Molecular Genetics and Metabolism 107 (2012) 394–402

Contents lists available at SciVerse ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Spectrum of neurological and survival outcomes in pyruvate dehydrogenase complex (PDC) deficiency: Lack of correlation with genotype

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ARTICLE INFO

Article history:
Received 28 July 2012
Received in revised form 31 August 2012
Accepted 1 September 2012
Available online 7 September 2012

Keywords:
Intellectual disability
Hypotonia
Seizures
Corpus callosum
Leigh syndrome
Ketogenic diet

ABSTRACT

Pyruvate dehydrogenase complex (PDC) deficiency is a relatively common mitochondrial disorder that primarily presents with neurological manifestations and lactic acidemia. We analyzed the clinical outcomes and neurological features of 59 consented symptomatic subjects (27 M, 32 F), who were confirmed to have PDC deficiency with defined mutations in one of the genes of PDC (*PDHA1*, n = 53; *PDHB*, n = 4; *DLAT*, n = 2), including 47 different mutations, of which 22 were novel, and for whom clinical records and/or structured interviews were obtained.

39% of these subjects (23/59) have died. Of these, 91% (21/23) died before age 4 years, 61% (14/23) before 1 year, and 43% (10/23) before 3 months. 56% of males died compared with 25% of females. Causes of death included severe lactic acidosis, respiratory failure, and infection. In subjects surviving past 6 months, a broad range of intellectual outcomes was observed. Of 42 subjects whose intellectual abilities were professionally evaluated, 19% had normal or borderline intellectual ability (CQ/IQ ≥ 70), 10% had mild intellectual disability (ID) (CQ/IQ 55–69), 17% had moderate ID (CQ/IQ 40–54), 24% had severe ID (CQ/IQ 25–39) and 33% had profound ID (CQ/IQ < 25). Assessment by parents was comparable. Of 10 subjects who reached age 12 years, 9 had had professional IQ assessments, and only 4 had IQs ≥ 70 (only 2 of these 4 had assessments after age 12 years). The average outcome for females was severe-to-profound ID, whereas that of males was mild-to-moderate ID.

Of subjects for whom specific neurological data were available, the majority had hypotonia (89%), and hypertonia or mixed hyper-/hypotonia (49%) were common. Seizures (57%), microcephaly (49%), and structural brain abnormalities including ventriculomegaly (67%) and agenesis, dysgenesis, or hypoplasia of the corpus callosum (55%) were common. Leigh syndrome was found in only 35%. Structural brain abnormalities were more common in females, and Leigh syndrome was more common in males. In a subgroup of 16 ambulatory subjects > 3.5 years in whom balance was evaluated, ataxia was found in 13. Peripheral neuropathy was documented in 2 cases but not objectively evaluated in most subjects.

Outcomes of this population with genetically confirmed PDC deficiency are heterogeneous and not distinctive. Correlations between specific genotypes and outcomes were not established. Although more females survive, related to the prevalence of X-linked *PDHA1* mutations, symptomatic surviving females are generally more severely impaired cognitively and have a different pattern of neurological impairment compared to males. Neonatal or infant onset of symptoms was associated with poor outcomes. Males with *PDHA1* mutations and low fibroblast PDC activity were less likely to survive beyond infancy. Recurrence rate in siblings of subjects with *PDHA1* mutation was less than 5%. Paradoxically, in this retrospective review, potential factors considered possibly relevant to development, such as *in vitro* PDC activity, specific mutations, use of ketogenic diets, supplements, or medications, were generally not confirmed to be significantly correlated with objective

Abbreviations: CIDEM, Center for Inherited Disorders of Energy Metabolism; CQ, cognitive quotient; DCA, dichloroacetate; MELAS, mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes; PDC, pyruvate dehydrogenase complex; TPP, thiamine pyrophosphate.

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http://dx.doi.org/10.1016/j.ymgme.2012.09.001

outcomes of survival or neuro-cognitive function. Therefore, the basis of variability of these outcomes remains largely undetermined.

1. Introduction

Pyruvate dehydrogenase complex (PDC) deficiencies are inherited disorders of mitochondrial energy metabolism typically characterized by lactic acidemia and damage to the central or peripheral nervous systems. PDC, located in the mitochondrial matrix, catalyzes oxidative decarboxylation of pyruvate to acetyl CoA, which can enter the tricarboxylic acid cycle to generate reduced substrates for the electron transport chain. As such, it is a key step in the generation of cellular energy from carbohydrates and certain amino acids.

PDC contains 5 enzymes, 3 catalytic and 2 regulatory, as well as 3 cofactors and a binding protein. The first catalytic enzyme, pyruvate dehydrogenase (E1), is a heterotetramer of alpha and beta subunits encoded by the *PDHA1* and *PDHB* genes binding thiamine pyrophosphate (TPP). E1, the rate limiting step in pyruvate oxidation, is activated by pyruvate dehydrogenase phosphatases, encoded primarily by the *PDP1* gene, and inactivated by pyruvate dehydrogenase kinases. The second catalytic enzyme is E2, dihydrolipoamide transacetylase, encoded by the *DLAT* gene. The third catalytic enzyme is E3, dihydrolipoyl dehydrogenase, a flavoprotein encoded by the *DLD* gene. E3 is not specific to PDC, but is shared by other 2-ketoacid dehydrogenase complexes, and thus deficiencies of E3 activity typically have consequences beyond those expected for isolated PDC deficiency. E3 is bound to E2 by the E3 binding protein, encoded by the *PDHX* gene. Recently, mutations in genes outside of the PDC complex have been found to account for some cases of deficiency of PDC and other enzymes [1–4].

The phenotypic spectrum of PDC deficiency is broad, including severe congenital lactic acidosis with neonatal death, hypotonia, developmental delay, encephalopathy and/or seizures, and childhood-onset intermittent ataxia or isolated neuropathy with normal cognitive functioning. Limited suggested genotype–phenotype correlations for the various genes associated specifically with PDC deficiency have been proposed in the literature (see Discussion for details). Mutations of *DLD* or genes involved in biosynthesis of TPP and lipoate that are not specific to PDC may result in a broader range of clinical manifestations, extending beyond the nervous system.

The Center for Disorders of Energy Metabolism (CIDEM) has served for many years as a major reference lab in North America for biochemical diagnosis of PDC deficiency. About 10 years ago, we embarked on a project to collect more information about as many of these cases as possible and to systematically compare phenotypic manifestations with determination of genotypes on an investigative basis. We reviewed the developmental, neurological, and survival outcomes of PDC deficient cases from whom we were able to obtain consent and clinical information, and in whom we identified mutations of genes specific to PDC. These cases are the subject of this report.

2. Methods

This investigation and consent process were approved by the Institutional Review Board of University Hospitals Case Medical Center. Potential subjects were initially identified as having PDC deficiency based on enzyme assay in blood lymphocytes, cultured fibroblasts, or skeletal muscle [5]. These requested clinical analyses were performed in the CIDEM laboratory at University Hospitals Case Medical Center and Case Western Reserve University. Subjects with deficient PDC activity (less than the 3rd percentile of control samples in at least one assay) were potentially eligible for the study (see supplementary Table S-1 for reference ranges). Affected cases or their families were contacted through their referring physicians and then directly to obtain informed consent. After consent and release of medical information were obtained, cultured fibroblasts, stored frozen lymphocytes or muscle, or in a few cases, additional blood samples, were analyzed by sequencing up to 6 genes associated with PDC (*PDHA1*, *PDHB*, *DLAT*, *DLD*, *PDHX*, and *PDP1*), until a pathogenic mutation was found or not found. Both cDNA and genomic exons were sequenced in most cases, as previously described [6,7]. Reference sequences used for identification of mutations reported here were: NM_000284.3 (*PDHA1*); NM_000925.2 (*PDHB*); and NM_001931.4 (*DLAT*).

In two cases, mutational analysis was done in clinical laboratories elsewhere (Baylor College of Medicine and the Hospital for Sick Children, Toronto) and copies of those reports were obtained.

A structured interview was conducted by one or more of the authors with a parent of the subjects and directly with 2 older subjects, which included detailed questions and scaled responses about birth history, the clinical course and process leading to a diagnosis of PDC deficiency, developmental and school achievements, neurological symptoms, prior imaging, other medical history, medication and supplement use, diet, response to interventions, family history, and cause(s) of death, if applicable. Requested medical records included physician notes, discharge summaries, physical, occupational, and speech therapy evaluations, laboratory and radiology reports, and autopsy reports, if applicable. The review of collected records was conducted in 2011–2012.

2.1. Selection of subgroup for analysis

Subjects were included in the survival and neurological outcomes analysis if they were symptomatic and had an identified presumably "pathogenic" (see below) mutation(s) in one of the 5 genes specifically associated with PDC deficiency (with the exception of *DLD*). Included subjects with (X-linked) *PDHA1* mutations were hemizygotes (males) or heterozygotes (females); those with mutations inherited in an autosomal recessive manner (e.g. *DLAT* or *PDHB*) were homozygotes or compound heterozygotes (Table S-2A). Factors supportive of the pathogenicity of mutations included prior reports of the mutation in association with PDC deficiency, severe deletions or premature chain terminations, and evolutionary conservation of nucleotides at sites of missense mutations. Novel missense mutations were characterized as "probable" if they met criteria for being probably pathogenic by *in silico* prediction via SIFT (http://sift.jcvi.org) and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2). Predicted pathogenicity was characterized as "confirmed" if the mutation also was previously reported, or resulted in exon skipping, in frame deletion, frameshift, or premature chain termination.(Table S-2A) In most cases of point mutations, additional *in silico* predictions of significantly altered protein structure of *PDHA1* mutations were based on two Protein Data Bank entries 1N14 and 3EXE (http://www.rcsb.org/pdb), and those of *PDHB* mutations were previously described [7–9]. (Table S-2C) Evaluation of these structural models of mutations by the Swiss pdb molecular viewer (http:/spdbv.vital-it.ch/) was based on disturbance of: 1) secondary or tertiary structure due to altered hydrogen bonds, salt bridges, hydrophobic interaction, or steric hindrance; 2) interaction with TPP, pyruvate, or Mg; 3) catalytic site; 4) phosphorylation site; or 5) intermolecular interactions. Known polymorphisms were excluded. Subjects were excluded if they did not have any symptoms attributable to PDC deficiency (e.g. an asymptomatic mutation-positive mother of a proband with a *PDHA1* mutation) or if they had expired without being tested for PDC activity or mutational analysis (including siblings of subjects).

2.2. Survival

Survival was determined from the parental questionnaire and medical records. Those subjects who were last reported alive and whose parents had consented to be re-contacted were called by telephone or sent a letter if more than 5 years had elapsed since last contact or a period longer than half the age of the child had elapsed. If the family could not be contacted, survival at the time of data analysis was characterized as "unknown".

2.3. Intellectual outcomes

Cognitive quotient or intelligence quotient was determined directly from results of intelligence testing, if available, and reported for the oldest age for which data were available. If specific cognitive quotient was not available, approximate cognitive quotient scores were calculated from reports of achievements by parents and clinicians by dividing the mean age at which a non-motor developmental milestone is typically achieved (by Denver II criteria) by the subject's age at achievement of that milestone. As subjects frequently had hypotonia, which impairs motor development even in intellectually normal children, verbal abilities (expressive and receptive) were employed as a surrogate for cognition if specific cognitive data were unavailable, rather than calculating a global developmental quotient. Data were collected separately from parent reports and from professional evaluations (e.g. physicians, psychologists, and physical, occupational, and speech therapists). Categories of intellectual outcomes included "normal" (CQ/IQ≥70), mild intellectual disability (CQ/IQ 55–69), moderate intellectual disability (CQ/IQ 40–54), severe intellectual disability (CQ/IQ 25–39) and profound intellectual disability (CQ/IQ)<25. Cognition was only assessed in infants 6 months of age or older. For purposes of analysis, CQ or IQ scores were converted to a 1 to 5 scale, with 1 representing profound disability and 5 representing normal intellect. Variations in categorical outcomes between different assessments were averaged. These numbers were rounded up to integral values for categorization.

2.4. Neurological outcomes

Neurological symptoms were queried in the parent questionnaire and recorded from any available medical records. Although specific, focused questions were asked on the parental questionnaire, the term "lack of balance," used to assess ataxia, remained open to interpretation. To reduce error resulting from these questions, only the responses of the parents of ambulatory children were counted towards the total number with ataxia, and confirmation by medical records was utilized when possible. Structural brain findings were obtained from radiology reports in most cases, usually MRI. Primary images were generally not available for review. In a few cases (N=4), structural brain findings were based on autopsy reports as well as imaging reports, and in 2 cases, structural brain findings were based solely on autopsy reports. If a finding could not be adequately assessed for a certain subject, that subject was not included in the analysis of a given finding. Of note, subjects reported as having Leigh syndrome represent both those with radiographically-defined Leigh syndrome (as per radiology reports) and well as those described by the referring clinician as having Leigh syndrome (on the basis of radiographic criteria with or without clinical criteria).

2.5. Data analysis

GraphPad software (http://www.graphpad.com/quickcalcs) was used to perform Fisher's exact test. Two-tailed Fisher's exact test was used to analyze 2×2 contingency tables containing 2 categorical variables (e.g. to compare the frequency of neurological outcomes of males versus females). The Kaplan-Meier survival curve (GraphPad Prism version 5.04 for Windows, GraphPad Software, La Jolla, California USA, www.graphpad.com) was used to graphically compare survival in males versus females. The log-rank (Mantel-Cox) test was used to compare the survival of males and females.

3. Results

3.1. Mutations

Contact was established with 144 families of potentially eligible PDC deficient subjects. DLD (E3) deficiency was excluded. Consent, clinical information, and a sample adequate for genetic analysis were obtained from 92 of these subjects. Mutational analysis identified a pathogenic mutation in 59 subjects from 59 families in *PDHA1* or in both alleles of *PDHB or DLAT*, who were included in this analysis of outcomes. No pathogenic mutations were identified in *PDHX* or *PDP1*. No pathogenic mutations in the 6 genes sequenced or only one mutation of *DLAT* (one subject) were identified in 33 subjects (36%) with low PDC activity in cells or tissues (Fig. 1). Those subjects were not included in this analysis.

Fifty-nine subjects (27 males, 32 females) met the criteria for inclusion in the survival and neurological outcomes analysis. These included (by self-identification) 41 Caucasian, 6 Hispanic, 4 African American, 3 Asian, 1 Iranian, 1 Arab, and 3 uncharacterized families. 90% (53/59) of these subjects had one mutation in *PDHA1*, 7% (4/59) subjects each had mutations in both alleles of *PDHB* (2 from consanguineous families), and 3% (2/59) subjects (not from consanguineous families) each had mutations in both alleles of *DLAT* (Table S-2A). These mutations were considered pathogenic by the criteria listed above, including the predicted consequences on protein structure (see Table S-2A). These mutations and their predicted protein structural consequences are included along with observed activity of PDC in fibroblasts, lymphocytes, and/or muscle for each subject (Table S-2A, B and C). A total of 47 different mutations were identified in these 59 symptomatic subjects. Seven of these mutations (previously reported) were found in more than one subject. These included in *PDHA1*, c.1133G>A (p.R378H) found in 5 male subjects, c.904C>T (p.R302C) in 4 females, c.1132C>T (p.R378C) in 2 males and 1 female, c.380G>A (p.R127Q) in 2 males, 491A>G (p. N164S) in 2 males, 787C>G (p.R263G) in 1 male and 1 female, and c.1142_1145dupATCA (p.W383fs*6) in 2 females. 22 of these 49 mutations have not been previously reported, to our knowledge.

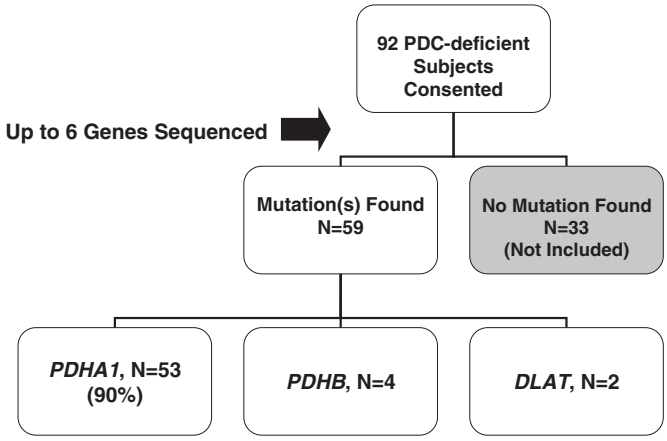

Fig. 1. Flowchart of subject selection. 92 eligible consented PDC-deficient subjects underwent sequencing of up to 6 genes (*PHDA1*, *PDHB*, *DLAT*, *DLD*, *PDHX*, and *PLP1*). 59 were found to have mutation(s) in one of these genes, and 33 were not found to have a mutation(s) (including subjects who only had one mutation in a gene with recessive inheritance). Of those with causative mutations, 53 had mutations in *PDHA1*, 4 had mutations in both alleles of *PDHB*, and 2 had mutations in both alleles of *DLAT*. Subjects with *DLD* mutations were excluded from the current analysis.

3.2. Family history

None of the subjects in this series were siblings of each other. Thirteen families reported a potential family history of possible PDC deficiency, of which 5 were confirmed or considered very probable. These included 5 total siblings of 2 subjects with *PDHB* mutations, who were born alive and had since expired) [7]. One subject with a *PDHA1* mutation had a first cousin with confirmed PDC deficiency. Two subjects' mothers had terminated 4 pregnancies because of positive prenatal diagnosis (3 pregnancies in one asymptomatic mother with a *PDHA1* mutation, and one pregnancy with *DLAT* deficiency). Mutational analysis of asymptomatic family members was not planned as a routine part of this study, but was performed in 8 mothers of subjects with *PDHA1* mutations in our affiliated clinical lab upon request (2 positive), and for confirmation of heterozygosity in both parents of 3 of the subjects with *PDHB* mutations (all positive) [7]. 26% (14/54) mothers reported a total of 16 miscarriages; 13 of these were mothers of a subject with a *PDHA1* mutation. Four pregnancies were terminated based on confirmed genetic prenatal diagnosis (3 with *PDHA1* mutations in one family). Out of 96 siblings of all subjects born alive, 43% were males. Out of 83 living siblings of the 53 subjects with *PDHA1* mutations, 43% were males. None of these living siblings were symptomatic. Therefore, the recurrence rate of confirmed or symptomatic PDC deficiency due to *PDHA1* mutations in siblings in this cohort was <5% (3/83).

3.3. Survival

The 59 subjects were born between 1980 and 2009. As of 2011, 39% (23/59) subjects were known to have died. Of these 23 subjects who died, 43% (10/23) died before 3 months of age, 61% (14/23) died before 1 year of age, and 91% (21/23) died before 4 years of age. (Fig. 2A) Only one subject died after age 10 years. Because of the unknown status of many subjects between 5 and 15 years, these are minimum estimates of mortality. There was a difference in overall survival between male and female subjects. Overall, 56% of males died (N = 15), and 25% of females died (N = 8) (Fig. 2B). Causes of death included severe lactic acidosis (N = 16), respiratory failure (N = 19), and infection (N = 4). In many cases, more than one cause of death was specified. Four of the deaths were directly associated with progressive neurological dysfunction. All subjects who died had been diagnosed with PDC deficiency before age 3 years.

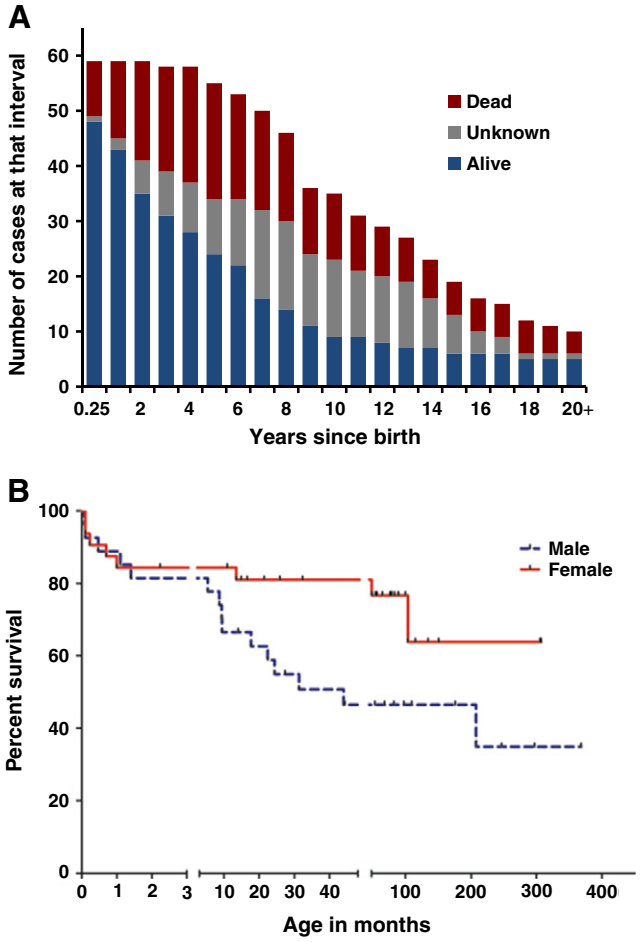

Fig. 2. (A) Survival of PDC deficient subjects with identified mutations. Percent of all 59 subjects in this series who might have reached a certain age (calculated from date of birth) divided into 3 groups (known to have died: red; known to be alive: blue; or unknown if alive or dead: grey). (B) Same data depicted in Kaplan–Meier survival curve format, shown separately for males (blue dotted line) and females (red solid line). Survival at end of curve reflects only subjects who reached or would have reached that age by the time of data analysis.

3.4. Intellectual outcomes

The cognitive quotients (CQ) or intellectual quotients (IQ) obtained directly or extrapolated from data provided by parents and those provided by professionals differed somewhat. Overall, the parents were more likely to describe achievements that resulted in the child being categorized as either profoundly intellectually disabled or of normal cognitive ability. Although assessment by parents was comparable, information provided by professional observers (some of which was observed directly and some of which was based on parental observations recorded by the professional) resulted in a more even distribution between the 5 categories of intellectual outcome. Divergent assessments by >1 professional observer sometimes resulted in intermediate categories. Of 42 subjects whose intellectual abilities were professionally evaluated, 19% (8/42) had normal or borderline intellectual ability (CQ/IQ ≥ 70), 10% (4/42) had mild intellectual disability (ID) (CQ/IQ 55–69), all of whom were categorized as mild-to-moderate, 17% (7/42) had moderate ID (CQ/IQ 40–54), including 2 subjects categorized as moderate-to-severe, 24% (10/42) had severe ID (CQ/IQ 25–39), including 4 subjects categorized as severe-to-profound, and 33% (13/42) had profound ID (CQ/IQ<25).

In contrast to their superior survival, females as a group had poorer intellectual outcomes than males as a group. By professional rating, males had an average intellectual outcome of moderate-to-mild intellectual disability, and females had an average intellectual outcome of profound-to-severe intellectual disability (Fig. 3). Of 10 subjects who reached age 12 years, 9 had had professional IQ assessments, and only 4 had IQs ≥ 70. (Only 2 of these 4 had assessments after age 12 years). Cognition roughly correlated with age at diagnosis of PDC deficiency, with those diagnosed earlier having generally poorer cognitive outcomes. Of those with a normal cognitive outcome, the earliest diagnosis of PDC deficiency had been made at 2.5 years of age. In the group of survivors, a diagnosis made after 4 years of age was significantly more likely to be associated with a professional rating of either normal cognition or mild cognitive disability than a diagnosis made before 4 years of age ($p<0.01$) by Fisher's exact test.

3.5. Neurological outcomes

Hypotonia was the most common neurological finding, seen in 89% (50/56) of subjects for whom this data was available. Hypertonia was reported in 49% (26/53) of subjects. 22 subjects had hypotonia, typically axial, combined with hypertonia, typically appendicular.

Seizures occurred in 57% (30/53, 15 F, 15 M). Reported seizure types included complex partial (N = 6: 4 F, 2 M), myoclonic (N = 5:

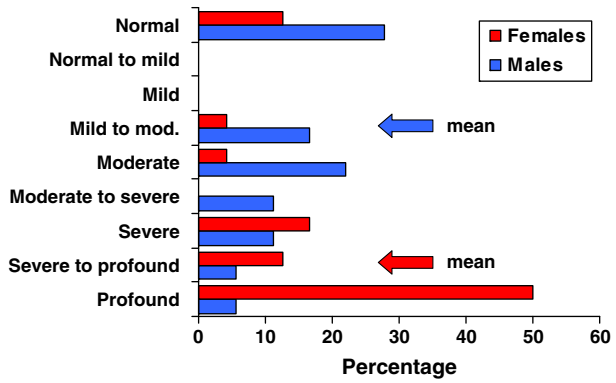

Fig. 3. Cognitive disability of surviving PDC deficient subjects with identified mutations. Number of male (blue) and female (red) subjects with a professional assessment of CQ/IQ in each category. Mean CQ/IQ is indicated by the blue (male subjects) and red (female subjects) arrows.

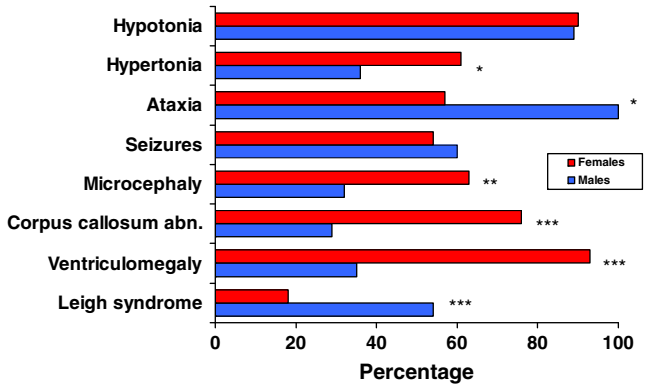

Fig. 4. Frequency of neurological outcomes in females and males. Among those subjects in whom neurological outcomes could be ascertained, percentage of females (red) and males (blue) with individual neurological and neuroimaging findings. P values (Fisher's exact test) reflect the differences in frequency between males and females, as follows: *signifies $p \leq 0.10$, **signifies $p < 0.05$, and ***signifies $p \leq 0.01$. Proportion of subjects with each outcome/subjects assessed: hypotonia: 26/29 F, 24/27 M; hypertonia: 17/28 F, 9/25 M; ataxia: 4/7 F, 9/9 M; seizures: 15/28 F, 15/25 M; microcephaly: 19/30 F, 8/25 M; corpus callosum abnormality: 22/29 F, 7/24 M; ventriculomegaly: 27/29 F, 8/23 M; Leigh syndrome: 5/28 F, 14/26 M.

4 F, 1 M), tonic (N = 4: 1 F, 3 M), and tonic–clonic (N = 3: 2 F, 1 M). Infantile spasms were reported in 3 female subjects. Multiple seizure types (3 or more) were reported in 4 female subjects, 2 of whom were described as having Lennox–Gastaut syndrome, and 3 other subjects (2 F, 1 M) had 2 seizure types. One female subject was described as having "petit mal" seizures. Seizure type was not specified or uncertain in 10 subjects (2 F, 8 M). Both generalized and focal EEG abnormalities were described. Subjects were prescribed a variety of antiepileptic drugs, generally corresponding to seizure type. Antiepileptic drugs included phenobarbital (N = 10), topiramate (N = 6), carbamazepine (N = 4), levetiracetam (N = 4), phenytoin (N = 2), zonisamide (N = 1), valproic acid (N = 1), lacosamide (N = 1), and rufinamide (N = 1). Benzodiazepines prescribed either regularly or as needed included clonazepam (N = 4), clobazam (N = 1), and lorazepam (N = 1). One subject with infantile spasms was treated with ACTH. Eight subjects were on no antiepileptic drugs (1 F, 7 M). 24/30 subjects with epilepsy were treated with ketogenic diets. Complete data were not available regarding the severity of subjects' epilepsy or the efficacy of these treatment regimens.

Ventriculomegaly was noted in 67% (35/52), corpus callosum abnormalities in 55% (29/53), and Leigh syndrome was reported in 35% (19/54). Corpus callosum abnormalities consisted of complete agenesis in 6 of these 29 subjects, partial agenesis in 9, and hypoplasia or thinning in 15. One subject had both partial agenesis and hypoplasia. Microcephaly was found in 49% (27/55) of subjects. Ataxia was reported in 13 out of 16 ambulatory subjects greater than 3.5 years of age, and could not be adequately assessed in other subjects. Fig. 4 compares of the frequency of these neurological outcomes in males and females.

Peripheral neuropathy was noted in 2 subjects, either subjectively reported in older subjects or demonstrated by nerve conduction studies. Dystonia was specifically reported in 6 subjects. Significant visual impairment (deficits not amenable to corrective lenses, including cortical visual impairment) was reported in 66% (31/47) of subjects, and hearing loss in 25% (11/44).

3.6. Variations of PDC activity and relationships to outcomes

PDC activity (activated-dephosphorylated and inactivated-phosphorylated) was measured in fibroblasts, lymphocytes, and/or muscle biopsies in all cases, and each patient had two or more separate assays of activity. There was a large variation of activity in these specimens, ranging from activity well within the reference range to undetectable. However, there was no significant overall correlation of this variation or mean PDC activities of the specimens assayed with the predicted severity of the identified mutation, either in males or females. We found a significant difference in survival of males with *PDHA1* mutations whose fibroblast PDC activity was ≥35% of the reference mean compared with those whose PDC activity was <35% of the mean ($p = 0.02$) (supplementary Fig. S-1A). This difference was not significant in females with *PDHA1* mutations (supplementary Fig. S-1B). There was no significant correlation of PDC activity in fibroblasts from males or females with *PDHA1* mutations and their intellectual outcome, described below (Fig. S-2). Within specimens from the same individual with *PDHA1* mutations, there were large variations of PDC activity, even within the same specimen (Table S-1B). This was evident in comparison of PDC activity in cultured skin fibroblasts and fresh blood lymphocytes from the same individual (supplementary Fig. S-3). Although these differences might be expected to correlate with potential PDC activity *in vivo*, we found no correlation with the maximum, the minimum, the degree of difference, or the mean fibroblast PDC activity and outcomes of survival and cognitive disability. We did find that these variations of PDC activity in sub-cultured skin fibroblasts from the same cell line were greater in females than males, as expected with *PDHA1* mutations (Fig. S-4). Some variation was also observed in males, which remains unexplained; similar variation was also found in males or females with autosomal mutations.

3.7. Genotype–phenotype

The large number of different mutations in this group of subjects did not permit significant analysis of the relationship between genotype and survival. For the group with the most common mutation in this data set, p.R378H in *PDHA1*, all males, 4 out of 5 subjects died. (The difference between this subgroup and the overall group is not statistically significant.) The ages at death for these 4 subjects varied widely, from 1.4 months to 3.6 years. One subject was alive at age 14 years with moderate intellectual disability, vision and hearing impairment, hypotonia, ventriculomegaly, and Leigh syndrome. Among these 5 males, mean cultured skin fibroblast PDC activity ranged from 10 to 28%, with the oldest survivor having 13% of the activity of the control mean. The highest PDC activity of these samples, 61% in blood lymphocytes (associated with 5% in muscle), was from a boy who paradoxically died at 6 weeks of age. Only 2 subjects in this cohort had the p.R263G mutation, which has been reported most frequently, accounting for about 4% of all reported cases [10]. One of these cases is a 20-year-old male with normal IQ. The other is a 12-year-old female, who is also functioning within the normal range of IQ. However, there is a large range of functional outcomes within reported cases with this same mutation,

ranging from another normally functioning adult male to severely disabled females and fatal outcomes in infant males [10–12].

3.8. Therapeutic interventions

We inquired about interventional experience for potential treatment of PDC deficiency, including dietary treatment (ketogenic), administration of thiamine and other natural product supplements, and drugs (especially dichloroacetate (DCA)). These results are summarized as a percentage of those cases in which such information could be ascertained, including dose ranges used, and the family's subjective assessment of the benefits of this intervention.

Thiamine was the most frequently employed intervention, with 68% (39/57) of subjects reporting some use. Doses ranged widely from 25 mg to 2000 mg/day (average dose: 174 mg; median dose: 150 mg/dl). Subjective impressions of the benefit of thiamine supplementation were "very helpful" in 25% (5/20) of responding families, "somewhat helpful" in 15%, and "uncertain" in 55% of subjects. No subject reportedly had a remarkable beneficial response to thiamine treatment.

The next most common intervention was dietary, with 60% (35/58) of the subjects reportedly having used a "ketogenic" diet at some time. Of these 35 subjects, 54% had used a "severe" (4:1) ketogenic diet, 9% of had used a "moderate" ketogenic diet (3:1), and 37% had used a "mild" ketogenic diet (2:1). Altogether, 83% (24/29) of responding families reported that use of a ketogenic diet was "very helpful", 10% said it was "somewhat helpful", and 7% reported that it was "not helpful"; responses were not consistently characterized as to how the ketogenic diet was helpful. Assessments of blood beta-hydroxybutyrate and/or acetoacetate or urine ketones were available in very few of these subjects. No significant correlation was found between use of ketogenic diets and survival or cognitive function outcomes, which is not surprising since use of ketogenic diets varied by degree of severity, duration, and age of administration. However, this intervention was associated with the most favorable subjective reports.

Because supplementation with carnitine is generally recommended in individuals receiving ketogenic diets (to replace increased urinary losses of acylcarnitines), we did not consider this to be a separate variable; 52% of subjects had used carnitine. Five subjects had used DCA, and one family reported that it was "very helpful". Coenzyme Q_{10} supplementation had been used in 24%. The data pertaining to coenzyme Q_{10} supplementation were insufficient to determine benefit.

4. Discussion

This study is the first to analyze survival and cognitive outcomes in a relatively large cohort of subjects with PDC deficiencies assessed longitudinally. Subjects were identified as having deficient PDC activity by our laboratory, but as a group received medical care throughout the United States and Canada, thus direct assessments of their status were made by a diverse group of clinicians and parent observers. The subjects reported here are a relatively homogeneous sample, in that all have detectable pathogenic mutations in genes directly associated with the pyruvate dehydrogenase complex. None are known to have large deletions, contiguous gene syndromes, or mutations in genes shared with other metabolic pathways.

We found significant mortality and neurological morbidity in our population of subjects. At least 39% of cases in our cohort had died before or during this study. A large group of neonates with severe lactic acidosis accounted for nearly half of the deaths in this study, and most of the deaths occurred at or before 4 years of age, with only 2 subjects dying after their fifth birthdays. There was a statistically significant difference in mortality between the males and females in our study, 56% versus 25% respectively ($p = 0.03$). This is accounted for by the high proportion of X-linked *PDHA1* mutations in this population. We presume that females with a substantial proportion of active X chromosomes bearing the normal allele would be relatively protected from the effects of a *PDHA1* mutation on the other allele. As evidenced by the 2 asymptomatic heterozygous carriers out of 8 mothers of the subjects with *PHDA1* mutations (tested by request for prenatal diagnosis), females with a *PDHA1* mutation may be asymptomatic, which we attribute to favorable skewing of X-inactivation [13,11,14].

However, the superior survival seen in female subjects is offset by their worse cognitive outcomes, as a group, compared with their male counterparts. Determinations of cognitive quotients are uncertain for subjects who died before 6 months of age, and were assessed in all subjects who survived past that period, regardless of whether or not they subsequently died. We presume that the worse cognitive outcomes in females as a group are a consequence of greater survival. We observed that, as a group, males with very low fibroblast PDC activity tend not to survive infancy, and some likely experience antenatal demise, as seen in *PDHA1* knock-out mice [15]. This may explain the higher proportion of female to male siblings of the subjects reported in this series. Females with X-linked mutations are more likely to survive, but may have severe disabilities. There are obvious exceptions to this generalization, with some severely disabled male survivors and high-functioning females, as well as severely affected females who did not survive infancy. There are also some surviving males with no intellectual disability and only isolated ataxia or neuropathy; these subjects may have a higher degree of residual PDC activity in the central nervous system, even though this was not evident by assays of PDC in available cells and tissues from these subjects.

We had initially anticipated exploring associations between the genotypes of these subjects and their clinical phenotypes. However, the large proportion of unique mutations (only 3 out of 47 mutations were seen in more than two individuals) limited these comparisons. The number of subjects sharing a common mutation is too small in this series for adequate comparative analysis. Previously reported series for the relatively more frequent missense mutations, such as p.R263G or p.R378H, have shown large variations of outcomes in subjects with these mutations and failed to establish distinguishable clinical phenotypic spectra associated with a particular mutation [10,16,14]. Furthermore, 22 of these 47 mutations are reported here for the first time, to our knowledge, and we do not have previously described clinical phenotypes for comparison. This high frequency of novel mutations of *PDHA1* and the relatively low frequency of affected siblings or excessive spontaneous termination of pregnancies of mothers of affected children would be consistent with a high rate of *de novo* mutations, as has been previously suggested [14,11,17].

We have compared our overall findings to those of previously published series (Table 1) [10,14,18]. Previously reported percentages of subjects with hypotonia did not exceed 58% in these series. One explanation for our higher percentage of 89% may be the retrospective design of our study, which detects hypotonia deemed present by any of a number of clinicians and therapists examining the subjects at separate times. Similarly, we found a greater number of subjects with hypertonia. We could not always distinguish different types of increased tone, such as rigidity, spasticity, and dystonia, from the parental questionnaires or medical records collected, and thus hypertonia serves as an encompassing category in our study. As seen with many other causes of cerebral impairment, hypertonia and hypotonia were frequently observed in the same subjects, specifically appendicular hypertonia with axial hypotonia.

We observed a similar proportion of subjects with developmental delay compared to other prior reports, when only outcomes for children who survived to the age of 6 months are included. This 80% does not include children with learning problems whose CQ or IQ scores were equal to or greater than 70; thus, this is likely an underestimation of the impact of PDC deficiency on cognition. We reported a greater percentage of subjects with reported seizures. Our percentages of subjects

Table 1
Reported clinical features in PDC deficiencies (%).

Clinical feature	Robinson et al. [18]	Patel et al. [10]	Imbard et al. [14]	This series
Hypotonia	53% (n[a] = 30)	46% (n = 371)	58% (n = 80)	89% (n = 56)
Hypertonia	13% (n = 30)		9%[b] (n = 80)	49% (n = 53)
Developmental delay	83%[c] (n = 23)	57% (n = 371)	69%[c] (n = 71)	81%[d,c] (n = 42)
Seizures	40% (n = 30)	26% (n = 371)	16% (n = 80)	57% (n = 53)
Ataxia[e]	17% (n = 30)	19% (n = 371)	20% (n = 80)	22%[f] (n = 59)
Ventriculomegaly[g]	85% (n = 20[h])	35% (n = 186)	38% (n = 76)	67% (n = 52)
Corpus callosum abnorm.	15% (n = 20[h])	31% (n = 186)	22% (n = 76)	55% (n = 53)
Leigh syndrome	15% (n = 20[h])	27% (n = 186)	12% (n = 76)	35% (n = 54)
Peripheral Neuropathy[e]	3% (n = 30)	7% (n = 371)	21% (n = 80)	3%[i] (n = 59)

[a] Number of subjects evaluated.
[b] Does not include dystonia.
[c] 6 Months and older.
[d] Developmental quotient < 70.
[e] Uncertain ascertainment.
[f] % of entire cohort, 81% of ambulatory subjects.
[g] Includes hydrocephalus and cortical atrophy.
[h] Subjects who had neuroimaging or autopsy data.
[i] Percent of entire cohort, 15% of those evaluated objectively or who could verbalize symptoms of peripheral neuropathy.

with ataxia or peripheral neuropathy were comparable to those reported by these other authors.

The frequency of ventriculomegaly varied widely between studies. Other neuroimaging abnormalities (corpus callosum abnormalities and Leigh syndrome) were also more frequent in our subjects than in those reported previously. Again, our retrospective data collection and the combination of reports from several sources may have contributed to this increased frequency, as likely did our practice of excluding all subjects who could not adequately be assessed from the denominators of our calculations. We surmise this may be a major contributor to our increased rates of clinical features compared to other authors [10,14].

A broad clinical classification has been proposed, divided into 4 neurological phenotypes, as follows [19]. In that categorization, symptomatic neonates typically present with lactic acidosis, with severe encephalopathy and hypotonia. Brain malformations are common in this group. Children of either sex presenting later in infancy may have severe psychomotor delay with or without early-onset epilepsy. Males presenting later in infancy may manifest Leigh syndrome, with episodic brainstem dysfunction. Childhood onset, described in males, may manifest as recurrent ataxia with axonal neuropathy. Our data roughly echoes these categories, but it is our impression that there is a broad spectrum of clinical features within PDC deficiency, and some cases do not fall into these specific descriptive categories. For example, a female subject in our study had childhood onset of symptoms with normal intellectual functioning, a presentation typically described in males.

The observational study design that we employed has several limitations. Although specific, focused questions were asked on the retrospective parental questionnaire, the responses remained somewhat subjective, and vulnerable to recall bias. Clinicians' objective examinations carried more weight than parental reports due to the more specific, and prospective, neurological data they provided, but clinicians, unlike parents, did not observe the subjects continuously. The data we examined was dependent on the procurement of medical records, usually from other institutions. Completeness of medical records varied from case to case. These same limitations applied to both male and female subjects, so no systematic bias would be expected to result from this limitation. Despite attempts at re-contacting them as described above, a significant proportion of subjects were not accounted for at the time of follow-up data analysis, but of those whose status is known, the majority of deaths occurred in early childhood. Thus, it seems appropriate to counsel parents that the chances of long-term survival in children older than age 4 are generally better, although occasionally mortality does occur.

We are not able to add anything significant to previous observations that genotype–phenotype correlations between different *PDHA1* mutations are extremely limited and not reliably prognostic [20]. The variability of severity seen with X-linked *PDHA1* mutations is to be expected in females as the result of different patterns of X-inactivation [13], but such variability exists in males as well and remains largely unexplained. A few individuals of both sexes who are mosaic for the *PDHA1* mutation also have been identified, and males with mosaicism may experience milder outcome than those without mosaicism [6]. However, mosaicism has not been documented in most males with variable outcomes associated with the same mutation. This variability was observed in the current series among 5 males with the R378H mutation, who differed significantly not only in their clinical outcomes but also with *in vitro* PDC activity, which was not correlated with outcome. The same is true for males with the most commonly reported (but not in this series) *PDHA1* mutation, p.R263G, with variable clinical outcomes and PDC activity in skin fibroblasts and other cells and tissues [10,21,22].

Not surprisingly, the spectrum of manifestations observed with recessively-inherited *PDHB* mutations is similar to that seen with *PDHA1* [7]. Reported *DLAT* mutations, also recessively inherited, have been associated with Leigh syndrome and episodic dystonia, but these findings are not exclusive to *DLAT* [23,24]. Likewise, reported cases of E3BP deficiency (due to homozygous or compound heterozygous mutations in *PDHX*), have been most commonly associated with lactic acidosis, delayed psychomotor development, and hypotonia, with apparently longer survival compared to other forms of PDC deficiency [25]. Outcomes in *PDP1* are also heterogeneous and correlated, in the few reported patients, with the severity of the mutation and its effect on the activity of the PDP1 enzyme [26,27]. It seems reasonable, given the generally poor correlation of clinical outcomes with specific mutations, that one should not expect categorical differences in the outcomes related to different genes that are specific to PDC.

Although we did not assess X-inactivation ratios of normal and mutated alleles in the female subjects, the overall activity of PDC in the samples analyzed should reflect the combined effects of both the severity of mutations and the degree of inactivation of the normal allele in that sample. However, it would not be expected that the pattern of X-inactivation in the cell or tissue sample analyzed from the female subjects should reflect that within the central nervous system of those subjects. Indeed, we found that PDC activity in subcultures of the same cultured fibroblast cell line generally varied more in females than in males.

The results of our observations have implications for genetic counseling. The recurrence rate of symptomatic PDC deficiency in siblings of a child with a *PDHA1* mutation was quite low (3/83 sibs, or <5%). This is likely due to the high frequency of *de novo PDHA1* mutations, but also may be due partly to variable penetrance in female siblings. None of the 4 *PDHA1* mutation-negative mothers had more than one affected child (which would have implied germline mosaicism). Prenatal testing is available, but in this series, relatively few mothers chose elected to pursue this. Genetic diagnosis is definitely more reliable than enzymatic testing for prenatal testing. However, genetic diagnosis, pre- or postnatally, presents prognostic uncertainty, particularly in females, who may be asymptomatic carriers of *PDHA1* mutations, mildly affected, or severely affected. Similarly, some males with the same *PDHA1* mutations have much milder symptoms than others. The best predictor of survival and cognitive outcome appears to be the age of diagnosis, with neonatal presentations typically associated with early death, and childhood-onset cases associated with better survival and functional status, as noted by other authors [14,19].

The relatively high frequency (36%) of mutations not found in this series of subjects who were known to be PDC-deficient by enzyme

analysis has previously been reported [11,14], although most reports of PDC mutations do not include cases in which no mutation was found. This observation implies that standard genomic exon sequencing analysis, if negative, should not be the only test employed for suspected patients, since we estimate that would detect only approximately 2/3 of PDC deficient cases. Further genetic investigation of PDC deficient cases detected by enzymatic activity likely will require more extensive testing designed to detect intronic splicing mutations, large deletions, mosaicism, and changes in genes that are not specific for PDC. For now, we recommend a combination of enzymatic assays and mutational analyses of PDC specific genes for comprehensive detection of all subjects suspected of having PDC deficiency.

This retrospective correlation of PDC activity, mutations, and outcomes was not designed to assess whether specific treatments were effective in improving outcomes. We did obtain limited information about frequency of use of various interventions and found that thiamine supplementation was most common, followed by ketogenic diet treatment, with relatively few subjects receiving DCA. This observation is not surprising, as thiamine is the most readily available, cheapest, easiest to administer, and has no reported adverse side effects, whereas ketogenic diets are the most difficult, and DCA is still an investigational drug of unproven benefit. Subjective retrospective assessments of the benefits of these various interventions were most favorable for ketogenic diet treatment, but we cannot confidently determine from these data benefit or lack thereof for any of these interventions.

Several reports have indicated that some cases of PDC deficiency are thiamine responsive, and this has been attributed to specific genotypes [28–31]. Two cases in this series had one of those mutations (p.R263G; one male and one female). Both were receiving thiamine, alive, and doing relatively well, but the boy was also receiving a ketogenic diet and the girl's mother, who carries the same mutation, is asymptomatic.

Ketogenic diet treatment has the theoretical benefit of bypassing PDC to provide an alternate source of acetyl-CoA as a substrate for oxidative phosphorylation. Experimental studies of ketogenic nutritional support in zebrafish and conditional murine models of PDC deficiency have shown significant benefits [32,33] Several case reports have supported this benefit, including comparison of cases of males with identical *PDHA1* mutations who received different degrees of ketogenic diet severity [34–36]. It is also clear from these prior reports and this series that ketogenic diet therapy does not reverse prior neurological damage and is not effective in sustaining survival in the most severe cases of PDC deficiency.

Dichloroacetate (DCA) is an inhibitor of pyruvate dehydrogenase kinases and a known activator of PDC, which in theory could be beneficial by maintaining activation of any residual PDC, which presumably is generally common to viable cases. Treatment with DCA also has been reported to be beneficial in individual cases of PDC deficiency [37]. In a double-blind, controlled, cross-over trial of children with various mitochondrial disorders, including PDC deficiency, administration of DCA was not beneficial overall in improving functional assessments or quality of life; retrospective subgroup analysis of cases with PDC deficiency ($n=11$) showed no improvement in their observed outcomes [38]. Use of DCA in older cases with MELAS has been associated with neuropathy, but younger children appear to be less sensitive to this side effect [39,40].

Although prospective controlled clinical trials of potential therapeutic interventions have been identified as a priority for mitochondrial disorders in general, this has not yet been done adequately for PDC deficiency. These observations of the natural history and variability of outcomes for PDC deficiency hopefully provide additional background necessary to planning such clinical trials, as well as providing updated information useful for genetic counseling. The most compelling challenge which has eluded our analysis of these data is identification of those critical factors that distinguish children who have severe outcomes from those who do far better. We speculate that this is related to the level of expression of residual PDC in the central nervous system, including the presumed variable effects of specific mutations, variable X-inactivation in females, and somatic mosaicism in some males. However, we are not able to confirm this directly or adequately identify other genetic or biochemical mechanisms that may contribute to variations in PDC activity or energy metabolism in general. Further investigation is needed to identify factors that contribute to this currently unpredictable variation. Development of feasible techniques for assessing metabolic flux through PDC and ATP production both *in vitro* as well as *in vivo* in organs and tissues under variable physiological conditions, especially within the central nervous system, would be valuable tools for such investigation.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ymgme.2012.09.001.

Acknowledgments

We are very indebted to support of this work from the Zachary Foundation and the Katie Foundation. We could not have done these analyses without the invaluable work of members of the CIDEM laboratory staff and the assistance and support of our institutional colleagues, especially Drs. Shawn McCandless, Charles Hoppel, and Matthew Warman. We wish to acknowledge the cooperation of many physicians who referred these cases to CIDEM, assisted us in establishing contact with the families, and provided medical records. Most importantly, we appreciate the great cooperation and concern of those families and individuals affected by PDC deficiency who shared their time and life stories that form the basis of this report.

References

[1] J.A. Mayr, P. Freisinger, K. Schlachter, B. Rolinski, F.A. Zimmermann, T. Scheffner, T.B. Haack, J. Koch, U. Ahting, H. Prokisch, W. Sperl, Thiamine pyrophosphokinase deficiency in encephalopathic children with defects in the pyruvate oxidation pathway, Am. J. Hum. Genet. 89 (2011) 806–812.
[2] J.A. Mayr, F.A. Zimmermann, C. Fauth, C. Bergheim, D. Meierhofer, D. Radmayr, J. Zschocke, J. Koch, W. Sperl, Lipoic acid synthetase deficiency causes neonatal-onset epilepsy, defective mitochondrial energy metabolism, and glycine elevation, Am. J. Hum. Genet. 89 (2011) 792–797.
[3] J.M. Cameron, A. Janer, V. Levandovskiy, N. MacKay, T.A. Rouault, W.H. Tong, I. Ogilvie, E.A. Shoubridge, B.H. Robinson, Mutations in iron–sulfur cluster scaffold genes NFU1 and BOLA3 cause a fatal deficiency of multiple respiratory chain and 2-oxoacid dehydrogenase enzymes, Am. J. Hum. Genet. 89 (2011) 486–495.
[4] A. Navarro-Sastre, F. Tort, O. Stehling, M.A. Uzarska, J.A. Arranz, T.M. Del, M.T. Labayru, J. Landa, A. Font, J. Garcia-Villoria, B. Merinero, M. Ugarte, L.G. Gutierrez-Solana, J. Campistol, A. Garcia-Cazorla, J. Vaquerizo, E. Riudor, P. Briones, O. Elpeleg, A. Ribes, R. Lill, A fatal mitochondrial disease is associated with defective NFU1 function in the maturation of a subset of mitochondrial Fe–S proteins, Am. J. Hum. Genet. 89 (2011) 656–667.
[5] D. Kerr, G. Grahame, G. Nakouzi, Assays of pyruvate dehydrogenase complex and pyruvate carboxylase activity, in: L.J.C. Wong (Ed.), Mitochondrial Disorders: Biochemical and Molecular Analysis, Humana/Springer, Clifton, N.J., 2012, pp. 93–119.
[6] K. Okajima, M.L. Warman, L.C. Byrne, D.S. Kerr, Somatic mosaicism in a male with an exon skipping mutation in PDHA1 of the pyruvate dehydrogenase complex results in a milder phenotype, Mol. Genet. Metab. 87 (2006) 162–168.
[7] K. Okajima, L.G. Korotchkina, C. Prasad, T. Rupar, J.A. Phillips III, C. Ficicioglu, J. Hertecant, M.S. Patel, D.S. Kerr, Mutations of the E1beta subunit gene (PDHB) in four families with pyruvate dehydrogenase deficiency, Mol. Genet. Metab. 93 (2008) 371–380.
[8] E.M. Ciszak, L.G. Korotchkina, P.M. Dominiak, S. Sidhu, M.S. Patel, Structural basis for flip-flop action of thiamin pyrophosphate-dependent enzymes revealed by human pyruvate dehydrogenase, J. Biol. Chem. 278 (2003) 21240–21246.
[9] M. Kato, R.M. Wynn, J.L. Chuang, S.C. Tso, M. Machius, J. Li, D.T. Chuang, Structural basis for inactivation of the human pyruvate dehydrogenase complex by phosphorylation: role of disordered phosphorylation loops, Structure 16 (2008) 1849–1859.
[10] K.P. Patel, T.W. O'Brien, S.H. Subramony, J. Shuster, P.W. Stacpoole, The spectrum of pyruvate dehydrogenase complex deficiency: clinical, biochemical and genetic features in 371 patients, Mol. Genet. Metab. 105 (2012) 34–43.
[11] E. Quintana, L. Gort, C. Busquets, A. Navarro-Sastre, W. Lissens, S. Moliner, M. Lluch, M.A. Vilaseca, M.L. De, A. Ribes, P. Briones, Mutational study in the PDHA1 gene of 40 patients suspected of pyruvate dehydrogenase complex deficiency, Clin. Genet. 77 (2010) 474–482.
[12] R. Bachmann-Gagescu, J.L. Merritt, S.H. Hahn, A cognitively normal PDH-deficient 18-year-old man carrying the R263G mutation in the PDHA1 gene, J. Inherit. Metab. Dis. (2009) (Short Report #181 online).

[13] H.H.M. Dahl, Pyruvate dehydrogenase $E_1\alpha$ deficiency: males and females differ yet again, Am. J. Hum. Genet. 56 (1995) 553–557.
[14] A. Imbard, A. Boutron, C. Vequaud, M. Zater, L.P. de, H.O. de Baulny, C. Barnerias, M. Mine, C. Marsac, J.M. Saudubray, M. Brivet, Molecular characterization of 82 patients with pyruvate dehydrogenase complex deficiency. Structural implications of novel amino acid substitutions in E1 protein, Mol. Genet. Metab. 104 (2011) 507–516.
[15] M.T. Johnson, S. Mahmood, S.L. Hyatt, H.S. Yang, P.D. Soloway, R.W. Hanson, M.S. Patel, Inactivation of the murine pyruvate dehydrogenase (Pdha1) gene and its effect on early embryonic development, Mol. Genet. Metab. 74 (2001) 293–302.
[16] C. Marsac, C. Binelli, I. Desguerre, M. Diry, F. Fouque, L. De Meirleir, G. Ponsot, S. Seneca, F. Poggi, J.M. Saudubray, M.T. Zabot, D. Fontan, W. Lissens, Biochemical and genetic studies of four patients with pyruvate dehydrogenase E1 alpha deficiency, Hum. Genet. 99 (1997) 785–792.
[17] W. Lissens, L. De Meirleir, S. Seneca, I. Liebaers, G.K. Brown, R.M. Brown, M. Ito, E. Naito, Y. Kuroda, D.S. Kerr, I.D. Wexler, M.S. Patel, B.H. Robinson, A. Seyda, Mutations in the X-linked pyruvate dehydrogenase (E1) alpha subunit gene (PDHA1) in patients with a pyruvate dehydrogenase complex deficiency, Hum. Mutat. 15 (2000) 209–219.
[18] B.H. Robinson, H. MacMillan, R. Petrova-Benedict, W.G. Sherwood, Variable clinical presentation in patients with defective E_1 component of pyruvate dehydrogenase complex, J. Pediatr. 111 (1987) 525–533.
[19] C. Barnerias, J.M. Saudubray, G. Touati, L.P. de, O. Dulac, G. Ponsot, C. Marsac, M. Brivet, I. Desguerre, Pyruvate dehydrogenase complex deficiency: four neurological phenotypes with differing pathogenesis, Dev. Med. Child Neurol. 52 (2010) e1–e9.
[20] J.M. Cameron, V. Levandovskiy, N. MacKay, I. Tein, B.H. Robinson, Deficiency of pyruvate dehydrogenase caused by novel and known mutations in the E1alpha subunit, Am. J. Med. Genet. A 131 (2004) 59–66.
[21] D.S. Kerr, S.A. Berry, M.M. Lusk, L. Ho, M.S. Patel, A deficiency of both subunits of pyruvate dehydrogenase which is not expressed in fibroblasts, Pediatr. Res. 24 (1988) 95–100.
[22] I.D. Wexler, S.G. Hemalatha, T.C. Liu, S.A. Berry, D.S. Kerr, M.S. Patel, A mutation in the $E_1\alpha$ subunit of pyruvate dehydrogenase associated with variable expression of pyruvate dehydrogenase complex deficiency, Pediatr. Res. 32 (1992) 169–174.
[23] R.A. Head, R.M. Brown, Z. Zolkipli, R. Shahdadpuri, M.D. King, P.T. Clayton, G.K. Brown, Clinical and genetic spectrum of pyruvate dehydrogenase deficiency: dihydrolipoamide acetyltransferase (E2) deficiency, Ann. Neurol. 58 (2005) 234–241.
[24] C.A. McWilliam, C.K. Ridout, R.M. Brown, R.C. McWilliam, J. Tolmie, G.K. Brown, Pyruvate dehydrogenase E2 deficiency: a potentially treatable cause of episodic dystonia, Eur. J. Paediatr. Neurol. 14 (2010) 349–353.
[25] R.M. Brown, R.A. Head, A.A. Morris, J.A. Raiman, J.H. Walter, W.P. Whitehouse, G.K. Brown, Pyruvate dehydrogenase E3 binding protein (protein X) deficiency, Dev. Med. Child Neurol. 48 (2006) 756–760.
[26] M.C. Maj, N. MacKay, V. Levandovskiy, J. Addis, E.R. Baumgartner, M.R. Baumgartner, B.H. Robinson, J.M. Cameron, Pyruvate dehydrogenase phosphatase deficiency: identification of the first mutation in two brothers and restoration of activity by protein complementation, J. Clin. Endocrinol. Metab. 90 (2005) 4101–4107.
[27] J.M. Cameron, M. Maj, V. Levandovskiy, C.P. Barnett, S. Blaser, N. MacKay, J. Raiman, A. Feigenbaum, A. Schulze, B.H. Robinson, Pyruvate dehydrogenase phosphatase 1 (PDP1) null mutation produces a lethal infantile phenotype, Hum. Genet. 125 (2009) 319–326.
[28] M. Di Rocco, L.D. Lamba, G. Minniti, U. Caruso, E. Naito, Outcome of thiamine treatment in a child with Leigh disease due to thiamine-responsive pyruvate dehydrogenase deficiency, Europ. J. Paediatr. Neurol. 4 (2000) 115–117.
[29] K. Narisawa, H. Endo, S. Miyabayashi, K. Tada, Thiamine responsive pyruvate dehydrogenase deficiency, J. Nutr. Sci. Vitaminol. (Tokyo) S21-1 (1992) 585–588.
[30] E. Naito, M. Ito, I. Yokota, T. Saijo, Y. Ogawa, Y. Kuroda, Diagnosis and molecular analysis of three male patients with thiamine-responsive pyruvate dehydrogenase complex deficiency, J. Neurol. Sci. 201 (2002) 33.
[31] E. Naito, M. Ito, I. Yokota, T. Saijo, J. Matsuda, Y. Ogawa, S. Kitamura, E. Takada, Y. Horii, Y. Kuroda, Thiamine-responsive pyruvate dehydrogenase deficiency in two patients caused by a point mutation (F205L and L216F) within the thiamine pyrophosphate binding region, Biochim. Biophys. Acta 1588 (2002) 79–84.
[32] M.R. Taylor, J.B. Hurley, H.A. Van Epps, S.E. Brockerhoff, A zebrafish model for pyruvate dehydrogenase deficiency: rescue of neurological dysfunction and embryonic lethality using a ketogenic diet, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 4584–4589.
[33] S. Sidhu, A. Gangasani, L.G. Korotchkina, G. Suzuki, J.A. Fallavollita, J.M. Canty Jr., M.S. Patel, Tissue-specific pyruvate dehydrogenase complex deficiency causes cardiac hypertrophy and sudden death of weaned male mice, Am. J. Physiol. Heart Circ. Physiol. 295 (2008) H946–H952.
[34] R.E. Falk, S.D. Cederbaum, J.P. Blass, G.E. Gibson, R.A. Pieter Kark, R.E. Carrel, Ketogenic diet in the management of pyruvate dehydrogenase deficiency, Pediatrics 58 (1976) 713–721.
[35] I.D. Wexler, S.G. Hemalatha, J. McConnell, N.R. Buist, H.H. Dahl, S.A. Berry, S.D. Cederbaum, M.S. Patel, D.S. Kerr, Outcome of pyruvate dehydrogenase deficiency treated with ketogenic diets. Studies in patients with identical mutations, Neurology 49 (Dec. 1997) 1655–1661.
[36] M.S. Saenz, L. Pickler, E. Elias, L. Fenton, D.S. Kerr, J. Van Hove, Resolution of lesions on MRI with ketogenic diet in pyruvate dehydrogenase deficiency, Mol. Genet. Metab. 99 (2010) 231–232.
[37] P.W. Stacpoole, C.L. Barnes, M.D. Hurbanis, S.L. Cannon, D.S. Kerr, Treatment of congenital lactic acidosis with dichloroacetate: a review, Arch. Pediatr. Adolesc. Med. 77 (1997) 535–541.
[38] P.W. Stacpoole, D.S. Kerr, C. Barnes, S.T. Bunch, P.R. Carney, E.M. Fennell, N.M. Felitsyn, R.L. Gilmore, M. Greer, G.N. Henderson, A.D. Hutson, R.E. Neiberger, R.G. O'Brien, L.A. Perkins, R.G. Quisling, A.L. Shroads, J.J. Shuster, J.H. Silverstein, D.W. Theriaque, E. Valenstein, Controlled clinical trial of dichloroacetate for treatment of congenital lactic acidosis in children, Pediatrics 117 (2006) 1519–1531.
[39] P. Kaufmann, K. Engelstad, Y. Wei, S. Jhung, M.C. Sano, D.C. Shungu, W.S. Millar, X. Hong, C.L. Gooch, X. Mao, J.M. Pascual, M. Hirano, P.W. Stacpoole, S. DiMauro, V. De, Dichloroacetate causes toxic neuropathy in MELAS: a randomized, controlled clinical trial, Neurology 66 (2006) 324–330.
[40] P.W. Stacpoole, The dichloroacetate dilemma: environmental hazard versus therapeutic goldmine - both or neither? Environ. Health Perspcet. 119 (2011) 155–158.