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Genetic landscape of primary mitochondrial diseases in children and adults using molecular genetics and genomic investigations of mitochondrial and nuclear genome

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Abstract

Background Primary mitochondrial diseases (PMD) are one of the most common metabolic genetic disorders. They are due to pathogenic variants in the mitochondrial genome (mtDNA) or nuclear genome (nDNA) that impair mitochondrial function and/or structure. We hypothesize that there is overlap between PMD and other genetic diseases that are mimicking PMD. For this reason, we performed a retrospective cohort study.

Methods All individuals with suspected PMD that underwent molecular genetic and genomic investigations were included. Individuals were grouped for comparison: (1) individuals with mtDNA-PMD; (2) individuals with nDNA-PMD; (3) individuals with other genetic diseases mimicking PMD (non-PMD); (4) individuals without a confirmed genetic diagnosis.

Results 297 individuals fulfilled inclusion criteria. The diagnostic yield of molecular genetics and genomic investigations was 31.3%, including 37% for clinical exome sequencing and 15.8% for mitochondrial genome sequencing. We identified 71 individuals with PMD (mtDNA $n=41$, nDNA $n=30$) and 22 individuals with non-PMD. Adults had higher percentage of mtDNA-PMD compared to children (p -value = 0.00123). There is a statistically significant phenotypic difference between children and adults with PMD.

Conclusion We report a large cohort of individuals with PMD and the diagnostic yield of urine mitochondrial genome sequencing (16.1%). We think liver phenotype might be progressive and should be studied further in PMD. We showed a relationship between non-PMD genes and their indirect effects on mitochondrial machinery. Differentiation of PMD from non-PMD can be achieved using specific phenotypes as there was a statistically significant difference for muscular, cardiac, and ophthalmologic phenotypes, seizures, hearing loss, peripheral neuropathy in PMD group compared to non-PMD group.

Keywords Primary mitochondrial diseases, Exome sequencing, Mitochondrial genome sequencing

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Background

Primary mitochondrial diseases (PMD) are one of the most common inherited metabolic disorders. They are due to pathogenic variants in the mitochondrial or nuclear genome that impair mitochondrial function and energy production. The prevalence of PMD is about 1 in 4000 live births. There are more than 500 different genetic defects causing PMD [1, 2].

Almost every cell in the human body has mitochondria which are the energy production machinery of the cells. The electron transport chain, located in the inner mitochondrial membrane, is crucial for oxidative phosphorylation and ATP production in the cell [3–5]. Molecular genetic defects of mitochondrial and nuclear genome disrupt the function of the five complexes in the electron transport chain, leading to impaired mitochondrial function and reduced energy production [5–8]. Functional and structural defects of mitochondria affect multiple organs, especially the high energy requiring organs such as brain, retina, cardiac and skeletal muscle, but also liver, kidney, endocrine and gastrointestinal systems. The phenotypes of PMD are on a spectrum ranging from prenatal or neonatal onset lethal disease affecting multiple organ systems to adult onset progressive external ophthalmoplegia. The disease onset is in childhood in two-thirds of the individuals with PMD [9, 10].

Mitochondrial disease criteria were applied for the diagnostic confirmation of suspected PMD prior to 2013 and new clinical criteria were developed in the absence of molecular genetics and genomic investigations recently [11–14]. Until recently, numerous investigations were applied to help in the diagnosis of PMD including biochemical investigations (e.g. lactate, pyruvate, amino acid and organic acid analyses), exercise test, muscle biopsy, brain magnetic resonance imaging (MRI), brain magnetic resonance spectroscopy (MRS) and lumbar puncture. Additionally, individuals with suspected PMD undergo echocardiography, endocrinological investigations, ultrasounds of liver and kidney, hearing tests and ophthalmologic exam to investigate if there is any other organ involvement. In the recent years, molecular genetics and genomic investigations have been applied to confirm underlying genetic diagnosis in individuals with suspected PMD [15–19]. As the mitochondrial DNA variant load can vary between tissues, mitochondrial DNA should be extracted from the most affected organ for molecular genetics and genomic investigations. Commonly used tissues include blood, muscle, buccal swabs, and skin fibroblasts [20–22]. Despite extensive investigations, the underlying genetic defect may not be identified. This may prematurely end the individuals' diagnostic journey, overmedicalize their care and potentially limit

access to appropriate treatments for the actual underlying genetic disease [13, 23].

We hypothesize that there is an overlap between PMD and other genetic diseases that mimic PMD. For this reason, we performed a retrospective cohort study to report the: (1) Genetic landscape of PMD in Alberta; (2) Diagnostic yield of mitochondrial genetics and genomic investigations; and (3) Comparison of phenotypes, biochemical features, muscle histopathology, electron transport chain activities, neuroimaging, and neurophysiological studies between individuals with PMD and with other genetic diseases mimicking PMD (non-PMD).

Methods

Alberta Research Information Services (ARISE) at the University of Alberta approved this study (Approval ID: Pro00112487). Northern Alberta Clinical Trials and Research Centre (NACTRC) approved this clinical research study for the use of Alberta Health Services Data (Approval ID: PRJ38205). We used different databases including FoxPro, Sunquest, Connect Care and Metabolic Genetics Clinic Databases and generated an Excel Database. Our inclusion criteria were: (1) All individuals with suspected PMD who were referred to our metabolic genetics clinic at the University of Alberta; and/or (2) All individuals who had any of the following molecular genetics and genomic investigations: targeted next generation sequencing panels, clinical exome sequencing, mitochondrial deletion/duplication testing, mitochondrial genome sequencing, and common mitochondrial variant testing.

We reviewed Electronic Patient Charts for the clinical features, biochemical investigations, cardiac assessments, neuroimaging features, and molecular genetics and genomic investigations. We entered all information into an Excel database (Microsoft Corp., Redmond, WA, U.S.A.).

Molecular genetics and genomic investigations using individual and/or parents' DNA samples were performed in clinical molecular genetics and genomic laboratories according to their methods. American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) variant classification guidelines for interpretation of genetic variants were applied [24, 25]. All variants in the Genome Aggregation Database (gnomAD v3.2.1) (<http://gnomad.broadinstitute.org/about>) for their allele frequency in the general population were searched [26].

We divided individuals into four groups to compare their phenotypes and genetic diagnoses including: (1) Group 1: individuals with genetically confirmed mtDNA-PMD; (2) Group 2: individuals with genetically confirmed nDNA-PMD; (3) Group 3: individuals with other genetic

diseases that are mimicking PMD (non-PMD); (4) Group 4: individuals with no confirmed genetic diagnosis. We analyzed individuals who had undergone muscle biopsy (e.g., histopathology and/or electron transport chain activity results).

We performed protein 3D structure prediction for wild type and variant protein structures for variants of uncertain significance (VUS) in nDNA-PMD genes for predicting the impact of VUS on the protein structure. We retrieved protein FAST-All (FASTA) sequences from the UniProt database and input them to the AlphaFoldColab2 program for 2D structure visualization and processing. We retrieved output data files from AlphaFoldColab2 and input to PyMol 2.5.5 Edu for 3D structure visualization of the protein structures. We assessed sequence coverage and confidence levels for reliability and validity of structures. We performed all imaging of structures in accordance with the sequence alignment of variant protein structure with wild-type protein structures. We assessed variant protein models for predicted change in structure and amino-acid interaction in relation to wild type. We labelled variant residues for each gene variant.

We performed statistical analysis using R statistical software (version 4.0.2). Results are given as mean \pm SD (range). Non-parametric Fisher's exact test was chosen to compare between groups as indicated where appropriate. Results were considered statistically significant with a two-tailed p-value of <0.05 .

Results

There were 403 individuals in our Excel database whom we identified from FoxPro, Sunquest, Connect Care and Metabolic Genetics Clinic Databases. Two-hundred-and-ninety-seven individuals fulfilled the inclusion criteria. The demographics of all individuals are summarized in Table 1. All genetic and genomic investigations and their diagnostic yield was summarized in Table 2. All individuals with their phenotypes and genotypes are summarized in Supplemental Table 2 1 (mtDNA-PMD), Supplemental Table 2 (nDNA-PMD), and Supplemental Table 3 (non-PMD). All individuals with no genetic diagnosis are summarized in Supplemental Table 4.

Ninety-three individuals had confirmed genetic diseases. The diagnostic yield of molecular genetics and genomic investigations was 31.3%. There were 71 individuals with PMD (mtDNA-PMD $n=41$, nDNA-PMD $n=30$), and 22 individuals with non-PMD. The number of individuals with mtDNA-PMD and nDNA-PMD is depicted in Fig. 1. There were 89 pathogenic/likely pathogenic single nucleotide variants in 45 genes in 98 individuals including 59 variants in 37 nuclear genes and 30 variants in eight mitochondrial genes. Additionally, there was one deletion spanning 137 nuclear

genes and 18 mitochondrial deletions spanning two to 10 genes. All nDNA and mtDNA variants in previously established disease genes and their ACMG variant classification are summarized in Supplemental Table 5. There were five individuals who had common pathogenic variant ($n=1$), multiple mtDNA deletions ($n=2$) and large mtDNA single deletions ($n=2$) with a $<20\%$ heteroplasmy rate in muscle. There were seven VUS in six genes in six individuals including *TRIP12* (OMIM# 604506), *NEFH* (OMIM#162230), *RRM2B* (OMIM#604712), *NLRP3* (OMIM#606416), *POLG* (OMIM#174763), and *RYR1* (OMIM# 180901). These include two genes causing nDNA-PMD (*POLG*, *RRM2B*) and four genes causing non-PMD (*TRIP12*, *NEFH*, *NLRP3*, *RYR1*). Despite that the phenotypes of these individuals matched with their genotypes, due to the VUS classification of these variants, definitive genetic diagnoses were not confirmed (Supplemental Table 5). Additionally, we identified *PLCH2* (OMIM#612836) candidate gene in one individual (Supplemental Table 5). None of these individuals were included into the diagnostic yield calculations or Supplemental Tables 1, 2 and 3.

One hundred-three individuals underwent muscle biopsy (children $n=24$ and adults $n=79$). PMD was confirmed in 25 of those individuals (mitochondrial $n=16$, nuclear $n=9$). Five of those individuals had non-PMD. We depicted the number of molecular genetics and genomic investigations of these individuals in Supplemental Fig. 1.

Phenotypes, biochemical features, and genotypes of individuals in group 1

This group includes 41 individuals with mtDNA-PMD (children $n=5$; adults $n=36$) from 40 families (Supplemental Table 5). We summarized their diagnoses, clinical features, biochemical features, neuroimaging, and genotypes in Supplemental Table 1. The number of individuals with different genetic diseases is depicted in Fig. 1. We included five individuals (Mito058; Mito214; Mito268; Mito282; Mito397) (Supplemental Table 1) with low heteroplasmy rate ($<20\%$) in different tissues (muscle $n=1$; urine $n=2$; blood $n=2$). Only one of those individuals with a single large mtDNA deletion and a low heteroplasmy in muscle (Mito282) had a negative clinical exome sequencing. Despite low heteroplasmy rates, they were included into the Supplemental Table 1 and the diagnostic yield calculations as there are several reports in the medical literature that we summarized in the discussion.

Chronic progressive external ophthalmoplegia (CPEO) (OMIM#530000) was the most common phenotype ($n=17$) in 41.5% of individuals with large mitochondrial DNA deletions. Muscle histopathology results revealed ragged red fibres ($n=10$), reduced cytochrome c oxidase

Table 1 Demographic information of all individuals is summarized in Table 1

| Demographics | Group 1 | Group 2 | Group 3 | Group 4 | Total |
|--------------------------|---|--|--|---|---|
| Numbers | Total (n=41) Adult (n=36) Children (n=5) | Total (n=30) Adult (n=13) Children (n=17) | Total (n=22) Adult (n=8) Children (n=14) | Total (n=204) Adult (n=140) Children (n=64) | Total (n=297) Adult (n=197) Children (n=100) |
| Male | Total=15 Adult=14 Children=1 | Total=13 Adult=7 Children=6 | Total=13 Adult=5 Children=8 | Total=83 Adult=48 Children=35 | Total=124 Adult=74 Children=50 |
| Female | Total=26 Adult=22 Children=4 | Total=17 Adult=6 Children=11 | Total=9 Adult=3 Children=6 | Total=121 Adult=92 Children=29 | Total=173 Adult=123 Children=50 |
| Current age (range) | Total=45.1±19.5 SD yrs (6 mo–77 yrs) Adult=50±15.2 SD yrs (21–77 yrs) Children=9.9±6.2 SD yrs (6 mo–15 yrs) | Total=19.8±16.3 SD yrs (4 mo–51 yrs) Adult=34.8±12.7 SD yrs (19–51 yrs) Children=8.4±6.6 SD yrs (4 mo–yrs) | Total=19.1±18 SD yrs (1–71 yrs) Adult=38.5±15.9 SD yrs (19–71 yrs) Children=8.1±5.02 SD yrs (1.5–15 yrs) | Total=33.1±22.5 SD yrs (6 wks–84 yrs) Adult=44.2±18.2 SD yrs (19–84 yrs) Children=8.8±5.5 SD years (6 wks–18 yrs) | Total=32.4±22.4 SD yrs (6 wks–84 yrs) Adult=44.4±17.6 SD yrs (19–84 yrs) Children=8.7±5.6 SD years (6 wks–18 yrs) |
| Age of onset (range) | Total=38.4±17.8 SD yrs (3 mo–69 yrs) Adult=38.4±17.8 SD yrs (18–69 yrs) Children=1.3±1.8 SD yrs (NB–4 yrs) | Total=9.5±15.4 SD yrs (NB–58 yrs) Adult=20.9±19.6 SD yrs (6 mo–49 yrs) Children=2.4±3.8 SD yrs (NB–14 yrs) | Total=4.4±7.8 SD yrs (NB–33 yrs) Adult=19.6±11 SD yrs (NB–33 yrs) Children=1.9±3.3 SD yrs (NB–10 yrs) | Total=20.9±20.8 SD yrs (0–74 yrs) Adult=31.4±19.6 SD years (NB–74 years) Children=3.0±3.7 SD yrs (NB–14 yrs) | Total=20±20.7 SD yrs (NB–74 yrs) Adult=31.1±19.4 SD yrs (NB–74 yrs) Children=2.6±3.6 SD years (NB–14yrs) |
| Age of diagnosis (range) | Total=38.8±18.7 SD yrs (5 mo–75 yrs) Adult=43.7±13.9 SD yrs (19–75 yrs) Children=4.1±4.4 SD yrs (NB–11 yrs) | Total=12.8±15.3 SD yrs (NB–59 yrs) Adult=24±17.8 SD yrs (2 mo–59 yrs) Children=4.7±4.7 SD yrs (NB–12 yrs) | Total=13.6±16.5 SD yrs (2 mo–65 yrs) Adult=28±18.9 SD yrs (2–65 yrs) Children=4.7±3.9 SD yrs (2 mo–11 yrs) | NA | Total=24.7±21.1 SD yrs (NB–75 yrs) Adult=36.4±17.9 SD yrs (2 mo–75 yrs) Children=4.5±4.06 SD yrs (NB–12 yrs) |
| Deceased | Total=5 Adult=4 Children=1 | Total=3 Adult=1 Children=2 | Total=5 Adult=1 Children=4 | Total=26 Adult=10 Children=16 | Total=39 Adult=16 Children=23 |
| Most common phenotypes | Total=Muscular (n=18) Adult=Muscular (n=16) Children=Neurodevelopmental (n=4) | Total=Muscular (n=17) Adult=Muscular (n=9) Children=Neurodevelopmental (n=11) | Total=Neurodevelopmental (n=17) Adult=Muscular (n=5) Children=Neurodevelopmental (n=13) | Total=Muscular (n=102) Adult=Muscular (n=77) Children=Neurodevelopmental (n=34) | Total=Muscular (n=150) Adult=Muscular (n=104) Children=Neurodevelopmental (n=62) |

Mo(s) = Month(s); NB = Newborn; NA = Not applicable; Wks = Weeks; Yr(s) = Year(s)

(COX) staining (n=7), and mitochondrial abnormalities in electron microscopy (n=10). 78% of individuals had a confirmed mtDNA-PMD by mitochondrial genome sequencing with or without deletion/duplication analysis and 87.5% of these genetic diagnoses were confirmed in muscle (n=15) or urine (n=14) mtDNA samples.

Phenotypes, biochemical features, and genotypes of individuals in group 2

This group includes 30 individuals with nDNA-PMD (children n=17; adults n=13) from 25 families with 35 pathogenic/likely pathogenic variants in 19 different genes (Supplemental Table 5). We summarized their

diagnoses, clinical features, biochemical features, neuroimaging, and genotypes in Supplemental Table 2. The number of individuals with different genetic diseases is depicted in Fig. 1.

The most common nDNA-PMD was dilated cardiomyopathy with ataxia syndrome (DCMA), also known as 3-methylglutaconic aciduria type V (OMIM#610198), due to biallelic pathogenic variants in *DNAJC19* (MIM#608977). There were seven affected individuals from four families. All individuals had elevated urine 3-methylglutaconic acid (if measured), and dilated cardiomyopathy or left ventricular dysfunction in echocardiography. Only one-third of individuals had ataxia.

Table 2 Diagnostic yield of molecular genetics and genomic investigations of all individuals are summarized in Table 2

| | Diagnostic yield of clinical exome sequencing <i>n</i> (%) | Diagnostic yield of next generation sequencing panels <i>n</i> (%) | Diagnostic yield of mitochondrial genome sequencing <i>n</i> (%) | Diagnostic yield of mitochondrial common variant testing <i>n</i> (%) |
|----------|--|--|---|---|
| Total | 33 (37.1%) | 9 (18.4%) | 32 (15.8%) Muscle mtDNA 17 (17.9%) Urine mtDNA 14 (16.1%) Blood mtDNA 4 (14.3%) BuccalmtDNA 2 (50%) | 8 (11.8%) |
| Adult | 13 (25.5%) | 3 (10.3%) | 28 (20.7%) Muscle mtDNA 15 (23.8%) Urine mtDNA = 13 (22.4%) Blood mtDNA 1 (5.9%) Buccal mtDNA 1 (50%) | 6 (12.7%) |
| Children | 20 (52.6%) | 6 (30%) | 4 (6%) Muscle mtDNA 2 (6.3%) Urine mtDNA 1 (3.4%) Blood mtDNA 1 (9%) Buccal mtDNA 1 (50%) | 2 (9.5%) |

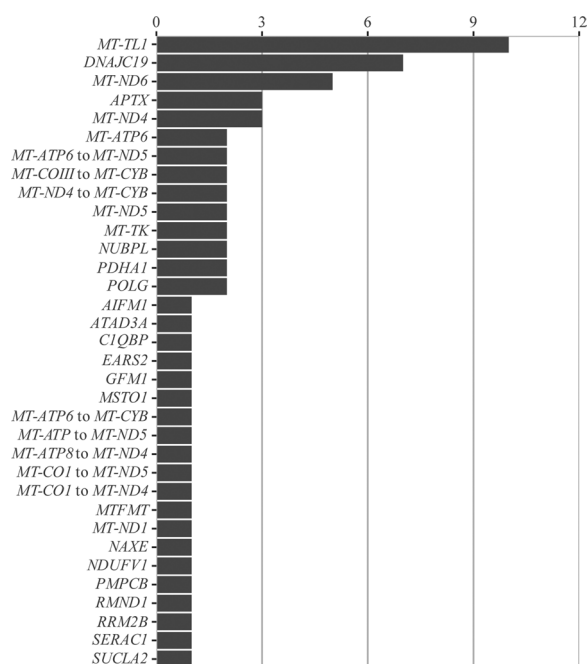


Fig. 1 Genetic landscape of mtDNA-PMD and nDNA-PMD is depicted in Fig. 1

Cerebellar atrophy was reported in two out of four individuals who underwent brain MRI. One out of three individuals had hepatic steatosis in liver ultrasound.

Muscle histopathology results revealed ragged red fibres (*n*=2), reduced COX staining (*n*=3) and mitochondrial abnormalities in electron microscopy (*n*=2).

63.3% of individuals had confirmed genetic diagnoses by clinical exome sequencing.

Phenotypes, biochemical features and genotypes of individuals in group 3

This group includes 22 individuals with non-PMD from 21 families with 22 pathogenic/likely pathogenic variants. We summarized their diagnoses, clinical features, biochemical features, neuroimaging, and genotypes of individuals with non-PMD in Supplemental Table 3.

Muscle histopathology results revealed ragged red fibres (*n*=1), reduced COX staining (*n*=3), and mitochondrial abnormalities in electron microscopy (*n*=2). 63.6% of individuals had confirmed genetic diagnoses by clinical exome sequencing.

Phenotypes, biochemical features and genotypes of individuals in group 4

There were 204 individuals with suspected PMD without a genetic diagnosis. We summarized their phenotypes, biochemical investigations and molecular genetics and genomic investigations in Supplemental Table 4 and Supplemental Fig. 2. These individuals underwent extensive molecular genomic investigations including mitochondrial genome sequencing (72%), clinical exome sequencing (25.5%) and next generation sequencing panels (15.7%). It is still likely that some of these individuals may have a mtDNA- or nDNA-PMD.

3D protein structure prediction

The *RRM2B*, and *POLG* VUS showed shifts in their protein structures compared to the wildtype, with movements of large peripheral alpha helices measuring 17.5 and 7.8 angstroms, respectively. The structural analysis of these VUS are depicted in Supplemental Figs. 3 and 4.

Comparison of individuals between groups

We compared Groups 1, 2, and 3 for their phenotypes, biochemical features, muscle histopathology, electron transport chain activities, neuroimaging, and neurophysiological studies. We summarized all these information and their statistical analysis in Supplemental Table 6 and Supplemental Table 7. We depicted all these information in Fig. 2 and Supplemental Fig. 5. We combined Groups 1 and 2 (all with PMD) and compared with Group 3 (Supplemental Table 7 and Fig. 3). There was a statistically significant difference for muscular, cardiac, and ophthalmologic phenotypes, seizures, hearing loss, peripheral neuropathy in the Groups 1+2 compared to Group 3. Whereas there was a statistically significant difference

for neurodevelopmental and gastrointestinal phenotypes, movement disorder and hypotonia in Group 3 compared to Group 1+2. The comparisons are depicted in Fig. 3 and Supplemental Table 7.

Discussion

We report 71 individuals with 26 different mitochondrial and nuclear single gene PMD and 14 different mitochondrial genome deletions in our study cohort. Adults had higher percentage (87.8%) of mtDNA-PMD compared to children (p -value = 0.00123) in our study, which has been previously reported that about 30% of mtDNA-PMD are children [27–31]. Muscle histochemistry was suggestive of PMD in 44% of individuals with mtDNA-PMD, but only in 20% of individuals with nDNA-PMD. There is a statistically significant difference for neurodevelopmental phenotype between children and adults with PMD (p = 0.0004021). Although there is no statistically significant difference for muscular phenotype between children and adults, this phenotype is more common in adults. It has been previously reported that adults are more likely

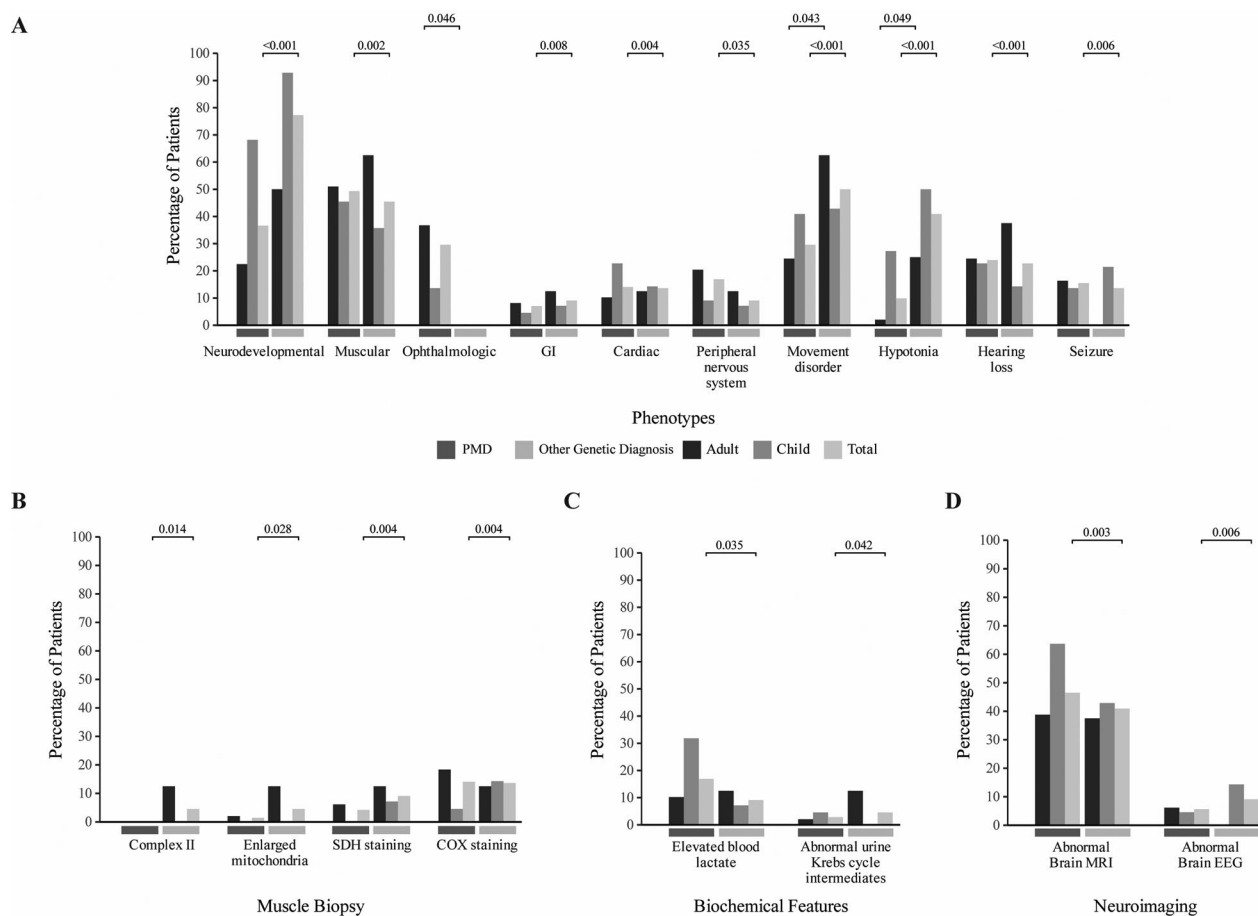


Fig. 2 Comparison of phenotypes, biochemical features, muscle histopathology, electron transport chain activities, neuroimaging, and neurophysiological studies between all groups is depicted in Fig. 2

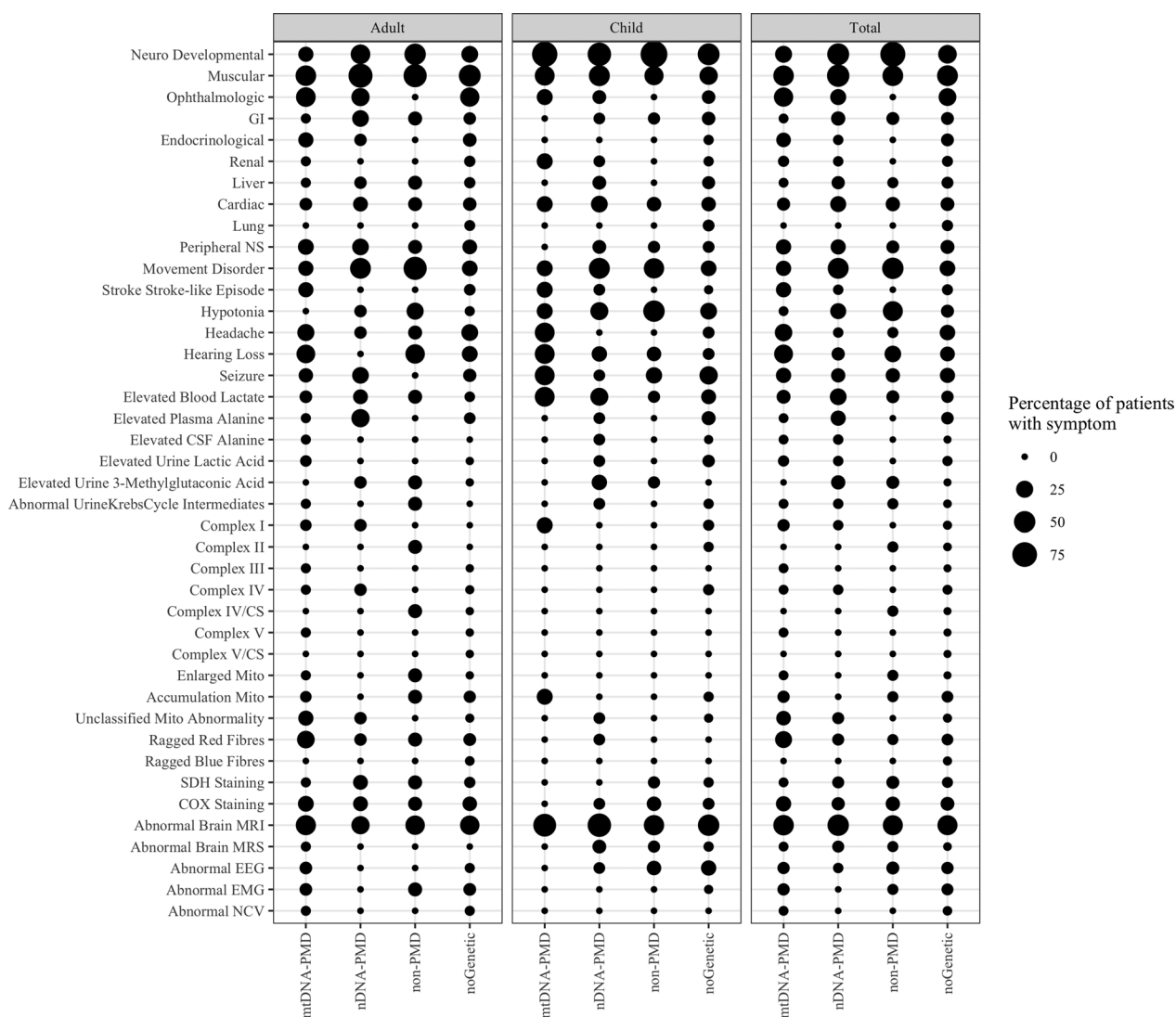


Fig. 3 Comparison of clinical, biochemical features, muscle biopsy, and neuroimaging results between PMD (Groups 1 + 2) and non-PMD group (Group 3) is depicted in Fig. 3

to present with classical PMD syndromes, whereas children present with non-specific [32] or neurodevelopmental phenotypes [32, 33]. We included four individuals with low heteroplasmy in either urine or in blood as they may have higher heteroplasmy in muscle. This was previously reported comparing muscle, urine and blood heteroplasmy rates that the muscle has the highest heteroplasmy rate [34–36]. We included two individuals: (1) one with a single large mtDNA deletion and a low heteroplasmy rate of 12% in muscle, who had normal clinical exome sequencing; (2) with multiple mtDNA deletions with a heteroplasmy rate of 20–30% as there were reports of low heteroplasmy in muscle for single or multiple mtDNA deletions [37–39]. We think that in

the future more individuals with low heteroplasmy rate in muscle with single or multiple mtDNA deletions might be reported as mtDNA-PMD.

The diagnostic yield of clinical exome sequencing for suspected PMD has been reported between 35 and 57% in six different studies including children and/or adults [29, 40–45]. There were 28 to 142 individuals included into those studies. The number of confirmed genetic diseases ranged between 14 and 42. The diagnostic yield of targeted next generation sequencing panels for suspected PMD has been reported between 2.5 and 41% in five different studies including children and/or adults [46–52]. The number of genes on those next generation sequencing panels ranged between six and 1598. There

were 42 to 450 individuals included in those studies. The number of confirmed genetic diseases ranged between six and 46 in those studies. The diagnostic yield of mitochondrial genome sequencing for suspected PMD has been reported between 2.4 and 35% in children and/or adults in four different studies [43, 47, 48, 52]. Two of those studies used blood samples and the diagnostic yields were between 7 and 12% [43, 48] and two of those studies used muscle samples and the diagnostic yields were between 2.4 and 35% [47, 52]. Detection of single mtDNA variants (m.3243A>G; m.8344A>G) [34, 36, 53] and single large-scale mtDNA deletions [34, 35] in urine were previously reported for the diagnosis of mtDNA-PMD. In a recent study, urine was sensitive to identify pathogenic mtDNA variants in nine out of 11 individuals with suspected PMD [54]. In our study, the diagnostic yield of clinical exome sequencing was 37.1%; the diagnostic yield of targeted next-generation sequencing panels was 18.4% and the diagnostic yield of mitochondrial genome sequencing was 15.8% including 17.9% in muscle mtDNA and 16.1% in urine mtDNA. To the best of our knowledge, we report for the first-time the diagnostic yield of urine mitochondrial genome sequencing in individuals with suspected PMD ($n=87$) in the diagnosis of mtDNA-PMD. We think that the availability of urine mitochondrial genome sequencing in molecular genetics and genomic laboratories may be used as a first line cost effective and non-invasive screening test in adult individuals with suspected PMD.

Despite a similar number of children and adults underwent mitochondrial genome sequencing and nuclear genetics and genomic investigations, there were 36 adults with mtDNA-PMD whereas 17 children with nDNA-PMD (p -value=0.00123). This is likely due to mitochondrial clonal expansion where pathogenic mitochondrial DNA variants (missense and deletions/duplications) replicate more than the wildtype mitochondrial DNA within a cell [55–57]. These pathogenic mitochondrial DNA variants accumulate over time and lead to a higher proportion of mitochondria carrying the same pathogenic variant within the cell and impair mitochondrial function [55–57]. There are several hypotheses for the mitochondrial clonal expansion. The random genetic drift hypothesis refers to pathogenic mitochondrial DNA variants accumulate by chance without selective advantage [58, 59]. The "survival of the smallest" [60] and "survival of the sickest" [61] hypotheses propose that smaller or less functional mitochondrial DNA molecules have a replicative advantage, either due to quicker replication or evasion of mitophagy. The negative feedback loop hypothesis proposes that reductions in mitochondrial DNA-encoded proteins lead to compensatory increases in mitochondrial DNA replication [62]. The perinuclear niche

hypothesis suggests localized cellular responses to mitochondrial dysfunction near cell nuclei drive mitochondrial DNA replication through retrograde stress signaling [63]. Finally, a decline in mitophagy due to aging is associated with the accumulation of damaged mitochondria, which may explain the clonal expansion of pathogenic mitochondrial DNA variants [64, 65]. mtDNA variants are more likely to be lost in rapidly dividing cells such as blood, intestinal epithelium, buccal mucosa, and urine [55, 66–69]. However, it has been reported in some individuals that large-scale mtDNA deletions clonally expand from birth in skeletal muscle fibres and neurons in post-mitotic cells [55, 56, 70]. In our study, 66.7% of variants in muscle samples were deletions in adults with mtDNA-PMD. Interestingly, a study investigated muscle fibers in one individual and found that the number of mitochondria was increased in muscle fibers who had high number of mitochondrial DNA deletions and marked electron transport chain deficiencies in those fibers compared to the muscle fibers with less mitochondrial DNA deletions [71]. Mitochondrial DNA heteroplasmy increases with age due to accumulation of pathogenic mitochondrial DNA variants [72–74]. This may also explain why children have less mtDNA-PMD diagnosis in our study as they may have heteroplasmy rates lower than the detection limit of 10%.

DCMA is one of the rare nDNA-PMD due to biallelic pathogenic variants in *DNAJC19*. Less than 100 individuals with DCMA have been reported in the medical literature to date since its first description in 2006. In 2021, the c.130-1G>C pathogenic *DNAJC19* variant was reported in 43 individuals with DCMA from a Hutterite population in Alberta, Canada [75, 76]. The detailed phenotypes, neuroimaging, and long-term outcome information was available for about half of those individuals. Only one individual had macrovesicular steatosis, and moderate fibrosis in liver biopsy [75]. In a recent study, one individual with DCMA had fatty liver changes in liver ultrasound and severe steatosis and fibrosis in liver biopsy [77]. To the best of our knowledge, we report the third individual with DCMA who has hepatic steatosis in the liver ultrasound and five new individuals with DCMA for the first time in the medical literature. Interestingly, *DNAJC19* plays a role in cardiolipin remodeling by binding with the prohibitin PHB2 molecule and modifying cardiolipin acylation resulting in the impairment of the integrity of the inner mitochondrial membrane [78–80]. Cardiolipin dysfunction impairs oxidative phosphorylation and increases the production of the reactive oxygen species (ROS) [80]. Additionally, hepatic steatosis is reported to be secondary to accumulation of fatty acids in liver cells and the exacerbation of oxidative stress and insulin resistance in PMD [81–83]. Mitochondrial ROS

formation causes non-alcoholic steatosis in PMD [84]. There have been reports of hepatic disease (e.g. steatosis, fibrosis, cirrhosis) in about 20% of individuals with more than 70 different PMD [85, 86]. In our study, 5.6% of individuals with nDNA-PMD and mtDNA-PMD had hepatic disease (hepatic steatosis $n=1$, hepatic cirrhosis $n=1$, unspecified hepatic disease $n=2$). It seems that there is a spectrum of liver phenotypes in PMD and ongoing oxidative stress, accumulation of fatty acids in liver and mitochondrial ROS formation result in a progressive hepatic disease leading to fibrosis and cirrhosis. For these reasons, we recommend close monitoring of liver in PMD for better understanding of the natural history of hepatic disease and management of disease morbidity.

There are other genetic diseases that mimic PMD [87]. Sometimes biochemical investigations cannot differentiate PMD from non-PMD [88]. Some of the non-PMD are spinal muscular atrophy (*SMN1*) [89], Friedreich ataxia (*FXN*) [90], Charcot-Marie-Tooth disease type 2 K (*GDAP1*) [89], hereditary spastic paraplegia 7 (*SPG7*), Wilson disease (*ATP7B*) [89], methylmalonic aciduria and propionic aciduria [91, 92], fatty acid oxidation disorders [93], argininosuccinic aciduria [94], purine and pyrimidine synthesis disorders [95], *BCAP31* associated encephalopathy [96] and riboflavin transporter deficiency (*SLC52A2*, *SLC52A3*) [97]. Some of these non-PMD are involved in mitochondrial energy metabolism and affect important co-factors for several enzymes such as Friedreich ataxia, riboflavin transporter deficiency, hereditary spastic paraplegia 7, and fatty acid oxidation disorders. Renal mitochondrial damage and altered mitochondrial energy metabolism was reported in propionic aciduria [98]. There is an overlap between PMD and non-PMD where the mitochondria are involved. Either accumulation or deficiency of organic molecules or cofactors affect the mitochondrial energy metabolism causing overlapping phenotypes (e.g., neurodevelopmental disorders, epilepsy, movement disorders) and biochemical features (e.g., elevated lactate, electron transport enzyme deficiencies) [87, 99–102]. There is an ongoing international collaboration for classification of metabolic genetic diseases [103, 104] that the list of PMD may be larger in the future. In our study cohort, 29% of individuals with confirmed genetic diseases had features suggestive of PMD (five with abnormal biochemical features suggestive of PMD). We summarized all causative genes causing and their protein–protein interactions with mitochondrial genes in our study cohort Supplemental Table 8 [44, 105–113]. There were 14 genes interacting with 32 mitochondrial genes associated with the electron transport chain, mitochondrial transcription regulation, mitochondrial ribosomal proteins, mitochondrial membrane transport and mitochondrial homeostasis (depicted in

Supplemental Fig. 6). It is important to remember that the confirmation of molecular genetic diagnosis in PMD is crucial for the implication of the prognosis and management decisions. A misdiagnosis of PMD may prematurely end the diagnostic odyssey, overmedicalize the care and potentially limit access to appropriate treatments for the actual underlying genetic diseases. It is important to know that other genetic diseases can mimic PMD.

Our study had several limitations including: 1) It is a retrospective cohort study; 2) There were no detailed molecular genetics and genomic investigations for different phenotypes in several individuals; 3) We did not have biochemical investigations, muscle histopathology and electron transport chain enzyme activity measurements in the majority of our study cohort; 4) Adult muscle biopsy is part of clinical care at our center, which may explain why there are fewer children with muscle biopsy compared to adults. Despite these limitations, we report a large cohort of individuals with PMD and provide diagnostic yield of different molecular genetics and genomic investigations for the genetic diagnosis of PMD.

In conclusion we report a 23.7% diagnostic yield of molecular genetics and genomic investigations for the diagnosis of PMD in our cohort. We report the diagnostic yield of urine (16.1%) mitochondrial genome sequencing for the first time. We also report 71 individuals with 26 different mtDNA and nDNA single gene PMD and 14 different mtDNA deletions. Interestingly mtDNA-PMD was significantly more common in adults. We showed that liver phenotype might be progressive leading to cirrhosis which needs to be studied further by close monitoring of liver in PMD. We were able to show a direct relationship between some of the non-PMD genes and their indirect effects on mitochondrial machinery.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13023-024-03437-x>.

Additional file 1.
Additional file 2.
Additional file 3.
Additional file 4.
Additional file 5.
Additional file 6.
Additional file 7.
Additional file 8.
Additional file 9.

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Author contributions

AA, DZ, SS MS: Reviewed charts, generated the database, drafted the manuscript, conducted the work, and read and approved the final version of the manuscript. AA and SS: Applied ACMG criteria for the variant classification. SB: Applied the statistical analysis, created figures, read and approved the final version of the manuscript. CL: Completed biochemical information entries into the database, read and approved the final version of the manuscript. SG-J and AC: provided information for patients included into the study, read and approved the final version of the manuscript. S.M-A: Planned, applied, and received funding, designed the study, analyzed the data, and drafted and revised the manuscript, read and approved the final version of the manuscript. All authors read and approved the final version of the manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate

The Research Ethics Office, Health Research Ethics Board, University of Alberta (Study ID: Pro00112487) approved this study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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