants detected in our

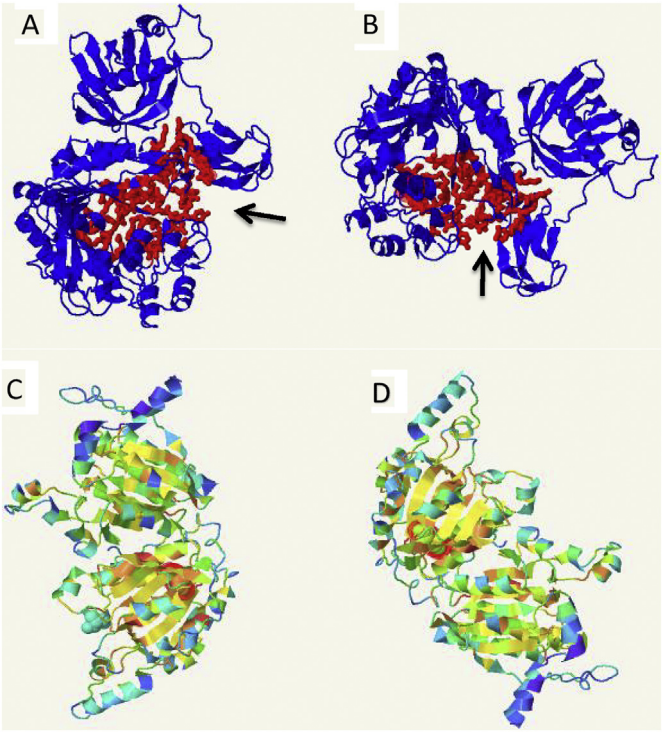

Fig. 2. Peptide modelling of 2 missense mutations in *PCCA* and *PCCB* genes; Arrows in A & B are showing predected active site (in red) in PCCA where A represent wild type PCCA and B represent PCCA with the mutation Gly142Asp. C is showing normal configration of PCCB while D is showing the configration with changing the amino acid Leu to His at position 388. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

cohort. In this gene we found 2 known pathogenic variants [26,29] and 5 novel homozygous sequence variants predicted to affect PCC function (Tables 1, 2). The novel pathogenic variants included two frameshift variants, two missense variants and one nonsense variant (each variant was found privately in one family only). The novel c.955C > T nonsense variant (p.Gln319*) was located near a previously characterized nonsense variant p.Arg313* [30] that was reported to show a significance response to different readthrough drugs [31]. Interestingly, the patient homozygous for the novel nonsense variant (p.Gln319*) showed a milder phenotype in comparison to other similar variants detected. However, the patient passed away at age of 4 years with cardiopulmonary arrest. The c.111_130del20 frameshift variant (p.Cys44Leufs*6) is the most premature truncating variant reported so far in *PCCA*. The other c.1375_1391dup17 frameshift variant is a complex insertion/duplication variant. The c.1375_1391dup17 frameshift variant is a complex insertion/duplication variant. This complex variant involves a duplication of 17 nucleotides in exon 16 of the *PCCA* gene starting at nucleotide 1375 and between the duplicated regions a large fragment with poly (A) rich sequence (~200 bp) is inserted. This variant is predicted to be disease-causing. Finally, the c.1288C > T nonsense variant (p.Arg430*) that has been reported previously [29] was found in two families of our cohort (Table 1).

In silico analysis suggested the pathogenicity of two of the novel (p.Thr89Arg and p.Val707Gly) missense variants detected in the study (Table 2). Both sequence variants were not found in our local database; SGP737 (http://shgp.kfshrc.edu.sa/bioinf/db/variants/mendliome/) nor found in the ExAC database or 1000 Genome. Among our PA cohort there were 59 patients (Table 1) with the pathogenic missense variant (c.425G > A; p.Gly142Asp). This pathogenic variant has been reported previously by colleagues in two PA patients in association with visual hallucinations [26] and by another group [8]. The variant p.Gly142Asp is located in the "A" subdomain of biotin carboxylase domain of the PCC enzyme. The A and C subdomains form the active site of PCCA subunit where p.Gly142 is part of active site and thus predicted to affect the catalysis or substrate binding [11]. Using fpocket2 program [32] where large pockets are frequently found to be the location of active site, the protein folding predicted for the p.Gly142Asp variant is showing a conformational change in the location of the active site of the protein when compared to normal folding (Fig. 2A & B). The p.Gly142Asp variant is the most common pathogenic variant in our cohort and would be considered as a founder pathogenic variant in Arabs.

Patients with the p.Gly142Asp variant presented with a moderate-severe phenotype including developmental delay, metabolic crisis, hypotonia and pancreatitis. Fig. 3A and B represent longitudinal data of two PA patients with a homozygous p.Gly142Asp variant. Biochemical measurements of ammonia (NH3), carbon dioxide (CO2), lipase enzyme level, and haemoglobin for eight years period for the first patient (Fig. 3A) are shown. The patient had frequent episodes of hyperammonemia, recurrent metabolic acidosis, mild anemia and infrequently elevated lipase levels as indicative of pancreatitis. The patient had a sudden death at 17 years of age while admitted with a mild crisis. The second patient (Fig. 3B) presented with recurrent hyperammonemia, metabolic acidosis, pancreatitis, mild anemia, intellectual disability, and attention deficit hyperactivity disorder (ADHD). The patient is currently 15 years old and still alive, and the corresponding data shown is for almost a 14 year period. Although most variants reported in *PCCA* and *PCCB* genes are private, we report here the first founder pathogenic variant in *PCCA* gene in our population.

3.2. Genotype-phenotype correlation for the missense variant (c.425G > A; p.Gly142Asp)

Although the overall clinical presentation of patients with this genotype is not too distinct from the reported classical clinical course of early onset PA [33–35], several observations are summarized below based on the longitudinal follow up of patients up to almost 20 years: a) all patients present in the neonatal period with symptoms of poor feeding, vomiting, lethargy, hypotonia, tachypnoea, variable metabolic acidosis compensated by respiratory alkalosis. The condition proceeds rapidly to acute encephalopathy characterized by variably depressed level of consciousness eventually leading to coma. Hyperammonemia is a constant feature in the neonatal period and can be severe enough to require hemofiltration. If treated early and aggressively the majority of the patients recover; b) and then present with an unpredictable recurrent metabolic crisis characterized with variable degree of hyperammonemia, metabolic acidosis, and pancreatitis with elevated lipase and amylase. Metabolic acidosis is often more severe when pancreatitis is present and requires longer period of hydration and hospitalization for recovery. Anemia, which often becomes chronic with acute exacerbations during crisis secondary to bone marrow suppression and frequent blood extraction, is commonly observed (Fig. 3A and B). Leukopenia and thrombocytopenia is often but not always observed, it usually appears a few days into crisis; c) presentation with only metabolic acidosis or pancreatitis is also observed. Presentation with only hyperammonemia is also observed, however much more commonly in older patients; d) another common observation is the dissociation of hyperammonemia and clinical symptoms as patients grow older. It seems that the tolerance of neurons to hyperammonemia increases with age. It is common to see patients with serum ammonia of over 200 μmol/l who are completely active with no symptoms or are oligosymptomatic with only nausea and a few episodes of vomiting; e) visual hallucinations and autism has also been reported in patients with this genotype [24,26] f) survival is usually limited to 20 years of age. Triphasic mortality is observed. Some patients die in the neonatal period due to severe crisis, although this is becoming less common with newborn screening and precautionary measures for at risk neonates,

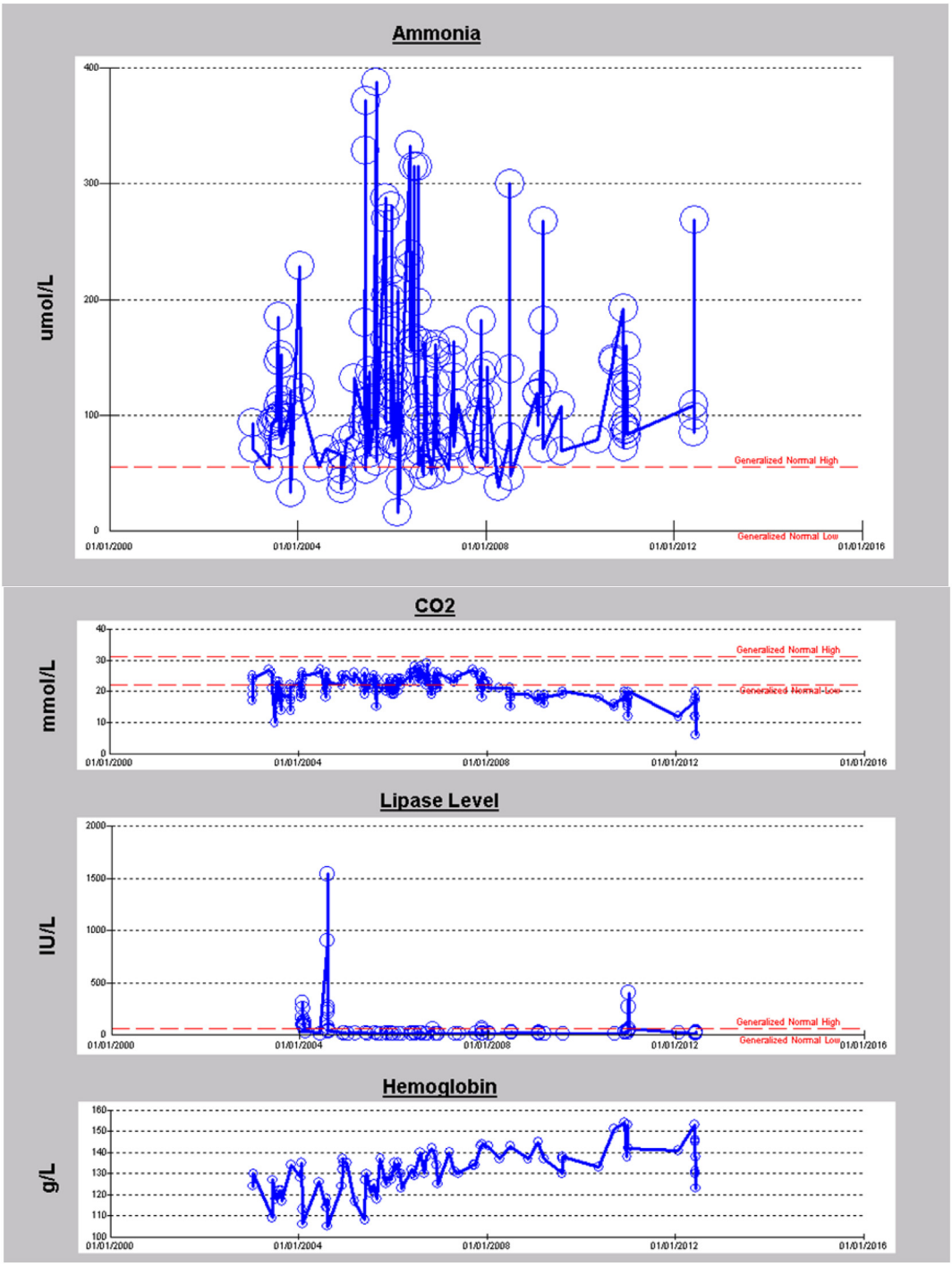

Fig. 3. A. 8 years longitudinal data for first patient with a homozygous p.Gly142Asp mutation in *PCCA* gene. B. 14 years longitudinal data for second patient with a homozygous p.Gly142Asp mutation in *PCCA* gene.

death around the first decade and then 2nd decade of life. The cause of death in the later years is usually a combination of poor overall health due to chronic disease, intercurrent infection often related to an indwelling central line, aspiration pneumonia, metabolic acidosis, pancreatitis, metabolic encephalopathy with hyperammonemia, stroke involving the basal ganglia and brain oedema. This relatively homogeneous PA patient population may provide an excellent opportunity for clinical trials for emerging therapies for this disease.

3.3. PCCB gene mutational analysis

Among our PA cohort there were 16 families with *PCCB* sequence variants (Table 1). All variants in affected individuals were in a homozygous state except one family where a compound heterozygous variant was found. Pathogenic variants detected in *PCCB* accounted for around 19% of all the variants detected in our cohort. A total of 9 variants were detected in *PCCB* in this cohort. Six of these variants are novel and not reported previously; four of which missense and 2 frameshift variants (Table 2). The c.90_108delins14 frameshift variant was found in one family and it involves the deletion of 19 nucleotides and

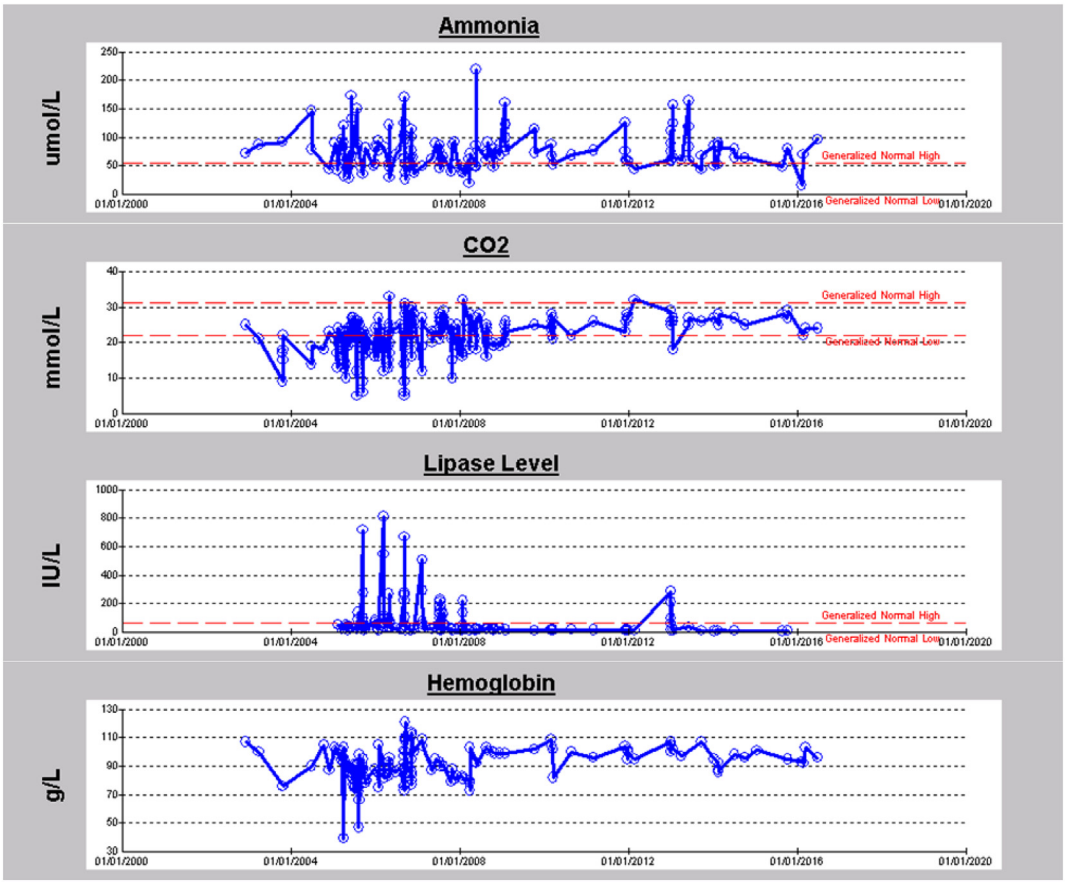

Fig. 3. (*continued*)

insertion of 14 nucleotides (gctgcatcgatgca), resulting in premature protein truncation (p. S30Rfs*25). The second novel c.518_543del26 frameshift variant (p.Leu173*) was also found in one family leading to protein truncation. Interestingly, Lévesque et al. [36] described a smaller deletion at same position c.517_518delTT (p.L173Gfs*56) causing truncation.

All missense variants detected were located at the C-terminus of propionyl-CoA carboxylase beta chain apart from the c.629 T > C variant (p.Leu210Pro), located at the N-terminus of the beta chain of PCC. The c.1163 T > A variant (p.Leu388His) was detected in two different families while 6 other novel *PCCB* variants were found in one family each. Modelling of p.Leu388His using Phyer2 showed configuration change of the protein in comparison to the normal protein (Fig. 2C & D).The novel c.866G > C missense variant (p.Arg289Pro) was detected in a compound heterozygous state with another reported c.990dupT frameshift variant (p.Glu331X) [37]. Regarding the novel c.1088C > T missense variant (p.Ser363Leu), the serine residue at position 363 (p.S363) is mutated to leucine in our cohort but was shown to be mutated to proline in another study [38]. The reported variant c.990dupT (p.Glu331*) was found in 7 families and accounted for 44% of variants in *PCCB*. In our cohort, there are 4 *PCCB* variants detected leading to premature protein truncation; Ser30Argfs*25, Arg113*, Leu173*, and Glu331*.

4. Conclusions

To date, this study is the largest to describe molecular basis of PA in the Saudi Arabian population. To summarize, all patients with identified variants in PA associated genes were from consanguineous marriages and most of the pathogenic variants identified were in the homozygous state. In our study, which included 84 families, pathogenic variants were detected in all cases. Such a high mutation detection rate would not be achieved without a comprehensive and confirmatory clinical assessment and biochemical analysis. All sequence variants detected were considered to be pathogenic; we carefully checked for the presence of additional variants in both genes (*PCCA* and *PCCB*) and confirmed the variant co-segregated, where parental samples were available. The allele frequency of all novel sequence variants was zero in all databases checked. Most missense variants detected in *PCCA* and *PCCB* in the altered amino acids in conserved regions of the protein. The p.Gly142Asp variant in *PCCA* is the most common cause of PA in our population followed by the Glu331* variant in *PCCB,* while all other variants detected were private familial variants. The p.Gly142Asp affects almost 70% of our PA patients and no such variant frequency has been reported in other populations studied so far [15,38–40]. The presence of a common pathogenic variant in *PCCA* signifies the homogeneity of Saudi Arabian population. The molecular genetic diagnosis of PA is a vital aid to the clinical management of families with PA, cascade carrier screening and prenatal testing for at-risk relatives.

In conclusion, in a Saudi Arabian cohort of families with PA we were able to detect disease causing variants in all families in the known propionic acidemia genes; *PCCA* and *PCCB*. Pathogenic variants in *PCCA* were the most common molecular genetic cause of PA in our cohort. Our study confirms the c.425G > A (p.Gly142Asp) variant to be the most common and a founder variant causing PA in the Saudi Arabian population. A clear insight into a genotype-phenotype correlation for this pathogenic variant is documented here.

Disclosure statement

The authors declare no conflicts of interest.

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