Expansion of the Phenotypic Spectrum of Propionic Acidemia with Isolated Elevated Propionylcarnitine

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Abstract We report three patients with elevations of propionylcarnitine (C3), one without elevations of 2-methylcitrate and 3-hydroxypropionate in urine organic acid analysis, and the other two showing only mild elevations, all of whom were subsequently confirmed to have propionic acidemia by molecular analysis of *PCCA* and *PCCB* genes. To date, they have had a mild clinical course. These cases illustrate the importance of considering high C3 as the only biochemical abnormality in a diagnosis of propionic acidemia. Since mild C3 elevations may be overlooked and considered non-diagnostic in isolation, we advise considering a diagnosis of propionic acidemia even in the absence of significant elevations 2-methylcitrate or 3-hydroxypropionate in urine organic acid analysis.

Abbreviations

2-MCA 2-Methylcitrate
3-OHP 3-Hydroxypropionate
C3 Propionylcarnitine

Introduction

Inborn errors of metabolism (IEMs) can have a severe impact on human health through the buildup of toxic metabolites resulting in serious, often fatal, diseases. Newborn screening (NBS) is used for early diagnosis of IEMs to avoid potentially catastrophic physical and neurological effects.

Propionic acidemia (PA) is an IEM affecting 1 in 50,000 to 1 in 100,000 individuals (Wikoff et al. 2007) resulting from a defect in the enzyme propionyl-CoA carboxylase (PCC). This enzyme is involved in metabolism of the branched chain amino acids, valine and isoleucine, and other propiogenic substrates. The clinical spectrum of PA is wide and ranges from an acute neonatal-onset to milder late-onset forms, and the clinical course is dominated by the risk of relapses of life threatening episodes of metabolic decompensation, with potential risk of death or further neurological damage. Patients with a mild form of PA on carnitine therapy have been described with severe and fatal hypertrophic cardiomyopathy in the absence of episodes of metabolic decompensation (Mardach et al. 2005). The underlying biochemical cause of the variability of neurological sequelae is not known.

The diagnosis of PA is based on the presence of elevated propionylcarnitine (C3) and possibly increased glycine and alanine in plasma and elevations in 2-methylcitrate (2-MCA) and 3-hydroxypropionate (3-OHP) with absence of

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methylmalonic acid (MMA) in urine organic acid analysis, as well as other metabolites derived from propionyl-CoA catabolism (Al-Dirbashi et al. 2014). Since C3 is not sensitive or specific, the diagnostic sensitivity is improved using acylcarnitine ratios (increased ratio of C3 to C2) (Turgeon et al. 2010).

Early diagnosis of PA is the key to effective management. Diagnosis of PA through NBS may lead to improved health outcomes with a lower mortality rate for affected children and lower stress for their parents (Grünert et al. 2012; Lücke et al. 2004; Waisbren et al. 2003). With the implementation of NBS for PA, a more favorable outcome has been observed when treatment is promptly started. The mainstay of the long-term treatment is a low-protein, high-energy diet and carnitine supplementation. Treatment with N-carbamylglutamate has been proved to reduce ammonia and glutamine in propionic acidemia patients (Mew et al. 2010). Liver transplantation has also been employed in PA and has been demonstrated to end hyperammonemic episodes (Silva et al. 2014).

Here, we describe three children with extremely mild PA whose diagnosis was initially based on isolated elevations of C3. By reporting these unusually mildly affected individuals, that lack metabolic abnormalities typically considered necessary for a diagnosis of PA, we expand the phenotypic spectrum of PA and raise the index of suspicion the clinician must have to capture the full spectrum of individuals affected with PA.

Case Reports

Patient 1

Patient 1 was a full-term healthy female who initially presented for abnormal second NBS with borderline C3 and elevated C3/C2. Confirmatory testing in the course of standard newborn screening follow-up, including plasma amino acid analysis, urine organic acid analysis, and plasma acylcarnitine analysis was performed. Initial plasma acylcarnitine analysis showed elevated C3 at 23,930 nmol/L (normal range 0–870 nmol/L). Qualitative urine organic acid analysis showed only moderate elevations in methylcitric acid and 3-hydroxypropionate, and plasma amino acids showed moderately increased glycine of 490 mmol/L (normal range 104–344 mmol/L). She was found to have hyperammonemia (183 μ mol/L, normal range 47–80 μ mol/L) and elevated lactate (3.7 mmol/L (normal range 0.2–2.0 mmol/L). She was admitted for management with IV dextrose fluids, IV sodium benzoate, and IV levocarnitine. Since her initial admission, she had no further episodes of hyperammonemia. At her 4-year follow-up, she was noted to have reached all her developmental milestones at

appropriate ages, and her growth was normal. She is currently managed with a protein-restricted diet, sodium benzoate, and levocarnitine. Apart from repeated isolated elevations in C3 on plasma acylcarnitine analysis and mild elevations of glycine, she shows no biochemical evidence of propionic acidemia with no detectable analytes suggestive of PA in repeated qualitative urine organic acid analyses. Sanger sequencing identified compound heterozygous pathogenic mutations in the *PCCA* gene in trans-configuration: c.223G>C (p. A75P) and c.923dupT (p. L308FfsX35) that have been previously identified and characterized (Campeau et al. 1999; Pérez et al. 2003; Yang et al. 2004). No mutations were identified in *PCCB*.

Patient 2

Patient 2 was a full-term male with no perinatal complications who had a normal initial but whose second NBS identified borderline elevated C3 and elevated C3/C2 at around 3 weeks of life (Table 1). He had been feeding with no issues at home, and there were no developmental concerns at the time of diagnosis. Confirmatory testing included urine organic acid analysis, plasma acylcarnitine analysis, and plasma amino acid analysis. The initial acylcarnitine analysis showed significant isolated elevation of C3 11,028 nmols/L, normal range: 0–870 nmols/L, while urine organic acids and plasma amino acids were unremarkable. He was started on a protein-restricted diet and levocarnitine. Molecular testing confirmed the diagnosis of PA by finding two heterozygous bi-allelic variants, c.562G>A (p. G188R) and c.1127G>A (p. R376H), in *PCCB* gene. The first mutation was previously reported (Pérez et al. 2003), while the second variant is a novel mutation predicted to be pathogenic (Table 2). At 20 months of age, there were no growth concerns, and he was developmentally normal (walking and two words sentences). He had two urine acylglycine analyses performed during routine follow-up, and on a single analysis, propionylglycine was very mildly elevated at 2.7 mmols/mol creatinine (normal range 0–2 mmols/mol creatinine), the other analysis was normal. No other metabolic abnormalities were detected on urine organic acid analysis performed three times (2-methylcitrate was not detected), and he has not suffered an acute metabolic decompensation to date.

Patient 3

Patient 3 was a full-term female who initially presented with an abnormal second NBS that showed elevated C3/C2 ratios but normal C3 levels. At 3 weeks of life, follow-up testing of urine organic acids revealed the presence of 2-methylcitrate and unremarkable plasma amino acids. The

Table 1 Biochemical and molecular findings from three patients with propionic acidemia

Current age	Patient 1 4 years old	Patient 2 20 months	Patient 3 3 years old
Newborn screens (NBS) nmol/L			
NBS 1 ^a (nmol/L)	C3 = 5,900 C3/C2 = 0.54	C3 = 6,340 C3/C2 = 0.38	C3 = 3,000 C3/C2 = 0.18
NBS 2 ^a (nmol/L)	C3 = 4,380 C3/C2: 0.99	C3 = 4,110 C3/C2 = 0.68	C3 = 3,890 C3/C2 0.48
Initial C3	40,240	11,028	2,440
Normal range: 0–870 nmol/L			
Average C3 at follow-up (excludes initial C3)	32,085 nmol/L	13,943 nmol/L	4,263 nmol/L
Most recent C3	23,930 nmol/L	19,602 nmol/L	3,185 mol/L
Urine 3-hydroxypropionic acid (3-OHP), qualitative	Modest elevation	Normal	Normal
Urine 2-methylcitrate (2-MCA), qualitative	Modest elevation	Normal	Modest elevation
Urine propionylglycine, targeted	N/A	2.7	N/A
Normal range: 0–2			
<i>PCCA</i> / <i>PCCB</i> sequence analysis	<i>PCCA</i> ^b : c.923dupT (p. L308FfsX35) c.223G>C (p. A75P)	<i>PCCB</i> ^c : c.562G>A (p. G188R) c.1127G>A (p. R376H)	<i>PCCA</i> ^b : c.1284+1G>A c.1999C>T (p. P667S)

N/A not performed

^aNewborns (1–6 days old) C3 $\geq$ 6,360 nmol/L (borderline), C3 $\geq$ 8,260 nmol/L (elevated) and C3/C2 $\geq$ 0.35 (elevated); newborns (7 or greater days old), C3 $\geq$ 4,040 nmol/L (borderline), C3 $\geq$ 5,490 nmol/L (elevated), C3/C2 $\geq$ 0.2 (elevated)

^b*PCCA*: NM_000282

^c*PCCB*: NM_000532

Table 2 Molecular profile and bioinformatic prediction of *PCCA* and *PCCB* novel variants

Gene	Amino acid changes	ExAC frequency	Homozygotes in ExAC	SIFT pred	Polyphen2 HDIV score	Polyphen2 HDIV pred	Mutation taster score	Radial SVM pred	CADD phred
<i>PCCB</i>	<i>PCCB</i> :NM_000532: exon11:c. G1127A: p. Arg376His	0.00003295	0	Deleterious	1.0	Damaging	1.000	D	32
<i>PCCA</i>	<i>PCCA</i> :NM_000282: exon22:c. C1999T: p. Pro667Ser	0	0	Deleterious	0.999	Damaging	1.000	D	20.3

acylcarnitine profile at this time revealed C3 of 4,930 nmol/L (normal range 0–1,120 nmol/L), and a normal plasma ammonia level. Her diagnosis was confirmed with molecular testing of *PCCA* which showed heterozygous bi-allelic variants: a known pathogenic variant, c.1284+1G>A (Campeau et al. 2001), and a novel likely pathogenic missense variant, c.1999C>T (p. Pro667Ser) (Table 2). She is managed on a protein restricted diet and levocarnitine. Currently at age three, she does not have any growth or developmental concerns, and she has reached all developmental milestones at the appropriate ages. She has no history of metabolic decompensation leading to hospitalizations, emergency room visits, or any minor or major illnesses.

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Discussion

In patients with PA, propionyl-CoA accumulates in the body, eventually causing downstream elevations in several compounds, including C3, 2-MCA, 3-OHP, and glycine. PA is included in the NBS core panel, to rule out a subset of C3-related metabolic disorders resulting from propionate accumulation (PA, methylmalonic aciduria (MMA), certain

cobalamin (Cbl) disorders). C3 is not specific for PA and a second-tier strategies analyzing methylmalonic acid, 3-hydroxypropionic acid, and 2-methylcitric acid from the initial DBS have been developed (la Marca et al. 2007; Lindner et al. 2008; Matern et al. 2007). Many laboratories use ratios of C3 to other acylcarnitine species (C2, C0, C16) or to methionine to improve specificity and sensitivity of C3 test (Wikoff et al. 2007).

In virtually all reported cases of PA including atypical and mild ones, the diagnosis is supported by concurrent abnormal acylcarnitine profile and urine organic acids that identify the disease-specific 2-MCA and 3-OHP (Al-Dirbashi et al. 2014; Weisfeld-Adams et al. 2010; Pérez-Cerdá et al. 2000). In this report, two individuals with compound heterozygous mutations in *PCCA/PCCB* present with significant C3 elevations in the absence of or only modest elevation in 2-MCA or 3-OHP, escaping the classic biochemical hallmarks for the PA diagnosis. Moreover, patient 1 showed a typical, albeit mild, biochemical profile of PA but without significant clinical symptoms.

All the mutations identified in the patients reported have been previously described with the exception of two novel mutations: one in *PCCB* c.1127G>A (p. Arg376His) and one in *PCCA* c.1999C>T (p. Pro667Ser). Both novel mutations are predicted pathogenic and are rare: the mutation in *PCCB* c.1127G>A showed a low frequency in ExAC (0.00003295) without homozygous patients while the mutation in *PCCA* c.1999C>T is not reported in ExAC (Table 2). Mutations in *PCCB* (c.1127G>A and c.1126C>T) in the same codon of the novel variant of patient 2 (c.1127G>T), and in *PCCA* (c.1999C>T and c.2002G>A) adjacent to the novel variant of patient 3 (c.1997 T>A), have been reported pathogenic in PA patients (Desviat et al. 2006; Kraus et al. 2012).

Most patients with PA showed nonatal onset, but later onset in adolescence or adulthood has been described (Lee et al. 2009). Recent studies indicated heart defects and renal failure as late-onset PA phenotypes (Lee et al. 2009; Pena et al. 2012; Vernon et al. 2013), suggesting disease progression still takes place in the mildest cases of PA. Close follow-up remains crucial to the well-being of these “mild” PA patients.

Our cases show a stable clinical course with a more favorable natural history (without metabolic decompensations), normal growth and a favorable neurocognitive development. We posit the lack of clinical manifestations could be due to the absence of significant elevations of potentially neurotoxic metabolites such as 2-MCA and 3-OHP. A potential pathophysiological correlation between high levels of 2-MCA and 3-OHP and neurological issues, for a secondary impairment of mitochondrial energy production, has already been described (Pérez-Cerdá et al. 1998; Schreiber et al. 2012).

Treatment in PA is accomplished mainly through dietary restriction, particularly of the protein substrates, and carnitine supplementation. The available treatments do not allow significant reduction of circulating 3-OHP, 2-MCA, glycine, and C3 (Pena and Burton 2012). All patients described here are on carnitine supplementation and a modified diet. The chronic treatment for these patients is necessary since the uncertainty about the long-term prognosis of PA, even in apparent milder cases such as theirs.

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Take-Home Message

The absence of 2-methylcitrate and 3-hydroxypropionate might be misleading in the diagnostic process for patients with propionic acidemia.

Authors' Contribution

GC and PSA were involved in conception and design of the study. TRD, KU, and NM analyzed and interpreted the data. QS, VRS, and WJC reviewed and revised critically the manuscript for important intellectual content. SHE supervised the work, coordinated the preparation of the work for publication, and is the corresponding author. All authors have approved the submission of the manuscript.

Competing Interest Statement

The authors declare no competing interests.

Ethics Statement

All procedures followed were in accordance with the ethical standards of the U.S. Department of Health and Human Services and were approved by the Baylor College of Medicine Institutional Review Board in accordance with Helsinki Declaration of 1975 as revised in 2000. This study was approved with a waiver of informed consent.

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