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ARTICLE

Potential relationship between genotype and clinical outcome in propionic acidaemia patients

C Pérez-Cerdá[1], B Merinero[1], P Rodríguez-Pombo[1], B Pérez[1], LR Desviat[1], S Muro[1], E Richard[1], MJ García[1], J Gangoiti[1], P Ruiz Sala[1], P Sanz[1], P Briones[2], A Ribes[2], M Martínez-Pardo[3], J Campistol[4], M Pérez[5], R Lama[6], ML Murga[7], T Lema-Garrett[8], A Verdú[9] and M Ugarte[1]

[1]Centro de Diagnóstico de Enfermedades Moleculares, Centro de Biología Molecular Severo Ochoa, CSIC-UAM, Madrid; [2]Instituto de Bioquímica Clínica, Corporación Sanitaria y CSIC, Barcelona; [3]Centro E Ramón y Cajal, Madrid; [4]Hospital San Juan de Dios, Barcelona; [5]Hospital Virgen del Rocío, Sevilla; [6]Hospital La Paz; [7]Hospital Príncipe de Asturias, Alcalá de Henares, Madrid; [8]Hospital Ntra Sra de Sonsoles, Avila; [9]Hospital Virgen de la Salud, Toledo, Spain

Propionic acidaemia (PA) is an autosomal recessive disorder caused by mutations in either of the *PCCA* or *PCCB* genes which encode the α and β subunits, respectively, of the mitochondrial enzyme propionyl-CoA carboxylase (PCC). In this work we have examined the biochemical findings and clinical outcome of 37 Spanish PA patients in relation to the mutations found in both *PCCA* and *PCCB* genes. We have detected 27 early-onset and 10 late-onset cases, showing remarkably similar biochemical features without relation to either the age of onset of the disease or the defective gene they have. Twenty-one of the patients have so far survived and three of them, now adolescents, present normal development. Different biochemical procedures allowed us to identify the defective gene in 9 PCCA deficient and 28 PCCB deficient patients. Nine putative disease-causing mutations accounting for 77.7% of mutant alleles were identified among PCCA deficient patients, each one carrying a unique genotypic combination. Of PCCB mutant alleles 98% were characterised. Four common mutations (ins/del, E168K, 1170insT and A497V) were found in 38/52 mutant chromosomes investigated, whereas the remainder of the alleles harbour 12 other different mutations. By examining the mutations identified both in *PCCA* and *PCCB* genes and the clinical evolution of patients, we have found a good correlation between certain mutations which can be considered as null with a severe phenotype, while certain missense mutations tend to be related to the late and mild forms of the disease. Expression studies, particularly of the missense mutations identified are necessary but other genetic and environmental factors probably contribute to the phenotypic variability observed in PA.
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Keywords: propionic acidaemia; mutation analysis; phenotype/genotype correlation

Introduction

Propionic acidaemia (MIM 232000, MIM 232050) is caused by deficiency of the mitochondrial biotin-dependent enzyme propionyl-CoA carboxylase (PCC; EC 6.4.1.3) that catalyses the carboxylation of propionyl-CoA to D-methylmalonyl-CoA.[1] In most affected patients the disease presents as a life-threatening ketoacidosis during the neonatal period and is associated with mental retardation and early death; in addition, some patients show a chronic late-onset form with milder symptoms and long survival rate. PCC is probably a dodecamer enzyme composed of α and β subunits (α6β6), with the α subunit containing the biotin prosthetic group.[2] PA results from mutations in either the *PCCA* or *PCCB* genes that encode for the α and β PCC subunits, respectively,[3]

Correspondence: Professor M Ugarte, Centro de Biología Molecular 'Severo Ochoa', Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain. Tel: +34917347011; Fax: +34917347797; E-mail: mugarte@cbm.uam.es
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defining two main intergenic complementation groups *pccA* and *pccBC*, the latter including two subgroups *pccB* and *pccC*.[4] The *PCCA* gene is located on chromosome 13q32[5] and the *PCCB* gene on chromosome 3ql3.3–q22.[5,6]

Prior to mutation analysis, identification of the responsible gene can be performed by complementation studies, by analysis of steady-state levels of PCC proteins, or by measurement of PCC activity in parents, as most parents of PCCB-deficient patients show normal levels of PCC activity rather than the 50% level expected of heterozygotes.[7] To date, 53 PA mutations have been reported on both *PCCA* and *PCCB* genes, as recently reviewed.[8] A great genetic heterogeneity is observed in the *PCCA* gene detecting no prevalent mutation in the patients investigated. However, a limited number of mutations in the *PCCB* gene accounts for most of the alleles investigated in both Caucasian and Oriental populations. Several reports compilating clinical and biochemical data have been published,[9–12] but the relationship between phenotype and genotype has not yet been examined.

In this work, we present the biochemical picture and clinical features of 37 Spanish PA patients in relation to the mutations found in the *PCCA* and *PCCB* genes, exploring a potential phenotype–genotype correlation.

Material and methods

Subjects

Thirty-seven Spanish PA patients from 35 unrelated families were included in this study. Table 1 lists information about family history and survival of patients. Twenty-seven patients were grouped in early-onset form when symptoms appeared within the first 3 weeks of life and 10 of them in late-onset form presenting after two months of life. Two patients diagnosed at birth (ALT and DVA) were included in the early-onset group due to a previous neonatally affected sib. Most early-onset patients exhibited clinical signs of the intoxication-type disease with vomiting, somnolence and/or coma, hypotonia and abnormal movements as myoclonic jerks and convulsions in some cases. The clinical manifestations in the 10 late-onset patients were more insidious and usually related to infections. When diagnosed, most patients had a remarkably similar biochemical phenotype, showing metabolic ketoacidosis, hyperammonaemia, hyperglycinaemia, hypocarnitinaemia (< 11 μmol/l) and the characteristic urinary organic acids pattern of PA. The most consistently organic acids found were methylcitric and 3-hydroxypropionic acids, 3-hydroxy-2-methylbutyric acid, tiglylglycine and propionylglycine. PCC deficiency was confirmed by direct assay of fibroblasts or lymphocytes. PCC activity was less than 5% of controls, except for one late onset case (OVE) who showed a clearly high residual activity of 11%. Clinical and biochemical details of some of these patients have already been described.[13–18]

Table 1 Family history and survival of 37 Spanish propionic acidaemia patients

	Early onset (newborn–20d)	*Late onset (2 m–5.5y)*	*% of total patients*
Number of patients	27 (73%)	10 (27%)	
Male/female	9/18	6/4	40.5/59.5
Age at diagnosis	26.9d (1d–5m)	23.5m (6m–6y)	
Consanguinity of parents	4	1	13
Unexplained family deaths	5	2	19
Affected siblings	2	0	5
Number of survivors	13	8	57
Present age of survivors	6.6y (15m–15.5y)	12.6y (2.5y–25y)	
Median survival age of deceased	16.7m (15d–6.5y)	16m; 5.5y	

d: days; m: months; y: years; ranges are in parentheses.

Samples

Lymphocytes were isolated from heparinized blood, and skin fibroblasts from patients, parents and controls were cultured by standard procedures. Whole blood and dried blood spots were also obtained as DNA source from controls, patients and families.

Genetic classification of PA patients

Carboxylase activities,[19] ^{3}H-biotin labelling experiments[3] and complementation studies[20] were performed as described earlier.

Molecular studies

Genomic DNA and total RNA were isolated from cultured skin fibroblasts by standard methods.[21,22] Detection of mutations in the *PCCA* gene were done by sequencing overlapping fragments of the complete *PCCA* coding sequence obtained by RT-PCR or of known exonic fragments as described.[23] *PCCB* mutations were analysed by direct cycle sequencing of each of the 15 *PCCB* exons using described primers.[20] Western blot analysis was performed according to standard procedures using affinity purified anti-β-PCC from immunised rabbits as primary antibody.[24] Northern blot analysis was carried out according to standard procedure as described.[23] The α and β subunit cDNAs were both kindly provided by Dr D Leclerc and Professor RA Gravel.

Results

Genetic classification of PA patients

Of 37 PA patients, 9 cases were identified as PCCA deficient and 28 (26 families) as PCCB deficient based on the following evidence.

Enzymatic findings in parents Carboxylase activities in fibroblasts or lymphocytes were measured in parents of 30 patients which resulted in identification of the defective gene in 27 of them. PCC activity in parents of 8 PCCA-deficient patients showed intermediate values with a diminished PCC/MCC ratio. On the other hand, parents of 19 patients with mutations in the *PCCB* gene, showed normal

PCC activity and PCC/MCC ratio as controls. The results of this study in parents' fibroblasts are summarised in Table 2. In three families bearing mutations in the *PCCB* gene, we have found discrepant results. Both parents of MRA and PRG presented intermediate values measured in fibroblasts and the mother of NPU also showed an intermediate PCC activity value measured in lymphocytes (data not shown).

^{3}H-Biotin labelling experiments With this procedure we have identified the defective gene in patients MRA, PRG and NPU whose parental PCC activities were uncertain and in seven patients with no parent sample available for enzymatic determination.

Three radioactive bands of 120 KDa, 74 KDa and 72 KDa corresponding to the biotin containing polypeptides of PC, α-MCC and α-PCC, respectively, were detected after SDS-PAGE and fluorography of a ^{3}H-biotin labelled extract of normal cells (Figure 1, lane C). Patients with defects in the *PCCA* gene showed no detectable band corresponding to the α chain of PCC (Figure 1, lanes 4, 6), except for case SAG who showed a faint band (Figure 1, lane 5), whereas patients bearing mutations in the *PCCB* gene had an α-PCC radioactive band similar to controls (Figure 1, lanes 1, 2 and 3).

Table 2 PCC activity in fibroblasts of parents of 20 patients with propionic acidaemia

	PCC[a] activity (pmol/min/ mg protein)	*Ratio PCC/MCC[b]*	*% of mean PCC*
PCCA deficient group			
Mean ± SD (*n*=12)	360±110	1.45±0.1	41±13
(range)	(198–500)	(1.2–1.6)	
PCCB deficient group			
Mean ± SD (*n*=24)	992±300	2.8±0.8	103±44
(range)	(500–2112)	(1.8–4.2)	
Parents MRA	400; 310		44; 35
Parents PRG	290; 240	1.8; 2.0	32; 27
Adult controls			
Mean ± SD (*n*=9)	893±300	2.5±0.3	100
(range)	(598–1272)	(2.0–2.8)	

[a]propionyl-CoA carboxylase; [b]β-methylcrotonyl-CoA carboxylase.

Complementation tests Studies were performed in 15 patient cell lines. Cells were fused with tester *pccA* and *pccBC* cell lines followed by examination of the ^{14}C-propionate incorporation rate (results not shown). The results obtained were in accordance with the previous assignment of patients to be PCCA or PCCB deficient patients performed by the above methods.

Mutation analysis and clinical outcome

A total of 9 and 16 different putative disease-causing mutations were detected in the *PCCA* and *PCCB* genes, respectively, accounting for 92.8% of all Spanish PA chromosomes.

The percentage of PCC activity, genotype and clinical features of nine PCCA deficient patients are given in Table 3. Among the nine mutations identified in the *PCCA* gene no prevalent one was found. Two nonsense (R288X and S537X), three splicing (1771IVS–2del9, 1824IVS + 3del4 and 1824IVS + 3insCT) and four missense mutations (R52W, A113T, I139T and G606R) were identified. Data on all these mutations have been reported elsewhere.[23,24] The I450V change, identified in two patients as homozygous, was present in 9% of controls, indicating a polymorphic variation. Until now, no change other than I450V polymorphism was found in patient DFH after sequencing the complete coding sequence. Four cases presented in the late-onset form of the disease and they are now alive, two of them (SAG and ESG) presenting a normal physical and mental development without dietary protein restrictions. Only one early-onset case (ANA) is alive but severely handicapped.

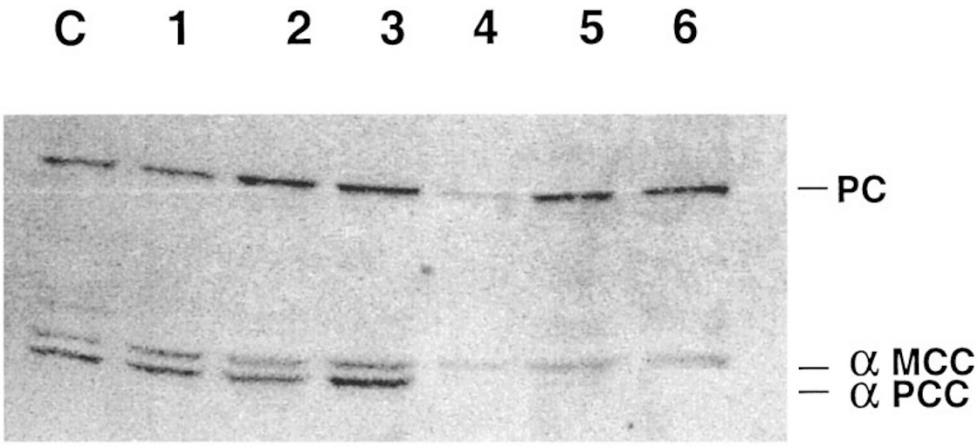

Figure 1 SDS-PAGE of ^{3}H-biotin labeled extracts of normal and PCC-deficient fibroblasts detected by fluorography. Radioactive bands correspond to the biotin-containing polypeptides of PC = pyruvate carboxylase; MCC = β-methylcrotonyl CoA carboxylase and PCC = propionyl CoA carboxylase. C, control cell line; Lanes 1–3: PCCB-deficient patients MRA, PRG and NPU; lanes 4–6: PCCA-deficient patients MPS, SAG and DFH respectively.

Table 3 Genotype and clinical outcome of nine PCCA-deficient patients

Patient (Year of dx[a])	*Type of presentation (Age onset/Age dx)*	*% PCC[b] activity*	*Genotype*	*Outcome*
MPS (1986)	Early (3d/7d)	0	R288X, R288X	Died 6.5y when varicella. Psychomotor delay, convulsions and feeding problems
DFH (1988)	Early (3d/8d)	0	I450V[P], I450V	Died 1y
ANA (1998)	Early (20d/24d)	0	S537X, S537X	Alive 1.5y. Severe psychomotor delay and hypotonia. Spastic tetraparesis. Weight p10th. LPD[c].C[d]
JCS (1983)	Early (2d/15d)	0	G606R+I450V, G606R+I450V	Died 3y
JJLF (1985)	Early (7d/20d)	1.2	1824IVS+3insCT, ND[e]	Died 25d
JMMO (1991)	Late (3m/14m)	4	A113T, A113T	Alive 9y. Mild psychomotor delay. Speech problems. Normal MRI (3y). Psoriasis. Weight p50th and height p90th. LPD.C
SAG (1986)	Late (15m/6y)	3.8	R52W, 1771IVS-2del9	Alive 18.5y. Normal development. Weight p90th and height p75th. NDPR[f]
MSH (1979)	Late (16m/17m)	1.3	1824IVS+3del4, 1824IVS+3del4	Alive 21y. Microcephaly at birth. IQ[g]: 59. Epilepsia crisis 11-13y. Retarded puberal development. Weight and height p90th. NDPR
ESG (1984)	Late (2m/7m)	4	I139T, ND	Alive 15y. Normal development. Weight p50th and height p75th. NDPR

[a]diagnosis; [b]propionyl-CoA carboxylase; [P]polymorphism; [c]low protein diet; [d]carnitine supplementation; [e]not detected; [f]no dietary protein restriction; [g]intelligence quotient.

Details about nine (32.1%) homozygous and 19 (67.8%) heterozygous PCCB-deficient patients are listed in Tables 4 and 5, respectively. It is noteworthy that PCCB homozygotes always present with early onset form. The mutation spectrum includes one insertion/deletion (1218del14ins12 or ins/del), two insertions (790–791insG and 1170insT), one nonsense mutation (W531X), two splice-site alterations (IVS1 + 3G- > C and IVS10–11del6) and ten missense mutations (R44P, S106R, G131R, E168K, G198D, R410W, M442T, A497V, R512C and L519P). Data on all these mutations have been reported elsewhere.[20,25] The four mutations most frequently found were ins/del (29%), E168K (19%), 1170insT (17%) and A497V (8%). Over 50% of the patients are alive, 12/22 early-onset and 4/6 late onset. All them show variable degrees of psychomotor delay and other systemic clinical complications except for three early-onset cases (PRG, JRC and PHG), who currently present good clinical development, and patient SVL who shows normal mental development at 13 years of age.

Western blot analysis of β-PCC subunit

As previously reported, a total absence of immunoreactive β-PCC was detected in all patients carrying mutations in the PCCA gene, except for case SAG in whom traces of β-subunit were detectable[23,24] and in the PCCB-deficient patients who were homozygous for ins/del or 1170insT mutations and in those patients who were compound heterozygotes for ins/del and R512C or 1170insT and L519P mutations.[26] For the remaining PCCB deficient patients, an unstable β-PCC subunit reduced in quantity or partially degraded was observed.[26]

Table 4 Genotype and clinical outcome of nine homozygous PCCB-deficient patients

Patient (Year of dx[a])	*Type of presentation (Age onset/Age dx)*	*% PCC[b] activity*	*Genotype*	*Outcome*
BLT (1986)	Early (3d/23d)	0.8	ins/del, ins/del	Died 27d
ALT (1989)	Early (/1d)	1.9	ins/del, ins/del	Alive 10y. Moderate psychomotor delay. Muscular hypotonia. Myocardiopathy. Weight p90th and height p50th LPD[c].C[d]
CBV (1995)	Early (2d/10d)	2.2	ins/del, ins/del	Alive 4y. Severe psychomotor delay. Severe hypotonia. S. West. Weight p50th and height p75th Gastrostomy. LPD.C
MRA (1996)	Early (7d/3m)	0	ins/del, ins/del	Alive 3y. Moderate psychomotor delay. Speech problems. LPD.C
PRG (1997)	Early (19d/21d)	1.7	E168K, E168K	Alive 2y. Normal development. Brunet-Lezine test: 24m. Weight and height p3th. LPD.C
APJ (1994)	Early (3d/1m)	1.2	1170insT, 1170insT	Alive 4.5y. Moderate psychomotor delay. Muscular hypotonia. MRI (2y): mild frontal atrophy. Weight p75th and height p90th. Gastrostomy. LPD.C
SSL (1993)	Early (5d/23d)	1.6	1170insT, 1170insT	Died 4m when gastroenteritis with SSS[e]
SVL (1986)	Early (2d/4d)	1.3	A497V, A497V	Alive 13y. Normal development. Muscular weakness. Normal MRI (9y). Gastrostomy. Weight p50th and height p10th. LPD.C
CTG (1979)	Early (2d/12d)	1.1	G198D, G198D	Died 9m

ins/del is 1218del14ins12 mutation.
[a]diagnosis; [b]propionyl-CoA carboxylase; [c]low protein diet; [d]carnitine supplementation; [e]scalded-skin syndrome.

Table 5 Genotype and clinical outcome of 19 heterozygous PCCB-deficient patients

Patient (Year of dx[a])	*Type of presentation (Apge onset/Age dx)*	*% PCC[b] activity*	*Genotype*	*Outcome*
AJA (1990)	Early (10d/10d)	0	ins/del, R44P	Alive 9.5y. IQ[c]: 70. Hypotonia. Partial epilepsia. Weight p75th and height p25th LD[d].C[e]
PVD (1991)	Early (20d/1m)	2.3	ins/del, G131R	Died 1.5m
VMP (1989)	Early (4d/14d)	5	ins/del, A497V	Alive 10y. Moderate mental retardation. MRI: discrete cortical atrophy. Weight p50th and height p25th. LPD.C.Ileu[f]
EAC (1995)	Early (20d/2m)	5	ins/del, R512C	Died 3y after febrile illness with diarrhoea
AG (1988)	Early (3d/7d)	0	ins/del, W531X	Died 15d. Congenital cardiopathy and hypothermia
NPU (1989)	Early (6d/5m)	3	ins/del, E168K	Alive 9.5y. Mild psychomotor delay. Tremor Myocardiopathy. LPD.C
MBG (1983)	Early (4d/6d)	0	E168K, IVS1+3G->C	Alive 15.5y. Moderate psychomotor delay. Muscular hypotonia. MRI: mild cortico-subcortical atrophy. Retarded puberal development. Weight p50th and height p10th. LPD.C.Ileu
DMS (1982)	Early (15d/2m)	1	E168K, 1170insT	Lost to follow up at 2y
JRC (1997)	Early (2d/5d)	1	1170insT, M442T	Alive 1.5y. Normal psychomotor development. Weight p75th and height p50th. LPD.C
PHG (1996)	Early (3d/15d)	0	1170insT, L519P	Alive 2.5y. Normal psychomotor development. Hypotonia. Weight and height p50th. LPD.C
LVA (1981)	Early (2d/10d)	0.6	1170insT, ND[g]	Died 2.5y after measles with SSS[h]
DVA (1994)	Early (/1d)	0[L]	1170insT, ND	Died 19d with SSS
CJF (1991)	Early (15d/2.5m)	1.2[L]	A497V, S106R	Died 4m with severe malnutrition and mycoardiopathy
JGG (1974)	Late (4m/16m)	1.5	ins/del, E168K	Alive 25y. Severely retarded. Myocardiopathy. LPD.C
RSC (1989)	Late (5m/6m)	0	ins/del, E168K	Died 16m
LGA (1991)	Late (2m/8m)	1.2	ins/del, E168K	Alive 8y. Psychomotor delay. Autism. Normal MRI (6y). LPD.C.Ileu
BJWG (1998)	Late (4m/22m)	1.1	E168K, 1170insT	Alive 2.5y. Mild psychomotor delay. Speech problems. Partial epilepsia. MRI (1y): arachnoid cyst in left temporal fossa. Weight p50th and height p97th. LPD.C
AML (1997)	Late (2m/7m)	2.4	E168K, 790-791insG+L17M	Alive 2y. Psychomotor delay. Hypotonia. Dystonic movements. Weight and height p3th. LPD.C.Ileu
OVE (1996)	Late (5.5y/5.5y)	11	IVS10-11del6, R410W	Died 5.5y after a metabolic stroke. Basal ganglia infarction

[a]diagnosis; [b]propionyl-CoA carboxylase; [c]intelligence quotient; [d]low protein diet; [e]carnitine supplementation; [f]isoleucine supplementation; [g]not detected; [h]scalded skin syndrome; [L]determined in lymphocytes.

Northern blot analysis

Northern blot analysis of total fibroblasts RNA was performed on six PCCA-deficient patients, all showing normal amounts of both α-PCC and β-PCC mRNA as previously reported.[23] In PCCB-deficient patients, we analysed the genetic compounds which showed no β-subunit by western blot, detecting no β-PCC mRNA in homozygous patients for ins/del or 1170insT mutations (Figure 2, lanes 1, 2) and normal in size and amount in those compound heterozygotes for ins/del and R512C or 1170insT and L519P mutations (Figure 2, lanes 3, 4). β chain mRNA in patients carrying E168K in homozygous fashion and those heterozygous for ins/del and G131R or ins/del and W531X was also normal (Figure 2, lanes 5–7).

Discussion

The aim of the present study was to investigate whether the different genotypes found in Spanish PA patients could be correlated to the clinical phenotypes of the disease.

To classify patients having defective *PCCA* or *PCCB* genes our first method was the measurement of PCC activity in

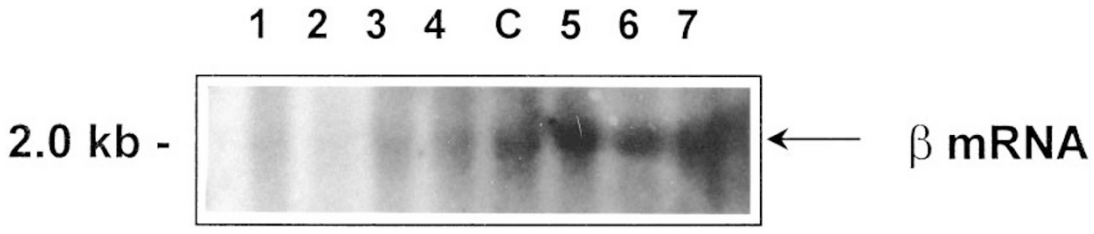

Figure 2 Northern blot analysis of 20 μg of total fibroblasts RNA from PCCB-deficient patients. C, control cell line. Lanes 1–4: patients MRA, APJ, PHG and EAC; lanes 5–7: patients PRG, PVD and AG, respectively.

parents, since the excess synthesis of β-subunit over α-subunit[27] allows a full measure of PCC activity in most parents of patients with β-subunit defects. In our study, the identification of the defective gene based on this approach correlated well with those obtained by ^{3}H-biotin labelling experiments and by complementation tests. In the five parents of PCCB-deficient patients with discrepant results, the intermediate PCC activity found could be explained by taking into account the interindividual variation in PCC activity which showed a normal wide range in both fibroblasts and lymphocytes, or as a result of the presence of stable dysfunctional β-subunits which could result in a lower PCC activity value. This could be the explanation for the data obtained in parents PRG and NPU bearing the E168K mutation.

Genotype–phenotype correlation

On the whole, propionic acidaemia is a very severe disease as illustrated by the number of deaths (15/37) and the number of retarded living patients (15/21); only six patients are developing normally. There is no correlation between clinical phenotype and the affected gene, since the proportion of deaths (44% in PCCA-deficient and 39% in PCCB-deficient patients) or retardations (33% in PCCA-deficient and 42% in PCCB-deficient patients) is similar in both molecular groups.

Our series of PCCA-deficient patients is characterised by the fact that each patient had a unique genotypic combination. Most patients showed a total absence of α and β-PCC subunits because the latter is intrinsically unstable and rapidly degrades if not complexed with α-subunit.[27] Detectable amounts of α and β subunits were only found in fibroblasts of patient SAG, a compound heterozygote for R52W and 1771IVS–2del9 mutations, the latter causing an in-frame deletion of 18 amino acids from the protein sequence.[23] Either allele could account for the residual traces of α and β-PCC subunits which could be related to the normal development of the patient who has always tolerated a regular protein diet. In addition, patient MSH, homozygous for the splicing mutation 1824IVS + 3de14 which cause the same in-frame deletion, also exhibits a relatively mild and long surviving course of the disease. The presumed severity of the nonsense mutations R288X and S537X, which would result in truncated proteins lacking functionally important domains, correlates well with the early presentation and severe phenotype of the patients carrying them. However, the functional consequences of the missense mutations A113T, I139T, and G606R cannot be clearly predicted without expression studies. The presence of the A113T and I139T changes were found to relate to a milder expression of the disease in patients JMMO and ESG. However, the homozygous patient for the G606R mutation also carrying the I450V polymorphism, presented neonatally and proved to be fatal. The polymorphic variation I450V is over-represented in PCCA-deficient patients, 16% versus 9% in controls,[24] and may contribute with different susceptibility to disease as has been described for the variant 625G->A and 511C->T alleles in ethylmalonic aciduria.[28]

Concerning the mutations found in PCCB-deficient patients, we have identified three (1170insT, ins/del and 790–791insG) which could be categorised as null mutations as they produce a premature stop codon. The two mRNA-minus mutations 1170insT and ins/del, resulting in non-detectable β-PCC subunit, are clearly related to a severe phenotype, since all homozygous patients for these mutations presented neonatally, and the four still alive are hypotonic and mentally retarded despite excellent compliance with therapy. Phenotype correlation with the mutations affecting splice site of exon 1 (IVS1 + 3G->C) and 11 (IVS10–11 del6) is difficult to ascertain because they are present only on one chromosome. However, it is worth noting the high residual PCC activity and the unusual manifestation of the disease in the compound heterozygote (OVE) with the R410W and IVS10–11del6 mutations,[17] the latter causing the loss of exon 11 that generates an in-frame transcript and therefore possibly would produce a partially functional protein. Among the 11 missense mutations identified, E168K which results in detectable β-PCC protein is currently the second most frequent in Spain. It has been detected in one homozygous and in 8 compound heterozygous patients, all of them functionally hemizygotes, as E168K is combined with a null mutation on the other allele. The presence of the E168K mutation seems to confer a less severe PA phenotype since five of these nine patients presented a late-onset form of the disease and seven of them are alive with variable developmental delay. The A497V mutation has been detected in three patients from a small village in central Spain. This fact may be explained by consanguinity–CJF and VMP are distally related- or by other factors such as genetic drift. The patient who is a homozygous carrier presents with excellent clinical evolution. Finally, we have identified three changes in exon 15 (R512C, L519P and W531X), which has been postulated to be a hot spot for mutations.[20] The results of a recent study using the *in vivo* mammalian two-hybrid system in COS cells show that these three mutations remove the ability to form β–β homodimers,[29] suggesting that the β-PCC carboxyl terminus could be part of a functional domain involved in homomeric association, and therefore mutations in this region would severely affect PCC enzyme, explaining the severity of the disease in patients carrying R512C and W531X mutations in combination with ins/del.

In summary, several conclusions can be drawn from the present study.

(1) We have identified most of the putative disease-causing mutations in our PA population which will allow an accurate and rapid prenatal diagnosis and carrier status detection for family members–the only reliable way to determine carriers of *PCCB* mutations.[18]

(2) In relation to the affected gene, a poor prognosis of the disease could be expected for the previously described PCCA-deficient patients who carried mutations that affect mRNA stability.[27,30] However, near half our PCCA-deficient patients presented with a mild phenotypic expression, probably as a result of the presence of normal amounts of α-subunit mRNA,[24] indicating a different effect of the mutations.

(3) We have found good correlation with certain mutations which can be considered as null with a severe phenotype, while other missense mutations tend to be related to the late and mild form of the disease.

Future expression studies will provide more insight into the effect of each mutation on the PCC enzyme and its possible relationship with the patient's phenotype. In any case, we believe that irrespective of the PA genotype, interindividual variations in enzymes involved in the secondary pathways which would prevent propionic acid and its toxic derivatives from accumulating, could be of importance in the phenotypic expression of the disease. This is in accordance with the now generally accepted idea of complexity in monogenic traits.[31]

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