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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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Abstract

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 retrospectively analyzed 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 screen (NBS), clinical presentation (symptomatic), or family history.

Results—The average age at initial evaluation and treatment was significantly younger for patients ascertained via an abnormal NBS versus those who were referred for clinical symptoms. Furthermore, the majority of individuals ascertained due to an abnormal NBS were asymptomatic at diagnosis compared with a minority of clinical presentations. A notable difference in frequency of metabolic acidosis at initial presentation was observed between those with abnormal NBS

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(12.5%, 2/16) versus clinical presentations (79%, 19/24). The frequency of hyperammonemia was similar in both groups.

Conclusion—Our data support the continued value of newborn screening 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 C3 acylcarnitines. Although expanded newborn screening has allowed for early diagnosis and treatment, long-term outcomes of individuals with PA, especially with respect to mode of ascertainment, remains unclear and would benefit from longitudinal study.

Keywords

Organic acidemia; newborn screening; C3 acylcarnitine; nonketotic hyperglycinemia; hyperammonemia; longitudinal follow up; natural history

Propionic acidemia (PA) is a rare inborn error of metabolism (IEM) 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 cycle¹. Propionyl-CoA carboxylase is a dodecamer comprised of α and β subunits, encoded by the *PCCA* and *PCCB* genes, respectively².

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 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 NBS leads to earlier diagnosis than symptomatic testing¹¹, but objective information regarding the impact of newborn screening (NBS) on age at evaluation and treatment, and long-term outcomes, is limited. The Inborn Errors of Metabolism Information System (IBEM-IS) is a multicenter collaborative database that collects longitudinal information since 2007 on metabolic conditions that are included in NBS. We analyzed available data regarding initial presentations for individuals with PA who participate 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 abnormal NBS will have earlier evaluations; (2) Is there a difference in types and frequency of symptoms at initial evaluation between the methods of ascertainment? We posit that ascertainment early in life results in fewer and less severe symptoms; and (3) Are 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 cases with PA enrolled in the IBEM-IS. Three cases that did not include information regarding initial presentation were excluded. 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 the University of Wisconsin at Madison.

Data regarding initial ascertainment, diagnostic testing, and clinical symptoms were evaluated. Clinical results were tabulated and analyzed based on three categories for initial ascertainment, which were indicated by the originating center. The three categories were abnormal newborn screen, clinical presentation (many cases in this cohort were ascertained prior to initiation of NBS by tandem mass spectrometry), or family history. In some cases, multiple modes of ascertainment were indicated by the originating center. These were reviewed and adjudicated to only one 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 under or over 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 values were made using Independent Samples Mann-Whitney U tests. Frequency comparisons were made using the Pearson Chi Square test or Fisher's Exact Test when expected cell counts were less than five. IBM SPSS Statistics for Windows, Version 23.0 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 de-identified prior to sharing with the author group.

The clinical classifications used for health status at initial presentation were: critically ill, GI complications, respiratory complications, neurological complications, neurological and GI complications, or asymptomatic. A patient was determined to be critically ill if the laboratories 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 utilizing the propionyl-CoA carboxylase mutation database maintained through the University of Colorado, <http://cbs.lf1.cuni.cz/pcc/pccmain.htm> (last accessed on 14 February 2016). Any alleles that had not been previously published 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>; SIFT, <http://sift.jcvi.org/>). The reference sequences utilized for these analyses are as follows: *PCCA* ENSG00000175198 (gene) and ENST00000376285 (transcript) and *PCCB* ENSG00000114054 (gene) and ENST00000251654 (transcript).

Results

Data for 58 cases with propionic acidemia entered into the IBEM-IS were evaluated. The data set represented a fairly balanced distribution with respect to sex (female 26/58). The majority of patients were Caucasian (44/58), 3/58 were Black/African American, 1/58 were American Indian, and 3/58 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 newborn screen (N=25), clinical presentation (N=26), or family history (N=7). Full clinical and molecular information is presented in Table I (available at www.jpeds.com).

We analyzed age at initial metabolic evaluation and initiation of treatment for PA among individuals in each group utilizing 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 cases (11/16) ascertained by NBS had metabolic evaluation and initiation of treatment on the same day (Figure 1). Of the remaining cases, four had a metabolic evaluation after initiation of treatment and one received treatment after initial metabolic evaluation (Figure 1). One case was excluded from these calculations because the reported value of 1065 days actually represented the age at which the subject enrolled in the IBEM-IS, and there was no additional information. The mean age at initial metabolic consultation for *clinical presentations* was 332 days (N=24) and the mean age at initiation of treatment (N=26) was 323 days. The majority of cases (21/24) ascertained by clinical presentation had metabolic evaluation and initiation of treatment on the same day (Figure 1). Of the remaining cases, two had metabolic consultation after treatment was started and one had metabolic consultation before receiving treatment (Figure 1). Cases 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 seven cases ascertained by family history had metabolic evaluation before initiation of treatment. Of the remaining cases, two had metabolic evaluation after initiation of treatment and two had initiation of treatment on the same day (Figure 1). No outliers were removed from the clinical presentation or family history groups despite large ranges in values, as the timeframes were consistent with the method of ascertainment.

The age at initial evaluation and initiation of treatment was younger for those ascertained by NBS versus other presentations (Figure 2). The age at initial metabolic evaluation and age at initial treatment were significantly younger for cases ascertained by abnormal NBS when compared with clinical presentation ($p=0.004$ and $p=0.011$, respectively). However, no difference in age at initial metabolic evaluation or initial metabolic treatment was observed for cases ascertained by abnormal NBS versus family history ($p=0.769$ and $p=0.221$, respectively).

Of the 33 cases ascertained by clinical presentations and family history, 15/33 were evaluated or treated by 30 days of age, and the remaining 18/33 after 30 days of age. Eleven of these 18 cases were evaluated or treated after one year of age (Table II; available at www.jpeds.com). Compared with the NBS group, significantly more individuals had late evaluations (18/33 versus 2/19, Chi-Square=9.87, $p=0.002$). In the clinical and family history cohorts, referrals older than 30 days of age received initial metabolic evaluation by subspecialist at an average age of 683 days (median 593, range 35–3030 days) and initial treatment at an average age of 624 days (median 480, range 35–2920 days). In contrast, the NBS group, comprising 2 critically ill individuals, had an average age at initial evaluation of 39 days (median 39, range 34–44) and the average age at initial treatment of 44 days

(median 44, range 44–44). One patient was evaluated and treated at 44 days of age. The other was evaluated at 34 days and the date of treatment initiation was not reported.

Symptoms and laboratory findings at presentation

We analyzed 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 21/25 cases ascertained by abnormal NBS. Approximately half (10/21) of the PA patients referred due to an abnormal NBS were critically ill at initial metabolic consultation. Symptoms and laboratory abnormalities during critical illness included lethargy/fluctuating alertness/encephalopathy (6/10), hypothermia (2/10), hyperammonemia (5/10), metabolic acidosis and hypoglycemia (2/10 each). One of 21 had non-critical GI complications, and the rest were asymptomatic at metabolic consultation.

Ten of the 26 patients ascertained by clinical presentation were critically ill at initial metabolic evaluation, as determined by clinical and laboratory findings. Symptoms and/or lab abnormalities included lethargy (7/10), coma (5/10), seizures (6/10), metabolic acidosis (9/10) and hyperammonemia (8/10). Several cases had metabolic acidosis without critical illness (Table I). Among the remaining 15 clinical presentations, 1 patient lacked data regarding health status and the other 14 had GI (6/26, primary symptom was vomiting), neurological (4/26, symptoms of lethargy or seizures), or respiratory complications (2/26). 1/26 had neurological and GI complications. No cardiac complications were reported at presentation.

Patients ascertained by family history were largely asymptomatic (6/7) at initial metabolic evaluation, except for 1 individual who had neurological complications.

Patients who presented clinically had metabolic acidosis at initial diagnosis more frequently than patients ascertained by abnormal NBS (19/24 vs. 2/16; $\chi^2=17.109$, $p<0.001$) or by family history (19/24 vs. 1/7, Fisher's Exact Test $p=0.004$). There was no significant difference in the frequency of hyperammonemia at initial presentation between patients who presented clinically versus by abnormal NBS (10/24 vs. 9/16; $\chi^2=0.819$, $p=0.366$).

Among presentations at greater than 30 days of age by family history or clinical symptoms, the most common symptoms were GI (5/18) or neurologic (4/18). Eighteen percent were critically ill at presentation. Over half (12/18) presented with metabolic acidosis, with or without hyperammonemia, including the only case that lacked clinical symptoms at presentation.

Of the seven Amish individuals, 4 had clinical presentations at an average age of 1.3 years, 2 were ascertained by family history in the neonatal period, and 1 was referred due to an abnormal NBS. Two of the four individuals with clinical presentation had respiratory symptoms, and 2 had GI symptoms. Both of the Amish cases that were ascertained by family history were asymptomatic, and the case ascertained by an abnormal NBS was asymptomatic but an elevated ammonia level was reported at initial evaluation.

Mean propionylcarnitine (C3) levels in dried blood spots for individuals ascertained via abnormal NBS were 18.06 μM (SD = 7.72 μM , range 3.4–33.31; N=20). Average C3:C2

ratio was 0.98uM (SD=0.57 μ M, range 0.28–2.35; N=17) and a C3:C16 ratio of 7.21uM (SD = 3.92 μ M, range 1.99–13.9; N=11). There was no difference in the mean C3 levels between critically ill and non-critically ill cases ascertained by abnormal NBS patients (data not shown).

Molecular diagnostics

Genotype information was available for twenty-six of the fifty-eight cases. Due to unresolved data queries, molecular results were excluded for four cases. All 26 cases that were genotyped reported two variant alleles. Seven cases reported two variants in *PCCA*, and nineteen of the cases had two variants in *PCCB*. In total, 52 variant alleles were reported, with approximately one quarter occurring in the *PCCA* gene (14/52), and the rest in *PCCB* (38/52).

Variant alleles were characterized as missense, nonsense, insertion or deletion, splicing, or unknown, in the case of insufficient information for assignment to any of the preceding categories. The most frequent type of variant was missense, observed in 27/52 alleles. Over one-half of the *PCCB* mutations were missense (23/38), but missense mutations were less common in *PCCA* (4/14). The most commonly observed mutation in the cohort was a missense in *PCCB*, c.1606A>G, occurring with an allelic frequency of 25% (13/52). Eight of these alleles were detected in the homozygous state in individuals of Amish background. A common *PCCB* mutation in the general Caucasian population is c.1218del14ins12¹² and was observed in our data set in 10% of the *PCCB* alleles (4/38). Ten variants were found to have not been previously reported in the literature or in open-source databases and are listed in Table IV.

To explore relationships between genotype and C3 acylcarnitine values in NBS samples, we compared C3 levels between individuals with *PCCA* or *PCCB* mutations. No significant differences were found between C3 acylcarnitine values and mutations in either *PCCA* or *PCCB* (p=0.414). We also tested for differences in C3 acylcarnitine levels and having at least one null mutation in either *PCCA* or *PCCB*; however, the differences in values observed in C3 acylcarnitine levels between individuals with at least one null mutation and those without null mutations did not reach statistical significance (p=0.259).

Discussion

We analyzed initial presentations in a cohort of 58 individuals with PA enrolled in the IBEM-IS and compared initial symptoms and time to evaluation based on one of three methods of ascertainment that were reported by the originating center (abnormal NBS, clinical presentation, or family history). The IBEM-IS captures age at initial evaluation by metabolic specialist and institution of treatment, but not age at presentation. Previous publications have reported that the vast majority of patients with propionic acidemia present in the neonatal period^{13–16}. In our dataset, less than half of cases with clinical presentations 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 had an initial metabolic evaluation after one year of age. Published data is sparse regarding symptoms and their frequencies in individuals who present after 1 year of

age^{15,16,14}. In our cohort, half of individuals with late presentations had GI and neurologic findings.

Mean age at consultation and initiation of treatment was significantly lower for patients who had NBS in comparison with clinical presentations. This result affirms the importance of NBS to allow for early detection and treatment. However, long-term follow-up data are required to evaluate if early detection and institution of treatment translate to significant long-term benefits in terms of survival or outcomes.

Approximately 40% of patients were deemed critically ill at initial metabolic consultation, regardless of type of ascertainment but most commonly in the neonatal period. The most commonly affected organ system at initial presentation was the gastrointestinal system, reflected by symptoms that included vomiting, diarrhea, hepatomegaly, failure to thrive, and gastrointestinal symptoms were more common at initial presentation than neurologic symptoms. These symptoms are similar to those previously described^{11, 14}. Non-critical 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 acidemias present with metabolic acidosis and hyperammonemia¹⁷. The accumulation of propionyl-CoA results in inhibitory effects on several metabolic pathways including the urea cycle, which likely is a consequence of suppression of N-acetyl glutamate synthetase, leading to a block in the first step of the urea cycle¹⁸. Typically, metabolic acidosis precedes hyperammonemia. In our cohort, metabolic acidosis at initial presentation was most commonly observed in patients ascertained by clinical presentation than by a positive NBS. However, we noted no significant difference between these two groups with regard to frequency of hyperammonemia. This 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 requires rapid evaluation and treatment even if the initial blood gas 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 thus far consist of missense mutations, small insertions and deletions, splicing mutations, and large genomic deletions or duplications¹⁹. We note that molecular testing was a frequently-employed adjunct to diagnostic evaluation. In all cases that had sequencing, two variant alleles were detected, with a number of previously unpublished alleles. A caveat to analysis is that phase is assumed to be *trans*, and laboratory interpretation of the variant was not included in the database. There was no correlation between the gene mutated and C3 acylcarnitine levels on NBS, or the presence of null mutations in either gene and C3 acylcarnitine levels on NBS. C3 acylcarnitine levels can be dependent on carnitine supplementation and clinical status when sample was drawn and therefore the actual level may not be directly related to genotype. In addition, residual propionyl-coA carboxylase enzyme activity may reflect genotype-phenotype correlations more accurately. However, less than half of the cases had

propionyl-coA carboxylase enzyme measurement, either in fibroblasts or lymphocytes, and a very small number of cases also had molecular testing. This trend may reflect the widespread availability of molecular testing for diagnostic purposes.

Limitations to this study include ascertainment bias, as 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, data 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 comparison of long-term outcomes by ascertainment method in the future.

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 individuals may present with hyperammonemia without metabolic acidosis, an uncommon observation, warranting rapid intervention and treatment. In addition, the results objectively demonstrate the value of NBS for diagnosing PA and instituting treatment early in life.

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Funding information is available at www.jpeds.com (Appendix 2). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Appendix 1

Additional members of the Inborn Errors of Metabolism Collaborative include

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Appendix 2

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

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

Table 1

Clinical, laboratory, and molecular information on all cases included in the series.

ID	Ascertainm ent	Days of Age at Initial Metabol ic Consult	Days of age at Initiati on of Treat ment	Days Between n Consult and Treatm ent	Clinical Category	Symptoms at initial metabolic contact	Gene	Lab abnormalities at first contact with metabolic specialist	Mutation/Variant 1	Mutation 1 Category & Prediction	Mutation/Varia nt 2	Mutation 2 Category & 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 & 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	–	–	Asympto matic	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				
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

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9#	Family history	57	7	-50	Asymptomatic	None	Unknown	No abnormal labs				
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 AST				
12	Clinical Presentation	690	690	0	GI	Dehydration Vomiting	Unknown	Low free and total carnitine				
13	Abnormal NBS	11	-	-	Asymptomatic	None	PCCB	No abnormal labs	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 Hypertiglyceridemia 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				
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 Hypertiglyceridemia Ketonuria				

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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 & ketonuria Low plasma total & free carnitine Metabolic acidosis Elevated liver enzymes Hematuria & proteinuria	c.398T>C p.L133P	Missense, MT: disease causing PP: probably damaging S: damaging	c.415C>T p.Q139X	Nonsense, previously reported
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

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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 labs done 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				
34	Family history	10	10	0	Asymptomatic	None	Unknown	No abnormal labs				
35	Family history	1	2	1	Asymptomatic	None	Unknown	Ketosis & ketonuria Metabolic acidosis				
36	Family history	22	22	0	Asymptomatic	None	Unknown	No abnormal labs				
37	Abnormal NBS	13	20	7	Asymptomatic	None	Unknown	Low BUN and creatinine, high potassium, calcium, and AST, low total protein.				
38	Abnormal NBS	–	–	–	Unknown		PCCB	No information	c.1606A>G p.N536D	Missense, previously reported	c.386_387delT TinsAAC p.F129X	Nonsense MT prediction: disease causing
39	Clinical Presentation Abnormal labs	–	35	–	Critical Illness	Coma Seizure Vomiting	Unknown	Metabolic acidosis				
40	Clinical Presentation Abnormal labs	10	10	0	Neurologic	Coma Poor feeding	Unknown	No information				

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43	Abnormal NBS Clinical presentation	8	8	0	Critical Illness	Hypotonia Infection/sepsis Lethargy Poor feeding	<i>PCCA</i>	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		<i>PCCA</i>	No information	c.1367G>T p.G456V	Missense MT prediction: disease causing PP: Probably damaging	c.600+1C>A	Splicing MT prediction: disease causing
45	Abnormal NBS Clinical presentation	3	2	–1	Critical Illness	Poor feeding	<i>PCCA</i>	Hyperammonemia Ketosis Lactic acidosis	c.1023dupT p.V342C[s*]19	Insertion, MT prediction: disease causing	Genomic deletion of exons 3–4	Deletion
46	<i>Clinical Presentation</i>	646	646	0	<i>Unknown</i>	<i>Unknown</i>		<i>No information</i>	<i>Negative for common mutation panel of 5 alleles (done 2004)</i>		<i>Negative for common mutation panel of 5 alleles (done 2004)</i>	
48	Clinical Presentation	30	30	0	GI	Vomiting Weight loss	<i>PCCA</i>	Metabolic acidosis	c.184-17_18delTG p.T62_S100del39	Splicing Previously reported	c.231+44_47de 1ATTT p.T62_S100del 39	Splicing Previously reported
49	Family history	–1	1	2	Asymptomatic	None	Unknown	No abnormal labs				
50	Clinical Presentation	3	3	0	Critical Illness	Poor feeding	<i>PCCA</i>	Hyperammonemia Hypoglycemia Metabolic acidosis	<i>Unresolved query on reported allele</i>		<i>Unresolved query on reported allele</i>	
51	<i>Clinical Presentation</i>	540	540	0	<i>GI</i>	<i>Developmental delay(s) Lethargy Vomiting</i>	<i>PCCA</i>	<i>No information</i>	<i>Unresolved query on reported allele</i>		<i>Unresolved query on reported allele</i>	
52	Abnormal NBS	5	5	0	Critical Illness	Lethargy Poor feeding	<i>PCCA</i>	No information	c.1591T>C p.S531P	Missense MT: disease causing PP: possibly damaging	Del exon 20–21	Unknown
53	Abnormal NBS	15	3	–12	Asymptomatic		<i>PCCA</i>	No information	c.1591T>C p.S531P	Missense MT: disease causing PP: possibly damaging	Del exon 20–21	Unknown
54	Abnormal NBS	17	17	0	Asymptomatic	None	<i>PCCB</i>	No information	c.683C>T p.P228L	Missense, previously reported	c.1218del14ins 12	Frameshift, previously reported
55#	<i>Clinical Presentation</i>	900	900	0	<i>GI</i>	<i>Lethargy Seizure Vomiting Diarrhea</i>	<i>PCCB</i>	<i>Metabolic acidosis Anemia</i>	c.1606 A>G p.N536D	<i>Missense, previously reported</i>	c.1606 A>G p.N536D	<i>Missense, previously reported</i>
56#	Abnormal NBS	29	29	0	Asymptomatic	None	<i>PCCB</i>	Hyperammonemia	c.1606 A>G p.N536D	Missense, previously reported	c.1606 A>G p.N536D	Missense, previously reported
57	<i>Clinical Presentation</i>	330	340	10	<i>GI</i>	<i>Developmental delay(s) Vomiting</i>	<i>Unknown</i>	<i>Low plasma total & free carnitine</i>				
58	Clinical Presentation Abnormal labs	7	0	–7	GI	Failure to thrive Hypotonia Vomiting	Unknown	Anemia				

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59	Abnormal NBS	–	–	–	Asymptomatic		<i>PCCA</i>	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

Italics: clinical or family history presentations at greater than 30 days of age.

Ascertainment in bold: primary ascertainment categorization, if more than one was provided.

DNA variants in bold: unpublished variants

#Cases identified as being Amish.

Table 2

Time to evaluation and treatment in individuals older than 30 days of age ascertained by clinical presentation and family history, N = 18.

	Initial metabolic consultation	Initiation of treatment
Range	35–3030 days	35–2920 days
Mean	683 days	624 days
Median	593 days	480 days

Table 3

Clinical presentation and specific symptoms. FTT: failure to thrive, DD: developmental delay.

Symptoms	Newborn Screen	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
Neuro+ GI	0	1	0
Asymptomatic	10	1	6
No data	4	1	0
Total	25	26	7

Table 4

PCCA and PCCB alleles that are not previously reported (in bold), with predicted effect on protein. I=intron, E=exon, MT: MutationTaster, PP: PolyPhen, S: SIFT.

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