Comparison of Methods of Initial Ascertainment in 58 Cases of Propionic Acidemia Enrolled in the Inborn Errors of Metabolism Information System Reveals Significant Differences in Time to Evaluation and Symptoms at Presentation

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Objectives To compare time to evaluation and symptoms at diagnosis of propionic acidemia (PA) by method of ascertainment, and to explore correlations between genotype and biochemical variables.

Study design Clinical symptoms, genotype, and biochemical findings were analyzed retrospectively in 58 individuals with PA enrolled in the Inborn Errors of Metabolism Information System (IBEM-IS) based on the type of initial ascertainment: abnormal newborn screening (NBS), clinical presentation (symptomatic), or family history.

Results The average age at initial evaluation and treatment was significantly younger in patients ascertained via abnormal NBS compared with those referred for clinical symptoms. Furthermore, the majority of individuals ascertained because of abnormal NBS were asymptomatic at diagnosis, compared with a minority of clinical presentations. A notable difference in the frequency of metabolic acidosis at initial presentation was observed between those with abnormal NBS (12.5%; 2 of 16) and those with an abnormal clinical presentation (79%; 19 of 24). The frequency of hyperammonemia was similar in the 2 groups.

Conclusion Our data support the continued value of NBS to identify individuals with PA, who are diagnosed and treated earlier than for other modes of ascertainment. There were no statistically significant correlations between genotype and NBS for C3 acylcarnitines. Although expanded use of NBS has allowed for early diagnosis and treatment, long-term outcomes of individuals with PA, especially with respect to mode of ascertainment, remain unclear and would benefit from a longitudinal study. *(J Pediatr 2017;180:200-5).*

Propionic acidemia (PA) is a rare inborn error of metabolism with autosomal recessive inheritance. The disorder is characterized by deficient propionyl-CoA carboxylase (PCC) enzyme, affecting catabolism of propiogenic amino acids and odd-chain fatty acids and impairing production of intermediates of the tricarboxylic acid cycle.[1] PPC is a dodecamer comprised of α and β subunits, encoded by the *PCCA* and *PCCB* genes, respectively.[2]

Incidence figures for PA range from 1 in 165 000 to 1 in 300 000, and the condition is more common in the Middle East and in Old Order Amish.[3-7] Early presentations occur in the neonatal period, and late-onset presentations occur at variable ages.[8-10] Data from European groups indicates that newborn screening (NBS) leads to earlier diagnosis than symptomatic testing,[11] but objective information regarding the impact of NBS on age at evaluation and treatment, and on long-term outcomes, is limited. The Inborn Errors of Metabolism Information System (IBEM-IS) is a multicenter collaborative database initiated in 2007 that collects longitudinal information on metabolic conditions included in NBS. We analyzed available data on the initial presentation of individuals with PA participating in the IBEM-IS to answer the following questions: (1) Can the method of ascertainment impact age at initial evaluation? We hypothesized that individuals with a abnormal NBS will have earlier evaluation; (2) Is there a difference in the type and frequency of symptoms at initial evaluation based on the method of ascertainment? We hypothesized that ascertainment early in life results in fewer and less severe symptoms; and (3) Are

GI	Gastrointestinal
IBEM-IS	Inborn Errors of Metabolism Information System
NBS	Newborn screening
PA	Propionic academia
PCC	Propionyl-CoA carboxylase

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there genotype–phenotype correlations? We hypothesized that null mutations lead to lower residual enzyme activity, which would result in higher NBS C3 acylcarnitine levels.

Methods

We reviewed available data for 61 patients with PA enrolled in the IBEM-IS. Three patients with no information on initial presentation were excluded. The data review was retrospective and included data entered between June 13, 2007, and November 6, 2015. The abstracted data did not include direct or indirect identifiers. This study was reviewed and granted exempt status by the Institutional Review Board for Clinical Investigations at Duke University and at the University of Wisconsin at Madison.

Data on initial ascertainment, diagnostic testing, and clinical symptoms were evaluated. Clinical results were tabulated and analyzed based on 3 categories for initial ascertainment, as indicated by the originating center: abnormal NBS, clinical presentation (many cases in this cohort were ascertained before the initiation of NBS by tandem mass spectrometry), and family history. In some cases, multiple modes of ascertainment were indicated by the originating center. These cases were reviewed and adjudicated to only 1 category based on timing and other clinical data. Early versus late evaluations were categorized based on whether the age at initial subspecialist visit or treatment was <30 days or >30 days of life, respectively. Descriptive statistics were used for quantitative results. Comparisons of age at initial metabolic evaluation, age at initial treatment, and C3 acylcarnitine values were done using the independent-samples Mann-Whitney *U* test. Frequency comparisons were made using the Pearson χ^2 test or Fisher exact test when expected cell counts were <5. SPSS for Windows, version 23.0 (IBM, Armonk, New York) was used for all statistical comparisons and graphical representations. Any discrepant data were clarified by direct query of the institution entering the data via the coordinating center (Michigan Public Health Institute), and responses were deidentified before being shared with the author group.

The clinical classifications used for health status at initial presentation were critically ill, gastrointestinal (GI) complications, respiratory complications, neurologic complications, neurologic and GI complications, and asymptomatic. A patient was deemed critically ill if the laboratory results and symptoms indicated a medical emergency. Such qualifications included combinations of lethargy, hypotension, hypotonia, poor feeding, encephalopathy, sepsis, and/or laboratory findings such as hyperammonemia. These qualifications were presumed to infer significant illness.

Published genotypes were cross-referenced using the PPC mutation database maintained through the University of Colorado (http://cbs.lf1.cuni.cz/pcc/pccmain.htm). Any alleles that had not been published previously were assessed for impact on structure and function using open-source software (Mutation Taster [www.mutationtaster.org], PolyPhen-2 [http://genetics.bwh.harvard.edu/pph2/index.shtml], or SIFT [http://sift.jcvi.org/]). The following reference sequences were used for these analyses: *PCCA*, ENSG00000175198 (gene) and ENST00000376285 (transcript); *PCCB*, ENSG00000114054 (gene) and ENST00000251654 (transcript).

Results

Data for 58 patients with PA entered into the IBEM-IS were evaluated. The dataset represents a fairly balanced distribution with respect to sex (females, n = 26; males, n = 32). The majority of patients were Caucasian (44 of 58), 3 were Black/African American, 1 was American Indian, and 3 were reported as mixed race. Seven patients lacked information regarding race. Seven individuals were also identified as Amish.

Ages at Initial Evaluation and Treatment

Cases were ascertained by abnormal NBS (n = 25), clinical presentation (n = 26), or family history (n = 7). Complete clinical and molecular information is presented in **Table I** (available at www.jpeds.com).

We analyzed the age at initial metabolic evaluation and initiation of treatment for PA among the patients in each group using descriptive statistics. Cases ascertained via abnormal NBS had a mean age at initial metabolic evaluation of 15 days (n = 19) and initiation of treatment of 12 days (n = 16). The majority of patients (11 of 16) ascertained by NBS had metabolic evaluation and initiation of treatment on the same day (**Figure 1**); of the remaining patients, 4 had a metabolic evaluation after initiation of treatment and 1 received treatment after initial metabolic evaluation (**Figure 1**). One patient was excluded from these calculations because the reported value of 1065 days actually represented the age at which the patient was enrolled in the IBEM-IS, and no additional information was available.

The mean age at initial metabolic consultation for clinical presentation was 332 days (n = 24), and the mean age at initiation of treatment (n = 26) was 323 days. The majority of patients (21 of 24) ascertained by clinical presentation had metabolic evaluation and initiation of treatment on the same day (**Figure 1**). Of the remaining patients, 2 had metabolic consultation after the start of treatment and 1 had metabolic consultation before the start of treatment (**Figure 1**).

Patients ascertained by family history had an average age at initial metabolic evaluation (n = 7) and initiation of treatment (n = 7) of 449 days and 427 days, respectively. Three of the 7 patients ascertained by family history had metabolic evaluation before the initiation of treatment, 2 had metabolic evaluation after the initiation of treatment, and 2 had metabolic evaluation on the day of initiation of treatment (**Figure 1**). No outliers were removed from the clinical presentation or family history groups despite large ranges in values, because the time frames were consistent with the method of ascertainment.

The ages at initial evaluation and initiation of treatment were younger for those patients ascertained by NBS versus other presentations (**Figure 2**). The age at initial metabolic evaluation and age at initial treatment were significantly younger for

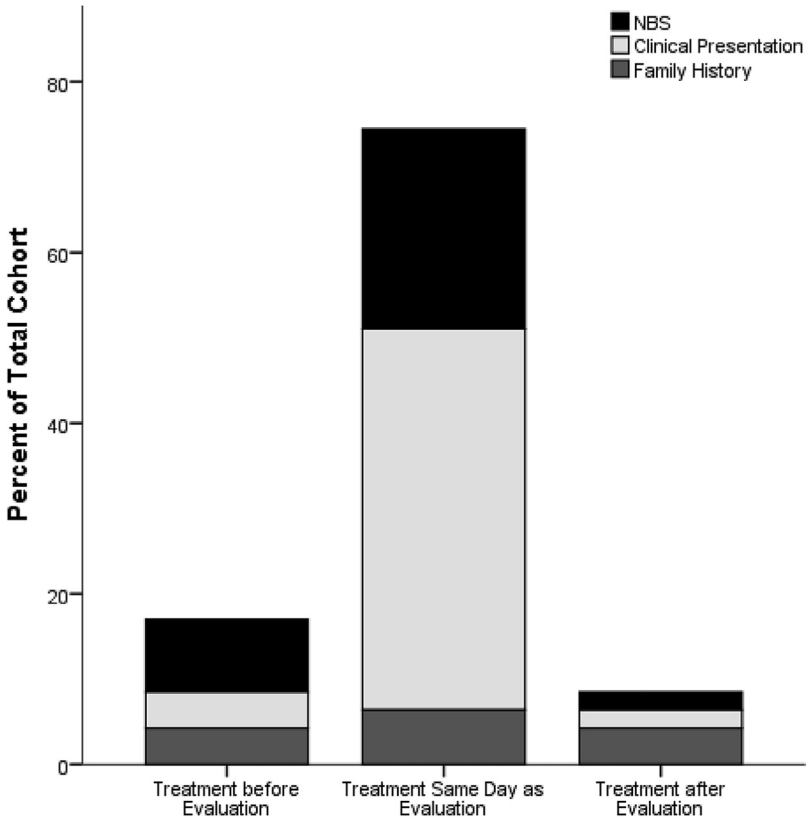

Figure 1. Percentage of the total cohort that received treatment before, at the same time as, or after initial metabolic evaluation, stratified by method of ascertainment (sum of all bars = 100%).

patients ascertained by abnormal NBS compared with patients ascertained by clinical presentation ($P = .004$ and $P = .011$, respectively); however, no difference in age at initial metabolic evaluation or initial metabolic treatment was observed for patients ascertained by abnormal NBS and those ascertained by family history ($P = .769$ and $P = .221$, respectively).

Of the 33 patients ascertained by clinical presentation and family history, 15 were evaluated or treated before 30 days of age and 18 were evaluated or treated after 30 days of age, including 11 evaluated or treated after 1 year of age (**Table II**; available at www.jpeds.com). Compared with the NBS group, significantly more individuals ascertained by clinical presentation and family history had late evaluation (18 of 33 vs 2 of 19; $\chi^2 = 9.87$; $P = .002$). In the clinical and family history cohorts, patients referred after 30 days of age underwent initial metabolic evaluation by a subspecialist at an average age of 683 days (median, 593 days; range, 35-3030 days) and received initial treatment at an average age of 624 days (median, 480 days; range, 35-2920 days). In contrast, the NBS group, which included 2 critically ill individuals, had an average age at initial evaluation of 39 days (median, 39 days; range, 34-44 days) and an average age at initial treatment of 44 days (median, 44 days; range, 44-44 days). One patient was evaluated and treated at 44 days of age; the other patients was evaluated at 34 days, and the date of treatment initiation was not reported.

Symptoms and Laboratory Findings at Presentation

We analyzed the patients' symptoms at initial presentation or evaluation with respect to method of ascertainment (**Tables I** and **III**). Information regarding symptoms at initial evaluation was available for 21 of 25 patients ascertained by abnormal NBS. Approximately one-half (10 of 21) of the patients referred because of an abnormal NBS were critically ill at the time of initial metabolic consultation. Symptoms and laboratory abnormalities during critical illness included lethargy/fluctuating alertness/encephalopathy (6 of 10), hypothermia (2 of 10), hyperammonemia (5 of 10), metabolic acidosis (2 of 10), and hypoglycemia (2 of 10 each). One of the 21 patients had noncritical GI complications, and the remainder were asymptomatic at the time of metabolic consultation.

Ten of the 26 patients ascertained by clinical presentation were critically ill at the time of initial metabolic evaluation, as determined by clinical and laboratory findings. Symptoms and/or laboratory abnormalities included lethargy (7 of 10), coma (5 of 10), seizures (6 of 10), metabolic acidosis (9 of 10), and hyperammonemia (8 of 10). Several patients had metabolic acidosis without critical illness (**Table I**). Among the remaining 15 patients, 1 patient lacked data regarding health status and the other 14 had GI (n = 6; vomiting as the primary symptom), neurologic (n = 4; lethargy or seizures), or respiratory complications (n = 2). One patient had both

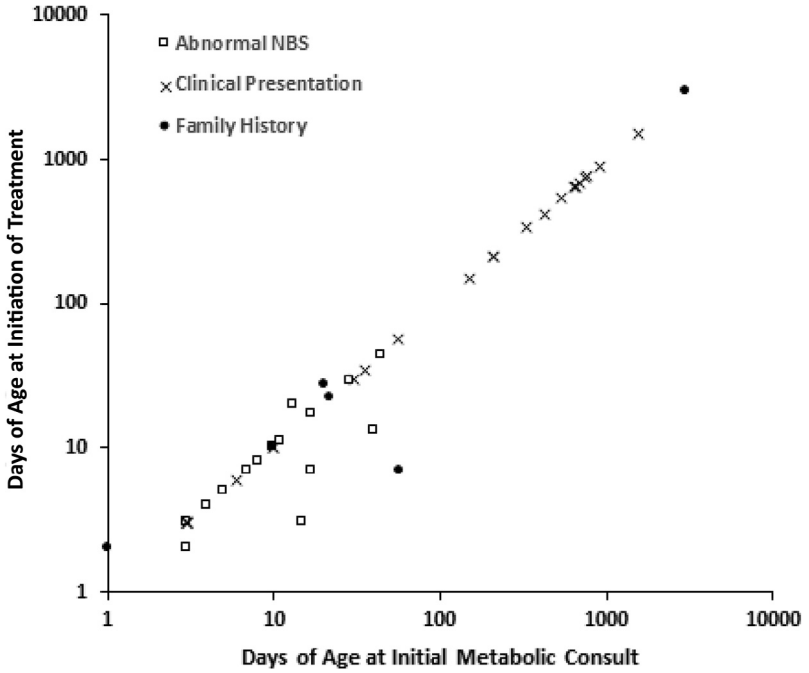

Figure 2. Comparison of days of age at initial metabolic consult vs days of age at initiation of treatment by ascertainment method. Reported values <1 (2 cases) are not shown.

neurologic and GI complications. No cardiac complications were reported at presentation.

Six of the 7 patients ascertained by family history were asymptomatic at the time of initial metabolic evaluation. One patient exhibited neurologic complications.

Metabolic acidosis at the time of initial diagnosis was detected more frequently in the patients ascertained by clinical presentation compared with those ascertained by abnormal NBS (19 of 24 vs 2 of 16; $\chi^2 = 17.109$; $P < .001$) or by family history (19 of 24 vs 1 of 7; $P = .004$, Fisher exact test). There was no significant difference in the frequency of hyperammonemia at initial presentation between the patients ascertained by clinical presentation and those ascertained by abnormal NBS (10 of 24 vs 9 of 16; $\chi^2 = 0.819$; $P = .366$).

Among the patients presenting at >30 days of age ascertained by family history or clinical presentation, the most common findings were GI (5 of 18) and neurologic (4 of 18) symptoms. Eighteen percent were critically ill at presentation. More than one-half (12 of 18) presented with metabolic acidosis, with or without hyperammonemia, including the 1 patient with no clinical symptoms at presentation.

Of the 7 Amish patients, 4 were ascertained by clinical presentation at an average age of 1.3 years, 2 were ascertained by family history in the neonatal period, and 1 was ascertained by abnormal NBS. Two of the 4 patients ascertained by clinical presentation had respiratory symptoms, and 2 had GI symptoms. Both of the Amish patients who were ascertained by family history were asymptomatic, and the patient ascertained by abnormal NBS was asymptomatic but had an elevated ammonia level at initial evaluation.

In the patients ascertained by abnormal NBS, the mean propionylcarnitine (C3) level in dried blood spots was 18.06 ± 7.72 μM (range, 3.4-33.31 μM) (n = 20), the mean C3:C2 was 0.98 ± 0.57 μM (range, 0.28-2.35 μM) (n = 17), and the mean C3:C16 was 7.21 ± 3.92 μM (range 1.99-13.9 μM) (n = 11). There was no difference in mean C3 level between the critically ill and non–critically ill patients ascertained by abnormal NBS (data not shown).

Table III. Clinical presentation and specific symptoms by ascertainment method

Symptoms	NBS	Clinical presentation	Family history
Critical illness	10	10	0
GI (vomiting, diarrhea, hepatomegaly, FTT)	1	7	0
Respiratory	0	2	0
Neurologic (hypotonia, DD, seizures)	0	4	1
Neurologic and GI	0	1	0
Asymptomatic	10	1	6
No data	4	1	0
Total	25	26	7

DD, developmental delay; *FTT*, failure to thrive.

Molecular Diagnostics

Genotype information was available for 26 of the 58 patients. Owing to unresolved data queries, molecular results were excluded for 4 patients. All 26 patients who were genotyped reported 2 variant alleles; 7 had 2 variants in *PCCA*, and 19 had 2 variants in *PCCB*. In total, 52 variant alleles were reported, with approximately one-quarter occurring in *PCCA* (n = 14) and the remainder occurring in *PCCB* (n = 38).

Table IV. *PCCA* and *PCCB* alleles not previously reported (bold), with predicted effect on protein

Ascertainment	Gene	Mutation 1/protein nomenclature	Mutation 1 type and prediction	Mutation 2/protein nomenclature	Mutation 2 type and prediction
NBS	*PCCB*	**c.1398 + 2delT**	Splicing, I13 MT prediction: disease-causing	c.1606A > G p.N536D	Missense, E15
Clinical presentation	*PCCB*	**c.596C > T** p.P199L	Missense, E6 MT prediction: disease-causing	**c.676A > G** p.T226A	Missense, E7 MT prediction: disease-causing
NBS	*PCCB*	**c.398T > C** p.L133P	Missense, E4 MT prediction: disease-causing; PP prediction: probably damaging; S prediction: damaging	c.415C > T p.Q139X	Nonsense, E4
NBS	*PCCB*	**c.1172-1173delTT** p.F391Cfs*2	Deletion, E11 MT prediction: disease-causing	**c.1172-1173delTT** p.F391Cfs*2	Deletion, E11 MT prediction: disease-causing
NBS	*PCCA*	**IVS14-1G > A** (c.1285-1G > A)	Splicing, I14 MT prediction: disease-causing	**IVS14-1G > A** (c.1285-1G > A)	Splicing, I14 MT prediction: disease- causing
NBS	*PCCA*	**c.1367G > T** p.G456V	Missense, E16 MT prediction: disease-causing; PP prediction: probably damaging	**c.600 + 1G > A**	Splicing, I7 MT prediction: disease- causing
NBS	*PCCA*	**c.1023dupT** p.V342Cfs*19	Insertion, E12	Genomic deletion of exons 3-4	Deletion, I2-4
NBS	*PCCA*	**c.1591T > C** p.S531P	Missense, E18 MT prediction: disease-causing PP prediction: possibly damaging	Del exon 20-21	Unknown

E, exon; *I*, intron; *MT*, MutationTaster; *PP*, PolyPhen; *S*, SIFT.
Bold indicates previously unreported alleles.

Variant alleles were characterized as missense, nonsense, insertion or deletion, splicing, or unknown (in the event of insufficient information for assignment to any of the other categories). The most frequent type of variant was missense, observed in 27 of 52 alleles. More than one-half of the *PCCB* mutations were missense (23 of 38), but missense mutations were less common in *PCCA* (4 of 14). The most commonly observed mutation in the cohort was a missense in *PCCB*, c.1606A > G, occurring with an allelic frequency of 25% (13 of 52). Eight of these 13 alleles were detected in the homozygous state in the Amish patients. A common *PCCB* mutation in the general Caucasian population, c.1218del14ins12,[12] was observed in >10% of the *PCCB* alleles (4 of 38) in our dataset. Ten variants were not previously reported in the literature or in open-source databases and are listed in **Table IV**.

To explore the relationships between genotype and C3 acylcarnitine values in NBS samples, we compared C3 levels in patients with *PCCA* mutations and those with *PCCB* mutations. We found no significant differences between the 2 groups ($P = .414$). We also tested for differences in C3 acylcarnitine levels in patients with at least 1 null mutation in either *PCCA* or *PCCB*, and found that the difference between patients with at least 1 null mutation and those with no null mutations did not reach statistical significance ($P = .259$).

Discussion

We analyzed initial presentations in a cohort of 58 patients with PA enrolled in the IBEM-IS and compared initial symptoms and time to evaluation based on 3 methods of ascertainment reported by the originating center (abnormal NBS, clinical presentation, and family history). The IBEM-IS captures age at initial evaluation by a metabolic specialist and institution of treatment, but not age at presentation. Previous studies have reported that the vast majority of patients with PA present during the neonatal period.[13-16] In our dataset, less than one-half of patients with PA ascertained by clinical presentation or family history were evaluated by a metabolic specialist in the first month of life. Furthermore, approximately one-third of cases ascertained by clinical presentation in the IBEM-IS underwent an initial metabolic evaluation after 1 year of age. Published data on symptoms and their frequency in individuals who present after age 1 year are sparse.[14-16] In our cohort, one-half of the patients with late presentation exhibited GI and/or neurologic symptoms.

The mean age at the time of consultation and initiation of treatment was significantly lower in the patients ascertained by NBS compared with those ascertained by clinical presentation. This finding affirms the importance of NBS to allow for early detection and treatment. Nonetheless, long-term follow-up data are needed to evaluate whether early detection and institution of treatment translate to significant long-term benefits in terms of survival or outcomes.

Approximately 40% of our patients were deemed critically ill at the initial metabolic consultation, regardless of the mode of ascertainment but most commonly during the neonatal period. The most commonly affected organ system at initial presentation was the GI system, reflected by such symptoms as vomiting, diarrhea, hepatomegaly, and failure to thrive. GI symptoms were more common at initial presentation than neurologic symptoms. These symptoms are similar to those described previously.[11,14] Noncritical presentations included subacute symptoms involving the GI system and neurologic system. These were also the most common presentations in cases referred after 30 days of age.

Most patients with severe organic acidemia present with metabolic acidosis and hyperammonemia.[17] The accumulation of PPC produces inhibitory effects on several metabolic pathways, including the urea cycle, likely as a consequence of

the suppression of N-acetyl glutamate synthetase, leading to a block in the first step of the urea cycle.[18] Typically, metabolic acidosis precedes hyperammonemia. In our cohort, metabolic acidosis at initial presentation was more commonly observed in patients ascertained by clinical presentation compared with those ascertained by NBS; however, there was no significant difference between these 2 groups in terms of frequency of hyperammonemia. This finding implies that several patients ascertained by NBS had hyperammonemia, but not metabolic acidosis, at initial presentation. The recognition that patients with PA may be hyperammonemic without metabolic acidosis is important for clinicians. Hyperammonemia necessitates rapid evaluation and treatment even if the initial blood gas analysis shows a normal pH. One caveat regarding this conclusion is that numerical values for ammonia level were not available through the IBEM-IS.

Individuals with PA are often compound heterozygotes for mutations in either the *PCCA* or *PCCB* gene. The types of mutations characterized to date include missense mutations, small insertions and deletions, splicing mutations, and large genomic deletions or duplications.[19] We note that molecular testing was a frequently used adjunct to diagnostic evaluation. In all patients who underwent sequencing, 2 variant alleles were detected, with a number of previously unpublished alleles. A caveat to this analysis is that phase was assumed to be *trans*, and laboratory interpretation of the variant was not included in the database. There was no correlation between the mutated gene and C3 acylcarnitine level on NBS, or between the presence of null mutations in either gene and C3 acylcarnitine levels on NBS. C3 acylcarnitine levels can be dependent on the patient's use of carnitine supplementation and clinical status when the sample was drawn, and thus the actual level might not be directly related to genotype. In addition, residual PCC activity may reflect genotype–phenotype correlations more accurately. However, less than one-half of the patients data on PCC levels in either fibroblasts or lymphocytes, and only a very small number of patients also had molecular testing data. This trend may reflect the widespread availability of molecular testing for diagnostic purposes.

Limitations of the present study include ascertainment bias, given that our analysis was based on a convenience cohort of individuals enrolled in the IBEM-IS. In addition, our analysis depended on the accuracy and completeness of the data entered, and required multiple rounds of clarification from the originating centers. Finally, a comprehensive dataset that allows for systematic evaluation of long-term outcomes in individuals with PA is lacking, and continued follow-up through the IBEM-IS will provide valuable longitudinal data for future comparisons of long-term outcomes by ascertainment method.

Despite the limitations, this retrospective analysis provides important new information about initial presentations based on a large cohort of individuals with PA. This study suggests that a number of patientss may present with hyperammonemia without metabolic acidosis, an uncommon observation warranting rapid intervention and treatment. In addition, our results objectively demonstrate the value of NBS for diagnosing PA and instituting treatment early in life. ■

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Appendix 1

Additional members of the Inborn Errors of Metabolism Collaborative are as follows:

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Appendix 2

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Table I. Clinical, laboratory, and molecular data

ID	Ascertainment	Age at initial metabolic consult, d	Age at initiation of treatment, d	Interval between consult and treatment, d	Clinical category	Symptoms at initial metabolic contact	Gene	Laboratory abnormalities at first contact with metabolic specialist	Mutation/ variant 1	Mutation 1 category and prediction	Mutation/ variant 2	Mutation 2 category and prediction
1	Clinical presentation	3	3	0	Critical illness	Hypothermia Hypotonia Lethargy Other	*PCCB*	Hyperammonemia Hypoglycemia Ketosis Metabolic acidosis Anemia Hyperglycemia Low plasma free carnitine	c.1218del14ins12	Frameshift, previously reported	c.1495C > T p.R514X	Nonsense, previously reported
2	Clinical presentation	3	3	0	Critical illness	Body odor Hypotonia Tachypnea Other	Unknown	Hyperammonemia Hypoglycemia Ketosis and ketonuria Metabolic acidosis				
3	Clinical presentation	6	6	0	Critical illness	Cerebral Edema Coma Dehydration Hypothermia Increased intracranial pressure Jaundice Lethargy Poor feeding Seizure Decreased pupillary response	Unknown	Metabolic acidosis Low plasma free carnitine				
4	Abnormal NBS	6	–	–	Asymptomatic	None	*PCCB*	Hyperammonemia	**c.1398 + 2delT**	Splicing MT prediction: disease-causing	c.1606A > G p.N536D	Missense, previously reported
5	*Clinical presentation; deceased*	*35*	*35*	*0*	*Critical illness*	*Apnea* *Body odor* *Coma* *Fatigue* *Hypertonia* *Jaundice* *Lethargy* *Poor feeding* *Renal failure-acute* *Seizure* *Vomiting*	*Unknown*	*Hyperammonemia* *Metabolic acidosis*				
*6**	*Clinical presentation*	*210*	*210*	*0*	*GI*	*Vomiting*	*Unknown*	*Metabolic acidosis*				

(*continued*)

Table I. Continued

ID	Ascertainment	Age at initial metabolic consult, d	Age at initiation of treatment, d	Interval between consult and treatment, d	Clinical category	Symptoms at initial metabolic contact	Gene	Laboratory abnormalities at first contact with metabolic specialist	Mutation/ variant 1	Mutation 1 category and prediction	Mutation/ variant 2	Mutation 2 category and prediction
7*	Family history	20	27	7	Asymptomatic	None	*PCCB*	No abnormal labs	c.1606A > G p.N536D	Missense, previously reported	c.1606A > G p.N536D	Missense, previously reported
*8**	***Clinical presentation;*** *Family history*	*150*	*150*	*0*	*Respiratory*	*Respiratory distress*	*PCCB*	*Metabolic acidosis*	*c.1606A > G p.N536D*	*Missense, previously reported*	*c.1606A > G p.N536D*	*Missense, previously reported*
9*	Family history	57	7	–50	Asymptomatic	None	Unknown	No abnormal lab values				
*10**	*Clinical presentation*	*652*	*652*	*0*	*Respiratory*	*Loss of muscle control* *Diarrhea* *Respiratory distress*	*Unknown*	*Metabolic acidosis*				
11	Abnormal NBS	11	11	0	Asymptomatic	None	Unknown	Elevated aspartate aminotransferase				
12	*Clinical presentation*	*690*	*690*	*0*	*GI*	*Dehydration* *Vomiting*	*Unknown*	*Low free and total carnitine*				
13	Abnormal NBS	11	–	–	Asymptomatic	None	*PCCB*	No abnormal lab values	c.331C > T p.R111X	Nonsense, previously reported	c.1606A > G p.N536D	Missense, previously reported
14	*Clinical presentation*	*56*	*56*	*0*	*Critical illness*	*Failure to thrive* *Lethargy* *Poor feeding* *Seizure* *Vomiting*	*unknown*	*Hyperammonemia* *Hypoglycemia* *Bone marrow suppression* *Hypertriglyceridemia* *Ketonuria*				
15	Abnormal NBS	4	4	0	Critical illness	Dehydration Failure to thrive Hypotonia Jaundice Lethargy	Unknown	Hyperammonemia Bone marrow suppression Hematuria Ketonuria				
16	*Clinical presentation*	*210*	*210*	*0*	*Neurologic*	*Developmental delay(s)* *Failure to thrive* *Fatigue* *Hypotonia* *Irritability* *Lethargy* *Poor feeding* *Seizure* *Vomiting*	*Unknown*	*Unknown*				
17	Abnormal NBS	1065	1065	0	Unknown	Unknown	Unknown	Unknown				

(*continued*)

Table I. Continued

ID	Ascertainment	Age at initial metabolic consult, d	Age at initiation of treatment, d	Interval between consult and treatment, d	Clinical category	Symptoms at initial metabolic contact	Gene	Laboratory abnormalities at first contact with metabolic specialist	Mutation/ variant 1	Mutation 1 category and prediction	Mutation/ variant 2	Mutation 2 category and prediction
18	*Clinical presentation*	*420*	*420*	*0*	*Neurologic*	*Cognitive Impairment Dehydration Developmental delay(s) Failure to Thrive Fatigue Hypotonia Lethargy Loss of developmental milestone(s) Poor feeding Poor growth Rickets Vomiting*	*Unknown*	*Metabolic acidosis Renal tubular acidosis Hypertriglyceridemia Ketonuria*				
19	*Clinical presentation*	*–*	*404*	*–*	*Critical illness*	*Coma Hypothermia Lethargy Respiratory distress*	*PCCB*	*Hyperammonemia Metabolic acidosis*	***c.596C > T*** *p.P199L*	*Missense MT prediction: disease-causing*	***c.676A > G*** *p.T226A*	*Missense, E7 MT prediction: disease-causing*
20	*Clinical presentation*	*1552*	*1528*	*–24*	*Asymptomatic*	*None*	*PCCB*	*Metabolic acidosis*	*c.1218del14ins12*	*Frameshift, previously reported*	*c.683C > T p.P228L*	*Missense, previously reported*
21	Abnormal NBS	4	4	0	Critical illness	Hyporeflexia Hypotonia Poor feeding Vomiting	*PCCB*	Hypoglycemia	c.386-387delTTinsAAC p.F129X	Nonsense MT prediction: disease-causing	c.1218del14ins12	Frameshift, previously reported
22	*Clinical presentation*	*760*	*760*	*0*	*Neurologic*	*Ataxia Confusion Lethargy Tachycardia Vomiting*	*PCCB*	*Hyperammonemia Hypoglycemia Metabolic acidosis Elevated liver enzymes Ketonuria*	*c.683C > T*	*Missense, previously reported*	*c.1398 + 1G > C*	*Splicing, previously reported*
23	*Family history*	*3030*	*2920*	*–110*	*DD/LD*	*Learning Disability*	*PCCB*	*Hyperglycemia*	*c. 683C > T p.P228L*	*Missense, previously reported*	*c.1398 + 1G > C*	*Splicing, previously reported*
24	**Abnormal NBS;** clinical presentation	3	3	0	Critical illness	Fluctuating level of alertness Hypothermia Hypotonia Lethargy Poor feeding Vomiting Hypotension	*PCCB*	Hyperammonemia Hypoglycemia Ketosis and ketonuria Low plasma total and free carnitine Metabolic acidosis Elevated liver enzymes Hematuria and proteinuria	**c.398T > C** p.L133P	Missense MT prediction: disease-causing; PP prediction: probably damaging; S prediction: damaging	c.415C > T p.Q139X	Nonsense, previously reported

(*continued*)

Table I. Continued

ID	Ascertainment	Age at initial metabolic consult, d	Age at initiation of treatment, d	Interval between consult and treatment, d	Clinical category	Symptoms at initial metabolic contact	Gene	Laboratory abnormalities at first contact with metabolic specialist	Mutation/ variant 1	Mutation 1 category and prediction	Mutation/ variant 2	Mutation 2 category and prediction
25	Clinical presentation	3	3	0	Critical illness	Coma Failure to thrive Fatigue Hypothermia Hypotonia Jaundice Lethargy Poor feeding Seizure Vomiting Abnormal breathing	*PCCB*	Hyperammonemia Hypoglycemia Metabolic acidosis	c.335G > A p.G112D	Missense, previously reported	c.1204delG	Deletion, previously reported
26	Abnormal NBS	10	10	0	GI	Fatigue Hypotonia Irritability Poor feeding Poor growth	Unknown	Low plasma total and free carnitine Elevated liver enzymes				
27	**Abnormal NBS;** clinical presentation	7	7	0	Critical illness	Dehydration Hypotonia Poor feeding Tachycardia	*PCCB*	Hyperammonemia Metabolic acidosis Ketonuria	**c.1172-1173delTT** p.F391Cfs*2	Deletion MT prediction: disease-causing	**c.1172-1173delTT** p.F391Cfs*2	Deletion MT prediction: disease-causing
28	Abnormal NBS	44	44	0	Critical illness	Hepatomegaly	PCCB	Hyperammonemia	Unresolved query on reported allele	Missense	c.683C > T p.P228L	Missense, previously reported
29	Abnormal NBS	–	–	–	Unknown	Unknown	Unknown	Hyperammonemia Hypoglycemia				
30	Abnormal NBS	34	–	–	Critical illness	Dysmorphism Hypothermia Lethargy Tachypnea Hypoglycemia	PCCA	No laboratory tests performed at initial visit	c.782A > G p.E261G	Missense, previously reported	c.923dupT p.L308fs*35	Insertion, previously reported
31	*Clinical presentation*	*750*	*750*	*0*	*Neurologic*	*Hypotonia* *Microcephaly* *Poor growth*	*Unknown*	*Metabolic acidosis*				
32	Abnormal NBS	17	7	–10	Critical Illness	Dermatitis Lethargy Poor feeding Vomiting Encephalopathy	Unknown	Elevated bilirubin				
33	Clinical presentation	10	10	0	Critical illness	Apnea Hypothermia Lethargy Poor feeding Seizure Intracerebellar bleed Encephalopathy	Unknown	Hyperammonemia Metabolic acidosis				

(*continued*)

Table I. Continued

ID	Ascertainment	Age at initial metabolic consult, d	Age at initiation of treatment, d	Interval between consult and treatment, d	Clinical category	Symptoms at initial metabolic contact	Gene	Laboratory abnormalities at first contact with metabolic specialist	Mutation/ variant 1	Mutation 1 category and prediction	Mutation/ variant 2	Mutation 2 category and prediction
34	Family history	10	10	0	Asymptomatic	None	Unknown	No abnormal lab values				
35	Family history	1	2	1	Asymptomatic	None	Unknown	Ketosis and ketonuria Metabolic acidosis				
36	Family history	22	22	0	Asymptomatic	None	Unknown	No abnormal lab values				
37	Abnormal NBS	13	20	7	Asymptomatic	None	Unknown	Low blood urea nitrogen and creatinine, high potassium, calcium, and aspartate aminotrasferase, low total protein				
38	Abnormal NBS	–	–	–	Unknown		*PCCB*	No information	c.1606A > G p.N536D	Missense, previously reported	c.386_387delTTinsAAC p.F129X	Nonsense MT prediction: disease-causing
39	***Clinical presentation;*** *abnormal lab values*	–	*35*	–	*Critical illness*	*Coma Seizure Vomiting*	*Unknown*	*Metabolic acidosis*				
40	**Clinical presentation;** abnormal lab values	10	10	0	Neurologic	Coma Poor feeding	Unknown	No information				
43	**Abnormal NBS;** clinical presentation	8	8	0	Critical illness	Hypotonia Infection/sepsis Lethargy Poor feeding	*PCCA*	Hyperglycinemia	**IVS14-1G > A** (c.1285-1G > A)	Splicing MT prediction: disease-causing	**IVS14-1G > A** (c.1285-1G > A)	Splicing MT prediction: disease-causing
44	Abnormal NBS	–	–	–	Unknown		*PCCA*	No information	**c.1367G > T** p.G456V	Missense MT prediction: disease-causing PP prediction: probably damaging	**c.600 + 1G > A**	Splicing MT prediction: disease-causing
45	**Abnormal NBS;** clinical presentation	3	2	–1	Critical illness	Poor feeding	*PCCA*	Hyperammonemia Ketosis Lactic acidosis	**c.1023dupT** p.V342Cfs*19	Insertion MT prediction: disease-causing	**Genomic deletion of exons 3-4**	Deletion
46	*Clinical presentation*	*646*	*646*	*0*	*Unknown*	*Unknown*		*No information*	*Negative for common mutation panel of 5 alleles (done 2004)*		*Negative for common mutation panel of 5 alleles (done 2004)*	
48	Clinical presentation	30	30	0	GI	Vomiting Weight loss	*PCCA*	Metabolic acidosis	c.184-17_18delTG p.T62_S100del39	Splicing, previously reported	c.231 + 44_47delATTT p.T62_S100del39	Splicing, previously reported

(*continued*)

Table I. Continued

ID	Ascertainment	Age at initial metabolic consult, d	Age at initiation of treatment, d	Interval between consult and treatment, d	Clinical category	Symptoms at initial metabolic contact	Gene	Laboratory abnormalities at first contact with metabolic specialist	Mutation/ variant 1	Mutation 1 category and prediction	Mutation/ variant 2	Mutation 2 category and prediction
49	Family history	–1	1	2	Asymptomatic	None	Unknown	No abnormal lab values				
50	Clinical presentation	3	3	0	Critical illness	Poor feeding	*PCCA*	Hyperammonemia Hypoglycemia Metabolic acidosis	*Unresolved query on reported allele*		*Unresolved query on reported allele*	
51	*Clinical presentation*	*540*	*540*	*0*	*GI*	*Developmental delay(s) Lethargy Vomiting*	*PCCA*	*No information*	*Unresolved query on reported allele*		*Unresolved query on reported allele*	
52	Abnormal NBS	5	5	0	Critical illness	Lethargy Poor feeding	*PCCA*	No information	**c.1591T > C** p.S531P	Missense MT prediction: disease-causing; PP prediction: possibly damaging	Del exon 20-21	Unknown
53	Abnormal NBS	15	3	-12	Asymptomatic		*PCCA*	No information	**c.1591T > C** p.S531P	Missense MT prediction: disease-causing; PP prediction: possibly damaging	Del exon 20-21	Unknown
54	Abnormal NBS	17	17	0	Asymptomatic	None	*PCCB*	No information	c.683C>T p.P228L	Missense, previously reported	c.1218del14ins12	Frameshift, previously reported
*55**	*Clinical presentation*	*900*	*900*	*0*	*GI*	*Lethargy Seizure Vomiting Diarrhea*	*PCCB*	*Metabolic acidosis Anemia*	*c.1606 A > G p.N536D*	*Missense, previously reported*	*c.1606 A > G p.N536D*	*Missense, previously reported*
56*	Abnormal NBS	29	29	0	Asymptomatic	None	*PCCB*	Hyperammonemia	c.1606 A > G p.N536D	Missense, previously reported	c.1606 A > G p.N536D	Missense, previously reported
57	*Clinical presentation*	*330*	*340*	*10*	*GI*	*Developmental delay(s) Vomiting*	*Unknown*	*Low plasma total and free carnitine*				
58	**Clinical presentation;** abnormal lab values	7	0	–7	GI	Failure to thrive Hypotonia Vomiting	Unknown	Anemia				
59	Abnormal NBS	–	–	–	Asymptomatic		*PCCA*	No information	c.1228C > T p.R410W	Missense, previously reported	c.1228C > T p.R410W	Missense, previously reported
60	Abnormal NBS	–	–	–	Asymptomatic		Unknown	No information				
61	Abnormal NBS	40	13	–27	Asymptomatic	None	PCCB	Other	c.1606A > G p.N536D	Missense, previously reported	c.1606A > G p.N536D	Missense, previously reported

MT, MutationTaster; *PP*, PolyPhen; *S*, SIFT.
Italics: clinical or family history presentations at >30 days of age. Ascertainment in bold: primary ascertainment categorization, if more than 1 was provided. DNA variants in bold: unpublished variants.
*Patients identified as Amish.

Table II. Time to evaluation and treatment in patients aged >30 days ascertained by clinical presentation and family history (n = 18)

	Initial metabolic consultation	Initiation of treatment
Range, d	35-3030	35-2920
Mean, d	683	624
Median, d	593	480