SERVICE UTILIZATION BY PROPIONIC ACIDEMIA PATIENTS

by

Amanda Jane Jacquart

B.S. in Human Biology, University of Wisconsin Green Bay, 2012

Submitted to the Graduate Faculty of

the Department of Human Genetics

Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Master of Science

University of Pittsburgh

2014

UNIVERSITY OF PITTSBURGH

GRADUATE SCHOOL OF PUBLIC HEALTH

This thesis was presented

by

Amanda Jacquart

It was defended on

April 4, 2014

and approved by

Advisor: Catherine M. Walsh Vockley, M.S., LCGC, Senior Genetic Counselor, Division of Medical Genetics, Children's Hospital of Pittsburgh of UPMC

Robin E. Grubs, Ph.D., LCGC, Assistant Professor, Co-Director of the Genetic Counseling Program, Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh

John W. Wilson, Ph.D., Assistant Professor, Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh

SERVICE UTILIZATION BY PROPIONIC ACIDEMIA PATIENTS

Amanda Jane Jacquart, M.S.

University of Pittsburgh, 2014

ABSTRACT

Propionic acidemia (PA) is a rare inborn error of metabolism (IBEM) identifiable by newborn screening (NBS). PA patients have variable clinical presentations and neonatal symptomology can be severe and potentially life threatening. Longitudinal data is being collected to learn more about the natural history of PA and current management of these patients. As care recommendations are being developed, it is important to understand patients' service utilization and current unmet needs.

This study describes the PA patient data from the Inborn Errors of Metabolism Information System (IBEM-IS), a database created to follow patients with conditions detected or detectable by NBS. Data from 46 PA subjects and 137 visits entered into IBEM-IS were analyzed for anticipated needs of PA patients based on the clinical spectrum and current published practice guidelines. Analysis of PA data in IBEM-IS revealed that the majority of the desired variables were absent. Thus, precise determination of service utilization by PA patients remains incomplete. There remains a need for comprehensive, uniform data collection and more detailed assessment of patients.

The original design of the study incorporated an interview of parents/guardians of PA patients to identify current service utilization for comparison to aggregate data in IBEM-IS. Due to recruitment challenges, two of three aims of this study were not achieved. The difficulties experienced with recruitment and collaborative work with a multi-center consortium are discussed.

This analysis of PA patient data in IBEM-IS augments the database and promotes future research studies. Learning more about service utilization and parents' perceived unmet needs for PA patients may also have implications for a broader group of IBEM identifiable by NBS. Further, the need to prove efficacy of NBS, as a public health program intended to decrease morbidity and mortality, can be supported by this analysis of outcomes and patients needs in those identified by NBS and those identified clinically. The improved long-term care and follow up resulting from focused research on current practices and needs in these patients will impact many individuals and their families.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

I would like to express my gratitude to the individuals who helped and advised me throughout the duration of my thesis. First, many thanks go to my committee members. I thank Cate Walsh Vockley for all of the time and energy she put into developing, executing, and reinventing my thesis when needed. Her advocacy for this project and her expertise with collaborative work was essential. I appreciate her willingness to take on this endeavor to serve as my mentor and her commitment to the completion of this project. I thank Dr. Robin Grubs for her continued support and guidance through the many challenges and obstacles faced during the implementation of this project. She played a pivotal role in the organization of a plan to regroup and move forward. I thank Dr. John Wilson for discussing the descriptive statistics of my project and engaging in thoughtful conversations with respect to study limitations and proposed solutions.

I am grateful for the Inborn Errors of Metabolism Collaborative (IBEMC) and their work in creating the Inborn Errors of Metabolism Information System (IBEM-IS). I thank the research coordinators who collected and entered data across the various participating centers and for the patients and families who graciously participated. I thank Susan Berry, M.D, the Principal Investigator for IBEM-IS, Sally Hiner for being a point of contact in the Collaborative, and Shaohui Zhai for her exhaustive efforts in supplying complete, functional data. I especially would like to thank Dr. Paul Levy, a principal investigator from Children's Hospital at

Montefiore in New York, for offering his services and writing an Excel macro to translate the data for my project.

The Human Genetics department has been a constant source of support throughout my educational career at the University of Pittsburgh. I am indebted to my program directors, the faculty, staff, and students for their willingness to lend a helping hand and their eagerness to be a mentor as well as a friend. Words cannot describe how thankful I am for my fellow genetic counseling classmates. I have been inspired and forever changed by each of these ten amazing women.

Lastly, I would like to thank my family and friends for their continuous love and support. Their overwhelming level of encouragement and understanding during this time has been a blessing. Special thanks to Ian Sleger, for being a source of strength and comfort throughout this journey.

1.0 INTRODUCTION

Propionic acidemia (PA) is an autosomal recessive inborn error of metabolism. PA is a rare organic acid disorder with an estimated worldwide incidence ranging from 1 in 300,000 to 1 in 165,000 live births. Mutations in either the *PCCA* or *PCCB* gene cause PA and lead to an accumulation of organic acids in the blood, urine, and tissue. The abnormal build-up of organic acids is toxic and can be life-threatening. Acute manifestations of PA such as vomiting, poor feeding, and lethargy can be observed shortly after birth in affected individuals and also later in life during times of metabolic crisis brought on by catabolic stressors. If these symptoms are left untreated seizures, and in some cases coma and death, can occur. Chronic manifestations of PA include failure to thrive, developmental delay, vomiting, protein intolerance, neurological manifestations including seizures, MRI abnormalities and intellectual disability and heart complications such as cardiomyopathy and cardiac rhythm abnormalities (Pena and Burton 2012).

Early diagnosis of PA had been associated with a lower mortality rate. However, a study done by Grünert et al. in 2012 compared the overall clinical outcomes of 20 PA patients who were diagnosed through newborn bloodspot screening (NBS) to 35 patients who were diagnosed by a selective metabolic screen because of clinical presentation or family history. The study did not find a significant difference between the two groups. There was little difference with regard to their clinical course, including the number of metabolic crises, physical and neurocognitive

development, and long-term complications (Grunert, Mullerleile et al. 2012). While early detection of PA is important, the current management and treatment of individuals affected with PA, even if initiated promptly, may not be significantly improving the patient's quality of life.

Due to the low incidence and heterogeneity of the condition, there were previously no clear guidelines for the medical management of patients with PA. To establish a standardized list of practice guidelines, the Children's National Medical Center in Washington, D.C. convened a group of healthcare professionals, researchers, and parents in January 2011. At the time of this meeting and in the published proceedings, it was acknowledged that there had only been limited improvement in understanding of the natural history of PA through collaborative studies to date. Additionally, it was stated that the handful of larger studies were limited by a lack of uniformity in data collection (Sass, Hofmann et al. 2004, Chapman and Summar 2012).

The Inborn Errors of Metabolism Collaborative (IBEMC) is a group of clinicians and research coordinators working together to compile information on patients who have one of the conditions detected or detectable by newborn screening (NBS). IBEMC created a multi-center database to follow and study patients with inborn errors of metabolism, called the Inborn Errors of Metabolism Information System (IBEM-IS). This resource was developed in recognition of the need to understand more about the natural history and clinical outcomes for patients with rare inborn errors of metabolism and to promote future studies and additional research (Berry, Jurek et al. 2010).

This present study assesses the PA subject data present in IBEM-IS to explore completeness of patient-specific content and individual and compiled healthcare utilization. To date, there has been insufficient data gathered from these patients and families regarding this information (Chapman and Summar 2012). Understanding what services PA patients and their

families are using and identifying potential unmet needs is important in the development of effective management and treatment protocols to improve clinical outcomes and quality of life.

2.0 HYPOTHESIS AND SPECIFIC AIMS

2.1 HYPOTHESIS

Hypothesis 1: The differences between the two sets of service utilization data collected (database vs. interviews) are due to patients accessing but not disclosing more services than are documented in IBEM-IS. Patients may find it difficult to communicate all or unmet service needs in the clinic setting, thus requiring more thorough assessment of needs through targeted questioning by healthcare professionals.

Hypothesis 2: The differences between the two sets of service utilization data collected are due to the current data collection and entry practices associated with IBEM-IS.

2.2 SPECIFIC AIMS

Aim 1: To analyze the service utilization data collected within the IBEM-IS patient registry as compared to anticipated needs based on the clinical spectrum of propionic academia (PA).

Aim 2: To interview parents/guardians of PA patients to identify current service utilization and unmet needs.

Aim 3: To compare parent/guardian stated service utilization to documented aggregate data in the IBEM-IS.

3.0 BACKGROUND AND SIGNIFICANCE

3.1 PROPIONIC ACIDEMIA

Propionic acidemia (PA) was first described by Dr. Barton Childs and his colleagues at Johns Hopkins Hospital in 1961 (Childs, Nyhan et al. 1961). PA is a rare, autosomal recessive inborn error of metabolism with an estimated worldwide incidence ranging from 1 in 300,000 to 1 in 165,000 live births (Pena and Burton 2012). PA can be found in individuals of all races and ethnicities. However, the condition appears to be more common in the Inuit population of Greenland, as well as in Japan, Saudi Arabia, and some Amish communities. With the implementation of NBS and tandem mass spectrometry, variability in the frequency of PA among countries has been observed. The disease frequency is reported to be 1 in 1000 in the Greenland Inuit, 1 in 17,400 in Japan, 1 in 27,264 in Saudi Arabia, 1 in 129,000 in the United States, and 1 in 250,000 in Germany (Desviat, Pérez et al. 2004).

3.1.1 Molecular Basis

Biallelic mutations in either the *PCCA* gene, located on chromosome 13q32.3, or the *PCCB* gene, located on chromosome 3q22.3, cause PA (Perez-Cerda, Merinero et al. 2000). The *PCCA* and *PCCB* genes work to encode the α and β subunits of the mitochondrial enzyme propionyl-CoA carboxylase (PCC). The enzyme is a heterododecamer of six α subunits and six β subunits

(Perez-Cerda, Merinero et al. 2000). PCC catalyzes the conversion of propionyl-CoA to D-methylmalonyl-CoA, which eventually enters the Krebs cycle as succinyl-CoA (Ugarte, Perez-Cerda et al. 1999). The function of PCC in the body is to play a role in the catabolism of isoleucine, threonine, methionine, and valine, odd-numbered-chained fatty acids, cholesterol, and other metabolites (Tahara, Kraus et al. 1993, Magdalena, Celia et al. 1999). Deficiency of the PCC enzyme results in an accumulation of propionic acid, free organic acids, and ammonia in the blood, urine, and tissue (Pena, Franks et al. 2012).

3.1.2 Clinical Manifestations

The clinical picture of PA is variable and natural history of the disorder is still being studied and understood. In a study done by Pena et al. in 2012, a survey of Propionic Acidemia Foundation (PAF) members was used for a cross-sectional retrospective review. The responses from 58 individuals were assessed to determine the frequency of reported complications. Seizures were reported as one of the most common complications, occurring in 41% of individuals. Nineteen percent of responders reported having cardiomyopathy, which occurred primarily in school-age children and adults, and was reported to be the cause of death in 70% of deceased patients (Pena and Burton 2012). A retrospective study done by Grünert et al. in 2013 examined the clinical outcome data of 55 PA patients from 16 European metabolic centers. Within the study cohort, over 85% of the patients had metabolic decompensation during the neonatal period and 75% had mental retardation with a median IQ of 55. Other clinical manifestations in their study population included hematologic abnormalities, cardiac diseases, feeding problems, and impaired growth (Grünert, Müllerleile et al. 2013).

In the majority of PA cases, symptoms appear within a few hours or days after birth. Nonspecific findings such as poor feeding, vomiting, hypotonia, and lethargy are often the first clinical features that are observed. More serious medical concerns, including heart abnormalities and neurological complications may occur. PA is classified as an intoxication-type disorder of organic acid metabolism and is characterized by the build-up of propionic acid resulting in episodes of vomiting, dehydration, and severe metabolic acidosis. The health status of PA patients worsens during times of increased metabolic demand. Episodes of fever, infection, vomiting, trauma, and psychological or physiological stress can precipitate metabolic acidosis in individuals with PA. It is thought that at times concurrent with catabolic stressors, affected individuals have higher metabolic rates than they can tolerate and therefore require acute management to diminish morbidity and mortality. (Chapman, Gropman et al. 2012). Symptoms of metabolic decompensation may include poor feeding, lethargy, failure to thrive, vomiting, and potentially coma and death if left untreated.

Individuals with PA are at risk for developing heart complications including cardiomyopathy and arrhythmias. Cardiomyopathy is a common, long-term complication of PA. It may resolve with time or progress to cardiac failure (Dionisi-Vici, Deodato et al. 2006). Several fatal cases of cardiomyopathy in PA patients have been reported (Mardach, Verity et al. 2005). However, the age of diagnosis of PA, level of metabolic control, or amount of residual enzymatic activity do not seem to correlate with the risk for cardiomyopathy (Pena, Franks et al. 2012). One recognized arrhythmia, prolonged QT interval, has been detected on electrocardiogram in multiple patients (Pena, Franks et al. 2012). This abnormality is associated with syncope and sudden death (Jameson and Walter 2008).

Other long-term complications of PA involve the central nervous system. Metabolic crises that occur during early childhood, resulting in the accumulation of toxic metabolites, pose damage to the central nervous system (Haberlandt, Canestrini et al. 2009). The neurological complications that have been reported in case studies include seizures, basal ganglia abnormalities, movement disorders, and brain atrophy. PA patients may suffer from number of different types of seizures including: generalized tonic-clonic, absence, atonic, focal, focal with generalization, and myoclonic. The age of onset of seizures is typically during early childhood, often between 7 days to 4 years of age (Pena, Franks et al. 2012). A study of 17 PA patients done by Haberlandt et al. concluded that electroencephalogram (EEG) abnormalities and epileptic seizures were found in 50% of patients, however they found no relationship between age of onset of PA symptoms or number of metabolic decompensations and the development and frequency of seizure episodes (Haberlandt, Canestrini et al. 2009).

Currently there is limited information regarding the developmental status of patients with PA. The developmental outcomes have been reported to range from individuals with reduced IQ and delayed cognitive skills to individuals who attend regular school without any additional support (Pena, Franks et al. 2012). The study by Grünert et al. in 2013 determined that neurologic outcome of PA patients can be quite compromised. The study showed that approximately three quarters of the study subjects had mental retardation and that there was a negative correlation between the subject's IQ and the number of metabolic decompensations the subject experienced. This was found to be the trend even when excluding the subject's age as a factor (Grünert, Müllerleile et al. 2013). To date, there has been no improvement in the neurologic outcomes in PA patients despite the advancements of therapeutic interventions and treatment protocols (Grunert, Mullerleile et al. 2012, Sindgikar, Rao et al. 2013).

Episodes of acute pancreatitis and recurrent acute pancreatitis have been reported in a small number of PA patients. Acute pancreatitis should be suspected in PA patients experiencing ongoing abdominal pain (Bultron, Seashore et al. 2008). During episodes of metabolic decompensation, neutropenia and thrombocytopenia have been reported. With the reported cases of hematologic abnormalities, it is thought that this might contribute to PA patients being at an increased risk for infection, however this remains uncertain (Pena, Franks et al. 2012).

Children with PA have a tendency to be small for age due to malnutrition and protein-restricted diet. Dysmorphic features that are typically seen in individuals with methylmalonic aciduria (MMA) can be seen in individuals with PA. These features include: frontal bossing, a widened depressed nasal bridge, epicanthal folds, long philtrum, and an upturned curvature of the lips (Burton 1998). Rare complications that can be associated with PA are optic atrophy, hearing loss, premature ovarian insufficiency (POI), chronic renal failure, and osteoporosis (Williams, Hurley et al. 2009, Lam, Desviat et al. 2011).

3.1.3 Diagnosis

A clinical diagnosis of PA is defined based on clinical manifestations and time of presentation. Generally, patients phenotypically fall into one of two groups, neonatal-onset and late-onset. A majority of individuals present with symptoms during the neonatal period and clinical findings can be fairly nonspecific. These features include vomiting, poor feeding, lethargy, and hypotonia within the first days of birth. If symptoms progress and are left untreated, seizures, metabolic acidosis and/or hyperammonemia, and in some cases coma and death, may occur (Grunert, Mullerleile et al. 2012). The clinical findings associated with late-onset PA, which occurs anytime after the neonatal period, include: failure to thrive, developmental delay, chronic

vomiting, protein intolerance, various neurological symptoms, and cardiomyopathy (Grunert, Mullerleile et al. 2012). Children and adults with PA can experience acute decompensation that is similar to the symptoms associated with the neonatal presentation. Often this is brought on by catabolic stressors such as infection, injury, or surgery.

Newborn screening (NBS) can identify infants with PA. An acylcarnitine profile performed by tandem mass spectrometry (MS/MS) on dried blood spots shows an elevated propionylcarnitine (C3) level in affected individuals (Chapman, Gropman et al. 2012). When infants are symptomatic before the NBS results are available, often the diagnosis is made due to the baby's clinical presentation. In symptomatic individuals, the testing of urine organic acids and plasma amino acids distinguishes PA from other organic acidemias. Elevated 3-hydroxypropionate and the presence of methylcitrate, tiglylglycine, and propionylglycine in the urine and elevated glycine in the blood are characteristic of PA patients (Sindgikar, Rao et al. 2013). To confirm a diagnosis of PA, PCC enzyme activity can be measured in peripheral blood leukocytes or cultured skin fibroblasts or molecular genetic testing can be completed.

Molecular studies for PA, including gene sequencing and deletion/duplication analysis, are currently available for both the *PCCA* and *PCCB* genes. The mutation detection rate using sequence analysis is approximately 80% in the *PCCA* gene and 99% in the *PCCB* gene. Use of deletion/duplication analysis, which identifies exonic or whole gene-deletions, increases the detection rate by approximately 20% in the *PCCA* gene; deletions in the *PCCB* gene have not been reported to date. If the mutations are known in a family, then DNA analysis can be preformed for prenatal diagnosis. If the mutations are unknown, then prenatal diagnosis of PA may be achieved via measurement of enzyme activity in cultured chorionic villi cells or amniotic fluid cells (Perez-Cerda, Perez et al. 2004). At present, preimplantation genetic diagnosis (PGD)

is a valid reproductive option for couples at risk of transmitting mutations in the *PCCA* or *PCCB* gene to their children (Alberola, Bautista-Llacer et al. 2011).

3.1.4 Genotype-Phenotype Correlations

Compound heterozygosity, either in the *PCCA* or the *PCCB* genes, occurs in the majority of affected individuals, making genotype-phenotype correlations challenging (Pena, Franks et al. 2012). In general, null alleles are associated with a severe form of PA due to complete absence of enzyme activity, and missense mutations are associated with milder forms of PA, presumably due to residual enzyme activity (Desviat, Pérez et al. 2004). Null alleles that have been reported in the literature include p.Arg313X and Ser562X in the *PCCA* gene and p.Gly94X and several small deletions/insertions and splicing mutations in the *PCCB* gene. The reported missense mutations are p.Ala138Thr, p.Ile164Thr, and p.Arg288Gly in the *PCCA* gene and p.Asn536Asp in the *PCCB* gene. There are exceptions to missense mutation causing mild disease: 3 *PCCB* missense mutations, p.Gly112Asp, p.Arg512Cys, and p.Leu519Pro, are reported to affect heterododecamer formation and result in undetectable PCC enzyme activity and therefore are associated with a severe form of PA (Muro, Pérez et al. 2001).

The PCC enzymatic activity resulting from different mutations is often the most useful indicator to establish severity and prognosis of the condition. However, establishing correlations among the mutations, the measured enzymatic activity and the clinical manifestations of PA is complicated by the various methods in which PCC activity can be measured (i.e. fibroblasts, leukocytes, and in vitro systems). Direct comparisons are difficult because each method may yield differing enzymatic activities (Pena, Franks et al. 2012). Consistency of testing systems is important when attempting these correlations.

In patients who have PA, *PCCB* mutations are found more frequently than *PCCA* mutations (Pena, Franks et al. 2012). The most common mutation in the *PCCB* gene in the Caucasian population, c.1218_1231del14ins12, represents 32% of mutant alleles (Tahara, Kraus et al. 1993, Perez-Cerda, Merinero et al. 2000). A missense mutation in the *PCCB* gene, c.1606G>A (p.Asn536Asp), is associated with a less severe form of PA and is commonly seen in some Amish communities, identified in Lancaster, PA (Desviat, Pérez et al. 2004, Strauss and Puffenberger 2009).The most common mutation in the *PCCB* gene in the Japanese population c.1304T>C (p.Y435C), represents 25% of mutant alleles in that group (Tahara, Kraus et al. 1993). In Japan, this particular mutation is associated with a more mild clinical presentation of PA and also has been seen in asymptomatic individuals (Yorifuji, Kawai et al. 2002).

3.1.5 Management Recommendations

In January 2011, the Children's National Medical Center in Washington, D.C. assembled a group of physicians, investigators, and parents to discuss practice guidelines and medical management options for patients with PA (Chapman and Summar 2012). This effort included a rigorous review of English-language published materials regarding PA, contribution from experts and robust debate of any discrepancies. As a result of this meeting, articles were published outlining the acute management, chronic management, and health supervision options for PA patients.

3.1.5.1 Acute Management

All patients with PA are susceptible to metabolic crises and must have an emergency protocol in place to initiate when they show early signs of metabolic decompensation. The recommendations for acute management of patients with PA are categorized using a step-wise approach. The first

step involves initial care and the stabilization of the potential critically ill patient. This intervention may apply to a patient with a known or suspected diagnosis and may take place in a healthcare facility that is not considered a metabolic center. This intervention focuses mainly on basic life support and obtaining vital signs, placing intravenous lines, and drawing baseline laboratory studies. Equally essential is reversal of catabolism, a major source of metabolic toxicity. This entails discontinuation of all sources of protein and provision of a non-protein calorie source (IV dextrose with electrolytes). Verification of newborn screening results for neonates should also be done (Chapman, Gropman et al. 2012).

The second step in acute management of the symptomatic PA patient is transport to an established metabolic center where more aggressive interventions can take place as needed. If the patient did not stabilize with the reversal of catabolism, then he/she would require some level of accelerated care. Depending on clinical circumstances, this might involve insulin drip, hemodialysis or extracorporeal membrane oxygenation (ECMO) in the hyperammonemic patient, delivery of ammonia-lowering medications, and carnitine supplementation, all done with careful monitoring. Reintroduction of protein should be done as early as possible to prevent protein deficiency from contributing to the decompensation (Chapman, Gropman et al. 2012).

Once the patient is stabilized, the third step involves preparation for discharge of the patient and transition from acute to chronic management, including deceleration of acute interventions, establishment of a home regimen, and parental training (Chapman, Gropman et al. 2012).

3.1.5.2 Chronic Management

The needs of PA patients will differ depending on the severity of the condition, thus chronic management and treatment protocols should be customized for each affected individual.

Therefore, not every individual with PA will need all of the medical treatment and surveillance options that were proposed for this patient population. The chronic management recommendations that have been established in the literature are centered on nutritional assessments and laboratory monitoring, neurology, cardiology, immunology, gastroenterology, ophthalmology, ancillary treatment, and liver transplant evaluation. Further information regarding the specific practice guidelines is outlined in Table 1 (Sutton, Chapman et al. 2012).

Table 1. Outline of the Chronic Management and Health Supervision of Individuals with Propionic Acidemia

Specialty Area	Practice Guidelines
Nutrition	Recommended nutrition assessments at routine outpatient visits - at least every 6 months • Albumin, ammonia, plasma amino acids (fasting 3-4 hours), prealbumin
	Optional nutrition assessments to consider: • Quantitative acylcarnitine profile • Urine methylcitric acid • Other general measures of nutrition as needed, based on medical history, dietary intake, and growth parameters o Such as 25-hydroxyvitamin d, iron, selenium, free fatty acids etc.
	Diet history review at each visit and adjustments to be made as needed: • Normal growth velocity for weight • Normal levels of serum albumin and prealbumin • Normal levels of plasma isoleucine, methionine, threonine and • Valine • Normal to elevated levels of plasma glycine
Therapeutic Services	Early initiation of physical, occupational, and speech therapy services, to continue throughout childhood
Neurology	Management of stroke-like episodes • Ensure adequate fluid and caloric intake • Symptomatic treatment of focal neurological deficits
	Evaluation for seizures • EEG at diagnosis and annually • Referral to child neurology if epileptiform activity is detected
Cardiology	Evaluation for cardiomyopathy • Echocardiogram at diagnosis and annually o As needed to evaluate shortness of breath, tachycardia or other signs and symptoms of cardiac failure
	Screening for Long OTC and other cardiac conduction defects • ECG annually

Table 1. Continued

	• 24-hour Holter annually • ECG and 24-hour Holter during episodes of syncope, fainting, etc.
Immunology	CBC with differential at diagnosis, annually, and as needed
	If neutropenia present, institute infection control precautions (isolation, gown and glove, etc.)
	Expectant management with judicious use of colony stimulating factors only in cases where neutropenia is not resolving or there is evidence of bacterial infection per day
Gastroenterology	Evaluation and management of acute pancreatitis • Vomiting, anorexia, abdominal pain and unexplained acidosis should prompt evaluation for pancreatitis o Serum amylase and lipase measurements • Episodes of acute pancreatitis in PA should be managed using fluids, short-term bowel rest, jejunal feeds, and pain management • When necessary, total parenteral nutrition can be used in a safe manner
Ophthalmology	Evaluation and management of optic atrophy • Yearly examination by an ophthalmologist o Determine visual acuity, visual examination of the anterior chamber, and dilated evaluation of the fundus • Treatment of decreased visual acuity
Ancillary Treatment	Carnitine supplementation • 200–300 mg L-carnitine/kg body weight/day divided 2–3 times • For acute hyperammonemia and recurrent metabolic decompensations, consider doses on the high end of the range (300mg/kg/day)
	Biotin supplementation • May consider biotin 5 mg daily • If no reduction in plasma propionylcarnitine discontinue biotin
	Pro-motility agents • Daily use of laxative at age/weight-appropriate doses
	Bactericidal therapy • Metronidazole 10–20 mg/kg/day divided t.i.d. may be considered in individuals refractory to other standard interventions
	Gastrostomy tube/button placement • May be considered especially in infants and young children
	Port-a-cath placement • May be considered with unreliable peripheral venous access
Liver Transplantation	In individuals with recurrent episodes of hyperammonemia or acidosis that are not adequately controlled with medical therapies, liver transplant may be considered
Referenced from "Chronic management and health supervision of individuals with propionic acidemia."(Sutton, Chapman et al. 2012)	

For the more rare complications that have been seen in patients with PA, additional screening options can include evaluations by audiology for hearing loss and gynecological evaluations for premature ovarian insufficiency (POI) in older females. Lastly, parents should be provided with emergency care information that can accompany the patient at all times. A Medic-alert system should also be in place.

3.2 THE INBORN ERRORS OF METABOLISM COLLABORATIVE (IBEMC)

The Inborn Errors of Metabolism Collaborative (IBEMC) is a group of clinicians and research coordinators from a number of metabolic centers across the United States who work together to compile clinical information and care needs regarding patients who have one of the conditions detected or detectable by NBS (Berry, Jurek et al. 2010). IBEMC was developed in recognition of the need to understand more about the natural history and clinical outcomes for patients with an inborn error of metabolism (IBEM) by creating a multi-center database to follow and study IBEM patients. This is called the Inborn Errors of Metabolism Information System (IBEM-IS). The goal of IBEMC in creating such a database of clinical information was to better understand the history and outcomes of IBEM patients that were identified by NBS as compared to those who were clinically diagnosed later in life. This collection of information is intended to aid in the development of clinical practice guidelines and long-term follow up protocols for this set of rare metabolic conditions, as well as contribute to development of effective therapeutics through identification of biomarkers and support of clinical trials development.

3.2.1 The Inborn Errors of Metabolism Information System (IBEM-IS)

The Inborn Errors of Metabolism Information System (IBEM-IS) was initiated in January 2007 and data was collected from IBEM patients and their families using a web-based, secure data collection platform, called DocSite (Berry, Jurek et al. 2010). The participating metabolic centers entered subject data into the DocSite platform until January 2013, when data collection was transitioned to a new data collection platform, Research Electronic Data Capture (REDCap). This change was made to provide data compatibility with the ongoing NIH-funded programs through the Newborn Screening Translational Research Network (NBSTRN). The Michigan Public Health Institute, the University of Minnesota, and participating clinical centers are funded to work together to manage and provide support for the IBEM-IS project (NIH-Michigan Public Health Institute HD10-019, Children's Hospital of Pittsburgh: New York-Mid-Atlantic Regional Collaborative funding).

The metabolic centers that participate in IBEM-IS have IRB-approved protocols that allow for obtaining consent from individuals or their representatives that permit collection of longitudinal, condition-specific information regarding the patient. The data collection tool was designed based on an extensive literature review and the current clinical practices of metabolic providers, with input and review from multiple metabolic specialists (Berry, Jurek et al. 2010). The data set developers acknowledge that some aspects of the patients' clinical care are uniform among participating centers and there are aspects that differ. For this reason, the collected data incorporates elements that reflect the variability of care at the participating centers.

With the DocSite data collection platform, there were two different data sets that were utilized to collect data from the participating subjects. Intake data sets were collected at the time of the patients' enrollment. Interval data sets were collected at each outpatient metabolic visit

including the initial enrollment visit. Information was gathered from the subjects and the general data elements recorded in the intake and interval data sets are outlined in Table 2.

Table 2. List of General Data Elements Entered into DocSite

Data Set	General Data Elements
Intake	Demographical information
	Socioeconomic status
	Family history
	Prenatal history
	Neonatal history
	Measurements at birth
	Newborn screening information
	Diagnostic testing information
	Past health history
	Emergency management protocols
	Nutritional information
	Other
Interval	Demographical information
	Socioeconomic status
	Measurements at visit
	Past health history
	Emergency management protocols
	Care coordination
	Developmental assessments
	Education services
	Home monitoring
	Laboratory studies
	Imaging studies
	Pharmacotherapy
	Nutritional information
	Other

Currently, the REDCap data collection platform works using branching logic such that specific questions will be displayed depending on values entered in previous questions. Information is obtained at the subjects' enrollment and at each follow-up visit at the participating center. The amount of information collected in REDCap is greater when compared to the information that was collected in DocSite. REDCap also allows for comments to be added at the

end of each data section and for specific sections (e.g., pregnancy, dialysis, and transplant) it directs the provider to complete an additional form. The general data elements that are collected in each data sheet are listed in Table 3.

Table 3. List of General Data Elements Entered into REDCap

Data Sheet	Data Section	General Data Elements
Intake	Intake demographics	Consent
		Demographic information
		Condition
		Care and other studies
		Education
		Ancestral origin, race and ethnicity
		Socioeconomics
		Medical coverage
		Language
	Intake family history	Family history
	Intake past health history	Prenatal history
		Pregnancy
		Neonatal history
		Birth measurements
		Health history
		Dialysis
		Transplants
		Other history
		Prior testing
		Eye exam
		Emergency management
	Intake newborn screening	Newborn screening
		Newborn hearing screen
	Intake initial testing	Symptoms at initial contact
		Diagnostic testing
		Genetic testing
		Parent laboratory studies
Visit	Visit demographics and history	Consent
		Care and other studies
		Education
		Medical coverage
		Family history
	Visit health history	Health status
		Sick visits
		Procedures

Table 3. Continued

		Pregnancy
		Dialysis
		Transplants
		Other procedures
	Visit findings	Visit measurements
	Visit ancillary care	Care coordination
		Emergency management
		Developmental assessment
		Education
	Visit lab studies	Biochemical labs
		Chemistry labs
		Hematology labs
		Liver labs
		Renal labs
		Miscellaneous labs
	Visit management and treatment pharmacotherapy	Pharmacotherapy
	Visit management and treatment nutrition	Nutrition
Additional forms	Pregnancy	First – eighth pregnancy
		Current pregnancy
	Dialysis	First – tenth dialysis treatment
	Transplant	First – fifth transplant

Anonymized subject data is available to the researchers from all of the participating centers. The use of the data for research is encouraged and participating centers each have benchmarks both for patient recruitment and data entry, as well as for submission of proposed research projects utilizing the accumulated data. In addition, proposals can be submitted for use of the data by both researchers involved in the IBEMC and from researchers not involved in the IBEMC. Standard operating procedures have been developed for review of submitted protocols and access to the compiled data. Two general types of research projects are thus far being developed to integrate use of IBEM-IS data. One type of project involves using the IBEM-IS for data mining purposes only. Data mining is the process of finding correlations or patterns among variables in a large database. The other type of project involves establishing an IBEM-IS study cohort using the subjects who have provided informed consent for recontact, followed by

recruitment among this cohort of patients for collection of additional relevant data (Berry, Jurek et al. 2010).

As of March 1, 2014, there are 24 centers across the United States with IRB approval to enroll patients in IBEM-IS (Appendix A). Over 1,500 subjects have been consented to participate and have their long-term progress documented. There are 43 metabolic conditions being followed (Appendix B) and currently 46 PA patients have been consented to participate in IBEM-IS.

4.0 MATERIALS AND METHODS

The study was designed as a combined analysis of IBEM-IS data and comparison to information obtained though telephone interviews of parents and/or guardians of individuals affected by PA. The research protocol was submitted to the University of Pittsburgh's Institutional Review Board (IRB) and was approved by expedited review procedure authorized under 45 CFR 46.110 (Appendix C). The University of Pittsburgh Medical Center (UPMC) has guidelines to ensure the confidentiality of electronic protected health information when acquired from UPMC for purposes of research under a protocol approved by a nationally accredited IRB. Therefore, to comply with these guidelines, the Center for Assistance in Research using eRecord (CARe) was contacted to request permission to access the Children's Hospital of Pittsburgh's eRecord and approval was obtained.

A proposal for this project was submitted to the IBEM-IS Research Proposal Review Team and was reviewed by a 5-member committee and brought to the entire Collaborative for final approval. Upon approval by IBEMC, access was granted to a list of data elements, which was requested for PA patient data collected from January 1, 2007 to January 1, 2014 (Appendix D).

4.1 RECRUITMENT PROCEDURES

A letter was sent to all IBEM-IS site coordinators outside of our institution, asking them to contact the parents/guardians of IBEM-IS-consented propionic acidemia patients who are 0-18 years of age, and who agreed to be re-contacted (Appendix E). The site coordinators were provided with the patient invitation letter and a template to use as a cover invitation letter to send to their patients (Appendix F and Appendix G). The site coordinators were asked to report to the UMPC IBEMC investigators with the number of participants that met inclusion criteria and the number of participants contacted regarding the study. If this report did not occur within two weeks, the site coordinators were recontacted to obtain this information. This continued every two weeks until a response was obtained. Per the patient invitation letter, if the child's parent/guardian expressed willingness to participate, they were instructed to contact the investigator to proceed with the study.

With approval by the CARe team at UPMC, the study team was able to obtain contact information using the clinical application, Cerner, for patients with propionic acidemia who are 0-18 years of age, already consented to the IBEM-IS protocol, who have agreed to be re-contacted as part of IBEM-IS, and who were seen at Children's Hospital of Pittsburgh of UPMC metabolic clinic. Two subjects were recruited via direct mailing of the invitation letter from the local PI and site coordinator (Appendix H). If the child's parent/guardian did not contact the study team within 2-3 weeks after receiving the invitation letter, then a follow-up call was made to assess his/her willingness regarding study participation.

4.2 INFORMED CONSENT

A script was designed to obtain consent of the child's parent/guardian over the phone. The verbal "Yes" or "No" to participate in the interview from the parent/guardian was to be documented (Appendix I). Review of components of the consent was to occur after contacting the parent/guardian via telephone. An overview of the study was to be presented to the parent by the investigator; including a description of the focus of the study on assessment of services and schooling received by the patient, anticipated questions about perceived unmet needs, and the questionnaire/survey nature of the study. Parents/guardians were to be informed that their participation is completely voluntary and that the decision to enroll or not to enroll would not impact the clinical care of their child at UMPC facilities. There was no compensation for this study. The parent/guardian was to be provided with as much time as needed to make the decision whether or not to participate in the study and the PI or co-investigator would address any questions or concerns they may have. Consent was only to be obtained by a listed investigator on the IRB application.

4.3 TELEPHONE INTERVIEW

Once the child's parent/guardian granted permission to be interviewed, the total duration of participation was expected to be within one telephone call and would last a minimum of 45 minutes. Interviews were to be audio recorded for transcription. Identifying information, such as the subject's name, was to be removed from the transcript. A telephone-conducted interview was preferred over a paper-based or computer survey to allow the respondents to elaborate on their

answers and to enable the interviewer to build rapport and more thoroughly explore responses to service utilization practices and unmet needs.

The investigator was to conduct the telephone interviews within the Medical Genetics office suite located at Children's Hospital of Pittsburgh of UPMC. The interview was to be guided by a list of questions regarding which healthcare services used by PA patients. This includes which healthcare providers they may be seeing, if they or their family members participate in or seek help from various resources and/or support groups, their experience in school and early intervention programs, the size of their family including the number of affected children, and level of education completed for both parents/guardians, in addition to other demographic factors (Appendix J).

4.4 TELEPHONE INTERVIEW DATA

The subjects were to be given a study subject number. Research information was to be stored by subject number in a password-protected file. Only individuals directly involved in the research study were to have access to the protected health information of the patients. Any paper research information (such as telephone interview documents) was to be kept in the locked Medical Genetics office suite, which is accessible only by swiping a badge.

The expectation was to enroll subjects and perform the data analysis in the time frame of one year. The total number of subjects to be enrolled into this research study at this site was estimated to be 25. This number is based on the 46 currently active PA patients that are being longitudinally followed in the IBEM-IS protocol. Based on the time available and the estimated likelihood that patients would participate, the plan was to interview up to 25 parent/guardians of

patients with PA. The data collected from the phone survey was to be compared to the aggregate data from the existing IBEM-IS databases. The results of this study were to be used solely for descriptive analysis. It is acknowledged that the study population is small and thus would not reach statistical power. This is typical of similar studies done with rare disease patient populations.

4.5 IBEM-IS DATA

A list of specific data variables from the DocSite data set was requested by this investigator and submitted to IBEMC for extraction in November 2013. The data elements were examined for content. Upon review of the extracted data, a modified research proposal was submitted to encompass the REDCap data set and the additional data variables contained within it. The REDCap data was extracted in February 2014 and a dataset with the combined data variables from DocSite and REDCap was provided to the investigators at UPMC at the end of February 2014. The data elements from the combined datasets were assessed for quality and content. Descriptive statistics were performed using Microsoft Excel. For certain parts of the analysis, missing and/or unknown data fields were excluded. IBM's SPSS Statistics version 21 software package was used for analysis on the remaining data variables.

5.0 RESULTS

5.1 DESCRIPTION OF THE PA SUBJECT DATA IN IBEM-IS

There are 46 patients with PA enrolled in IBEM-IS. From June 2007 to December 2013 there have been a total of 137 visits entered into the database. Data was collected from IBEM-IS-consented patients at their routine metabolic visits at their participating center. The patients did not necessarily return for clinic visits on an annual or semi-annual basis to have data collected, but rather returned as needed or as prescribed by their health care providers. Over the 6 years, some subjects have been lost to follow-up and new subjects have been enrolled.

5.1.1 Demographical Information

A little over half of the subjects are male (24/46) and just less than half are female (22/46) IBEM-IS. A majority of the patients were 0-5 years of age (30%), with the second most frequent group being 11-15 years of age (24%) at intake. The proportion of subjects in other age groups are as follows: 11% were 6-10 years of age, 11% were 16-20 years of age, 11% were 21-30 years of age, 7% were 31-40 years of age and 4% were 41-50 years of age. One (2%) of the subjects did not have an age reported and/or documented at intake (Table 4, Figure 1).

Table 4. Percent of Subjects in Each Age Group Reported at Intake

Age	Number of Subjects	Percent
0-5 years	14	30%
6-10 years	5	11%
11-15 years	11	24%
16-20 years	5	11%
21-30 years	5	11%
31-40 years	3	7%
41-50 years	2	4%
Unknown	1	2%
	Total: 46	

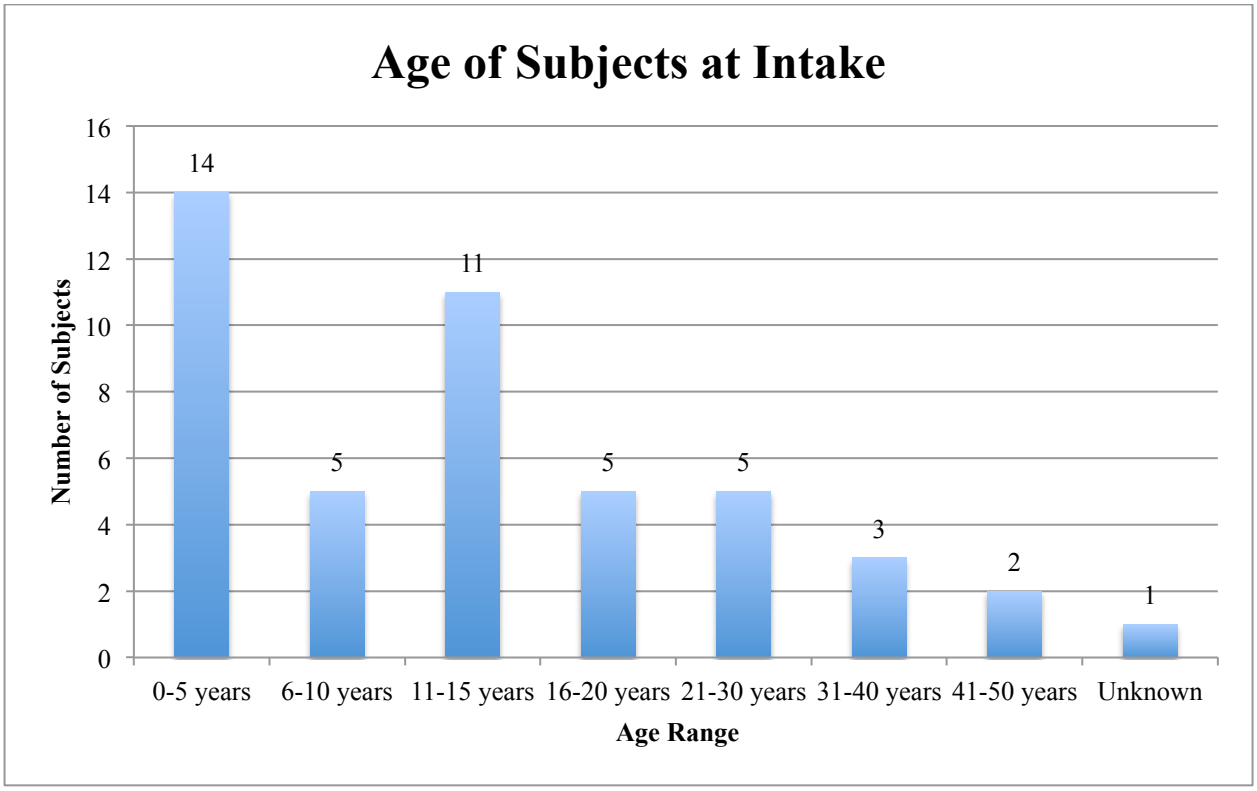

Figure 1. Age of Subjects at Intake

In 2007, three PA patients were enrolled in IBEM-IS. In subsequent years, three PA patients were enrolled in 2008, eight were enrolled in 2009, four were enrolled in 2010, nine were enrolled in 2011, ten were enrolled in 2012, and six were enrolled in 2013. The patients' ages at the time of intake are variable within each year and ranges from 0-50 years of age overall (Figure 2).

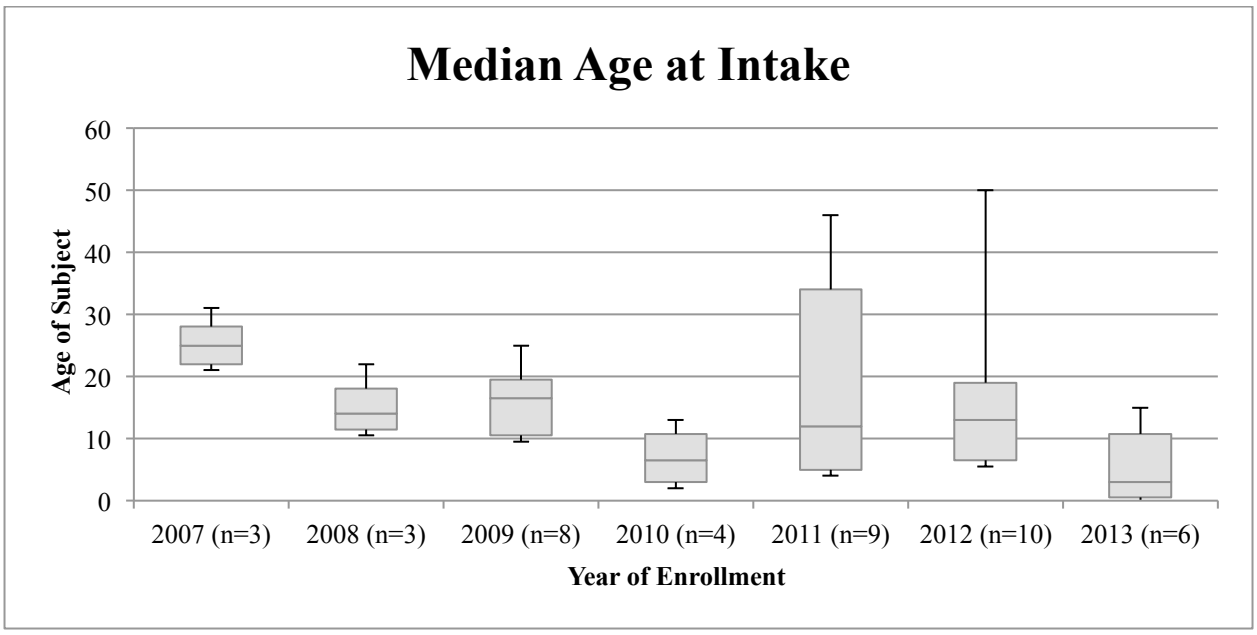

Figure 2. Median Age of Subjects at Intake for Each Year of Enrollment

Seventy-four percent of the subjects were reported to be Caucasian, 9% were not specified, 7% were Hispanic or Latino, 6% were biracial or multiracial, 2% were African American, and 2% were classified as other (Figure 3). Ethnicity of each patient was also recorded and is illustrated in Figure 4. Of note, 5 subjects were reported to belong to the Amish community. Ethnicity was not further specified for 20 Caucasian individuals. Twelve subjects were reported to have a European background, one subject is Arabic, and no Asian PA subjects were enrolled (Figure 4).

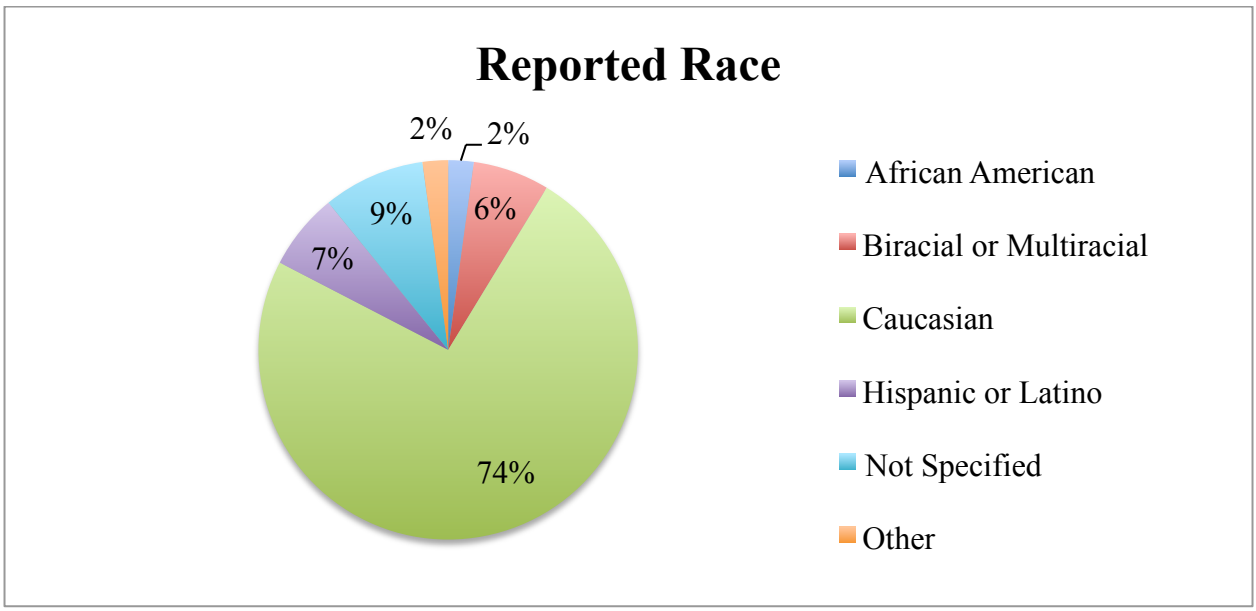

Figure 3. Reported Race

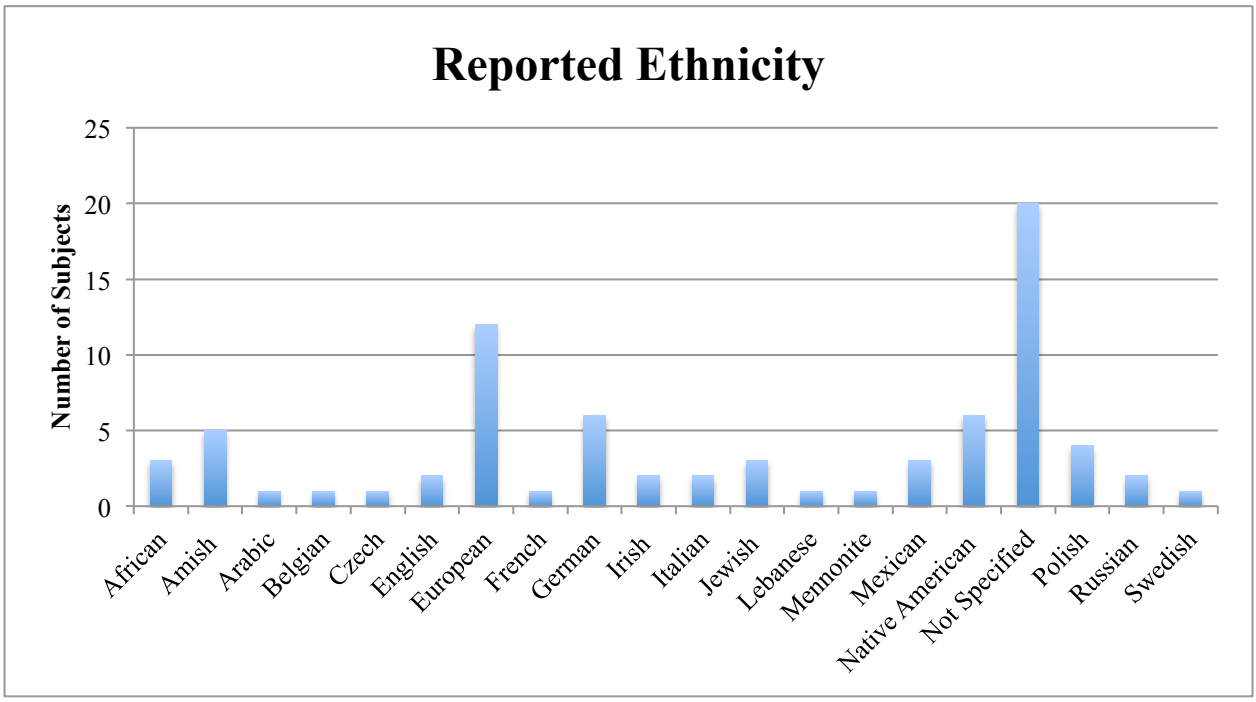

Figure 4. Reported Ethnicity

Almost half (22/46) of the data concerning maternal and paternal education status is missing or unknown in the dataset. For those with education status reported, a majority of the subjects' parents completed some level of a college degree. For 5 subjects, the highest level of

parental education was reported as 1-8 years; all 5 reported to belong to the Amish community (Figure 5).

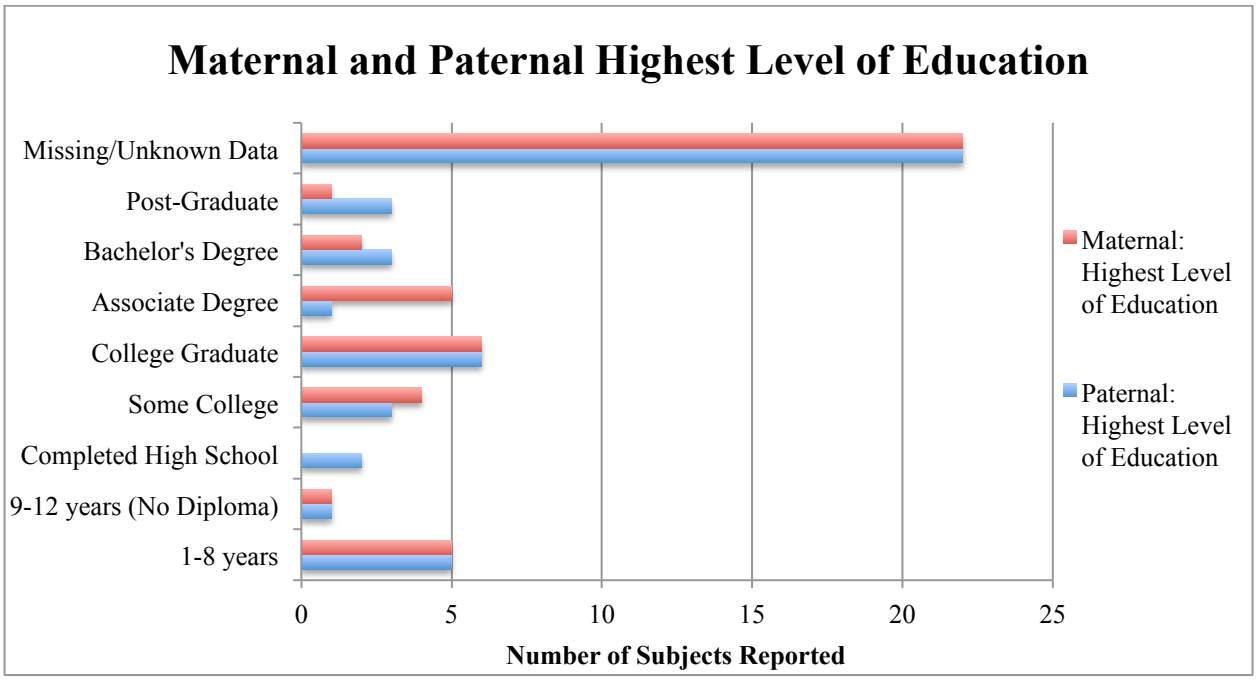

Figure 5. Reported Maternal and Paternal Highest Level of Education

Due to the small sample size, a Fisher's exact test was performed to evaluate the likelihood that the reported parent's level of education and the number of services a child utilizes are independent variables. The null hypothesis was that the variables are independent, while the alternative was that there is a correlation between parental level of education and number of services reported to be utilized (i.e. the higher the level of education the greater number of services reported). This test did not find statistical significance (p-value = 0.321) (Appendix K, Tables 18 and 19).

Fifty-six percent of the data concerning current insurance status is missing and/or unknown. The remaining data is almost equally distributed among commercial/private insurance (19%) and State/Federal insurance (17%). Medicaid used in addition to another type of insurance

was reported in 6% of subjects. One subject reported using the State Children with Special Health Needs (CSHN) Program (Figure 6).

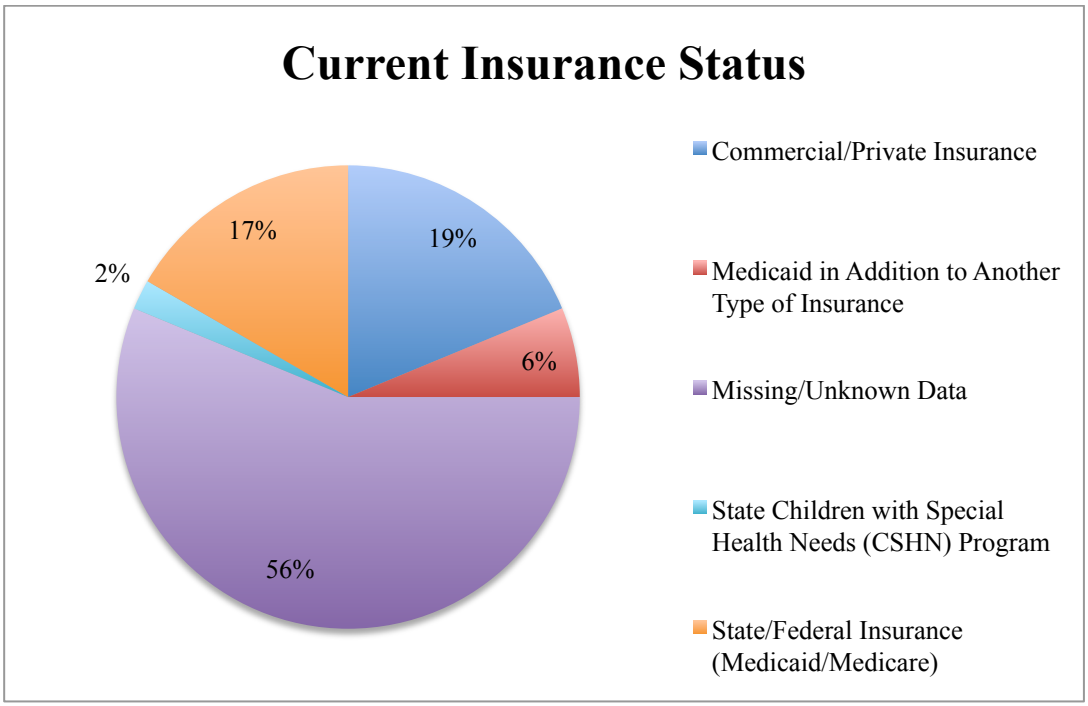

Figure 6. Current Insurance Status

A Fisher's exact test was performed to evaluate the likelihood that the type of insurance and the number of services a child utilizes are independent variables. The null hypothesis was that the variables are independent, while the alternative was that there is a correlation between type of insurance and number of services reported to be utilized (i.e. commercial/private insurance the greater number of services reported). This test did not find statistical significance (p-value = 1.000) (Appendix K, Tables 20 and 21).

5.1.2 Method of Diagnosis in PA Subjects

Forty-one percent of subjects were diagnosed with PA due to their clinical presentation and 35% of subjects were diagnosed by NBS. Other patients were brought to attention because of a sibling

with the same condition (11%) or the subjects had missing and/or unknown data (7%). Three of the subjects were documented in IBEM-IS has having more than one method of initial diagnosis (4% were diagnosed via abnormal NBS and clinical presentation and 2% were diagnosed clinical presentation and sibling of a patient with PA). It is unknown if the methods were concurrent at initial diagnosis. For this reason, those subjects were differentiated and placed in their own category (Table 5).

Table 5. Method of Initial Diagnosis in Subjects

Initial Diagnosis of this IBEM found by:	Number of Subjects	Percent
Abnormal NBS	16	35%
Abnormal NBS and Clinical Presentation	2	4%
Clinical Presentation	19	41%
Clinical Presentation and Sibling of Patient with PA	1	2%
Missing/Unknown Data	3	7%
Sibling of Patient with PA	5	11%
	Total: 46	

The subjects were excluded that had either missing or unknown data for the initial diagnosis and missing data for the number of days from birth to initiation of intervention and/or at time of initial face-to-face metabolic consultation. It was found that in the remaining 33 subjects, the median number of days of age from birth to initiation of intervention for PA diagnosed via abnormal NBS, clinical presentation, and sibling with PA was 9, 180, and 10 respectively. The maximum number of days of age from birth to initiation of intervention for PA diagnosed via abnormal NBS, clinical presentation, and sibling with PA was 2 years 11 months, 4 years 2 months, and 8 years respectively (Table 6).

Table 6. Method of Diagnosis and Median Days of Age from Birth to Initiation of Intervention for PA

Initial Diagnosis of this IBEM Found by:	Number of Subjects	Median Days of Age From Birth to Initiation of Intervention for PA	Minimum	Maximum
Abnormal NBS	10	9	3	1065
Clinical Presentation	18	180	3	1528
Sibling of Patient with PA	5	10	2	2920

The Wilcoxon rank-sum test was used as a nonparametric alternative to the two-sample *t*-test to compare the number of days of age from birth to initiation of intervention for subjects diagnosed via abnormal NBS and subjects diagnosed via clinical presentation. The null hypothesis was that there is no difference in the number of days of age from birth to initiation of intervention for subjects diagnosed via abnormal NBS and subjects diagnosed via clinical presentation. While the subjects diagnosed via clinical presentation ranked higher for the number of days than the subjects diagnosed via abnormal NBS, this test did not find statistical significance (p-value = 0.156) (Appendix L, Tables 22 and 23).

The median number of days of age at time of initial face-to-face metabolic consultation for PA diagnosed via abnormal NBS, clinical presentation, and sibling with PA was 12, 180, and 20 respectively. The maximum number of days at time of initial face-to-face metabolic consultation for PA diagnosed via abnormal NBS, clinical presentation, and sibling with PA was 2 years 11 months, 4 years 3 months, and 8 years 3 months respectively (Table 7).

Table 7. Method of Diagnosis and Median Days at Time of Initial Face-to-Face Metabolic Consultation

Initial Diagnosis of this IBEM Found by:	Number of Subjects	Median Days at Time of Initial Face-to-face Metabolic Consultation	Minimum	Maximum
Abnormal NBS	10	12	4	1065
Clinical Presentation	18	180	3	1552
Sibling of Patient with PA	5	20	1	3030

The Wilcoxon rank-sum test was used to compare the number of days of age at time of initial face-to-face metabolic consultation for subjects diagnosed via abnormal NBS and subjects diagnosed via clinical presentation. The null hypothesis was that there is no difference in the number of days of age at time of initial face-to-face metabolic consultation for subjects diagnosed via abnormal NBS and subjects diagnosed via clinical presentation. While the subjects diagnosed via clinical presentation ranked higher for the number of days than the subjects diagnosed via abnormal NBS, this test did not find statistical significance (p-value = 0.239) (Appendix L, Tables 24 and 25). Similar analyses were not done looking at the group of subjects with a sibling with PA because of small sample size (n=5).

For each of the 33 subjects, the number of the days at time of initial face-to-face metabolic consultation was subtracted from the number of days from birth to initiation of intervention and the absolute value was tallied. This was done to determine whether the two numbers differed for each subject, and to help elicit the meaning of "intervention" in this dataset. For the majority of the subjects (24/33), the day of intervention was the same day as the time of initial face-to-face metabolic consultation. The remaining 9 subjects had differences reported between the two days. Six subjects reported a difference of less than 2 weeks: one subject differed by 1 day, two subjects differed by 7 days, two subjects differed by10 days, and one subject differed by 12 days. Three subjects reported a much greater difference in days between intervention and metabolic consultation: one subject differed by 24 days, one subject differed by 50 days, and one subject differed by 110 days (Figure 7).

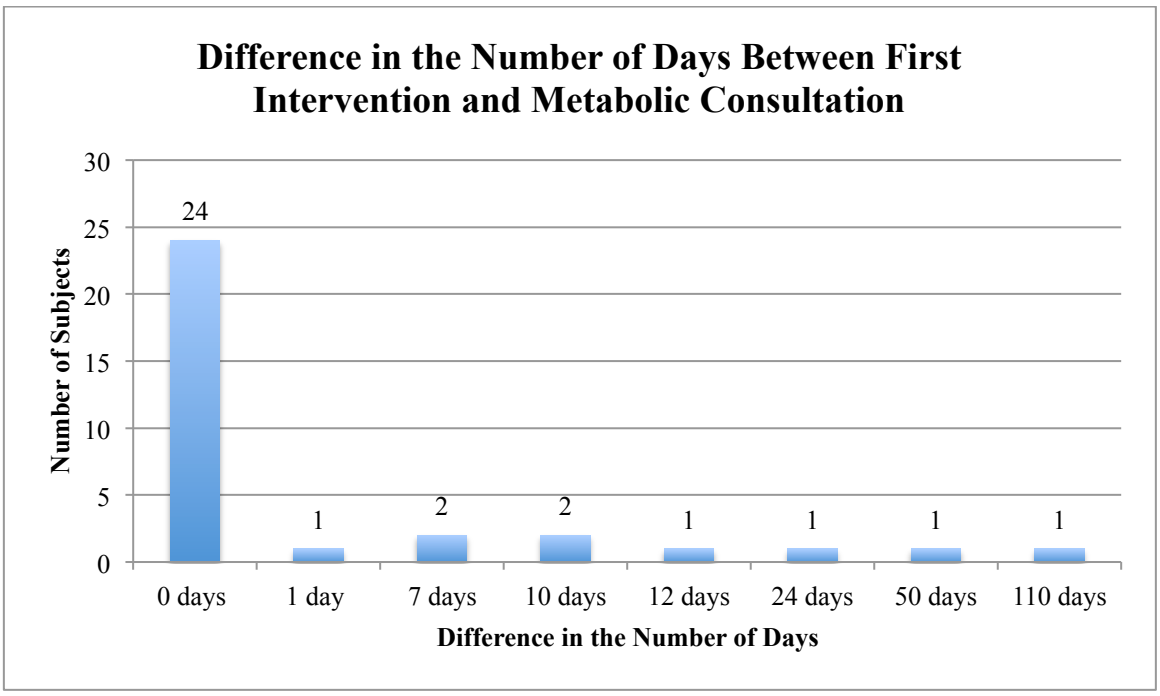

Figure 7. Difference in the Number of Days Between First Intervention and Metabolic Consultation

To assess the timing of intervention in the 33 subjects, the number of the days at time of initial face-to-face metabolic consultation was subtracted from the number of days from birth to initiation of intervention. The majority of subjects (73%) had the initiation of intervention occur on the same day as their first face-to-face metabolic consultation. Negative numbers corresponded with the initiation of intervention for PA occurring "before" the first face-to-face metabolic consultation took place, this was seen in 15% of the subjects. Positive numbers corresponded with the initiation of intervention for PA occurring "after" the first face-to-face metabolic consultation took place, this was seen in 12% of the subjects (Figure 8).

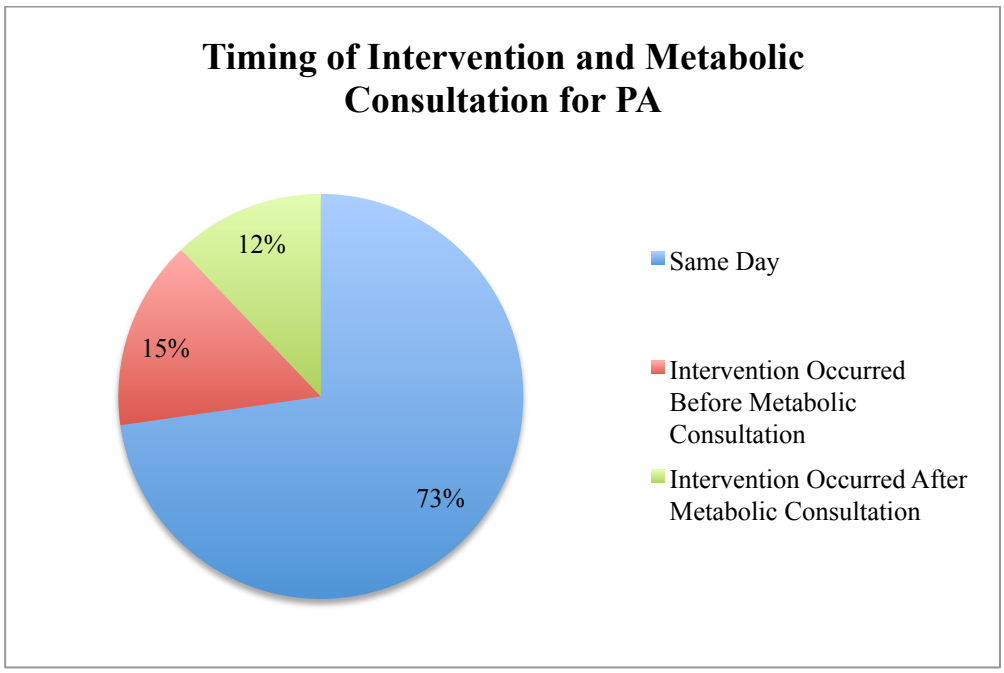

Figure 8. Timing of Intervention and Face-to-face Metabolic Consultation

5.1.3 Mutation Status and Genetic Counseling of PA Subjects

Genotype information was available on 17 subjects. Seventy-one percent (12/17) of the subjects had mutations in the *PCCB* gene. Of the 10 possible alleles for *PCCA,* 3 could not be identified, and 4 had not been previously described in the literature. Of the 24 possible alleles *PCCB*, 7 had not been previously described in the literature. Complete genotype information was available for 6 subjects (one subject with *PCCA* gene mutations and 5 subjects with *PCCB* gene mutations). A majority (4/6) of these subjects were compound heterozygotes. Two subjects (subjects 25 and 28) were homozygous for the missense N536D allele in the *PCCB* gene that has been identified in the Amish community in Lancaster, PA and associated with a milder phenotype. These subjects also reported being Amish as their ethnicity. One subject (subject 13) was a compound heterozygote for a nonsense and a missense mutation in the *PCCB* gene, one subject (subject 16)

was a compound heterozygote for an exon skipping and a frameshift mutation in the *PCCA* gene, and two subjects (subjects 14 and 29) were compound heterozygotes for different missense and frameshift mutations in the *PCCB* gene. Reported mutations were found in the current published literature, The Human Gene Mutation Database (HGMD), as well as database for *PCCA* and *PCCB* gene mutations, maintained by Jan Kraus, PhD at http://cbs.lf1.cuni.cz/pcc/pccmain.htm (last accessed on March 25, 2014) (Table 8 and Table 9).

Table 8. Subjects Documented with Mutations in the *PCCA* gene

Patient ID	Allele 1	Allele 1: Mutation Description	Allele 2	Allele 2: Mutation Description
10	c.1023dupT	---	Deletion exon 3-4	Large genomic deletion[a] (Desviat, Sanchez-Alcudia et al. 2009)
16	c.782A>G p.E261G	Exon 17 skipping[b] (Desviat, Clavero et al. 2006)	c.923dupT p. L308fs	Insertion/deletion resulting in a frameshift mutation and stop codon (Desviat, Pérez et al. 2004)
19	c.1591T>C	---	Not Identified	---
20	c.1591T>C	---	Not Identified	---
40	c.231+47_50delTATT variant of unknown significance in intron 3	---	Not Identified	---

[a]Expression analysis in a eukaryotic system was performed for the deletion involving exons 3–4 involving 39 amino acids. The results demonstrated a total absence of residual activity of the protein, confirming its pathogenicity (Desviat, Sanchez-Alcudia et al. 2009).

[b]Novel mutation reported in the literature

---Mutation has not been previously reported in the literature

Table 9. Subjects Documented with Mutations in the *PCCB* gene

Patient ID	Allele 1	Allele 1: Mutation Description	Allele 2	Allele 2: Mutation Description
11	c.483C>T	---	c.683C>T p.P228L	Missense mutation (Desviat, Pérez et al. 2004)
13	c.331C>T p.R111X	Nonsense mutation (Sanchez-Alcudia, Perez et al. 2012)	c.1606A>G p. N536D	Missense mutation[a] (Desviat, Pérez et al. 2004)
14	c.1218del14ins12 p.E407fs	Insertion/deletion resulting in a frameshift mutation and stop codon[b] (Desviat, Pérez et al. 2004)	c.683C>T p.P228L	Missense mutation (Desviat, Pérez et al. 2004)
25	c.1606A>G p. N536D	Missense mutation[a] (Desviat, Pérez et al. 2004)	c.1606A>G p. N536D	Missense mutation[a] (Desviat, Pérez et al. 2004)
28	c.1606A>G p. N536D	Missense mutation[a] (Desviat, Pérez et al. 2004)	c.1606A>G p. N536D	Missense mutation[a] (Desviat, Pérez et al. 2004)
29	c.335G>A p. G112D	Missense mutation[c] (Desviat, Pérez et al. 2004)	c. 1204delG p. A402fs	Insertion/deletion resulting in a frameshift mutation and stop codon (Desviat, Pérez et al. 2004)
31	c.683C>T p.P228L	Missense mutation (Desviat, Pérez et al. 2004)	IVS13+1G>C	---
32	c.683C>T p.P228L	Missense mutation (Desviat, Pérez et al. 2004)	IVS13+1G>C	---
35	c.398T>C	---	c.415C>T p.Q139X	Nonsense mutation http://cbs.lf1.cuni.cz/pcc/pccmain.htm
36	c.386-387delTTinsAAC	---	c.1218del14ins12 p.E407fs	Insertion/deletion resulting in a frameshift mutation and stop codon [b] (Desviat, Pérez et al. 2004)
41	Deletion of GGGCATCATCCGGC at bases c.1218_1231	---	c.1495C>T p.R499X	Nonsense mutation (Desviat, Pérez et al. 2004)
44	c.1398+2delT	---	c.1606A>G p. N536D	Missense mutation[a] (Desviat, Pérez et al. 2004)

[a] Associated with a less severe form of PA. Seen in some Amish communities, identified in Lancaster, PA (Desviat, Pérez et al. 2004, Strauss and Puffenberger 2009)

[b] Most frequent mutant allele reported in individuals of northern European origin (Tahara, Kraus et al. 1993, Perez-Cerda, Merinero et al. 2000).

[c] Affects heterododecamer formation and is associated with undetectable PCC enzyme activity and the severe phenotype (Muro, Pérez et al. 2001)

---Mutation has not been previously reported in the literature

The clinical information documented in IBEM-IS for the 6 subjects with complete genotype information is listed in Table 10. Out of all the variables requested, the only variables with documented information was age, ethnicity, number of hospitalizations prior to intake in IBEM-IS, number of days of age from birth to initiation of intervention for PA, the method of initial diagnosis by which the IBEM was found, and echocardiogram results obtained prior to intake in IBEM-IS. None of the subjects reported any other health care services received currently or community resources currently received. Only one subject, Subject 13, reported a list of providers seen at the metabolic visit, which included a physician, dietician, genetic counselor, and nurse. None of the subjects reported having any birth defects or comorbidities, with the exception being Subject 28, who was reported to have asthma.

Table 10. Subjects with Complete Genotype and the Clinical Information Documented in IBEM-IS

Patient ID	Gene	Allele	Classification Based on Literature	Age at Intake (years)	Ethnicity	Number of Hospitalizations Prior to Intake in IBEM-IS	Days of Age:		Initial Diagnosis of this IBEM Found by:	Echocardiogram Results Obtained Prior to Intake in IBEM-IS
							Birth to Initiation of Intervention	Initial Face to Face Metabolic Consultation		
13	*PCCB*	p.R111X p.N536D	Severe Mild	3	European	0	Unknown	Unknown	Abnormal NBS	N/A
14	*PCCB*	p.P228L p.E407fs	Mild Severe	36	Not Specified	5	1528	1552	Clinical Presentation	Normal
16	*PCCA*	p.E261G p.L308fs	Novel Severe	2	Not Specified	2	Unknown	134	Abnormal NBS	Normal
25	*PCCB*	p.N536D p.N536D	Mild Mild	12	Amish	Unknown	150	150	Abnormal NBS	N/A
28	*PCCB*	p.N536D p.N536D	Mild Mild	18	Amish	0	27	20	Sibling of Patient with PA	Normal
29	*PCCB*	p.G112D p.A402fs	Severe Severe	15	European	10	3	3	Clinical Presentation	Normal

Ninety-six percent (44/46) of the PA subjects in IBEM-IS reported having genetic counseling for their condition. For one subject, the data for this variable was missing and/or unknown and one subject reported that genetic counseling was not provided (Figure 9).

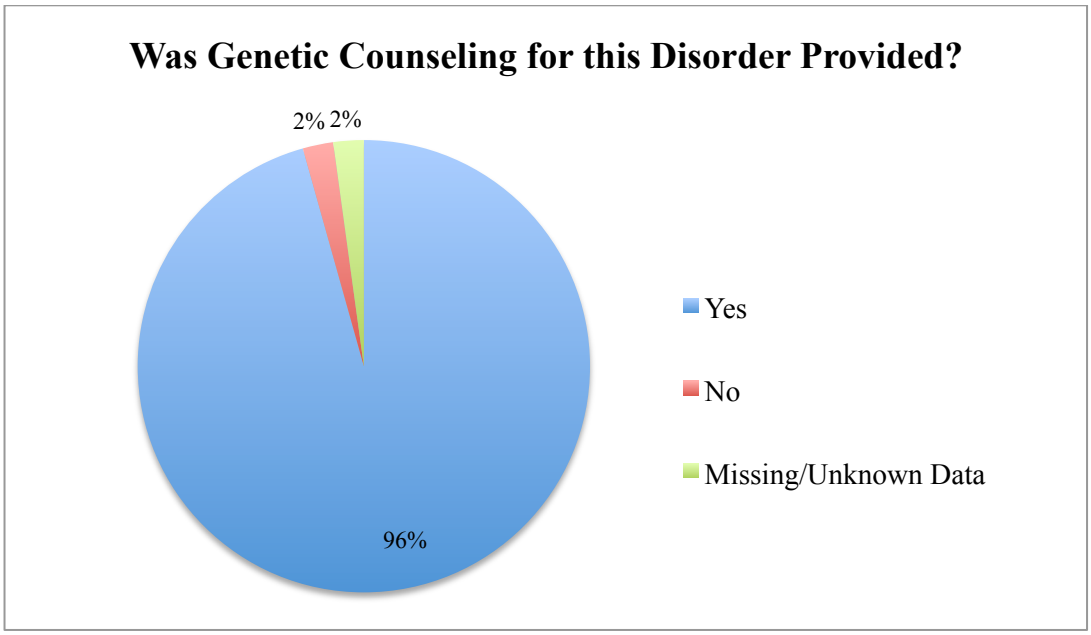

Figure 9. Was Genetic Counseling for this Disorder Provided?

5.1.4 Analysis of Service Utilization Data

Intake/consent visits for each subject were isolated and assessed for service utilization data content. The data variables of interest are the reported "other health care services received currently", the "community resources received currently", and "providers seen at this metabolic visit". This set of data (Table 11) incorporates the 28 subjects with only one visit documented in IBEM-IS and the first visit of the 18 subjects with multiple (2 or more) visits documented in the database. A majority (67-76%) of the desired data was missing or unknown.

Table 11. Content of the IBEM-IS Data at Intake

	Number of Subjects with Reported/Documented Data	Percent	Number of Subjects with Missing/Unknown Data	Percent
Other Heath Services Received Currently	14	30%	32	70%
Community Resources Received Currently	11	24%	35	76%
Providers Seen at this Metabolic Visit	15	33%	31	67%

There are 18 subjects with more than one visit documented in IBEM-IS. These subjects and subsequent visits were isolated to assess for service utilization data content. The data variables of interest are the reported "other health care services received currently", the "community resources received currently", and "providers seen at this metabolic visit". This set of data (Table 12) incorporates 18 subjects with a total of 106 follow-up visits documented in the database. The number of visits entered in IBEM-IS for each subject ranges from 2 to 18. A majority (83-89%) of the health services and provider data was reported and/or documented. Approximately half (46%) of the community resources data was missing or unknown.

Table 12. Content of the IBEM-IS Data for "Follow-up" Subjects

	Number of Visits with Reported/Documented Data	Percent	Number of Visits with Missing/Unknown Data	Percent
Other Heath Services Received Currently	88	83%	18	17%
Community Resources Received Currently	57	54%	49	46%
Providers Seen at this Metabolic Visit	94	89%	12	11%

All of the visits with missing or unknown data for the follow-up subjects were excluded and the only remaining variables with reported and/or completed data were analyzed. The percent of each heath service was calculated by dividing the number of times the particular health service was reported by the total number of health service reports. The health services that

were reported the most were cardiology (21%), occupational therapy (13%), physical therapy (12%), neurology (11%), speech-language therapy (8%), and ophthalmology (8%). All of the other health services were reported with a rate equal to or less than 5% (Table 13).

Table 13. Health Services Reported by PA Subjects in IBEM-IS

Other Health Services Received Currently	Number of Times Reported	Percent
Audiology	3	1%
Behavioral/Developmental Pediatrics	1	0.36%
Cardiology	59	21%
Dentistry	7	3%
Dietitian	11	4%
Feeding Therapy	1	0.36%
Gastroenterology	5	2%
Hematology	2	1%
Home Health Care	1	0.36%
Nephrology	3	1%
Neurology	30	11%
None	14	5%
Occupational Therapy	37	13%
Ophthalmology	21	8%
Orthopedics	7	3%
Other	4	1%
Physical Therapy	34	12%
Preschool	2	1%
Primary Care Provider	4	1%
Pulmonology	6	2%
Speech-Language Therapy	22	8%
Surgery	1	0.36%
Urology	1	0.36%
	Total: 276	

The percent of each community resource was calculated by dividing the number of times the particular community resource was reported by the total number of community resource reports. For community resources received currently, “none” (41%) and “other” (8%) were reported a majority of the time. The community resources that were reported the most were preschool (11%), social services – developmental disability (11%), social services – medical

(8%), and daycare (7%). All of the other community resources were reported with a percent equal to or less than 5% (Table 14).

Table 14. Community Resources Reported by PA Subjects in IBEM-IS

Community Resources Received Currently	Number of Times Reported	Percent
Daycare	5	7%
Family Support Group Related to this IBEM	2	3%
Family Support - Other	2	3%
Head Start	2	3%
None	31	41%
Nutritional Services (WIC/MAC)	4	5%
Other	6	8%
Preschool	8	11%
Social Services - County	2	3%
Social Services - Developmental Disability	8	11%
Social Services - Medical	6	8%
	Total: 76	

The percent utilization for each provider was calculated by dividing the number of times the particular provider was reported by the total number of provider reports. All 18 subjects reported seeing a physician and dietitian. The amount of times these providers were reported is comparable between the two (37% for dietitian and 36% for physician). The other providers were reported to be utilized less in comparison with a rate equal to or less than 7% (Table 15).

Table 15. Providers Reported by PA Subjects in IBEM-IS

Providers Seen at this Visit	Number of Times Reported	Percent
Dietitian	89	37%
Genetic Counselor	17	7%
Nurse	13	5%
Nurse Practitioner	12	5%
Physician	87	36%
Social Worker	10	4%
	Total: 228	

Variability has been observed in the number of times subjects reported utilizing a particular service, resource, and/or provider. Across the different services that were reported, some subjects reported utilizing the service once and other subjects reported using the same service multiple times. The tables in Appendix M-O reflect this observation in the follow-up visit data. For instance, 12 subjects reported receiving services from cardiology and cardiology has been reported 59 times. However, each subject did not utilize this services equally: 4 subjects reported twice, 2 subjects reported three times, 1 subject reported four times, 2 subjects reported five times, 1 subject reported nine times, 1 subject reported ten times, and 1 subject reported 12 times.

To assess the number of times services were reported per year, as some subjects were in IBEM-IS for longer periods of time than others and may report using more services, the data was reorganized. From the original 46 subjects and 137 visits that were entered in IBEM-IS, all of the visits with missing and/or unknown data were eliminated. This resulted in a sample size of 23 subjects with a total of 93 visits. The six services that were observed to be reported the most were cardiology, occupational therapy, physical therapy, neurology, speech-language therapy, ophthalmology, and none. There was no health service utilization data for years 2007 and 2008. In years 2009-2013, the most services were reported in 2012 and the least services were reported in 2009. The sample size for each year was determined by the total number of subjects participating in IBEM-IS that year and does not reflect the number of newly enrolled subjects per year (Figure 10).

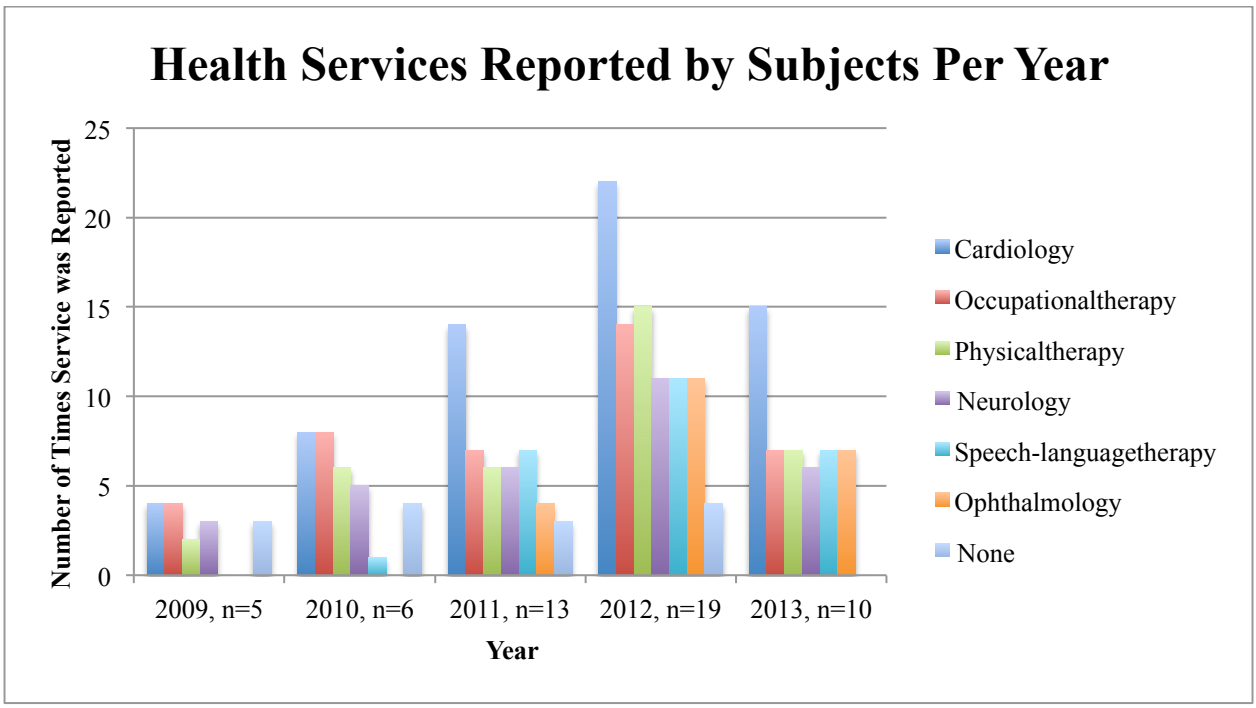

Figure 10. Health Services Reported by Subjects Per Year

The number of subjects enrolled each year was determined. The total number of subjects is considered to be 44 because 2 subjects did not have an intake visit included in the data provided to the investigator. The number of newly enrolled subjects was then compared to the total number of visits that were entered into IBEM-IS to infer what proportion of the data is attributed to newly enrolled subjects, as they have less time to be followed in IBEM-IS and therefore may have fewer opportunities to report using services (Table 16).

Table 16. Proportion of New Subject Data Entered Per Year

	2007	2008	2009	2010	2011	2012	2013	Total
Number of Newly Enrolled Subjects	3	3	8	5	9	10	6	44
Total Number of Visits Entered	3	3	17	18	26	43	27	137
Proportion of New Subject Data Entered	1.00	1.00	0.47	0.28	0.35	0.23	0.22	0.32

5.2 IDENTIFICATION OF CURRENT SERVICE UTILIZATION

Current service utilization in PA patients was not identified because of the inability to recruit subjects to interview for the study. At the investigator's site, the Children's Hospital of Pittsburgh of UMPC, two out of the total three PA patients being followed at the institution fit the inclusion criteria for the study. The parents of both patients were sent invitation letters in the mail directly from the investigator. Two weeks after the letters were mailed, the patients were called via telephone to inquire about participation. After leaving voicemail messages, contact was made with the parents of both patients and reasons for lack of response were communicated to this investigator. One patient's family had recently moved and had not received the letter as of the time of the call and the mother asked to call the investigator when she had more time. The other patient's father was out of town for the previous two weeks and did not know about the study. The family then moved to a different state and follow-up was lost. In the end, both parents did not return the investigators call to participate in the telephone interview.

To recruit PA subjects from the other 26 sites participating in IBEM-IS, an e-mail was sent to each of the co-investigators and research coordinators using a contact list that was provided by IBEMC. The e-mail contained an introduction to the study, an explanation of IBEMC's approval, an attached letter explaining the coordinators' role in the study, an attached letter of invitation for the parents/guardians of patients that meet inclusion criteria, a request to send the invitation letter in a timely manner, an attached cover letter for their convenience, and a request to reply back with the number of patients to whom the invitation letter was sent. The responses from each site (de-identified) are listed in Table 17.

After the first e-mail, only 4 out of the 26 sites responded (15%). One site only had one patient they were following and the letter was sent in the mail. Another site reported that all of

their PA patients were over 18 years of age. Two sites stated that they were no longer participating in IBEMC.

A second e-mail was sent to the 22 sites that did not respond, two weeks after the initial correspondence, as a reminder. Seven more sites responded (42%): one site reported that they were not following any PA patients in their clinic, two sites distributed the letter to one patient, and one site distributed the letter to 4 patients. The three other sites stated that their IRB required them to write a study addendum in order to send the invitation letter to their patients and that they would report back pending their submission. Only one of these sites responded, indicated that their IRB did not require an addendum, and reported that the invitation letter was sent to 4 PA patients.

A third point of contact (telephone and/or e-mail) was made, two weeks after the second e-mail, by the co-investigator of this study who is also the IBEM-IS site coordinator at the Children's Hospital of Pittsburgh of UPMC. This form of contact was made in attempt to facilitate a higher response rate from other IBEM-IS site coordinators. Out of the 9 sites that were contacted, 6 did not respond, one sent out 2 invitation letters, one reported that they were no longer participating in IBEMC, and one site had 1 PA patient that had not been consented to IBEM-IS.

After three separate attempts were made to contact the 26 sites, only 14 (54%) sites responded. Out of the 14 responding sites, only 6 (43%) sites had available PA patients to recruit. A total of 13 invitation letters were sent to eligible PA patients. At the closure of the enrollment period on December 31, 2013 none of the invited PA patients called to express interest in participation.

Table 17. Site Coordinator Response Log

Site	First E-mail	Second E-mail	Third Contact	Date Responded	Response	Number of Patients
A	10/15/13	----------	----------	10/15/13	Not currently participating	0
B	10/15/13	10/29/13	----------	10/30/13	No PA Patients	0
C	10/15/13	10/29/13	11/13/13	----------	----------	----------
D	10/15/13	10/29/13	11/13/13	----------	----------	----------
E	10/15/13	10/29/13	11/13/13	----------	----------	----------
F	10/15/13	10/29/13	----------	----------	----------	----------
G	10/15/13	10/29/13	----------	----------	----------	----------
H	10/15/13	10/29/13	----------	10/29/13	IRB addendum approved	4
I	10/15/13	10/29/13	----------	10/31/13	Invitation letters sent	4
J	10/15/13	----------	----------	10/15/13	All PA patients over 18	0
K	10/15/13	----------	----------	10/15/13	Invitation letters sent	1
L	10/15/13	10/29/13	----------	----------	----------	----------
M	10/15/13	10/29/13	11/13/13	----------	----------	----------
N	10/15/13	10/29/13	----------	----------	----------	----------
O	10/15/13	10/29/13	----------	----------	----------	----------
P	10/15/13	10/29/13	----------	----------	----------	----------
Q	10/15/13	10/29/13	----------	10/29/13	Pending IRB addendum	----------
R	10/15/13	10/29/13	11/13/13	11/15/13	Not currently participating	0
S	10/15/13	10/29/13	----------	10/29/13	Pending IRB addendum	----------
T	10/15/13	10/29/13	----------	11/1/13	Invitation letters sent	1
U	10/15/13	----------	----------	10/15/13	No longer participating	0
V	10/15/13	10/29/13	11/13/13	----------	----------	----------
W	10/15/13	10/29/13	----------	11/6/13	Invitation letters sent	1
X	10/15/13	10/29/13	11/13/13	----------	----------	----------
Y	10/15/13	10/29/13	11/13/13	11/13/13	Invitation letters sent	2
Z	10/15/13	10/29/13	11/13/13	12/2/13	1 PA patient, not consented	0

5.3 COMPARISON OF SERVICE UTILIZATION DATA

With the inability to recruit participants for the study, the interview of parent/guardians of PA patients was not done. Without the interview of parents and/or guardians, current service

utilization information in PA patients was not assessed. Therefore the parent-stated service utilization data could not be compared to the documented aggregate data in IBEM-IS.

6.0 DISCUSSION

6.1 DESCRIPTION OF THE PA SUBJECT DATA IN IBEM-IS

6.1.1 Demographical Information

Approximately half of the subjects entered in IBEM-IS were male and half were female. The equal distribution of affected males and females is characteristic of the autosomal recessive inheritance seen in this condition.

The majority of subjects fell within 0-20 years of age and a handful of subjects were 21 years of age and older, with the oldest subject being 50 years of age. The preponderance of subjects 0-20 years of age in IBEM-IS may be due to the focus on the pediatric population among the participating metabolic centers that are primarily located within pediatric hospitals. However, the progress made in the treatment of PA has improved life expectancy and therefore more patients are surviving later in life (Dionisi-Vici, Deodato et al. 2006). This may explain the upper age range of PA subjects in IBEM-IS.

PA is a pan-ethnic condition, however it appears to be more common in several populations worldwide (Desviat, Pérez et al. 2004). The populations with higher incidences of PA are the Inuit population of Greenland, some Amish communities, Saudi Arabians, and the Japanese. Very few PA subjects entered in IBEM-IS originated from one of these populations.

Five subjects were reported to belong to the Amish community, one subject reported to be Arabic, and no Japanese or Greenland Inuit subjects were enrolled. This occurrence may be attributable to the area of the Untied States from which subjects were recruited and enrolled. The 14 states participating in IBEM-IS are as follows: Illinois, Indiana, Kentucky, Michigan, Minnesota, Missouri, Nebraska, New Jersey, New York, Ohio, Oklahoma, Pennsylvania, South Dakota, and Wisconsin. The participating centers are located in a combination of select states from the Region 4 Genetics Collaborative, the Heartland Genetics and Newborn Screening Collaborative, and New York-Mid-Atlantic Consortium (NYMAC) for Genetic and Newborn Screening services. It is likely that the population of these states from the Midwest and Mid- and South-Atlantic do not have significant enough numbers of individuals of Greenlandic, Saudi Arabian, and Japanese ancestry to allow for occurrence of this rare disorder in these groups.

Over half of the data regarding parental highest level of education and current insurance status was missing and/or unknown in IBEM-IS, therefore the interpretation of this data is limited. A Fisher's exact test did not determine a correlation between level of parental education or type of insurance and the number of health services reported to be utilized and had a lack of statistical significance.

6.1.2 Method of Diagnosis in PA Subjects

Initial diagnoses of PA can be made by an abnormal NBS, clinical presentation, and/or having a sibling with PA. The number of days of age from birth to initiation of intervention for PA and days at time of initial face-to-face metabolic consultation were documented for each subject. The median number of days to initiation of intervention and days at time of consultation diagnosed via abnormal NBS, clinical presentation, and sibling with PA varied. While the subjects

diagnosed via clinical presentation appeared to have a higher number of days than the subjects diagnosed via abnormal NBS, the Wilcoxon rank-sum test did not find statistical significance and therefore a precise interpretation regarding differences between these two groups cannot be made.

The median number of days until intervention and at first consultation was similar in patients diagnosed via abnormal NBS and those having a sibling with PA. This may be the case for both of these groups of patients because the subjects were brought to medical attention earlier in life because of the test results and/or family history and were not yet symptomatic. The median number of days until intervention and at first consultation was largest for the patients diagnosed by clinical presentation, which reflects the known variability of this condition (Grunert, Mullerleile et al. 2012).

The minimum number of days from birth to initiation of intervention and at first consultation for PA is similar among each method of diagnosis: 1-4 days of life. This is representative of the prompt identification and diagnosis of PA with newborns that are symptomatic within hours or days after birth and those subjects who were brought to attention earlier in life due to abnormal newborn screening results and/or family history.

The maximum number of days of age from birth to initiation of intervention and at first consultation for PA was varied for each group. The maximum number of days was the largest for a patient with a sibling with PA. This subject may be an outlier for this group and it is possible that he or she was only brought to attention because of the family history and still was not symptomatic at 8 years of age. Asymptomatic PA, as observed by Wolf et al. (1979) in a 13-year-old girl, seems to be rare (Wolf, Paulsen et al. 1979, Grunert, Mullerleile et al. 2012). In this case, the subject may have been identified through diagnosis of a symptomatic younger sibling.

Additionally, a subject from the abnormal NBS group was first brought to attention at 3 years of age. This is the only subject that was greater than 45 days of age in this group and it is unclear as to the reason for late intervention other than a late onset of symptoms. It may reflect differences in how the various centers define "intervention," as well.

Due to the possibility that the definition of "intervention" may be different for the various healthcare professionals entering data in IBEM-IS, the differences in the number of days from birth to initiation of intervention and the number of the days at time of initial face-to-face metabolic consultation was determined. To some healthcare professionals, "intervention" may mean the time in which the patient first sees a metabolic physician due to the identification of PA. To others, "intervention" may mean the time in which the patient receives medical treatment, either for screening/prevention of PA symptoms or for clinical symptoms associated with PA. In the majority of the cases, the day of intervention was the same day as the time of initial face-to-face metabolic consultation. This suggests that initiation of intervention and first metabolic consultation are regarded as the same. In the remaining cases that had differences reported between the two days, some of them only differed by 1-2 weeks. This observation may be attributed to scheduling matters. A possible scenario may be a child presented to the emergency room due to metabolic decompensation and received treatment, and then he or she was scheduled for an outpatient metabolic visit 1-2 weeks later. In a few cases, the number of days differed considerably. One subject differed by 50 days and one subject differed by 110 days. The interpretations for these finings are challenging, however the difference in the number of days may reflect less severe symptomology not requiring immediate treatment or consultation, insurance coverage issues and or other obstacles experienced in obtaining follow-up care. Additionally, for cases that had differences reported between the two days, there did not seem to

be consistency in the timing of interventions in regard to initial metabolic consultation. Nonetheless, the clinical heterogeneity and variable age of onset described in the literature for PA patients are evident in range of days until intervention and at first metabolic consultation observed in each group (Grunert, Mullerleile et al. 2012, Grünert, Müllerleile et al. 2013).

6.1.3 Mutation Status PA Subjects

Limited information regarding genotype information was available for the PA subjects in IBEM-IS. Only 37% (17/46) of subjects had their mutation status reported. Of the 34 possible alleles in these 17 patients, 3 could not be identified and 11 had not been previously described in the literature. Seventy-one percent of the subjects had mutations in the *PCCB* gene. This is consistent with what is reported in the literature for PA patients; *PCCB* mutations are found more frequently than *PCCA* mutations (Pena, Franks et al. 2012). Complete genotype information was only available for 13% (6/46) subjects, thus making genotype/phenotype correlations challenging.

The subjects' mutation status was entered in IBEM-IS without standardized and uniform use of nomenclature. Some subjects' mutations were documented on a DNA level and others were documented on a protein level. In some cases, the specific mutation was transcribed from the laboratory report in a descriptive format and did not use standard mutation nomenclature. Additionally, the gene in which the mutation was found was not clarified for all of the subjects. A number of inferences had to be made for the gene. Either the mutation was reported previously in the literature in the *PCCA* or the *PCCB* gene, or the subject already had one mutation identified in one allele of the *PCCA* or *PCCB* gene. Given all of these areas of potential error, the

classification and interpretation of the *PCCA* and *PCCB* mutations for each subject was challenging.

Compound heterozygosity was observed in the majority of the 17 subjects with reported mutations (15/17), regardless of whether both mutations had been previously described in the literature. Therefore, the determination of phenotypic severity in these subjects is challenging because of the different genotype-phenotype correlations associated with each mutation type. In general, mutations characterized as null alleles, which comprise mutations predicted by the nature of the DNA change (nonsense mutations, out-of-frame deletions and insertions, splicing mutations resulting in frameshifts) and those determined experimentally (mutations without detectable enzyme activity), are associated with more severe forms of PA. Missense mutations retaining partial activity are associated with milder forms of PA (Desviat, Pérez et al. 2004). The PCC enzymatic activity resulting from different mutations is often the most useful indicator to establish severity and prognosis of the condition (Pena, Franks et al. 2012), however only one PA subject in IBEM-IS had his/her enzyme level recorded, thus enzyme activity cannot be utilized in this study for correlative purposes. In future studies, the Polymorphism Phenotyping v2 (Poly-Phen2) tool may be used to predict the possible impact of an amino acid substitution on the structure and function of *PCCA* and *PCCB* genes.

Among the subjects who had complete genotype information, Subject 29 had the *PCCB* missense mutation, G112D. This particular mutation is reported in the literature to affect heterododecamer formation and is associated with undetectable PCC enzyme activity and the severe phenotype (Muro, Pérez et al. 2001). This subject had a frameshift mutation (A402fs) on the second allele (Desviat, Pérez et al. 2004). By having compound heterozygosity (G112D/A402fs) for two “severe” or null alleles, this genotype would be associated with a more

severe phenotype. Subject 29 was diagnosed via clinical presentation and was 3 days old at intervention and first face-to-face contact with a metabolic physician. This subject was enrolled in IBEM-IS at 15 years of age and reported 10 hospitalizations and a normal echocardiogram prior to intake; other clinical information is missing and/or unknown. While the subject had a higher number of hospitalizations prior to intake, they occurred over 15 year time period; without knowing more about these episodes, it is difficult to determine whether they correlate with clinical severity.

Subject 13 is a compound heterozygote for nonsense and missense (R111X/N536D) mutations in the *PCCB* gene and Subject 14 is a compound heterozygote for missense and frameshift (P228L/E407fs) mutations in the *PCCB* gene. These subjects would be considered functionally hemizygotes, as the "severe" or null allele is combined with a "mild" or missense allele (Perez-Cerda, Merinero et al. 2000). Subject 13 was diagnosed via abnormal NBS and did not have any hospitalizations prior to intake in IBEM-IS at 3 years of age; other clinical information is missing and/or unknown. Therefore genotype/phenotype correlations cannot be made in this subject. Subject 14 was diagnosed via clinical presentation and was 4 years and 2 months old at intervention and 4 years and 3 months old at first face-to-face contact with a metabolic physician. This subject was enrolled in IBEM-IS at 36 years of age and reported 5 hospitalizations and a normal echocardiogram prior to intake; other clinical information is missing and/or unknown. Given the limited number of hospitalizations at age 36, it may be possible that this subject has less severe symptomology, but supporting data are limited.

Subject 16 is a compound heterozygote for both exon skipping and frameshift (E261G/L308fs) mutations in the *PCCA* gene. The E261G allele is a novel mutation reported in the literature and a specific genotype-phenotype correlation has not been made (Desviat, Clavero

et al. 2006). Subject 16 was diagnosed via abnormal NBS, was 34 days of age at first face-to-face contact with a metabolic physician and had 2 hospitalizations and a normal echocardiogram prior to intake in IBEM-IS at 2 years of age; other clinical information is missing and/or unknown. Precise genotype/phenotype correlations cannot be made in this subject at this time.

Only two subjects have homozygous mutations in the *PCCB* gene. Subject 25 and Subject 28 were reported to have 2 copies of the missense, N536D, allele that has been identified in the Amish community in Lancaster, PA (Desviat, Pérez et al. 2004, Strauss and Puffenberger 2009). This mutation has been reported to be associated with a milder phenotype, therefore it is anticipated that these two subjects would have less severe symptomology. Subject 25 was diagnosed via abnormal NBS, was approximately 3 months of age at intervention and first face-to-face contact with a metabolic physician; other clinical information is missing and/or unknown. Subject 28 was diagnosed by having a sibling with PA, was seen by a metabolic physician (at 20 days of age) prior to intervention at 27 days of age, did not have any hospitalizations prior to intake in IBEM-IS at 18 years of age, and reportedly had a normal echocardiogram; other clinical information is missing and/or unknown. Precise genotype/phenotype correlations cannot be made in these two subjects because of the lack of clinical information, however homozygosity for the N536D allele may still be suggestive of the less severe outcome in these two adolescent subjects. In particular, Subject 28 did not have any hospitalizations at 18 years of age and had a normal echocardiogram, thus suggesting more mild disease.

6.1.4 Analysis of Service Utilization Data

6.1.4.1 Content of the IBEM-IS Data at Intake

A majority of the data in IBEM-IS was missing and/or unknown for all of the desired variables for this study. Sixty-seven to seventy-six percent of the health service, community resource, and provider data for each subject's intake/consent visit was missing and/or unknown. A possible explanation as to why a large amount of the data is missing and/or unknown may be due to the process by which the data is currently being exported for statistical use. Through observation and reference to the original IBEM-IS protocol, it seems that when multiple visits are completed on a single day (i.e. an "intake" visit and an "interval" visit are to be collected on the day of enrollment) there is a dropout of some of the initial variables. Therefore, some of the desired variables for this study are considered missing and/or unknown because all of the information from both the "intake" visit and first "interval" visit were not included with the first measurement date. Efforts are being made to clarify the situation and allow for full access to this data.

6.1.4.2 Content of the IBEM-IS Data for "Follow-up" Subjects

The amount of missing and/or unknown data improved when the 18 "follow-up" subjects and their 106 visits were isolated from the dataset. A majority (83%) of the health services data was reported and/or documented. At this time, only limited interpretation of health service utilization for PA patients in IBEM-IS can be made. There may be other possible explanations for the differences in the number of times a particular service is reported. It is important to note in the following interpretations that services utilized are not weighted per individual, thus data may be biased due to certain services being used more by a small number of subjects. In these particular

cases, a precise distinction cannot be made between subjects enrolled in IBEM-IS for longer periods of time, subjects with more severe disease, and subjects that are more compliant with the proposed management guidelines. The tables representing the number of times a service is reported by a certain number of subjects are located in Appendix M-O.

Overall, a high degree of variability was observed in the number of times subjects reported utilizing a particular service. Differences in the number of times a particular service was reported per subject may be due to the timeframe he or she was enrolled in IBEM-IS. Some subjects have been in IBEM-IS for more years than others. Those subjects who were enrolled for a longer period of time had more opportunities to report the types and number of times health services were utilized. Additionally, subjects with multiple visits entered in IBEM-IS are following-up with the participating metabolic center more often and may be more likely to follow-up with other health services more often, as well. Therefore it is possible that the years with the greatest proportion of newly enrolled subjects would have the least amount of services reported. This could be an explanation for the lack of service utilization data in 2007 and 2009. In the years 2012 and 2013 the proportion of new subjects entered into IBEM-IS was the least (0.23 and 0.22 respectively, Table 13); and as expected health services were reported the most in 2012 and 2013 overall (Figure 10). This observation may also reflect that the majority of "intake" visits for newly enrolled subjects were seen to have missing and/or unknown data for health services, and therefore the least amount of services would be reported in the years with the most "intake" visits (i.e. newly enrolled subjects).

Some subjects may require more extensive follow-up with a particular service due to the severity of the condition or specific symptomatology. Thus, the greater number of times a subject reports utilizing a certain service may be an indication of more severe symptomology/disease.

The services that were reported the most were cardiology, occupational therapy, physical therapy, neurology, speech-language therapy, and ophthalmology. The more common, chronic complications of PA including failure to thrive, developmental delay, various neurological symptoms, and cardiomyopathy are exemplary of the need for these services. Additionally, these particular services fit well within the clinical spectrum of PA and management guidelines reported in the literature. However, other recommended health services such as gastroenterology, immunology, and transplantation services were reported less often (Sutton, Chapman et al. 2012). Only one subject reported using gastroenterology on 5 separate occasions. Immunology and transplantation services were not reported by any of the PA subjects in IBEM-IS (Appendix M). The reason for this may be that these specific services are associated with the more rare complications of PA such as acute pancreatitis, neutropenia, and the need for liver transplantation (Dionisi-Vici, Deodato et al. 2006, Sutton, Chapman et al. 2012).

There were instances in which a subject reported receiving a service only once and no other subjects reported using the same service. This was observed in the following health services: behavioral/developmental pediatrics, feeding therapy, home health care, surgery, and urology (Appendix M). Single reporting of a service may be attributed to the subject utilizing the particular service and then determining that the service is no longer needed. This may also be explained by a patient only having one visit documented in IBEM-IS and therefore he or she only had one opportunity to report this service.

In further assessment of the content or amount of missing data for the 18 "follow-up" subjects, it was seen that almost half (46%) of the community resources data was still missing or unknown. Out of the subjects that had data documented for this variable, the response was "none" 41% of the time. Additionally, for the cases in which a specific community service was

reported more often it was attributed to only 1 or 2 subjects (Appendix N). The amount of missing and/or unknown data for the community resources received by the subject may be due to limited investigation of these components of care during routine metabolic appointments, either due to time constraints or because it is not part of the current medical management guidelines (Sutton, Chapman et al. 2012). Additionally, the question of community resources may not be raised in medical visits, assuming that it is addressed through the patients' school system or other social services. However, if the data in IBEM-IS is truly representative of what PA patients are currently utilizing in terms of resources, it may be possible that families are unaware of the local community resources for which they are eligible. This uncertainty may have been clarified through the parent/guardian interviews, and speaks to the need for interaction with a social worker or genetic counselor in routine metabolic visits. Almost all (44/46) of the participants reported that genetic counseling was provided for their diagnosis of PA, however genetic counselors made up only 7% of the total reports for the "providers seen at this visit". One of the many competencies of genetic counselors is to identify community resources and advocate for clients (Uhlmann, Schuette et al. 2009). This particular skillset would be useful at the time of diagnosis and also at each follow-up visit with the family to provide updated information in the context of changing clinical issues.

A large majority (89%) of the "providers seen at this visit" data was reported and/or documented. All of the 18 "follow-up" subjects reported seeing a physician and dietician at their visit documented in IBEM-IS (Appendix O). This illustrates that the physician and dietitian are regarded as an integral part of the metabolic team.

6.2 IDENTIFICATION OF CURRENT SERVICE UTILIZATION AND COMPARISON OF SERVICE UTILIZATION DATA

As a consequence of not being able to recruit participants for the study, the interview of parent/guardians of PA patients could not performed. By lacking data from the interview, current service utilization information in PA patients could not be assessed. Therefore the parent-stated service utilization data was not available to be compared to the documented aggregate data in IBEM-IS. The last two aims of the study were not achieved because of the inability to recruit subjects to interview for the study. The barriers to subject recruitment are discussed below.

6.3 BARRIERS TO SUBJECT RECRUITMENT

It is evident that much of the planned comparative work for this study could not be done due to difficulties encountered in recruiting patients/parents for the interview component of the study. Multiple barriers in study enrollment were the focus of a paper by Peters-Lawrence et al. (2012). A specific barrier to patient recruitment in rare disease research is a limited pool of available subjects. In addition, the amount of resources available to support the project can play a part in the success of study enrollment. Limited research staff, the lack of partnership with community organizations specific to the condition of interest, and the lack of formal recruitment strategies incorporating the use of media were all listed as potential barriers in subject recruitment (Peters-Lawrence, Bell et al. 2012).

Recruitment is one of the most challenging aspects of a clinical research study. Successful recruitment of patients is contingent on 4 factors: the design of the study,

collaboration with other healthcare professionals, characteristics of the study population, and the recruitment strategies put into place (Patel 2003, Peters-Lawrence, Bell et al. 2012). It is necessary that the design and method of the study be established at an early stage. The design and method can include factors such as appropriate length of the enrollment period, thoughtful inclusion/exclusion criteria for participants, expected duration of active participation by the subjects, and the risks and benefits of participation. Communication between/among collaborators is essential. All parties involved need to know exactly what they will be asked to do, their particular role in the study, the timeframe within which they are expected to work, and the results of study. It is important to be cognizant of the characteristics of the target patient population and to be flexible with potential participants. With rare disease research, investigators must acknowledge potential obstacles in subject enrollment. Lastly, successful subject recruitment rests primarily on the specific recruitment strategies implemented and the distribution of invitations to participate in the study (Patel 2003). To identify potential barriers of subject recruitment for this study, each of the four components of successful recruitment are examined.

6.3.1 Design of the Study

For this project, recruitment was particularly hindered because the design of the study included a short enrollment period. Originally, active enrollment of subjects was to begin July 2013, however the approval of the protocol by both the IRB and IBEMC took several months and therefore delayed the project. IBEMC only accepts IRB-approved protocols for submission and, in this study, after the protocol was approved by IBEMC several modifications then needed to be submitted to the IRB. Active enrollment of subjects was pursued from October 15, 2013 until

December 31, 2013 and this two-and-a-half month period was not sufficient to accomplish the goals of this study. There was not sufficient time available to thoroughly exhaust all efforts of communication with the various site coordinators and the 2 potential subjects from the Children's Hospital of Pittsburgh of UPMC.

The inclusion criteria for the study were any IBEM-IS-consented PA patients, who were 0-18 years of age, and who agreed to be re-contacted. The age restriction that was placed on the target population for this study excluded 15 out of the 46 PA patients that were entered in IBEM-IS because they were 18 years of age and older. This was originally done because large sections of the telephone interview focused on interventions made within the school system and allowed the parent/guardians to elaborate on their answers concerning their satisfaction with the services offered and utilized.

The intent stated in the invitation letters and the telephone consent form was that the duration of the telephone interview would be a minimum of 45 minutes. Although the total duration of participation was to be within one telephone call, it is possible that some subjects did not wish to speak on the phone for a longer period of time and this may had deterred them from participating in the study. Additionally, establishing contact with participants by telephone might require several attempts at different times and on different days, thus making the coordination of a telephone-based interview even more challenging (Patel 2003).

There were no physical risks associated with this project. However, the interview may raise questions or concerns about the specialty health services that the child is currently receiving. The interview questions were drafted to address all of the potential services and resources and may not be applicable to all participants. The parents are encouraged to discuss these concerns with their child's healthcare team. And interviews have the potential for causing

individuals to become uncomfortable discussing personal matters. The study was not designed to provide any direct benefits to the parent/guardian or their child. The primary benefit would have only been an increased knowledge base for the treatment of PA patients generally.

6.3.2 Collaboration with Other Healthcare Professionals

Precise and detailed instructions were provided to each of the site coordinators, however almost half of the sites did not respond after three attempts at contact were made. The reason for the poor response rate is not entirely known. However, given tight staffing limits and lack of financial remuneration for this study, it may have been given low priority among the participating centers' many responsibilities. Among the sites that did respond, a number indicated that they were no longer participating in IBEM-IS. Some responders stated that the site coordinator from the contact list was no longer working for their institution and thus that the initial contact letter was misdirected and not re-directed appropriately. Overall, there were challenges in obtaining current contact information for site coordinators and participating centers. There is a need to maintain up-to-date information within a public forum for those who wish to conduct research using IBEM-IS data.

Additional means of communication, except for email, were not available to this investigator; however, the study was discussed by the Children's Hospital of Pittsburgh site coordinator on a number of occasions during routine IBEMC team conference calls and during a face-to-face team meeting in the fall of 2013. Additional telephone and face-to-face conversations with individual site coordinators may have prompted a better response. Research suggests that widespread support from the organization in which the study is being implemented is important (Peters-Lawrence, Bell et al. 2012).

A major challenge that was faced in collaborating with other centers was differences in IRB protocols among the centers. With internal IRB approval of protocols, unless all participating centers originally submitted protocols with the same criteria, each center must write amendments and submit modifications to their own IRB if protocol changes are made, particularly as it relates to recontacting patients, as occurred in this study. This process drastically slowed down progress when attempting to recruit IBEM-IS-consented subjects from other institutions. It has been suggested that moving to a single, umbrella IRB for collaborative projects would help overcome such challenges (Marsolo 2012).

6.3.3 Study Population

Propionic acidemia is defined as a "rare" or "orphan" disease, affecting fewer that 650 per 1,000,000 people in the US. In fact, it more correctly could be termed an "ultra-rare" disease, a term more recently applied to conditions that affect fewer than 20 per 1,000,000 people in the US. There is a low prevalence of individuals with the disease and the research and treatments related to the specific condition are sparse (Griggs, Batshaw et al. 2009). Research in rare diseases is often hindered by inadequate subject recruitment. For this reason, there is a need for multi-center collaborations to identify and recruit PA patients (Griggs, Batshaw et al. 2009). Currently at the Children's Hospital of Pittsburgh of UMPC, there are a total of three PA patients. Two of these fit into the inclusion criteria for the study, being 0-18 years of age, and one patient had just turned age 18 at the time of enrollment. Active participation by 26 other IBEM-IS centers was necessary for adequate subject enrollment.

6.3.4 Recruitment Strategies

It was necessary to go through a third party (i.e. the metabolic center known to the patients) to recruit subjects in order to maintain patient confidentiality. Further, individual patients are not identified to all Collaborative participants, in compliance with HIPAA regulations for personal health information. While confidentiality was maintained with our recruitment strategy, it posed a significant barrier to informing participants about the present study. Without participation by the metabolic centers and site coordinators affiliated with IBEM-IS, it was not possible to contact patients, inform them about the details of the study and invite subjects to participate in the study. The ability to use different methods of communication with potential participants, including mailed letters, telephone calls, e-mail correspondence, or face-to-face contact may have helped to increase the participation rate (Patel 2003).

6.4 STUDY LIMITATIONS

6.4.1 The Inborn Errors of Metabolism Information System (IBEM-IS)

The amount of missing and/or unknown data in the database posed a limitation in the analysis of the data. The amount data present in the database may be due to the process by which the data is currently being entered in IBEM-IS and exported for statistical use. The data present in IBEM-IS is information that is extracted from patients' medical records. Site coordinators and health professionals from the various participating centers enter the information into the database. If the necessary information is not documented in the subject's medical record, then it is not possible to

enter it into IBEM-IS. The site coordinators are thus potentially limited in their ability to abstract information and to enter complete sets of data for each subject if the data is not included in the clinical record

The data that is available to be entered into IBEM-IS is dependent on a number of different factors. The amount of information within in a patient's medical record is predominately determined by the provider with his or her visit documentation. Some providers may not dictate all of the patients' information that was discussed at each visit, especially with respect to other health service utilization and community resources received. Also, in the cases in which a particular subject is routinely following up with his/her provider, the provider may not document which health services are being used at each visit because this information is already understood and known to the provider. Patients may also not feel the need to report which health services are currently being used at each metabolic visit and therefore, the result is an underreporting of services.

A limitation in the analysis of the IBEM-IS data is that subjects who are first entered into IBEM-IS may or may not be new to the participating metabolic center. Data is collected from each subject at enrollment and is labeled as the intake visit even when the subject has been following-up with the metabolic center for a number of years. Therefore, the information documented for each subjects' intake visit cannot be regarded as baseline information. As the subjects are followed over time, clinical visits may focus on unique issues and not be as comprehensive as early visits. Thus, all of the components of the IBEM-IS surveys may not be addressed during follow-up visits. Additionally, the information present in IBEM-IS is based on opportunistic data collection practices and subjects are not obligated to report data on a routine

basis. For this reason, the data in IBEM-IS may not be truly representative of complete longitudinal information.

Another limitation in the analysis of the IBEM-IS data was the number of subtle differences in documentation among participating centers. There was not consistency in documentation especially for data fields in which information was "written-in" such as ethnicity, mutation description, and specific comorbidities. In some cases, the data needed to be rewritten, reorganized, and categorized for harmonization. Given that IBEM-IS is a relatively new database for the collection of clinical information for patients with an inborn error of metabolism, it seems possible that there are some challenges in the process by which the data is exported for statistical use. In particular, it seems that when multiple visits are completed on a single day (i.e. an "intake" visit and an "interval" visit are to be collected on the day of enrollment per protocol) there is a dropout of some of the initial variables. Therefore, some of the desired variables for this study may be considered missing and/or unknown. Efforts are being made to clarify the situation and allow for full access to this data.

6.4.2 DocSite vs. REDCap

Challenges were faced in the merging of the data within DocSite and REDCap because the two databases have different data collection platforms. The data reporting practices in DocSite require the user to type in the information for all available fields, while REDCap is built with a more sophisticated entry process. The data entry in REDCap involves branching logic and a series of pull-down menus. Also, the two data collection platforms contained different variables and different titles for variables that they had in common.

In addition to the differences in data entry between the two databases, there were also differences in the data output. DocSite data was exported with the data variables listed in a word format. Each visit had its own row and every variable had its own column. There was a new column for every additional data entry per variable. The REDCap data was exported in a coded format (0=yes, 1=no, etc.), which required the use of a data dictionary. Each visit had its own row and every potential entry for each variable had its own column. There was a need to develop and write macros to translate the data in REDCap for qualitative analysis purposes, causing additional unanticipated delays in being able to manipulate the data.

As a result of the aforementioned difficulties, there were limitations in the analysis of the combined data. One limitation was the shortened list of variables provided for analysis. The full list of desired variables was not provided in a combined format because the merging of the databases excluded some variables that were not present in both databases. As noted, another limitation was the amount of time for analysis of the combined data. Managing the data output from REDCap and merging the two databases required a substantial amount of time to be completed, thus taking away from the time spent on analysis.

6.4.3 Telephone Interviews of PA Subjects

Telephone interviews were to be conducted with parents and/or guardians of PA patients to determine which healthcare services PA patients are using and to identify the parent's current perception of unmeet needs in his/her child. While the second aim of this study was not achieved, potential benefits and limitations in the implementation of telephone interviews are discussed.

Interview methods allow investigators to explore matters in greater depth and can be a useful tool in gathering large amounts of information from respondents. This approach is particularly useful for the types of questions that were to be asked in this study. Intended questions were aimed at the parents' satisfaction with the care that their child receives, their experience in accessing treatment, how well the healthcare providers understand their child's condition, if any providers were especially responsive to their child's needs, and if they felt that any of their child's medical needs were not being adequately addressed. Therefore, through an in-depth interview and open dialogue, comprehensive data would have been collected from the participating parents. With this information, unmet needs of the patient would have been discerned. Furthermore, telephone interviews typically attain higher response rates than postal questionnaires (Sibbald, Addington-Hall et al. 1994).

A potential limitation of telephone interviews may be the nature of the responses obtained by such a method and a potential for bias. Some studies suggest that responses from interviewees may be influenced by the relationship between the interviewer and the responder and therefore are presumably more susceptible to a social desirability response bias (Patel 2003). Other studies conclude that the nature of responses differs little between face-to-face, telephone, and postal administration of questionnaires (Sibbald, Addington-Hall et al. 1994). These possibilities can be assessed in future comparative studies (below). It had been observed that the nature of responses obtained in an interview or survey can vary based on the individual reporting the information. Parent or guardian-reported data was examined in a study by Bailey et al. (2010). It was determined that variability in accuracy exists in the information gathered from parents or guardians. The data that was presumably the most accurate was information that can only be reported by parents or information perceptual in nature. Such factors were living

conditions, relationships, recreational activities, quality of life, and family stress (Bailey, Raspa et al. 2010). Recall bias can also be a limitation in parent or guardian-reported data. It can be difficult for parents to remember all of the details about events, medical interventions, and specialty health care visits after many years have passed. The study hoped to address these possibilities by comparing the parent-reported information to the data recorded into IBEM-IS from the review of medical records.

6.5 FUTURE STUDIES

Due to the inability to recruit participants for the study in a shortened time frame, continuation of the proposed study including conducting the telephone interview of parents/guardians of PA patients to discern health service utilization and unmet needs is desirable. Alternative steps can be taken in future studies to facilitate the recruitment process. Future studies may include PA patients of all ages to expand the target population. Separate interview documents can be created to address the needs of each age group (i.e. early intervention services, school-related services, transitional services, and adult services). It would be interesting to learn which services PA patients require at different stages of life. The list of interview questions could be drafted to be concise for the variables pertaining to each age group, thus shortening the interview time with the subject and also allowing for a greater number of respondents. Additionally, there may be consideration of recruiting PA patients outside of IBEM-IS for interview purposes. With this approach, future areas of study would focus on analyzing the interview/questionnaire data only without a comparison to the data collected in IBEM-IS. Future researchers may use a similar approach as the study done by Pena et al. in 2012, by distributing a survey among Propionic

Acidemia Foundation (PAF) members or a similar organization. Investigators may also consider alternative approaches to telephone interview that might help improve recruitment, such as e-mail correspondence and online forums to accommodate for participants with busy schedules.

Additional questions remain relating to health and community-based service utilization by PA patients and their families. Future studies may place more of an emphasis on the community resources that are available to PA patients and their families and work to gather information and to inform families of suitable support resources. After current service and resource utilization information is obtained from PA patients, further exploration into potential regional and ethnic differences in healthcare utilization would be a compelling area of research. Such factors may affect availability of and access to multidisciplinary healthcare and community-based services. Although limited by a small patient population, future research may focus on identifying the potential differences in service utilization and the community resources offered to PA patients across the various metabolic centers in the United States. Also, due to the higher incidence of PA in certain Amish communities, it would be interesting to learn which services this specific patient population has access to and, out of these services, which ones members of the Amish community find most useful.

7.0 CONCLUSION

This present study examined the PA patient data collected in IBEM-IS with respect to the anticipated needs of PA patients based on the clinical spectrum and compared them to anticipated needs based on current practice guidelines reported in the literature. The assessment of the PA data in IBEM-IS showed that the majority of the necessary variables were missing and/or unknown. Thus, only a preliminary interpretation of service utilization in PA patients was performed.

All of the PA subjects in IBEM-IS with documented follow-up visits reported seeing a physician and dietician. This acknowledges that the physician and dietitian are regarded as integral parts of the metabolic team. The specialty health services that were reported the most in PA patients with documented follow-up visits were cardiology, occupational therapy, physical therapy, neurology, speech-language therapy, and ophthalmology. These particular services fit well within what is needed based on the clinical spectrum of PA and the management guidelines reported in the literature (Sutton, Chapman et al. 2012). The more common, chronic complications of PA including failure to thrive, developmental delay, various neurological symptoms, and cardiomyopathy are exemplary of the need for the aforementioned services (Pena, Franks et al. 2012). However, other recommended health services, such as gastroenterology, were seldom reported, and immunology and transplantation services were not reported by any of the PA subjects in IBEM-IS. The reason for this may be that these specific

services are needed to address the more rare complications of PA such as acute pancreatitis, neutropenia, and liver transplantation due to recurrent episodes of hyperammonemia or acidosis not adequately controlled with medical therapies (Sutton, Chapman et al. 2012). Current information regarding community resources for PA subjects in IBEM-IS is limited. It is possible that these resources are not routinely documented as part of medical management because they are not part of the current medical management guidelines. However, if the data in IBEM-IS is truly representative of community resource utilization in PA patients, it may be possible that families are unaware of the local community resources for which PA patients are eligible. This highlights the need for a social worker or genetic counselor to be part of routine metabolic visits.

The two remaining aims were not achieved in this study due to challenges in subject recruitment. The original intent was to interview parents and/or guardians of PA patients to identify current service utilization and to compare the parent/guardian-reported service utilization data to aggregate data in IBEM-IS. There are two possible outcomes of the data comparison 1) Patients are accessing health services as reported to IBEM-IS or 2) Patients are accessing more health services than are documented in IBEM-IS. In the later scenario, the differences between the two sets of service utilization data may be due to patients accessing but not disclosing more services than are documented in IBEM-IS. On the other hand, the differences may be due to the current data collection and entry practices in the IBEM-IS. We had hoped to elucidate the actual cause from our analysis of collected data.

Despite the limitations due to the absence of subject recruitment, the analysis of the information collected from PA patients in IBEM-IS helps to build upon the existing database and will assist in promoting future research studies. By identifying the amount of missing and/or unknown information present in the database and by touching upon the correlations observed in

service utilization to date, the results of this study may aid in further development of effective longitudinal studies of PA patients. In addition, the study may provide insight into whether current patients are accessing and utilizing clinical and community based services and whether additional emphasis needs to be placed on these services by clinicians. Due to the rarity and heterogeneity of the condition, there is little known about the current health services being utilized by patients with PA and thus a continued need for uniform data collection practices and more detailed assessment of these patients. With knowledge of the specific health services most useful to PA patients and by identifying which services they are not accessing there is advancement in establishing care recommendations across various centers nationwide.

APPENDIX A

FACILITIES WITH IRB APPROVAL TO ENROLL PATIENTS IN IBEM-IS

State	Facility
AR	Children's Hospital - Genetics
IA	University of Iowa Children's Hospital
IL	Ann and Robert H. Lurie Children's Hospital of Chicago
IL	University of Illinois at Chicago
IN	Indiana University Department of Medical and Molecular Genetics
KY	University of Louisville
MD	Johns Hopkins
MI	University of Michigan
MI	Wayne State University
MN	University of Minnesota
MO	University of Missouri
NC	Duke University
NE	University of Nebraska
NJ	Hackensack University
NY	Children's Hospital at Montefiore
NY	New York Medical College
NY	University of Rochester
NY	Women and Children's Hospital of Buffalo
OH	Cincinnati Children’s Hospital Medical Center
OH	Nationwide Children's Hospital
OK	Saint Francis Hospital
OK	University of Oklahoma
SD	Sanford Children's Specialty Clinic
WI	Medical College of Wisconsin
WI	University of Wisconsin
WV	West Virginia University

APPENDIX B

METABOLIC CONDITIONS DOCUMENTED IN IBEM-IS

Amino Acidemias	Maple syrup urine disease
	Homocystinuria (CBS, MTHFR, Cbl D variant 1, Cbl E, Cbl G
	Tyrosinemia
	Arginemia
	Argininosuccinate acidemia (Yorifuji, Kawai et al.)
	Citrullinemia Type I (argininosuccinate synthetase)
	Citrullinemia Type II (citrin deficiency)
	Hypermethioninemia
	Defects of biopterin cofactor biosynthesis/regeneration
	Hyperphenylalaninemia/phenylketonuria
Fatty Acid Oxidation Disorders	Carnitine uptake deficiency (CUD)
	CACT deficiency
	CPT-1 deficiency
	CPT-2 deficiency
	SCAD deficiency
	SBCAD deficiency
	MCAD deficiency
	LCHAD deficiency
	Trifunctional protein deficiency
	VLCAD deficiency
	Dienoyl-CoA reductase deficiency
	Medium-chain ketoacyl-CoA thiolase deficiency (MCKAT)
	Glutaric acidemia type II
	Medium/Short-chain L-3-hydroxy acyl-CoA dehydrogenase deficiency (M/SCHAD)
Organic Acidemias	2-methylbutyryl-CoA dehydrogenase deficiency
	Isovaleric acidemia
	Glutaric acidemia type I
	Isobutyryl-CoA dehydrogenase deficiency
	3-MCC deficiency
	2-methyl 3-hydroxybutyryl CoA dehydrogenase (2M3HBA) deficiency
	Holocarboxylase synthetase deficiency
	3-methylglutaconic aciduria type I
	Beta-ketothiolase deficiency
	Succinyl CoA-3-keto transferase (SCOT) deficiency
	3-hydroxy 3-methylglutaryl (HMG) CoA lyase deficiency
	Propionic Acidemia
	MMA (Mut-, Mut0, cbl A, cbl B, cbl D variant 2)
	MMA + Hcy (Cbl C, Cbl D, Cbl F, Transcobalamin II)
	Malonic acidemia
Non-MS/MS Conditions	Biotinidase deficiency
	GALT deficiency
	Galactokinase (GALK) deficiency galactosemia
	UDP-galactose-4-epimerase (GALE) deficiency galactosemia

APPENDIX C

IRB APPROVAL LETTER

University of Pittsburgh
Institutional Review Board

3500 Fifth Avenue
Ground Level
Pittsburgh, PA 15213
(412) 383-1480
(412) 383-1508 (fax)
http://www.irb.pitt.edu

Memorandum

To: Georgianne Arnold, MD
From: Christopher Ryan, PhD, Vice Chair
Date: 10/14/2013
IRB#: MOD13050590-02 / PRO13050590
Subject: Service Utilization- Proprionic Acidemia

The University of Pittsburgh Institutional Review Board reviewed and approved the requested modifications by expedited review procedure authorized under 45 CFR 46.110.

Modification Approval Date: 10/14/2013
Expiration Date: 8/5/2014

Please note that it is the investigator's responsibility to report to the IRB any unanticipated problems involving risks to subjects or others [see 45 CFR 46.103(b)(5)]. Refer to the IRB Policy and Procedure Manual regarding the reporting requirements for unanticipated problems which include, but are not limited to, adverse events. If you have any questions about this process, please contact the Adverse Events Coordinator at 412-383-1480.

The protocol and consent forms, along with a brief progress report must be resubmitted at least one month prior to the renewal date noted above as required by FWA00006790 (University of Pittsburgh), FWA00006735 (University of Pittsburgh Medical Center), FWA00000600 (Children's Hospital of Pittsburgh), FWA00003567 (Magee-Womens Health Corporation), FWA00003338 (University of Pittsburgh Medical Center Cancer Institute).

Please be advised that your research study may be audited periodically by the University of Pittsburgh Research Conduct and Compliance Office.

APPENDIX D

DATA REQUESTED FOR THE STUDY

IBEM-IS Data Requested for the Study

Propionic Acidemia Intake:

- **Demographics**
  - Specify ethnicity if ethnicity is listed as "other", enter N/A if not applicable
- **Socioeconomic Status**
  - Maternal education: highest level of education
  - Paternal education: highest level of education
  - Is patient/primary caregiver proficient in written English?
  - Is patient/primary caregiver proficient in spoken English?
- **Diagnostic Testing**
  - Molecular Testing: Common or target mutation panel
  - Molecular Testing: Full sequencing
  - Mutation description: Allele 1 (format example 985A>G)
  - Mutation description: Allele 2 (format example 985A>G)
- **Past Health History**
  - Number of hospitalizations prior to Intake in IBEM-IS
  - Initial diagnosis of this IBEM-IS found by:
  - Days of age from birth to initiation of intervention for this IBEM (365 x yrs or 30 x months or counted days), enter 99999 if unknown
  - Symptom(s) at time of initial metabolic contact
  - Days of age at time of initial face to face metabolic consultation (365 x yrs or 30 x months or counted days), enter 99999 if unknown
  - Was genetic counseling for this disorder provided?
  - Echocardiogram results obtained prior to Intake: enter date of echo and explain results, enter N/A if not applicable
  - Identify specific birth defects
  - List specific comorbidities (enter N/A if not applicable, enter unknown if missing information)

Propionic Acidemia Interval:

- **Demographics**
  - Patient has attended an outpatient metabolic visit during the past 12 months?
  - Metabolic follow up status
  - Primary care status
- **Socioeconomic Status**
  - Current insurance status
- **Past Health History**
  - Has patient had surgical procedure(s) since the last outpatient metabolic visit?
  - Has patient received dialysis since the last outpatient metabolic visit
  - List specific comorbidities (enter N/A if not applicable, enter unknown if missing information)
- **Emergency Management**
  - Number of ER visits since last metabolic visit
  - Number of hospital admission (total) since last metabolic visit
- **Care Coordination**
  - Other health services received currently
  - Community resources received currently
  - Providers seen at this metabolic visit
  - Other providers seen at this metabolic visit (enter N/A if not applicable)
- **Developmental Assessment**
  - Developmental screening occurred at this visit?
  - Developmental milestones achieved at this time?
  - If developmental milestone(s) not achieved, which one(s) were not achieved?
  - If developmental milestone(s) not achieved, was patient referred for further developmental evaluation?
  - Was neuropsychological evaluation done since last outpatient visit? (If yes, complete Neuropsych Survey)

- Overall neuropsychological testing impression (from most recent neuropsych evaluation)
- Are behavioral concerns suspected as this time?
- If behavioral concerns are suspected at this time, explain (enter N/A if no behavioral concerns suspected)
- If behavioral concerns are suspected at this time, was patient referred for further evaluation?

- **Education**
  - Was patient referred for Special Education evaluation at this time?
  - Are Special Education services received by this patient currently?
  - Special Education services are received currently: age (in years) child qualified for services?
  - Reason Special Education services are received currently?
- **Laboratory Studies**
  - Molecular Testing: Common or targeted mutation panel done at this visit (enter specific mutation(s) on Intake Survey)?
  - Molecular Testing: Full sequencing done at this visit (enter specific mutation(s) on Intake Survey)?
- **Imaging Studies**
  - Abdominal imaging done since last outpatient metabolic visit?
  - Cardiac imaging done since last outpatient metabolic visit?
  - Musculoskeletal imaging done since last outpatient metabolic visit?
  - Dexa scan since last outpatient metabolic visit (z-score >-2), specify site?
  - Neurological imaging done since last outpatient metabolic visit?
  - Renal/pelvic/genital imaging done since last outpatient metabolic visit?
  - Other imaging (indicate type of imaging and if WNL or Abn) done since last outpatient metabolic visit?, enter N/A if not applicable
- **Nutrition**
  - Method of payment for low protein foods, if prescribed
  - Method of payment for metabolic formula, if prescribed
  - If other nutritional supplementation is taken (explain), enter N/A if not applicable

RedCap Data Requested for the Study

- **Demographics Information (page 2)**
  - Age
  - Societal sex
- **Condition (page 2)**
  - Patient condition category
  - Specify organic acid disorder diagnosis for the patient
  - Patient disorder identification method
  - Family member with this condition
- **Care and Other Studies (page 2)**
  - Miles from home to primary care
  - Miles from home to specialty care
  - Specify type of primary care provider
  - Specify medical home
  - Specify medical home-other, specify
- **Education (page 3)**
  - Maternal education
  - Paternal education
  - Patient education
  - Special education services received prior to intake
  - Age patient qualified for special education services
- **Ancestral Origin, Race and Ethnicity (page 4)**
  - Ancestral Origin
  - Ancestral Origin-Africa
  - Ancestral Origin-Asia
  - Ancestral Origin-Europe
  - Ancestral Origin-North America
  - Ancestral Origin-South America
  - Ancestral Origin-Oceania
  - Ancestral Origin-Other
  - Race
  - Patient is Hispanic or Latino
- **Medical Coverage (page 5)**
  - Medical coverage at time of intake
  - Medical coverage at intake-Patient assistance program, specify
  - Medical coverage at intake-Other, specify
- **Language (page 6)**
  - Primary language spoken at home
  - Written/web-based information on this condition provided to the patient/primary caregiver in his/her primary language
- **Prenatal History (page 16)**
  - Prenatal diagnosis done for this condition
  - Form of prenatal diagnosis
- **Neonatal history (page 17)**
  - Congenital anomalies
    - Type of congenital anomalies
  - Neonatal complications
    - Type of neonatal complications
- **Health History (page 19)**
  - Patient has had an outpatient specialty visit
  - Days of age from birth until intervention for this condition
  - Days of age from birth until first seen by subspecialist
  - History of premature ovarian insufficiency (POI)
  - History of coenzyme Q10 or OXPHOS deficiency

    - History of renal failure
- **Dialysis (page 19)**
    - Dialysis (any type) prior to intake
- **Transplants (page 19)**
    - Transplant prior to intake
- **Other History (page 19)**
    - Cardiomyopathy prior to intake
    - Hospitalizations prior to intake
    - Number of hospitalizations prior to intake related to this condition
    - Number of hospitalizations prior to intake not related to this condition
    - Genetic counseling provided
    - Provider of genetic counseling
    - Comorbidities at time of intake
- **Prior Testing (page 20)**
    - Echocardiogram prior to intake
    - Neurological imaging prior to intake
    - History of a seizure disorder
- **Eye Exam (page 21)**
    - Eye exam performed prior to intake
    - Eye exam findings
- **Status at time of NBS report to Specialist (page 26)**
    - Patient symptoms at initial contact
- **Genetic Testing (page 32)**
    - Type of genetic/genomic testing
    - Gene(s) associated with PROP PCCA PCCB Other
    - Gene(s) associated with PROP-other, specify
    - PCCA: Specify allele 1
    - PCCA: Specify allele 2
    - PCCB: Specify allele 1
    - PCCB: Specify allele 2
    - Other: Specify allele 1
    - Other: Specify allele 2
- **Education (page 34)**
    - Education status has changed since the last visit
- **Care and Other Studies (page 34)**
    - Providers seen at this visit
    - Providers seen at this visit, other- specify
- **Medical Coverage (page 34)**
    - Medical coverage at visit
    - Medical coverage at visit-Patient assistance program, specify
    - Medical coverage at visit-Other, specify
- **Health Status (page 36)**
    - Current comorbidities
- **Sick Visits (page 36)**
    - Sick visits since last outpatient visit
    - Number of sick visits
    - Reason for sick visit 1-10
    - Patient was admitted to the hospital as a result of sick visit 1-10
    - Number of inpatient days for sick visit 1-10
- **Procedures (page 40)**
    - Anesthesia since last visit
    - Surgeries since last visit
- **Dialysis (page 41)**
    - Dialysis (any type) since the last outpatient metabolic visit

- **Care Coordination (page 44)**
  - Other health services currently received
  - Specify other current health services
  - Specify other current health services-other, specify
  - Specify type of primary care provider
  - Community resources currently received
  - Specify current community resources
  - Specify current community resources-other, specify
  - Specify current family support
  - Specify current family support-other, specify
  - Specify medical home
  - Specify medical home-other, specify
  - Specify current social services
  - Specify current social services-other, specify
- **Developmental Assessment (page 45)**
  - Developmental assessment done at this visit
  - Developmental status
  - Severity of atypical development
  - Referred for further developmental assessment
  - Type of provider/service to whom patient was referred for developmental assessment
  - Type of provider/service to whom patient was referred for developmental assessment-other, specify
  - Neuropsychometric evaluation performed since last visit
  - Patient has mental health concerns
  - Referred for further mental health assessment
  - Type of provider/service to whom patient was referred for mental health assessment
  - Type of provider/service to whom patient was referred for mental health assessment-other, specify
  - Behavioral concerns
  - Referred for further behavioral assessment
  - Type of provider/service to whom patient was referred for behavioral assessment
  - Type of provider/service to whom patient was referred for behavioral assessment-other, specify
- **Education (page 47)**
  - Special education assessment recommended
  - Reason special education services received
  - Reason special education services received-other, specify
  - Special education category
  - Special education, other- specify
- **Nutrition (page 65)**
  - Number of special metabolic formulas recommended/prescribed
  - Method of payment for special metabolic formula 1-3
  - Modified low protein foods recommended/prescribed
  - Method of payment for modified low protein foods
- **Number of different episodes during which dialysis (any type) was used (page 151)**
- **Number of organ transplants received (page 164)**

APPENDIX E

SITE COORDINATOR LETTER

Children's Hospital of Pittsburgh of UPMC

Children's Hospital Drive
45th Street and Penn Avenue
Pittsburgh, PA 15201
www.chp.edu

Dear **[Site Coordinator Name]**,

You are receiving this letter because you are a site coordinator for the Inborn Errors of Metabolism Information System (IBEM-IS) research registry at **[location]**.

A new study has been reviewed and approved by IBEMC to review and assess the information being gathered about propionic acidemia patients regarding service utilization. We are looking at what services are currently being used, how they are being reported and recorded during clinic visits, and whether there are services that might still be needed by patients and families. Participation in the study will involve a telephone interview of the parents/guardians of children with propionic acidemia who have consented to participate in IBEM-IS. This interview will take about 45 minutes and will involve a series of questions regarding resources and services that the patient uses. A copy of the interview script and questionnaire are available upon request (see contact information below).

The subjects who will be approached about their willingness to be interviewed are the parents/guardians of patients with a diagnosis of propionic acidemia who are 0-18 years of age, and who as part of their consent to IBEM-IS, have agreed to be re-contacted about new related research that is of interest to propionic acidemia patients and their families.

A letter of invitation to the parents/guardians of the designated patients is attached. As a site coordinator, if you would please send this letter to your patients that meet the above criteria in a timely manner, it would be greatly appreciated.

This study is being conducted by Georgianne Arnold, MD, the Principal Investigator of the IBEM-IS research registry in Pittsburgh, PA. The interview is being conducted by Amanda Jacquart, candidate for a Master's degree in Genetic Counseling from the University of Pittsburgh. Please contact Amanda or Cate Walsh Vockley, MS, CGC to disclose the number of patients you have who meet the inclusion criteria for the study and to confirm that the enclosed letter of invitation has been sent to the parents/guardians of these patients.

Thank you for your participation in this effort.

Sincerely,

Georgianne L. Arnold, MD
Clinical Director
Division of Medical Genetics
Children's Hospital of
Pittsburgh of UPMC

Cate Walsh Vockley, MS, CGC
Senior Genetic Counselor
Division of Medical Genetics
Children's Hospital of
Pittsburgh of UPMC
Phone: (412) 692-7349
catherine.walshvockley@chp.edu

Amanda J. Jacquart, BS
Graduate Student Researcher
Children's Hospital of
Pittsburgh of UPMC
Phone: (412) 692-6770
amanda.jacquart@chp.edu

APPENDIX F

SITE COORDINATOR COVER LETTER TO PATIENTS

YOUR INSTITUTION'S LETTERHEAD

[Address]
[Address]
[Address]
[Address]

Today's Date, 2013

Dear Parent/Guardian of **[Patient Name]**,

Thank you for participating in the Inborn Errors of Metabolism Information System (IBEM-IS) research registry at **[Institution Name]**. As part of the registry, you agreed to be re-contacted about new related research studies that are of interest to propionic acidemia patients and their families.

Please read the enclosed invitation letter from our partners at the Children's Hospital of Pittsburgh of UPMC for details concerning a new study. If you wish to participate in this study or if you have additional questions, the contact information for the research team at the Children's Hospital of Pittsburgh can be found in the invitation letter and it is also listed below:

Cate Walsh Vockley, MS, CGC
Senior Genetic Counselor
Division of Medical Genetics
Children's Hospital of Pittsburgh of UPMC
Phone: (412) 692-7349
catherine.walshvockley@chp.edu

Amanda J. Jacquart, BS
Graduate Student Researcher
Children's Hospital of Pittsburgh of UPMC
Phone: (412) 692-6770
amanda.jacquart@chp.edu

Thank you for considering participation in this study.

Sincerely,

[Site Coordinator Name]
[Credentials]
[Etc.]

APPENDIX G

PATIENT INVITATION LETTER

Children's Hospital of Pittsburgh of UPMC

Children's Hospital Drive
45th Street and Penn Avenue
Pittsburgh, PA 15201
www.chp.edu

[Address]
[Address]
[Address]
[Address]

Dear Parent/Guardian of **[Patient Name]**,

You are receiving this letter because your child is currently participating in the Inborn Errors of Metabolism Information System (IBEM-IS) research registry at **[Institution Name]**. As part of the registry, you agreed to be re-contacted about new related research studies that are of interest to propionic acidemia patients and their families.

A new study is being done to review the information regarding service utilization that has been gathered as a part of the IBEM-IS research registry. This study will focus on children 0-18 years of age who have propionic acidemia. We are looking at what services are currently being used, how they are being reported and recorded during clinic visits, and whether there are services that might still be needed by patients and families. Participation is this study will involve a telephone interview of the parent/guardian with our study coordinator. This interview will take about 45 minutes and will involve a series of questions regarding resources and services that your child uses.

There are no physical risks associated with this project, and this study is not designed to provide any direct benefits to you or your child. This interview may, however, raise questions or concerns about your child's current needs that you may wish to discuss with your child's healthcare team.

All responses to the interview questionnaire are confidential. Information will include your child's age, but your child and you will not be identifiable in any other way. Your participation is voluntary, and you may withdraw from this project at any time.

This study is being conducted by Georgianne Arnold, MD, the Principal Investigator of the IBEM-IS research registry in Pittsburgh, PA. The interview will be conducted by Amanda Jacquart, a candidate for a Master's degree in Genetic Counseling at the University of Pittsburgh, under Dr. Arnold's supervision. Please contact Amanda by calling her at 412.692.6770 to confirm your willingness to participate in this study, or if you have additional questions. Thank you for considering this opportunity to help us improve patient care.

Sincerely,

Georgianne L. Arnold, MD
Clinical Director
Division of Medical Genetics
Children's Hospital of
Pittsburgh of UPMC

Cate Walsh Vockley, MS, CGC
Senior Genetic Counselor
Division of Medical Genetics
Children's Hospital of
Pittsburgh of UPMC
Phone: (412) 692-7349
catherine.walshvockley@chp.edu

Amanda J. Jacquart, BS
Graduate Student Researcher
Children's Hospital of
Pittsburgh of UPMC
Phone: (412) 692-6770
amanda.jacquart@chp.edu

APPENDIX H

CHP PATIENT INVITATION LETTER

Children's Hospital of Pittsburgh of UPMC

Children's Hospital Drive
45th Street and Penn Avenue
Pittsburgh, PA 15201
www.chp.edu

[Address]
[Address]
[Address]
[Address]

Dear Parent/Guardian of **[Patient Name]**,

You are receiving this letter because your child is currently participating in the Inborn Errors of Metabolism Information System (IBEM-IS) research registry at Children's Hospital of Pittsburgh of UPMC. As part of the registry, you agreed to be re-contacted about new research studies that are of interest to propionic acidemia patients and their families.

A new study is being done to review the information regarding service utilization that has been gathered as a part of the IBEM-IS research registry. This study will focus on children 0-18 years of age who have propionic acidemia. We are looking at what services are currently being used, how they are being reported and recorded during clinic visits, and whether there are services that might still be needed by patients and families. Participation is this study will involve a telephone interview of the parent/guardian with our study coordinator. This interview will take about 45 minutes and will involve a series of questions regarding resources and services that your child uses.

There are no physical risks associated with this project, and this study is not designed to provide any direct benefits to you or your child. This interview may, however, raise questions or concerns about your child's current needs that you may wish to discuss with your child's healthcare team.

All responses to the interview questionnaire are confidential. Information will include your child's age, but your child and you will not be identifiable in any other way. Your participation is voluntary, and you may withdraw from this project at any time.

This study is being conducted by Georgianne Arnold, MD, the Principal Investigator of the IBEM-IS research registry in Pittsburgh, PA. The interview will be conducted by Amanda Jacquart, a candidate for a Master's degree in Genetic Counseling at the University of Pittsburgh, under Dr. Arnold's supervision. Please contact Amanda by calling her at 412.692.6770 to confirm your willingness to participate in this study, or if you have additional questions. Thank you for considering this opportunity to help us improve patient care.

Sincerely,

Georgianne L. Arnold, MD
Clinical Director
Division of Medical Genetics
Children's Hospital of
Pittsburgh of UPMC

Cate Walsh Vockley, MS, CGC
Senior Genetic Counselor
Division of Medical Genetics
Children's Hospital of
Pittsburgh of UPMC
Phone: (412) 692-7349
catherine.walshvockley@chp.edu

Amanda J. Jacquart, BS
Graduate Student Researcher
Children's Hospital of
Pittsburgh of UPMC
Phone: (412) 692-6770
amanda.jacquart@chp.edu

APPENDIX I

TELEPHONE CONSENT SCRIPT

Telephone Consent Script:

Thank you for agreeing to be re-contacted concerning new research that is being done with propionic acidemia patients who are registered with the Inborn Errors of Metabolism Information System (IBEM-IS).

The purpose of this research study is to assess the information being gathered as a part of IBEM-IS from propionic acidemia patients and their families regarding service utilization. The outcome of this study will identify additional services that may be beneficial to propionic acidemia patients.

The study will involve a telephone interview of parents of the propionic acidemia patient population that already consented to the IBEM-IS protocol. This interview will take a minimum of 45 minutes.

While there are no physical risks associated with this project, this study is not designed to provide any direct benefits to you or your child. This interview may raise questions or concerns about your child's current needs that you may wish to discuss with your child's healthcare team. The interview will be audio recorded so that the PI can listen to the recording for data analysis. The audio recordings will be saved as MP3 files and the recordings will be transcribed into Word documents. The files will be maintained on a password-protected computer and will be kept until the conclusion the study and then destroyed. All responses are confidential, and results will be kept in a password-protected file. Information will include your child's age, but your child will not be identifiable in any other way. Your participation is voluntary, and you may withdraw from this project at any time. Even after this interview has concluded, you can later inform us that you have decided to withdraw from this study. However, the information that we have collected before you tell us that you are withdrawing will continue to be used.

If you are willing to participate, I am going to ask a series of questions regarding resources and services that your child uses. The purpose of this interview is to assess propionic academia patient's current needs and to determine if there are any that are not being met. It is anticipated that the results of this study will assist in the medical management of individuals with propionic academia.

Are you interested in participating in this study and do I have permission to ask you these questions?

YES NO

In the first section of this interview, I'll ask whether or not your child is seen by a variety of service providers. If not, I'll ask why and provide you with optional responses. If yes, I'll ask how old your child was at the first visit, how often your child uses this service, and how important this service is to your child's care. Note that not all of the providers and services listed are essential for every child who has propionic academia.

Do you have any questions before we begin?

APPENDIX J

TELEPHONE INTERVIEW

**After the telephone consent script has been read and verbal consent has been obtained and documented.*

Before we start the interview, please tell me your relationship to the child.

_____

What is your child's age? _____

At what age was your child diagnosed with propionic academia (PA)? _____

The following lists of services and providers are arranged alphabetically.

1.1a Routine Healthcare Providers

Does your child see the following healthcare providers and/or services?	If no, why not?	If yes, how old was your child at the first visit?	If yes, how often?	How important is this provider to your child's care?
Dietitian o Yes o No	o Not recommended o None available o Unaware of service availability o Too expensive o Concern about medical insurance o Child does not need this service o Other		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months o Every year o As needed o Other – Specify	o Very important o Somewhat important o Not important
Genetic Counselor o Yes o No	o Not recommended o None available o Unaware of service availability o Too expensive o Concern about medical insurance o Child does not need this service o Other		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months o Every year o As needed o Other – Specify	o Very important o Somewhat important o Not important
Geneticist o Yes o No	o Not recommended o None available o Unaware of service availability o Too expensive o Concern about medical insurance o Child does not need this service o Other		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months o Every year o As needed o Other – Specify	o Very important o Somewhat important o Not important

Mental health therapist (eg.psychologist, or counselor) o Yes o No	o Not recommended o None available o Unaware of service availability o Too expensive o Concern about medical insurance o Child does not need this service o Other		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months o Every year o As needed o Other – Specify	o Very important o Somewhat important o Not important
Metabolic Physician o Yes o No	o Not recommended o None available o Unaware of service availability o Too expensive o Concern about medical insurance o Child does not need this service o Other		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months o Every year o As needed o Other – Specify	o Very important o Somewhat important o Not important
Nurse o Yes o No	o Not recommended o None available o Unaware of service availability o Too expensive o Concern about medical insurance o Child does not need this service o Other		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months o Every year o As needed o Other – Specify	o Very important o Somewhat important o Not important
Nurse Practitioner o Yes o No	o Not recommended o None available o Unaware of service availability o Too expensive o Concern about medical insurance o Child does not need this service o Other		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months o Every year o As needed o Other – Specify	o Very important o Somewhat important o Not important
Physical/ Rehabilitative Medicine o Yes o No	o Not recommended o None available o Unaware of service availability		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months	o Very important o Somewhat important o Not important

	o Too expensive o Concern about medical insurance o Child does not need this service o Other		o Every year o As needed o Other – Specify	
Physician's Assistant o Yes o No	o Not recommended o None available o Unaware of service availability o Too expensive o Concern about medical insurance o Child does not need this service o Other		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months o Every year o As needed o Other – Specify	o Very important o Somewhat important o Not important
Primary Care Physician (PCP) o Yes o No	o Not recommended o None available o Unaware of service availability o Too expensive o Concern about medical insurance o Child does not need this service o Other		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months o Every year o As needed o Other – Specify	o Very important o Somewhat important o Not important
Social Worker o Yes o No	o Not recommended o None available o Unaware of service availability o Too expensive o Concern about medical insurance o Child does not need this service o Other		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months o Every year o As needed o Other – Specify	o Very important o Somewhat important o Not important

1.1b Are there any additional routine healthcare providers that your child sees?

_____
_____
_____
_____

1.1c What is your level of satisfaction with the care your child received from these routine healthcare providers?

o Very Satisfied
o Somewhat Satisfied
o Neither Satisfied or Dissatisfied
o Somewhat Dissatisfied
o Very Dissatisfied

1.1d What was your personal experience in accessing treatment from these routine healthcare providers?

- o Easy to Access
- o Somewhat Easy to Access
- o Difficult to Access
- o Extremely Difficult to Access
- o Is there anything else that you would like to add concerning your personal experience in accessing services?

_____
_____
_____
_____

1.1e How well do these routine healthcare providers understand your child's diagnosis of propionic acidemia?

- o Very Well
- o Well
- o Well Enough
- o Not Well
- o Not at all

1.1f How well did these routine healthcare providers incorporate your child's diagnosis of propionic acidemia into their clinical management of him/her?

- o Very Well
- o Well
- o Well Enough
- o Not Well
- o Not at all

1.1g Were any of these routine healthcare providers especially responsive or unresponsive to your child's needs?

- o No
- o Yes, please explain:

_____
_____
_____
_____

1.1h Is there anything else about your experience with these routine healthcare providers that you would like to share?

_____
_____
_____
_____

1.1i Are there any medical needs that your child has that you feel are not being adequately addressed by the providers we discussed?

- o No
- o Yes, please explain:

_____
_____
_____
_____

1.2a Healthcare Specialty Services

Does your child see the following healthcare specialty services?	If no, why not?	If yes, how old was your child at the first visit?	If yes, how often?	How important is this service to your child's care?
Audiology o Yes o No	o Not recommended o None available o Unaware of service availability		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months	o Very important o Somewhat important o Not important

	○ Too expensive ○ Concern about medical insurance ○ Child does not need this service ○ Other		○ Every year ○ As needed ○ Other – Specify	
Behavioral/ Developmental Pediatrics ○ Yes ○ No	○ Not recommended ○ None available ○ Unaware of service availability ○ Too expensive ○ Concern about medical insurance ○ Child does not need this service ○ Other		○ Only one time ○ 1 or more times/week ○ 1 or more times/month ○ Every 6 months ○ Every year ○ As needed ○ Other – Specify	○ Very important ○ Somewhat important ○ Not important
Cardiology ○ Yes ○ No	○ Not recommended ○ None available ○ Unaware of service availability ○ Too expensive ○ Concern about medical insurance ○ Child does not need this service ○ Other		○ Only one time ○ 1 or more times/week ○ 1 or more times/month ○ Every 6 months ○ Every year ○ As needed ○ Other – Specify	○ Very important ○ Somewhat important ○ Not important
Dermatology ○ Yes ○ No	○ Not recommended ○ None available ○ Unaware of service availability ○ Too expensive ○ Concern about medical insurance ○ Child does not need this service ○ Other		○ Only one time ○ 1 or more times/week ○ 1 or more times/month ○ Every 6 months ○ Every year ○ As needed ○ Other – Specify	○ Very important ○ Somewhat important ○ Not important
Endocrinology ○ Yes ○ No	○ Not recommended ○ None available ○ Unaware of service availability ○ Too expensive ○ Concern about medical insurance ○ Child does not need this service ○ Other		○ Only one time ○ 1 or more times/week ○ 1 or more times/month ○ Every 6 months ○ Every year ○ As needed ○ Other – Specify	○ Very important ○ Somewhat important ○ Not important

Gastroenterology o Yes o No	o Not recommended o None available o Unaware of service availability o Too expensive o Concern about medical insurance o Child does not need this service o Other		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months o Every year o As needed o Other – Specify	o Very important o Somewhat important o Not important
Hematology/ Oncology o Yes o No	o Not recommended o None available o Unaware of service availability o Too expensive o Concern about medical insurance o Child does not need this service o Other		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months o Every year o As needed o Other – Specify	o Very important o Somewhat important o Not important
Nephrology o Yes o No	o Not recommended o None available o Unaware of service availability o Too expensive o Concern about medical insurance o Child does not need this service o Other		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months o Every year o As needed o Other – Specify	o Very important o Somewhat important o Not important
Neurology o Yes o No	o Not recommended o None available o Unaware of service availability o Too expensive o Concern about medical insurance o Child does not need this service o Other		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months o Every year o As needed o Other – Specify	o Very important o Somewhat important o Not important
Neuropsychology o Yes o No	o Not recommended o None available o Unaware of service availability		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months	o Very important o Somewhat important o Not important

	○ Too expensive ○ Concern about medical insurance ○ Child does not need this service ○ Other		○ Every year ○ As needed ○ Other – Specify	
Ophthalmology ○ Yes ○ No	○ Not recommended ○ None available ○ Unaware of service availability ○ Too expensive ○ Concern about medical insurance ○ Child does not need this service ○ Other		○ Only one time ○ 1 or more times/week ○ 1 or more times/month ○ Every 6 months ○ Every year ○ As needed ○ Other – Specify	○ Very important ○ Somewhat important ○ Not important
Orthopedics ○ Yes ○ No	○ Not recommended ○ None available ○ Unaware of service availability ○ Too expensive ○ Concern about medical insurance ○ Child does not need this service ○ Other		○ Only one time ○ 1 or more times/week ○ 1 or more times/month ○ Every 6 months ○ Every year ○ As needed ○ Other – Specify	○ Very important ○ Somewhat important ○ Not important
Otolaryngology (ENT: ear, nose, and throat) ○ Yes ○ No	○ Not recommended ○ None available ○ Unaware of service availability ○ Too expensive ○ Concern about medical insurance ○ Child does not need this service ○ Other		○ Only one time ○ 1 or more times/week ○ 1 or more times/month ○ Every 6 months ○ Every year ○ As needed ○ Other – Specify	○ Very important ○ Somewhat important ○ Not important
Psychiatry ○ Yes ○ No	○ Not recommended ○ None available ○ Unaware of service availability ○ Too expensive ○ Concern about medical insurance ○ Child does not need this service ○ Other		○ Only one time ○ 1 or more times/week ○ 1 or more times/month ○ Every 6 months ○ Every year ○ As needed ○ Other – Specify	○ Very important ○ Somewhat important ○ Not important

Pulmonology ○ Yes ○ No	○ Not recommended ○ None available ○ Unaware of service availability ○ Too expensive ○ Concern about medical insurance ○ Child does not need this service ○ Other		○ Only one time ○ 1 or more times/week ○ 1 or more times/month ○ Every 6 months ○ Every year ○ As needed ○ Other – Specify	○ Very important ○ Somewhat important ○ Not important
Surgery ○ Yes ○ No	○ Not recommended ○ None available ○ Unaware of service availability ○ Too expensive ○ Concern about medical insurance ○ Child does not need this service ○ Other		○ Only one time ○ 1 or more times/week ○ 1 or more times/month ○ Every 6 months ○ Every year ○ As needed ○ Other – Specify	○ Very important ○ Somewhat important ○ Not important
Transplant Evaluation (kidney, heart, and/or liver) ○ Yes ○ No	○ Not recommended ○ None available ○ Unaware of service availability ○ Too expensive ○ Concern about medical insurance ○ Child does not need this service ○ Other		○ Only one time ○ 1 or more times/week ○ 1 or more times/month ○ Every 6 months ○ Every year ○ As needed ○ Other – Specify	○ Very important ○ Somewhat important ○ Not important
Transplant Reception (kidney, heart, and/or liver) ○ Yes ○ No	○ Not recommended ○ None available ○ Unaware of service availability ○ Too expensive ○ Concern about medical insurance ○ Child does not need this service ○ Other		○ Only one time ○ 1 or more times/week ○ 1 or more times/month ○ Every 6 months ○ Every year ○ As needed ○ Other – Specify	○ Very important ○ Somewhat important ○ Not important

1.2b Are there any other healthcare specialty services that provide care for you child?

_____
_____
_____
_____

1.2c What is your level of satisfaction with the care your child received from these healthcare specialty services?

- o Very Satisfied
- o Somewhat Satisfied
- o Neither Satisfied or Dissatisfied
- o Somewhat Dissatisfied
- o Very Dissatisfied

1.2d What was your personal experience in accessing these healthcare specialty services?

- o Easy to Access
- o Somewhat Easy to Access
- o Difficult to Access
- o Extremely Difficult to Access
- o Is there anything else that you would like to add concerning your personal experience in accessing services?

_____
_____
_____
_____

1.2e How well do these healthcare specialty services understand your child's diagnosis of propionic acidemia?

- o Very Well
- o Well
- o Well Enough
- o Not Well
- o Not at all

1.2f How well did these healthcare specialty services incorporate your child's diagnosis of propionic acidemia into their clinical management of him/her?

- o Very Well
- o Well
- o Well Enough
- o Not Well
- o Not at all

1.2g Were any of these specialty services especially responsive or unresponsive to your child's needs?

- o No
- o Yes, please explain:

_____
_____
_____
_____

1.2h Is there anything else about your experience with these specialty services that you would like to share?

_____
_____
_____
_____

1.2i Are there any medical needs that your child has that you feel are not being adequately addressed by the providers we discussed?

- o No
- o Yes, please explain:

_____
_____
_____
_____

1.3a Therapeutic Services

Does your child participate in or seek help from the following?	If no, why not?	If yes, how old was your child at the first visit?	If yes, how often?	How important is this service to your child's care?

Occupational Therapy ○ Yes ○ No	○ Not recommended ○ None available ○ Unaware of service availability ○ Too expensive ○ Concern about medical insurance ○ Child does not need this service ○ Other		○ Only one time ○ 1 or more times/week ○ 1 or more times/month ○ Every 6 months ○ Every year ○ As needed ○ Other – Specify	○ Very important ○ Somewhat important ○ Not important
Physical Therapy ○ Yes ○ No	○ Not recommended ○ None available ○ Unaware of service availability ○ Too expensive ○ Concern about medical insurance ○ Child does not need this service ○ Other		○ Only one time ○ 1 or more times/week ○ 1 or more times/month ○ Every 6 months ○ Every year ○ As needed ○ Other – Specify	○ Very important ○ Somewhat important ○ Not important
Respiratory Therapy ○ Yes ○ No	○ Not recommended ○ None available ○ Unaware of service availability ○ Too expensive ○ Concern about medical insurance ○ Child does not need this service ○ Other		○ Only one time ○ 1 or more times/week ○ 1 or more times/month ○ Every 6 months ○ Every year ○ As needed ○ Other – Specify	○ Very important ○ Somewhat important ○ Not important
Speech-language Therapy ○ Yes ○ No	○ Not recommended ○ None available ○ Unaware of service availability ○ Too expensive ○ Concern about medical insurance ○ Child does not need this service ○ Other		○ Only one time ○ 1 or more times/week ○ 1 or more times/month ○ Every 6 months ○ Every year ○ As needed ○ Other – Specify	○ Very important ○ Somewhat important ○ Not important

1.3b Are there any additional therapeutic services that provide care for your child?

_____
_____
_____
_____

1.3c What is your level of satisfaction with the care your child received from these therapeutic services?

- o Very Satisfied
- o Somewhat Satisfied
- o Neither Satisfied or Dissatisfied
- o Somewhat Dissatisfied
- o Very Dissatisfied

1.3d What was your personal experience in accessing these therapeutic services?

- o Easy to Access
- o Somewhat Easy to Access
- o Difficult to Access
- o Extremely Difficult to Access
- o Is there anything else that you would like to add concerning your personal experience in accessing services?

_____
_____
_____
_____

1.3e How well do these therapeutic services understand your child's diagnosis of propionic acidemia?

- o Very Well
- o Well
- o Well Enough
- o Not Well
- o Not at all

1.3f How well did these therapeutic services incorporate your child's diagnosis of propionic acidemia into their clinical management of him/her?

- o Very Well
- o Well
- o Well Enough
- o Not Well
- o Not at all

1.3g Were any of these therapeutic services especially responsive or unresponsive to your child's needs?

- o No
- o Yes, please explain:

_____
_____
_____
_____

1.3h Is there anything else about your experience with these therapeutic services that you would like to share?

_____
_____
_____
_____

1.3i Are there any medical needs that your child has that you feel are not being adequately addressed by the services we discussed?

- o No
- o Yes, please explain:

_____
_____
_____
_____

1.4a Home Health Services

Does your child participate in or seek help from the following?	If no, why not?	If yes, how old was your child at the first visit?	If yes, how often?	How important is this service to your child's care?
Home Health Care o Yes o No	o Not recommended o None available o Unaware of service availability o Too expensive o Concern about medical insurance o Child does not need this service o Other		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months o Every year o As needed o Other – Specify	o Very important o Somewhat important o Not important
Medical Home o Yes o No	o Not recommended o None available o Unaware of service availability o Too expensive o Religious reason o Concern about medical insurance o Child does not need this service o Other		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months o Every year o As needed o Other – Specify	o Very important o Somewhat important o Not important
Nutritional Services (WIC/MAC) o Yes o No	o Not recommended o None available o Unaware of service availability o Too expensive o Concern about medical insurance o Child does not need this service o Other		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months o Every year o As needed o Other – Specify	o Very important o Somewhat important o Not important
Personal Care Attendant (PCA) o Yes o No	o Not recommended o None available o Unaware of service availability o Too expensive o Religious reason o Concern about medical insurance o Child does not need this service o Other		o Only one time o 1-3 times/week o 1-2 times/month o Every 6 months o Every year o As needed o Other – Specify	o Very important o Somewhat important o Not important

Public Health Nurse o Yes o No	o Not recommended o None available o Unaware of service availability o Too expensive o Concern about medical insurance o Child does not need this service o Other		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months o Every year o As needed o Other – Specify	o Very important o Somewhat important o Not important
Respite Care o Yes o No	o Not recommended o None available o Unaware of service availability o Too expensive o Concern about medical insurance o Child does not need this service o Other		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months o Every year o As needed o Other – Specify	o Very important o Somewhat important o Not important

1.4b Are there any additional home health services that your child receives?

_____
_____
_____
_____

1.4c What is your level of satisfaction with the home health services your child receives?

o Very Satisfied
o Somewhat Satisfied
o Neither Satisfied or Dissatisfied
o Somewhat Dissatisfied
o Very Dissatisfied

1.4d What was your personal experience in accessing these home health services?

o Easy to Access
o Somewhat Easy to Access
o Difficult to Access
o Extremely Difficult to Access
o Is there anything else that you would like to add concerning your personal experience in accessing services?

_____
_____
_____
_____

1.4e How well do these home health services understand your child's diagnosis of propionic acidemia?

o Very Well
o Well
o Well Enough
o Not Well
o Not at all

1.4f How well did these home health services incorporate your child's diagnosis of propionic acidemia into their management of him/her?

o Very Well
o Well
o Well Enough
o Not Well
o Not at all

1.4g Were any of these home health services especially responsive or unresponsive to your child's needs?

o No
o Yes, please explain:

_____
_____
_____
_____

1.4h Is there anything else about your experience with these home health services that you would like to share?

_____
_____
_____
_____

1.4i Are there any medical needs that your child has that you feel are not being adequately addressed by the services we discussed?

o No
o Yes, please explain:

_____
_____
_____
_____

1.5a School Related and Support Services

Do you or your child participate in or seek help from the following?	If no, why not?	If yes, how old was your child at the first visit?	If yes, how often?	How important is this service to you and your child's care?
Daycare o Yes o No	o Not recommended o None available o Unaware of service availability o Too expensive o Concern about medical insurance o Child does not need this service o Other		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months o Every year o As needed o Other – Specify	o Very important o Somewhat important o Not important
Family Support - Other o Yes o If yes, which one _____ o No	o Not recommended o None available o Unaware of service availability o Too expensive o Concern about medical insurance o Child does not need this service o Other		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months o Every year o As needed o Other – Specify	o Very important o Somewhat important o Not important

Head Start o Yes o No	o Not recommended o None available o Unaware of service availability o Too expensive o Concern about medical insurance o Child does not need this service o Other		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months o Every year o As needed o Other – Specify	o Very important o Somewhat important o Not important
Preschool o Yes o No	o Not recommended o None available o Unaware of service availability o Too expensive o Concern about medical insurance o Child does not need this service o Other		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months o Every year o As needed o Other – Specify	o Very important o Somewhat important o Not important
Social Services (County, Medical, Developmental Disability) o Yes o No	o Not recommended o None available o Unaware of service availability o Too expensive o Concern about medical insurance o Child does not need this service o Other		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months o Every year o As needed o Other – Specify	o Very important o Somewhat important o Not important
Support groups/self help groups related to PA o Yes o If yes, which one _____ o No	o Not recommended o None available o Unaware of service availability o Too expensive o Concern about medical insurance o Child does not need this service o Other		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months o Every year o As needed o Other – Specify	o Very important o Somewhat important o Not important
Waivered Services (CAC/CADI waiver, other waivers) o Yes o No	o Not recommended o None available o Unaware of service availability		o Only one time o 1 or more times/week o 1 or more times/month o Every 6 months	o Very important o Somewhat important o Not important

	o Too expensive o Concern about medical insurance o Child does not need this service o Other		o Every year o As needed o Other – Specify	

1.5b Are there any additional services that your child receives?

_____
_____
_____
_____

1.5c What is your level of satisfaction with the school related and support services your child receives?

o Very Satisfied
o Somewhat Satisfied
o Neither Satisfied or Dissatisfied
o Somewhat Dissatisfied
o Very Dissatisfied

1.5d What was your personal experience in accessing these services?

o Easy to Access
o Somewhat Easy to Access
o Difficult to Access
o Extremely Difficult to Access
o Is there anything else that you would like to add concerning your personal experience in accessing services?

_____
_____
_____
_____

1.5e How well do these services understand your child's diagnosis of propionic acidemia?

o Very Well
o Well
o Well Enough
o Not Well
o Not at all

1.5f How well did these services incorporate your child's diagnosis of propionic acidemia into their management of him/her?

o Very Well
o Well
o Well Enough
o Not Well
o Not at all

1.5g Were any of these services especially responsive or unresponsive to your child's needs?

o No
o Yes, please explain:

_____
_____
_____
_____

1.5h Is there anything else about your experience with these services that you would like to share?

_____
_____
_____
_____

1.5i Are there any medical needs that your child has that you feel are not being adequately addressed by the services we discussed?

o No
o Yes, please explain:

_____

_____
_____
_____

1.6 How well do you think your child's various healthcare providers communicate with each other?

- o Very Well
- o Well
- o Well Enough
- o Not Well
- o Not at all
- o Is there anything else that you would like to add concerning the communication between your child's healthcare providers?

_____
_____
_____
_____

1.6 Did you discuss the services that you use with your healthcare team?

- o Yes
- o No, why not? Please explain:

_____
_____
_____
_____

1.7 Did your child spend any time in the newborn intensive care unit?

- o No
- o Yes, for how long? _____

Script:

Now I am going to ask you questions regarding your child's school experience. Some of these questions will have specific answers and other questions will be asking for your opinion.

Do you have any questions before we begin?

2. Early Intervention Programs and Schooling

1. Did your child receive any pre-school services through early intervention or Child Find programs?
- o No
- o Yes, at what age? ___ ___

2. If yes, what was your personal experience in accessing early intervention services?
- o Easy to Access
- o Somewhat Easy to Access
- o Difficult to Access
- o Extremely Difficult to Access

3. If your child did receive pre-school services, what kinds of services were provided? (Choose all that apply)
- o Counseling
- o Educational Instruction
- o Occupational Therapy
- o Physical Therapy
- o Speech Therapy
- o Other

4. What is your level of satisfaction with the services that were provided?

o Very Satisfied	o Neither Satisfied or Dissatisfied	o Somewhat Dissatisfied
o Somewhat Satisfied		o Very Dissatisfied

o Is there anything else that you would like to add concerning your personal experience in accessing services?

_____
_____
_____
_____

5. Is your child school aged?
o No
o Yes

6. If your child is school aged, what kind of school does he/she attend?
o Public
o Private
o Home-schooled
o Other (specify): _____

7. What grade is your child in? _____

8. Does your child receive special services?
o Yes, he/she has IEP (Individualized Education Plan)
o Yes, he/she has a 504 plan
o Yes, other (specify): _____
o No, he receives no services
o Unsure

9. In what kind of classrooms does your child work?
o Regular
o Special education classes with some main streaming
o Special education classes with no main streaming
o Unsure

10. In general, what kind of difficulties, if any, does your child have at school?
o None
o Access problems or problems getting around to classes
o Attention Deficit Disorder
o Behavior Problems
o Cognitive Impairment
o Hyperactivity/Impulsivity
o Learning Disabilities (specify): _____
o Making friends
o Problems fitting in
o Other (specify): _____

11. If your child does receive services through school what are they? (Choose all that apply)
o Occupational Therapy
o Personal Aide
o Physical Therapy
o Psychological Services (i.e., counseling, social skills training, behavior management programs)
o Special Education Services (to address specific learning problems)
o Speech and Language Therapy
o Other (specify): _____

12. How well does school staff understand your child's diagnosis?
- o Very Well
- o Well
- o Well Enough
- o Not Well
- o Not at all

13. Did the school staff demonstrate an interest in or eagerness to learn about propionic acidemia?
- o Yes, very much so
- o Somewhat
- o Not at all

14. Of the following people outside of the school, who has intervened with the school on behalf of your child so that appropriate services could be established or continued? (Choose all that apply)
- o Primary Care Doctor
- o Metabolic Healthcare Provider
- o Mental Health Therapist
- o Nurse
- o Social Worker
- o Special Education Advocate or Consultant
- o Special Education Lawyer
- o None of the above
- o Other (specify): _____

15. How would you describe the level of support you and your child currently receive from school?
- o Very High
- o High
- o Acceptable
- o Low
- o Very Low

16. At what school level did you experience the most support?
- o Pre-school
- o Elementary (K-6)
- o Middle School (6-8)
- o High School (9-12)

17. At what school level did you experience the least support?
- o Pre-school
- o Elementary (K-6)
- o Middle School (6-8)
- o High School (9-12)

18. Of the following people inside school, who provides support and understanding of your child's needs at school: (Choose all that apply)
- o Aide
- o Counselor / Social Worker
- o Occupational Therapist
- o Physical Therapist
- o Principal
- o Resource Teacher
- o School Nurse / Health Aide
- o Speech Therapist
- o Teacher
- o Other (specify): _____

19. What has been the most difficult challenge you faced while getting your child through school?

_____
_____
_____
_____
_____

20. Do you have any additional comments about your child's school experience?

_____
_____
_____

_____
_____

21. Are there any medical needs that your child has that you feel are not being adequately addressed by the early intervention programs, school services, and school staff?
- o No
- o Yes, please explain:

  _____
  _____
  _____
  _____

Script:

Now I am going to ask you several questions regarding your family demographics.
Do you have any questions before we begin?

3. Family Demographics

1. In what type of area is your home located, for example, urban/city, suburban, etc.?
- o Urban
- o Suburban
- o Rural
- o Other (Specify): _____

2. What is the size of your family?

Number of children: _____

Number of children with propionic academia: _____

3. Do you have access to the Internet?
- o No
- o Yes

4. If yes, where do you have access?
- o In your home
- o At work/office
- o At public library
- o Other (Specify): _____

5. Level of education completed for each Parent/Guardian

Maternal Education:
- o Unknown
- o 8th grade/less
- o 9th-12th grade, no diploma
- o High school graduate or GED completed
- o Some college credit but no degree
- o Associate degree (e.g., AA, AS)
- o Bachelor's degree (e.g., BA, AB, BS)
- o Master's degree (e.g., MA, MS, MEng, MEd, MSW, MBA)
- o Doctorate (e.g., PhD, EdD) or Professional degree (e.g., MD, DDS, DVM, LLB, JD)

Paternal Education:
- o Unknown
- o 8th grade/less
- o 9th-12th grade, no diploma
- o High school graduate or GED completed
- o Some college credit but no degree
- o Associate degree (e.g., AA, AS)
- o Bachelor's degree (e.g., BA, AB, BS)
- o Master's degree (e.g., MA, MS, MEng, MEd, MSW, MBA)
- o Doctorate (e.g., PhD, EdD) or Professional degree (e.g., MD, DDS, DVM, LLB, JD)

6. What type of health insurance coverage do you have?
- o None
- o HMO
- o Other managed care plan (including preferred provider organizations (PPO's) and Point of Service (POS) plans).
- o Medicaid/State sponsored programs
- o Military
- o Self-pay
- o Traditional insurance plans (BlueCross / BlueShield, etc.)
- o Don't Know or remember
- o Other (Specify): _____

Closing Script:

Thank you for participating in this telephone interview. Your answers will help us better understand the services and resources that are the most useful to patients with propionic acidemia.

If you have any additional questions or comments concerning this study please don't hesitate to contact myself or Cate Walsh Vockley.

My contact information is:
Phone: (412) 692-6770
Email: amanda.jacquart@chp.edu

Cate's Contact information is:
Phone: (412) 692-7349
Email: catherine.walshvockley@chp.edu

My contact information as well as Cate's can also be found on the invitation letter that you received in the mail.

APPENDIX K

FISHER'S EXACT TEST

Table 18. Highest Level of Education * Total Number of Health Services Reported Crosstabulation

			Total Number of Health Services Reported			Total
			Less than 10	10 to 20	20+	
Highest Level of Education Between Mother and Father	Post-Graduate	Count	1	1	0	2
		Expected Count	1.3	.3	.3	2.0
	Associate Degree to College Graduate	Count	5	0	2	7
		Expected Count	4.7	1.2	1.2	7.0
	9-12 years (no diploma) to Some College	Count	2	1	0	3
		Expected Count	2.0	.5	.5	3.0
Total		Count	8	2	2	12
		Expected Count	8.0	2.0	2.0	12.0

Table 19. Highest Level of Education * Total Number of Health Services Reported Fisher's Exact Test

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	4.536[a]	4	.338	.321		
Likelihood Ratio	5.854	4	.210	.265		
Fisher's Exact Test	4.441			.321		
Linear-by-Linear Association	.080[b]	1	.777	1.000	.486	.181
N of Valid Cases	12					

a. 9 cells (100.0%) have expected count less than 5. The minimum expected count is .33.

b. The standardized statistic is -.283.

Table 20. Type of Insurance * Total Number of Health Services Reported Crosstabulation

			Total Number of Health Services Reported			Total
			Less than 10	10 to 20	20+	
Type of Insurance	State/Federal Insurance (Medicaid/Medicare)	Count	5	2	2	9
		Expected Count	4.8	1.6	2.6	9.0
	Commercial/Private Insurance	Count	4	1	3	8
		Expected Count	4.2	1.4	2.4	8.0
Total		Count	9	3	5	17
		Expected Count	9.0	3.0	5.0	17.0

Table 21. Type of Insurance * Total Number of Health Services Reported Fisher's Exact Test

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	.588[a]	2	.745	1.000		
Likelihood Ratio	.594	2	.743	1.000		
Fisher's Exact Test	.754			1.000		
Linear-by-Linear Association	.225[b]	1	.635	.793	.419	.190
N of Valid Cases	17					

a. 6 cells (100.0%) have expected count less than 5. The minimum expected count is 1.41.

b. The standardized statistic is .475.

APPENDIX L

WILCOXON RANK-SUM TEST

Table 22. Days of Age from Birth to Initiation of Intervention Mean Rank by Diagnosis Method

	Diagnosis Method	N	Mean Rank	Sum of Ranks
Days of Age from Birth to Initiation of Intervention	Abnormal NBS	10	11.55	115.50
	Clinical Presentation	18	16.14	290.50
	Total	28		

Table 23. Days of Age from Birth to Initiation of Intervention Rank Test Statistics

	Days of Age from Birth to Initiation of Intervention
Mann-Whitney U	60.500
Wilcoxon W	115.500
Z	-1.419
Asymp. Sig. (2-tailed)	.156
Exact Sig. [2*(1-tailed Sig.)]	.160[b]

a. Grouping Variable: Diagnosis Method

b. Not corrected for ties.

Table 24. Days of Age at Time of Initial Face-to-Face Metabolic Consultation Mean Rank by Diagnosis Method

	Diagnosis Method	N	Mean Rank	Sum of Ranks
Days of Age at Time of Initial Face-to-Face Metabolic Consultation	Abnormal NBS	10	12.05	120.50
	Clinical Presentation	18	15.86	285.50
	Total	28		

Table 25. Days of Age at Time of Initial Face-to-Face Metabolic Consultation Rank Test Statistics

	Days of Age at Time of Initial Face-to-Face Metabolic Consultation
Mann-Whitney U	65.500
Wilcoxon W	120.500
Z	-1.177
Asymp. Sig. (2-tailed)	.239
Exact Sig. [2*(1-tailed Sig.)]	.245[b]

a. Grouping Variable: Diagnosis Method

b. Not corrected for ties.

APPENDIX M

VARIABILITY OBSERVED IN THE NUMBER OF TIMES OTHER HEALTH SERVICES WERE REPORTED PER "FOLLOW-UP" SUBJECT

Other Health Services Received Currently	Number of Times Reported Per Subject												Number of Subjects	Total Number Reported
	1	2	3	4	5	6	7	8	9	10	12	16		
Audiology	0	0	1	0	0	0	0	0	0	0	0	0	1	3
Behavioral/Developmental Pediatrics	1	0	0	0	0	0	0	0	0	0	0	0	1	1
Cardiology	0	4	2	1	2	0	0	0	1	1	1	0	12	59
Dentistry	0	1	0	0	1	0	0	0	0	0	0	0	2	7
Dietitian	2	1	1	1	0	0	0	0	0	0	0	0	5	11
Feeding Therapy	1	0	0	0	0	0	0	0	0	0	0	0	1	1
Gastroenterology	0	0	0	0	1	0	0	0	0	0	0	0	1	5
Hematology	2	0	0	0	0	0	0	0	0	0	0	0	2	2
Home Health Care	1	0	0	0	0	0	0	0	0	0	0	0	1	1
Nephrology	1	1	0	0	0	0	0	0	0	0	0	0	2	3
Neurology	2	2	1	0	1	0	0	0	0	0	0	1	7	30
None	4	1	0	0	0	0	0	1	0	0	0	0	6	14
Occupational Therapy	1	0	1	0	2	0	1	0	0	0	0	1	6	37
Ophthalmology	0	2	2	0	1	1	0	0	0	0	0	0	6	21
Orthopedics	0	1	0	0	1	0	0	0	0	0	0	0	2	7
Other	1	0	1	0	0	0	0	0	0	0	0	0	2	4
Physical Therapy	1	1	1	0	1	0	1	0	0	0	0	1	6	34
Preschool	0	1	0	0	0	0	0	0	0	0	0	0	1	2
Primary Care Provider	4	0	0	0	0	0	0	0	0	0	0	0	4	4
Pulmonology	1	0	0	0	1	0	0	0	0	0	0	0	2	6
Speech-Language Therapy	0	0	3	0	1	0	0	1	0	0	0	0	5	22
Surgery	1	0	0	0	0	0	0	0	0	0	0	0	1	1
Urology	1	0	0	0	0	0	0	0	0	0	0	0	1	1

APPENDIX N

VARIABILITY OBSERVED IN THE NUMBER OF TIMES COMMUNITY RESOURCES WERE REPORTED PER "FOLLOW-UP" SUBJECT

	Number of Times Reported Per Subject									
Community Resources Received Currently	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Number of Subjects**	**Total Number Reported**
Daycare	0	0	0	0	1	0	0	0	1	5
Family Support - Other	0	1	0	0	0	0	0	0	1	2
Family Support Group Related to this IBEM	0	1	0	0	0	0	0	0	1	2
Head Start	0	1	0	0	0	0	0	0	1	2
None	2	4	2	0	0	0	1	1	10	31
Nutritional Services (WIC/MAC)	2	1	0	0	0	0	0	0	3	4
Other	2	0	0	1	0	0	0	0	3	6
Preschool	1	1	0	0	1	0	0	0	3	8
Social Services - County	0	1	0	0	0	0	0	0	1	2
Social Services - Developmental disability	0	0	1	0	1	0	0	0	2	8
Social Services - Medical	2	0	0	1	0	0	0	0	3	6

APPENDIX O

VARIABILITY OBSERVED IN THE NUMBER OF TIMES PROVIDERS WERE REPORTED PER "FOLLOW-UP" SUBJECT

	Number of Times Reported Per Subject															
Providers Seen at this Visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**18**	**19**	**Number of Subjects**	**Total Number Reported**
Dietitian	2	5	4	0	2	0	0	1	1	2	0	0	1	0	18	89
Genetic Counselor	2	1	1	0	0	0	0	0	0	1	0	0	0	0	5	17
Nurse	6	1	0	0	1	0	0	0	0	0	0	0	0	0	8	13
Nurse Practitioner	0	1	2	1	0	0	0	0	0	0	0	0	0	0	4	12
Physician	2	6	4	0	1	0	1	0	0	3	0	0	0	1	18	87
Social Worker	2	1	0	0	0	1	0	0	0	0	0	0	0	0	4	10

BIBLIOGRAPHY

Alberola, T. M., R. Bautista-Llacer, X. Vendrell, E. Garcia-Mengual, M. Pardo, M. Vila and C. Calatayud (2011). "Case report: birth of healthy twins after preimplantation genetic diagnosis of propionic acidemia." J Assist Reprod Genet 28(3): 211-216.

Bailey, D. B., M. Raspa and M. G. Olmsted (2010). "Using a parent survey to advance knowledge about the nature and consequences of fragile X syndrome." Am J Intellect Dev Disabil 115(6): 447-460.

Berry, S. A., A. M. Jurek , C. Anderson and K. Bentler (2010). "The inborn errors of metabolism information system: A project of the Region 4 Genetics Collaborative Priority 2 Workgroup." Genetics in Medicine 12.

Bultron, G., M. R. Seashore, D. S. Pashankar and S. Z. Husain (2008). "Recurrent acute pancreatitis associated with propionic acidemia." J Pediatr Gastroenterol Nutr 47(3): 370-371.

Burton, B. K. (1998). "Inborn errors of metabolism in infancy: a guide to diagnosis." Pediatrics 102(6): E69.

Chapman, K., A. Gropman, E. MacLeod, K. Stagni, M. Summar, K. Ueda, N. Ah Mew, J. Franks, E. Island, D. Matern, L. Pena, B. Smith, V. Sutton, T. Urv, C. Venditti and A. Chakrapani (2012). "Acute management of propionic acidemia." Mol Genet Metab 105(1): 16-25.

Chapman, K. and M. Summar (2012). "Propionic acidemia consensus conference summary." Mol Genet Metab 105(1): 3-4.

Childs, B., W. L. Nyhan, M. Borden, L. Bard and R. E. Cooke (1961). "Idiopathic hyperglycinemia and hyperglycinuria: a new disorder of amino acid metabolism. I." Pediatrics 27: 522-538.

Desviat, L., B. Pérez, C. Pérez-Cerdá, P. Rodríguez-Pombo, S. Clavero and M. Ugarte (2004). "Propionic acidemia: mutation update and functional and structural effects of the variant alleles." Mol Genet Metab 83(1-2): 28-37.

Desviat, L. R., S. Clavero, C. Perez-Cerda, R. Navarrete, M. Ugarte and B. Perez (2006). "New splicing mutations in propionic acidemia." J Hum Genet 51(11): 992-997.

Desviat, L. R., R. Sanchez-Alcudia, B. Perez, C. Perez-Cerda, R. Navarrete, R. Vijzelaar and M. Ugarte (2009). "High frequency of large genomic deletions in the PCCA gene causing propionic acidemia." Mol Genet Metab 96(4): 171-176.

Dionisi-Vici, C., F. Deodato, W. Röschinger, W. Rhead and B. Wilcken (2006). "'Classical' organic acidurias, propionic aciduria, methylmalonic aciduria and isovaleric aciduria: long-term outcome and effects of expanded newborn screening using tandem mass spectrometry." Journal of inherited metabolic disease 29(2-3): 383-389.

Griggs, R., M. Batshaw, M. Dunkle, R. Gopal-Srivastava, E. Kaye, J. Krischer, T. Nguyen, K. Paulus, P. Merkel and N. Rare Diseases Clinical Research (2009). "Clinical research for rare disease: opportunities, challenges, and solutions." Molecular genetics and metabolism 96(1): 20-26.

Grünert, S., S. Müllerleile, L. De Silva, M. Barth, M. Walter, K. Walter, T. Meissner, M. Lindner, R. Ensenauer, R. Santer, O. Bodamer, M. Baumgartner, M. Brunner-Krainz, D. Karall, C. Haase, I. Knerr, T. Marquardt, J. Hennermann, R. Steinfeld, S. Beblo, H.-G. Koch, V. Konstantopoulou, S. Scholl-Bürgi, A. van Teeffelen-Heithoff, T. Suormala, W. Sperl, J. Kraus, A. Superti-Furga, K. Schwab and J. Sass (2013). "Propionic acidemia: clinical course and outcome in 55 pediatric and adolescent patients." Orphanet journal of rare diseases 8: 6.

Grunert, S. C., S. Mullerleile, L. de Silva, M. Barth, M. Walter, K. Walter, T. Meissner, M. Lindner, R. Ensenauer, R. Santer, O. A. Bodamer, M. R. Baumgartner, M. Brunner-Krainz, D. Karall, C. Haase, I. Knerr, T. Marquardt, J. B. Hennermann, R. Steinfeld, S. Beblo, H. G. Koch, V. Konstantopoulou, S. Scholl-Burgi, A. van Teeffelen-Heithoff, T. Suormala, W. Sperl, J. P. Kraus, A. Superti-Furga, K. O. Schwab and J. O. Sass (2012). "Propionic acidemia: neonatal versus selective metabolic screening." J Inherit Metab Dis 35(1): 41-49.

Haberlandt, E., C. Canestrini, M. Brunner-Krainz, D. Möslinger, K. Mussner, B. Plecko, S. Scholl-Bürgi, W. Sperl, K. Rostásy and D. Karall (2009). "Epilepsy in patients with propionic acidemia." Neuropediatrics 40(3): 120-125.

Jameson, E. and J. Walter (2008). "Cardiac arrest secondary to long QT(C)in a child with propionic acidemia." Pediatr Cardiol 29(5): 969-970.

Lam, C., L. R. Desviat, C. Perez-Cerda, M. Ugarte, B. A. Barshop and S. Cederbaum (2011). "45-Year-old female with propionic acidemia, renal failure, and premature ovarian failure; late complications of propionic acidemia?" Mol Genet Metab 103(4): 338-340.

Magdalena, U., P. r.-C. Celia, R. g.-P. Pilar, R. D. Lourdes, P. r. Bel□n, R. Eva, M. Silvia, C. Eric, O. Toshihiro and A. G. Roy (1999). "Overview of mutations in thePCCA andPCCB genes causing propionic acidemia." Human Mutation 14.

Mardach, R., M. Verity and S. Cederbaum (2005). "Clinical, pathological, and biochemical studies in a patient with propionic acidemia and fatal cardiomyopathy." Molecular genetics and metabolism 85(4): 286-290.

Marsolo, K. (2012). "Approaches to facilitate institutional review board approval of multicenter research studies." Med Care 50 Suppl: S77-81.

Muro, S., B. Pérez, L. Desviat, P. Rodríguez-Pombo, C. Pérez-Cerdá, S. Clavero and M. Ugarte (2001). "Effect of PCCB gene mutations on the heteromeric and homomeric assembly of propionyl-CoA carboxylase." Mol Genet Metab 74(4): 476-483.

Patel, M. X. (2003). "Challenges in recruitment of research participants." Advances in Psychiatric Treatment 9.

Pena, L. and B. Burton (2012). "Survey of health status and complications among propionic acidemia patients." American journal of medical genetics. Part A 158A(7): 1641-1646.

Pena, L., J. Franks, K. A. Chapman, A. Gropman, N. Ah Mew, A. Chakrapani, E. Island, E. MacLeod, D. Matern, B. Smith, K. Stagni, V. R. Sutton, K. Ueda, T. Urv, C. Venditti, G. M. Enns and M. L. Summar (2012). "Natural history of propionic acidemia." Mol Genet Metab 105(1): 5-9.

Perez-Cerda, C., B. Merinero, P. Rodriguez-Pombo, B. Perez, L. R. Desviat, S. Muro, E. Richard, M. J. Garcia, J. Gangoiti, P. Ruiz Sala, P. Sanz, P. Briones, A. Ribes, M. Martinez-Pardo, J. Campistol, M. Perez, R. Lama, M. L. Murga, T. Lema-Garrett, A. Verdu and M. Ugarte (2000). "Potential relationship between genotype and clinical outcome in propionic acidaemia patients." Eur J Hum Genet 8(3): 187-194.

Perez-Cerda, C., B. Perez, B. Merinero, L. R. Desviat, P. Rodriguez-Pombo and M. Ugarte (2004). "Prenatal diagnosis of propionic acidemia." Prenat Diagn 24(12): 962-964.

Peters-Lawrence, M., M. Bell, L. Hsu, I. Osunkwo, P. Seaman, M. Blackwood, E. Guillaume, R. Bellevue, L. Krishnamurti, W. Smith, C. Dampier, C. Minniti and N. Sickle Cell Disease Clinical Research (2012). "Clinical trial implementation and recruitment: lessons learned from the early closure of a randomized clinical trial." Contemporary clinical trials 33(2): 291-297.

Sanchez-Alcudia, R., B. Perez, M. Ugarte and L. R. Desviat (2012). "Feasibility of nonsense mutation readthrough as a novel therapeutical approach in propionic acidemia." Hum Mutat 33(6): 973-980.

Sass, J. O., M. Hofmann, D. Skladal, E. Mayatepek, B. Schwahn and W. Sperl (2004). "Propionic acidemia revisited: a workshop report." Clin Pediatr (Phila) 43(9): 837-843.

Sibbald, B., J. Addington-Hall, D. Brenneman and P. Freeling (1994). "Telephone versus postal surveys of general practitioners: methodological considerations." Br J Gen Pract 44(384): 297-300.

Sindgikar, S. P., S. Rao, R. D. Shenoy and N. Kamath (2013). "Biochemical basis of heterogeneity in acute presentations of propionic acidemia." Indian J Clin Biochem 28(1): 95-97.

Strauss, K. A. and E. G. Puffenberger (2009). "Genetics, medicine, and the Plain people." Annu Rev Genomics Hum Genet 10: 513-536.

Sutton, V. R., K. A. Chapman, A. L. Gropman, E. MacLeod, K. Stagni, M. L. Summar, K. Ueda, N. Ah Mew, J. Franks, E. Island, D. Matern, L. Pena, B. Smith, T. Urv, C. Venditti and A. Chakarapani (2012). "Chronic management and health supervision of individuals with propionic acidemia." Mol Genet Metab 105(1): 26-33.

Tahara, T., J. P. Kraus, T. Ohura, L. E. Rosenberg and W. A. Fenton (1993). "Three independent mutations in the same exon of the PCCB gene: differences between Caucasian and Japanese propionic acidaemia." J Inherit Metab Dis 16(2): 353-360.

Ugarte, M., C. Perez-Cerda, P. Rodriguez-Pombo, L. R. Desviat, B. Perez, E. Richard, S. Muro, E. Campeau, T. Ohura and R. A. Gravel (1999). "Overview of mutations in the PCCA and PCCB genes causing propionic acidemia." Hum Mutat 14(4): 275-282.

Uhlmann, W. R., J. L. Schuette, B. M. Yashar and ebrary Inc. (2009). A guide to genetic counseling. Hoboken, N.J., Wiley-Blackwell,: xxii, 624 p.

Williams, Z. R., P. E. Hurley, U. E. Altiparmak, S. E. Feldon, G. L. Arnold, E. Eggenberger and L. J. Mejico (2009). "Late onset optic neuropathy in methylmalonic and propionic acidemia." Am J Ophthalmol 147(5): 929-933.

Wolf, B., E. P. Paulsen and Y. E. Hsia (1979). "Asymptomatic propionyl CoA carboxylase deficiency in a 13-year-old girl." J Pediatr 95(4): 563-565.

Yorifuji, T., M. Kawai, J. Muroi, M. Mamada, K. Kurokawa, Y. Shigematsu, S. Hirano, N. Sakura, I. Yoshida, T. Kuhara, F. Endo, H. Mitsubuchi and T. Nakahata (2002). "Unexpectedly high prevalence of the mild form of propionic acidemia in Japan: presence of a common mutation and possible clinical implications." Human genetics 111(2): 161-165.