

Endocrine Disorders in Primary Mitochondrial Disease

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Context: Endocrine disorders are common in individuals with mitochondrial disease. To develop evidence-based screening practices in this high-risk population, updated age-stratified estimates of the prevalence of endocrine conditions are needed.

Objective: To measure the point prevalence of selected endocrine disorders in individuals with mitochondrial disease.

Design, Setting, and Patients: The North American Mitochondrial Disease Consortium Patient Registry is a large, prospective, physician-curated cohort study of individuals with mitochondrial disease. Participants (n = 404) are of any age, with a diagnosis of primary mitochondrial disease confirmed by molecular genetic testing.

Main Outcome Measures: Age-specific prevalence of diabetes mellitus (DM), abnormal growth and sexual maturation (AGSM), hypoparathyroidism, and hypothyroidism.

Results: The majority of our sample was pediatric (<18 years; 60.1%), female (56.9%), and white (85.9%). DM affected 2% of participants aged <18 years [95% confidence interval (CI): 0.4% to 5.7%] and 24.4% of adult participants (95% CI: 18.6% to 30.9%). DM prevalence was highest in individuals with mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes syndrome (MELAS; 31.9%, of whom 86.2% had the m.3243A>G mutation). DM occurred more often with mitochondrial DNA defects (point mutations and/or deletions) than with nuclear DNA mutations (23.3% vs 3.7%, respectively; $P < 0.001$). Other prevalence estimates were 44.1% (95% CI: 38.8% to 49.6%) for AGSM; 0.3% (95% CI: 0% to 1.6%) for hypoparathyroidism; and 6.3% (95% CI: 4% to 9.3%) for hypothyroidism.

Conclusion: DM and AGSM are highly prevalent in primary mitochondrial disease. Certain clinical mitochondrial syndromes (MELAS and Kearns-Sayre/Pearson syndrome spectrum disorders) demonstrated a higher burden of endocrinopathies. Clinical screening practices should reflect the substantial prevalence of endocrine disorders in mitochondrial disease.

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Abbreviations: AGSM, abnormal growth and sexual maturation; CI, confidence interval; CPEO, chronic progressive external ophthalmoplegia; DM, diabetes mellitus; GH, growth hormone; KSS, Kearns-Sayre syndrome; LHON, Leber hereditary optic neuropathy; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes syndrome; MERRF, myoclonic epilepsy with ragged red fibers; mtDNA, mitochondrial DNA; NAMDC, North American Mitochondrial Disease Consortium; nDNA, nuclear DNA; NIH, National Institutes of Health; OR, odds ratio; tRNA, transfer RNA.

Freeform/Key Words: diabetes mellitus, growth, hypothyroidism, mitochondrial disease

Primary mitochondrial disease encompasses a wide range of multisystem disorders caused by pathogenic genetic variants that lead to impairment of the mitochondrial respiratory chain and defective energy production [1, 2]. Clinical manifestations are highly heterogeneous and may occur at any age. Organs with substantial energy requirements (brain, heart, kidneys, skeletal muscle, and many endocrine organs) are frequently affected [1–5]. The minimal estimated prevalence of primary mitochondrial disease is 1:~4300 [4].

Mitochondrial disease genetics is complex. Abnormalities can occur in two genomes: the ~16.6-kb mitochondrial DNA (mtDNA) and/or the >250 recognized nuclear disease genes (nuclear DNA; nDNA) that encode constituents of the mitochondrial respiratory chain and/or proteins that contribute to physiologic mitochondrial function [1, 4]. The classification of mitochondrial diseases based on genetic diagnoses can be challenging, as individuals with identical pathogenic variants often exhibit a broad spectrum of clinical features. Mitochondrial disease categories are often defined phenotypically, according to commonly co-occurring clinical features [2, 4].

Despite the clinical heterogeneity of mitochondrial diseases, endocrine disorders are consistently encountered. The underlying mechanisms by which endocrine abnormalities occur are complex and incompletely understood. Overall, a lack of adenosine triphosphate production and/or increased oxidative stress in endocrine cells with impaired mitochondria may lead to failure of hormonal synthesis and/or secretion. Abnormal cellular signaling and calcium handling have also been implicated in the pathogenesis of mitochondrial disease endocrinopathies [5]. The best characterized comorbid endocrine condition in primary mitochondrial disease is diabetes mellitus (DM). DM has been strongly linked to the mtDNA m.3243A>G mutation, the most common genetic mutation underlying mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS) syndrome; however, DM is also observed in association with other mutations and other clinical forms of mitochondrial disease including Kearns-Sayre syndrome (KSS) [3, 6, 7]. Additional endocrine disorders that have been variably reported in mitochondrial disease include hypoglycemia, poor growth, short stature and growth hormone (GH) deficiency, hypoparathyroidism, hypothyroidism, adrenal insufficiency, pancreatic insufficiency, hypogonadism, and dyslipidemia [1, 5, 8–10]. Especially for conditions other than DM, most of the descriptions of endocrine disorders in mitochondrial disease are based on single case reports or small case series. Current prevalence estimates are insufficient to support the development of evidence-based screening guidelines for endocrine disorders other than DM in this likely high-risk population.

The objective of this study was to measure the point prevalence of selected endocrine disorders in a large cohort of patients with primary mitochondrial disease. Specifically, we leveraged a recently established resource to study this set of conditions, the National Institutes of Health (NIH)–supported North American Mitochondrial Disease Consortium (NAMDC) Patient Registry [11], which systematically collects data with respect to the presence or absence of several commonly encountered endocrine conditions. With this study, we aimed to enrich our understanding of previously observed clinical associations between diverse endocrinopathies and mitochondrial disease. These estimates will inform future studies focused on determining optimal screening strategies for endocrine disorders in individuals with primary mitochondrial disease and quantify the burden of undiagnosed endocrinopathies. Endocrine conditions are important to identify because they are often amenable to specific therapies; thus, prompt recognition and treatment would be expected to improve patient outcomes.

1. Methods

A. Participants

We included individuals from the NAMDC Patient Registry, an institutional review board-approved clinical registry of patients with suspected or confirmed mitochondrial diseases. We

confined our analyses to individuals with molecular genetic diagnoses confirming primary mitochondrial disease. The registry is a prospective, ongoing cohort study initiated in December 2010. Individuals of any age with suspected or confirmed mitochondrial diseases are enrolled through one of the 16 participating NAMDC clinical research sites. Referred patients are evaluated by NAMDC physicians and research staff members, who obtain informed consent and assent, facilitate the collation and review of medical history data and diagnostic test results, and enter data in a centralized electronic registry. Participants in the NAMDC registry are assigned a research diagnosis of mitochondrial disease on the basis of clinical, biochemical, and/or molecular genetic testing data that are determined according to standardized, consensus-based diagnostic criteria developed by NAMDC mitochondrial disease clinical experts. Clinical information on each participant is updated by each referring site annually [11–13].

B. Inclusion and Exclusion Criteria

Our analyses were restricted to participants with a diagnosis of primary mitochondrial disease confirmed by molecular genetic testing at any age from December 2010 to June 2016 inclusive. For the current study, a data use request was submitted to and reviewed by the NAMDC Data Use Committee, who provided the data for analysis. The extent and type of genetic testing performed, as well as interpretation of results, was at the discretion of the participants' NAMDC collaborating investigators. All genetic testing results are reviewed by an internal NAMDC clinical expert committee that determines the pathogenicity of genetic results and assigns a definite molecular genetic diagnosis [11]. We excluded participants who had not undergone genetic testing and/or in whom genetic testing did not disclose a diagnosis and who had missing sex and/or age information at NAMDC enrollment. All participants gave informed consent and/or assent to be part of the registry. No participant-identifying information was included in the request.

C. Demographics

We noted the participants' age at mitochondrial disease diagnosis, sex, and race/ethnicity. Age at mitochondrial disease diagnosis was classified into four categories: <18 years (pediatric), 18 to 44 years (young adult), 45 to 64 years (adult), and ≥ 65 years (older adult). When the age at diagnosis was not noted, the statistic was left as "unknown." We also reported age at NAMDC registry enrollment using the same categories. Race/ethnicity was participant-reported as white, Asian, Hispanic or Latino, black/African American, multiracial, American Indian/Alaska native, native Hawaiian/Pacific Islander, or other/unknown.

D. Molecular Genetic Diagnoses Classification

Genetic diagnoses were classified first as nDNA or mtDNA gene disorders causing primary or secondary mitochondrial disease. Next, nDNA and mtDNA pathogenic mutations were classified into primary mitochondrial disease subcategories according to previously published organizing schema [2, 4, 14–21], and as detailed in Supplemental Table 2.

E. Clinical Mitochondrial Syndromes

Currently, 24 clinical syndromes are reported in the NAMDC registry, listed in Supplemental Table 3 [11]. Physicians could also note clinical mitochondrial disease syndromes other than these 24 prespecified syndromes via "free text" entry.

F. Endocrine Conditions

Although individuals with mitochondrial disorders can have a range of endocrine disorders, the NAMDC data collection form systematically asks about the presence or absence of four

specific endocrine conditions: DM, hypogonadotropic hypogonadism, hypoparathyroidism, and hypothyroidism. It also specifically assesses constitutional symptoms, including growth delay, abnormal pubertal growth spurt, short stature, thinness, and cachexia. Each of these conditions is noted to be present, absent, or not assessed/unknown. Because the NAMDC data collection instrument is a comprehensive multisystem assessment, only selected conditions with substantial expected prevalence were assessed systematically in this way [22]. Age at onset of the endocrine condition was also noted if known.

In addition, free-text reporting of other endocrine conditions was permitted. Investigators reported either laboratory abnormalities or diagnoses. Because many of the designations regarding abnormal growth and development capture overlapping pathophysiologic processes, we designated a summary variable, “abnormal growth and sexual maturation” (AGSM), which included hypogonadotropic hypogonadism as well as all the previously noted constitutional symptoms. Also, free-text reports of GH deficiency or other hypogonadism were included in the AGSM summary variable. Because GH deficiency is of particular interest to clinicians as a treatable cause of abnormal growth, it was reported separately as well.

To permit the collation of summary statistics, other free-text conditions or endocrine laboratory findings were either assigned to a primary variable when appropriate (*e.g.*, “low thyroid level” was included as indicative of hypothyroidism) or reported separately (*e.g.*, “adrenal insufficiency” and “dyslipidemia”).

G. Statistical Analyses

Descriptive statistics for demographics and characteristics of the cohort were generated as frequencies and percentages.

G-1. Prevalence estimates

The overall point prevalence was calculated separately for DM, AGSM, and hypothyroidism. Specifically, for each condition, the prevalence was calculated by dividing the number of subjects with a specified endocrine manifestation by the number of subjects in the entire cohort, excluding subjects with missing/unknown information regarding each specified manifestation. We calculated 95% confidence intervals (CIs) for prevalence estimates using the binomial exact method.

Age-specific and sex-specific prevalence estimates were also generated for DM, AGSM, and hypothyroidism. Age-specific prevalence was based on the age at registry enrollment, categorized into pediatric (<18 years) or adult. Multivariable logistic regression was used to assess for prevalence differences between subgroups of age and sex and to adjust for confounding associations of age or sex. We used the presence or absence of the specified endocrine condition as the outcome variable and age and sex as input variables.

G-2. Associations with genotypes and/or clinical syndromes

Prevalence estimates were calculated for genetic disease-causing mutation subtypes and for clinical syndromes. To generate clinically informative estimates, we specifically focused on clinical syndromes that were most highly represented in the NAMDC cohort and/or clinical syndromes with a high previously reported burden of endocrine manifestations, including: MELAS, chronic progressive external ophthalmoplegia (CPEO), and KSS/Pearson syndrome mtDNA deletion spectrum [1, 5, 23]. However, for these individually rare and primarily mtDNA-based syndromes, numbers in some subgroups remained small. Multivariable logistic regression was used to assess for prevalence differences between mutually exclusive mtDNA and nDNA mutations (*i.e.*, individuals with both types of mutations were excluded from these analyses), adjusting for confounding effects of age or sex. Again, we used the presence or absence of the specified endocrine condition as the outcome variable and age, sex, and mutation type as input variables. For endocrine conditions not assessed directly by the

NAMDC instrument (*i.e.*, free-text conditions), we reported their described frequencies. Additional prevalence estimates were not performed for these because information about free-text conditions was not collected systematically. For regression analyses, a two-sided *P* value <0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics software, version 24.

H. Supplemental Analysis

To elucidate the burden of endocrine disease in patients with clinical primary mitochondrial disease who have not received a molecular genetic diagnosis, we generated supplemental analyses of the original cohort (*i.e.*, those with and without molecular genetic diagnoses) and then stratified the original cohort by molecular genetic diagnosis. Supplemental Table 1A summarizes key demographic characteristics. Supplemental Table 1B shows the age- and sex-specific prevalence estimates.

2. Results

A. Demographics

During the analysis period, 634 individuals with biochemical, clinical, or molecular evidence consistent with a definite mitochondrial disease were enrolled in the NAMDC registry; 425 of these individuals had a molecular diagnosis. We excluded 21 subjects because of missing sex and/or age at NAMDC enrollment. Thus, the total sample size for our analyses was 404 subjects. The majority of our sample was <18 years of age (60.1%; *n* = 243) at the time of diagnosis with mitochondrial disease; only two subjects were ≥65 years at diagnosis. The sample was 56.9% female (*n* = 230), and 85.9% of individuals self-identified as white (*n* = 347). Demographic results are summarized in [Table 1](#).

B. Molecular Genetic Diagnoses

Pathogenic nDNA variants accounted for 39.1% (158 of 404; 95% CI: 34.3% to 44.1%), and mtDNA defects accounted for 56.4% (228 of 404; 95% CI: 51.4% to 61.3%) of genetic diagnoses. The remaining 4% (*n* = 18) included both nDNA and mtDNA pathogenic variants ([Table 1](#)). The most common pathogenic variant was m.3243A>G (23.5% of all genetic diagnoses; 95 of 404; 95% CI: 19.5% to 28%). The m.3243A>G mutation was linked mostly to the MELAS phenotype (81.1%; 77 of 95), and the remainder was linked to diabetes and deafness (*n* = 6), maternal-inherited deafness (*n* = 3), multisystemic syndrome (*n* = 5), myopathy (*n* = 1), or encephalopathy (*n* = 1) or had no specified clinical syndrome (*n* = 2). Pathogenic variants in *POLG*, an nDNA-encoded gene whose product is critical for mtDNA replication and repair, were the most common nDNA pathogenic variants reported (27.8% of exclusive nDNA pathogenic variants; 44 of 158 individuals). Supplemental Table 2 details the molecular genetic diagnoses in the present analysis according to the enrolling site investigators.

C. Associated Clinical Mitochondrial Syndromes

A total of 389 subjects (96.3% of the cohort) were assigned to a clinical syndrome. The most common clinical syndromes were MELAS (25.4%; 99 of 389) and Leigh syndrome (17.7%; 69 of 389). Supplemental Tables 3 and 4 detail the subgroups of mitochondrial syndromes as well as the mitochondrial syndromes that were not included in the primary classification schema, respectively.

D. Prevalence of Endocrine Disorders

The results are summarized in [Table 2](#).

Table 1. Demographics and Characteristics of Our Cohort

	Frequency (n = 404)	Percentage (95% CI)
Age at mitochondrial disease diagnosis		
<18 y	243	60.1
18–44 y	95	23.5
45–64 y	27	6.7
≥65 y	2	0.5
Unknown	37	9.2
Age at enrollment in the NAMDC Patient Registry		
<18 y	187	46.3
18–44 y	128	31.7
45–64 y	72	17.8
≥65 y	17	4.2
Sex		
Female	230	56.9
Male	174	43.1
Race/Ethnicity		
White	347	85.9
Asian	16	4
Hispanic or Latino	16	4
Black/African American	8	2
Multiracial	7	1.7
American Indian/Alaska Native	3	0.7
Native Hawaiian/Pacific Islander	1	0.2
Other/Unknown	6	1.5
Type of associated pathogenic molecular genetic mutation		
Mutation of nuclear DNA	158	39.1 (34.3–44.1)
Mitochondrial DNA defects	228	0.4 (51.4–61.3)
Mutation(s) in both	18	4.5(2.7–7)
Presence of a clinical mitochondrial syndrome diagnosis		
Diagnosis identified	389	96.3
Undetermined/unknown	15	3.7

1. **DM.** The overall prevalence of DM in primary mitochondrial disorders was 14.7% (52 of 353; 95% CI: 11.2% to 18.9%). DM affected 2% of pediatric participants aged <18 years (3 of 152), and 24.4% of adult participants (49 of 201). The increasing prevalence of DM with age was statistically significant [odds ratio (OR): 1.06 for each year of age increase; $P < 0.001$; 95% CI of OR: 1.04 to 1.08]. We did not detect sex-specific differences in DM prevalence. The mean age at DM diagnosis was 32.5 years (standard deviation = 15.5 years; range, 4 to 60 years). Approximately 47.6% of affected patients were diagnosed with DM after a mitochondrial disease diagnosis; 26.2% and 26.2% were diagnosed with DM before and at diagnosis of mitochondrial disease, respectively.
2. **AGSM.** The overall prevalence of AGSM was 44.1% (150 of 340; 95% CI: 38.8% to 49.6%). AGSM was more prevalent in participants <18 years of age (57.6%; 87 of 151). The association of AGSM with a younger age was statistically significant (OR: 0.98 for each year of age increase; $P < 0.001$; 95% CI of OR: 0.97 to 0.99). Hypogonadism was noted in 2% of participants (7 of 346; 95% CI: 0.8% to 4.1%).
3. **Hypothyroidism.** The overall prevalence of hypothyroidism was 6.3% (22 of 352; 95% CI: 4% to 9.3%). The prevalence increased with age (OR: 1.03 for each year of age increase; $P = 0.005$; 95% CI of OR: 1.01 to 1.05).
4. **Other.** Hypoparathyroidism was noted in 0.3% of participants (1 of 347; 95% CI: 0% to 1.6%). The frequencies of other reported conditions (e.g., dyslipidemia and adrenal insufficiency) were noted.

Table 2. Age- and Sex-Specific Frequency and Prevalence of Endocrine Diseases in the Cohort (n = 404)

Endocrine Disorder	Overall Prevalence (f/n) [95% CI]	Sex-Specific		Age-Specific ^a	
			Prevalence (f/n) [95% CI]		Prevalence (f/n) [95% CI]
Diabetes mellitus	14.7% (52/353) [11.2%–18.9%]	F	18.2% (37/203) [13.2%–24.2%]	Pediatric (<18 y)	2% (3/152) ^b [0.4%–5.7%]
		M	10% (15/150) [5.7%–16%]	Adult	24.4% (49/201) ^b [18.6%–30.9%]
Atypical growth and sexual maturation	44.1% (150/340) [38.8%–49.6%]	F	40.1% (77/192) [33.1%–47.4%]	Pediatric (<18 y)	57.6% (87/151) ^b [49.3%–65.6%]
		M	49.3% (73/148) [41%–57.7%]	Adult	33.3% (63/189) ^b [26.7%–40.5%]
Hypothyroidism	6.3% (22/352) [4%–9.3%]	F	8.4% (17/202) [5%–13.1%]	Pediatric (<18 y)	2.6% (4/152) ^c [0.7%–6.6%]
		M	3.3% (5/150) [1.1%–7.6%]	Adult	9% (18/200) ^c [5.4%–13.9%]

Other Reported Endocrine Disorders in the Cohort

Adrenal insufficiency (n = 3)
 Precocious puberty (n = 5)
 Dyslipidemia (n = 5)
 Dysglycemia (n = 5)
 Growth hormone deficiency (n = 6)
 Gestational diabetes (n = 1)
 Hyperthyroidism (n = 2)
 Hyperparathyroidism (n = 2)
 Hypoparathyroidism (n = 1)
 Calcium disturbances (n = 1)
 Osteopenia/osteoporosis (n = 2)
 Polycystic ovary syndrome (n = 2)
 Abnormal menses (n = 2)

Abbreviations: f, frequency; F, female; M, male; n, total; SE, standard error.

^aPrevalence is based on age at enrollment in the NAMDC Patient Registry.

^b $P \leq 0.001$, difference between age groups adjusted for sex. Logit (diabetes) = -0.96 (SE: 0.6) + $[0.5$ (SE: 0.34) if females] – $[2.7$ (SE: 0.29) if pediatric age]. Odds of (diabetes) = 0.23 + $[1.7$ (95% CI: 0.85–3.2) if females] + $[0.07$ (95% CI: 0.02–0.22) if pediatric age]. Logit (AGSM) = -0.5 – $(0.25$ if females) + $(0.97$ if pediatric age). Odds of (AGSM) = 0.58 + $[0.78$ (95% CI: 0.5–1.2) if females] + $[2.6$ (95% CI: 1.7–4.1) if pediatric age].

^c $P < 0.05$, difference between age groups adjusted for sex. Logit (hypothyroidism) = -2.9 + $(0.86$ if females) – $(1.2$ if pediatric age). Odds of (hypothyroidism) = 0.05 + $[2.4$ (95% CI: 0.8–6.6) if females] + $[0.3$ (95% CI: 0.1–0.9) if pediatric age].

E. Genotype-Phenotype Associations

The results are summarized in Supplemental Table 5.

1. **DM.** DM occurred more often with pathogenic mtDNA variants (23.3%) than with nDNA variants (3.7%) ($P = 0.001$, accounting for age and sex; OR: 5.1; 95% CI of OR: 1.9 to 14). DM was most prevalent in MELAS (31.9%; 29 of 91) and in myoclonic epilepsy with ragged red fibers (MERRF; 16.7%; 2 of 12). DM was unlikely to be associated with Leigh syndrome (0%; 0 of 53). We calculated DM prevalence for specific genetic diagnoses where it was frequently observed in previous studies: DM prevalence was 39.8% in subjects with the m.3243A>G mutation (35 of 88), 9.1% in those with the m.8344A>G mutation (1 of 11), 7.1% in those with the m.11778G>A mutation (1 of 14), and 2.8% in those with *POLG* mutations (1 of 36).

2. *AGSM*. AGSM occurred in similar proportions in mtDNA variants (40.3%) compared with nDNA mutations (48.4%) ($P = 0.65$, accounting for age and sex; OR: 0.9; 95% CI of OR: 0.6 to 1.4). AGSM was most prevalent in Leigh syndrome (61.8%; 34 of 55) and MELAS (50%; 45 of 90). Lower AGSM prevalence was noted in *POLG*-related diseases (17.1%; 6 of 35), and Leber hereditary optic neuropathy (LHON; 9.5%; 2 of 21).
3. *Hypothyroidism*. Hypothyroidism occurred more often in mtDNA defects (8.5%) than in nDNA mutations (2.9%), but this trend was not statistically significant ($P = 0.2$, accounting for age and sex; OR: 2.2; 95% CI of OR: 0.7 to 6.9). Hypothyroidism was most prevalent in MERRF (15.4%; 2 of 13) and MELAS (12.2%; 11 of 90).

F. Endocrinopathies in KSS/Pearson Syndrome, CPEO, and MELAS

We performed a focused analysis of endocrine manifestations in MELAS, CPEO, and KSS/Pearson syndrome spectrum. In individuals with MELAS, DM prevalence was 31.9% (29 of 91; 95% CI: 22.5% to 42.5%), where 86.2% of individuals with the diabetic MELAS phenotype had an associated m.3243A>G mutation ($n = 25$). Other endocrinopathies in subjects with the MELAS phenotype included ASGM (50%; 45 of 90; 95% CI: 39.3% to 60.7%), hypothyroidism (12.2%; 11 of 90; 95% CI: 6.3% to 20.8%), and dyslipidemia ($n = 3$). KSS/Pearson syndrome spectrum was present in six subjects, with reported endocrine manifestations of DM ($n = 1$), AGSM ($n = 3$), hypoparathyroidism ($n = 1$), and adrenal insufficiency ($n = 1$). For patients with CPEO/CPEO plus mtDNA deletion spectrum disorder, reported endocrine manifestations were DM (6.3%; 1 of 16) and AGSM (25%; 4 of 16).

G. Supplemental Analysis

This is summarized in Supplemental Tables 1A and 1B. Overall, the prevalence of endocrine conditions in the entire cohort of primary mitochondrial disease was comparable to that in the subset of those with genetic confirmation. The prevalence of DM in adults and in females was higher in individuals with genetic diagnoses compared to individuals without genetic diagnosis (18.2% vs 7.7% in females, and 24.4% vs 11.3% in adults, respectively); this was the only statistically significant difference noted between those with genetic confirmation and those without genetic testing.

3. Discussion

This study characterized specific endocrine disorders in a well-defined, physician-curated cohort of individuals with genetically defined primary mitochondrial disease in North America. Our results confirm and enrich our understanding of previously reported clinical associations. Specifically, we identified a high prevalence of DM, particularly among those with mtDNA pathogenic mutations. AGSMs were commonly found across most clinical categories of primary mitochondrial disease. In addition, certain clinical mitochondrial syndromes (MELAS and KSS/Pearson syndrome spectrum) demonstrated a higher burden of endocrine disorders (including DM, AGSM, hypothyroidism, dyslipidemia, hypoparathyroidism, and/or adrenal insufficiency).

A. DM

Overall, the age-specific DM prevalence in this diverse cohort of individuals with mitochondrial disease was more than twice as high as the prevalence in the US population. In the pediatric age range (<18 years), DM prevalence was 2% (95% CI: 0.4% to 5.7%) compared with 0.05% to 0.2% in the general population [24]; however, small numbers of

children affected with DM in our cohort make the pediatric prevalence estimates less precise. Among adults aged ≥ 18 years with mitochondrial disease, the prevalence was 24.4% (95% CI: 18.6% to 30.9%) compared with the reported 14.3% (95% CI: 12.2% to 16.8%) for total diabetes (both diagnosed and undiagnosed) and the reported 9.1% (95% CI, 7.8% to 10.6%) for diagnosed diabetes in the US population [25]. Of those adult subjects with diabetes, 23.4% were thin, which is atypical for type 2 diabetes, the most common type in adults in the United States.

DM prevalence was high in those with the common m.3243A>G mutation (39.8%; 35 of 88; 95% CI: 29.5% to 50.8%), similar to previously reported estimates [3]. This mutation in the *MT-TL1* [mitochondrially encoded transfer RNA (tRNA) Leucine 1] gene affects the tertiary structure of mitochondrial tRNA, resulting in impaired synthesis of all 13 mtDNA-encoded structural subunits of the mitochondrial respiratory chain and overall mitochondrial dysfunction [21]. The diabetogenic effect of the m.3243A>G mutation is thought to be related to a decrease in glucose-induced insulin secretion by pancreatic beta-cells (which depends on the closure of the adenosine triphosphate-sensitive potassium channel) and premature aging of these cells [26]. The prevalence of DM in m.8344A>G [an *MT-TK* (mitochondrially encoded tRNA lysine) gene mutation causal of the MERRF clinical syndrome] was 9.1% (1 of 11; 95% CI: 0.2% to 41.3%), similar to the previously reported prevalence of 10% [3]. DM prevalence in *POLG* disorders (a nuclear gene encoding proteins involved in mtDNA replication and repair) was 2.8% (1 of 36; 95% CI: 0.1% to 14.5%), lower than previous reports in the literature (11%) [3]; differences may stem from the predominantly pediatric age range represented in our studies.

B. AGSM

The prevalence of growth and sexual development abnormalities was high, reported for more than half of pediatric participants. This is consistent with previously reported associations between poor growth and mitochondrial disorders [1, 5, 8]. Exact growth-failure prevalence is challenging to discern, given differing definitions and reporting biases. Among those with mitochondrial disorders, short stature, defined as a height at least two standard deviations below the mean for population reference values, reportedly occurs in 25% to 100% of patients, depending on the series and subtypes [1, 8, 27, 28]. Abnormally low body weight and stunting have also been observed in individuals with mitochondrial disorders [8], but again, adequately powered prevalence estimates are lacking. Abnormal growth was most often noted in Leigh syndrome (61.8%; 34 of 55; 95% CI: 47.7% to 74.6%) and MELAS (50%; 45 of 90; 95% CI: 39.3% to 60.7%); it was less likely to be noted in *POLG*-related diseases (17.1%; 6 of 35; 95% CI: 6.6% to 33.6%) and LHON (9.5%; 2 of 21; 95% CI: 1.2% to 30.4%). LHON is primarily thought of as an eye disease [4], but the presence of AGSM in 9.5% of our cohort suggests that patients with LHON may have multisystem involvement beyond the eye affecting growth.

Growth-failure etiology is likely multifactorial and, besides poor weight gain, includes a myriad of medical comorbidities beyond endocrinopathies that affect these patients, including central nervous system, cardiac, gastrointestinal, and renal diseases. Although GH deficiency has been reported [1, 5], establishing this diagnosis usually requires provocative testing. Systematic studies are needed to understand the true burden of GH deficiency in primary mitochondrial disease. In addition, low insulin-like growth factor 1 level, reflecting either GH deficiency and/or insulin-like growth factor 1 deficiency, is an important potential phenotypic feature that might explain the abnormal anthropometric profiles. Also, short stature in individuals with mitochondrial disease may result from the action of hormones such as GDF15, which can interfere with the action of GH; this may reflect a coordinated homeostatic response to inadequate energy availability to support normal growth [29]. In the NAMDC cohort studied, six individuals were reported to have GH deficiency.

C. Thyroid Disease

Hypothyroidism was reported in 6.3% of participants (22 of 352; 95% CI: 4% to 9.3%), which is comparable to the overall hypothyroidism prevalence in the US population (4.6%) [30]. To our knowledge, there are no previously reported estimates of thyroid disorder prevalence in mitochondrial disease. Few associations with specific nuclear-encoded genetic defects have been noted, particularly in *TANGO2* and *PTRH2* mutations, but insights about the presence of a causal mechanism between mitochondrial disease and thyroid dysfunction are lacking [1, 5, 31, 32]. The prevalence of hypothyroidism increased with age in our cohort ($P = 0.005$) and was more common in females (though this result did not reach statistical significance, with $P = 0.1$), also similar to what was observed in the US population [30]. The incidence in pediatric participants may be slightly higher than the background prevalence (2.6% vs 0.1% to 1.6%, respectively), but overall low rates in both this cohort and the population make comparisons more challenging [33, 34].

D. Endocrinopathies in KSS/Pearson Syndrome, CPEO, and MELAS

In MELAS, DM prevalence was substantial (31.9%) and was consistent with previous reports [3]. Also, a high prevalence of other endocrinopathies was noted in subjects with the MELAS phenotype, including ASGM (50%), hypothyroidism (12.2%), and dyslipidemia ($n = 3$).

For KSS and Pearson syndrome, we summarized endocrine manifestations in the disorders combined because they typically are considered to be conditions along a clinical spectrum with similar mtDNA deletion-based genetic etiology [2, 4]. Despite a limited number of subjects in our cohort ($n = 6$), multiple endocrine manifestations were noted in KSS/Pearson, including DM ($n = 1$), AGSM ($n = 3$), hypoparathyroidism ($n = 1$), and adrenal insufficiency ($n = 1$). Among 16 subjects with reported CPEO/CPEO plus mtDNA deletion spectrum disorder, reported endocrine manifestations were DM (6.3%; 1 of 16) and AGSM (25%; 4 of 16).

E. Supplemental Analysis

Our analysis of the entire cohort of primary mitochondrial disease showed that endocrine conditions remained consistently prevalent in individuals with clinical evidence of the disease, regardless of whether a molecular genetic diagnosis has been made. The prevalence of DM in adults and in females was higher in individuals with genetic diagnoses than in those without genetic testing (18.2% vs 7.7% in females and 24.4% vs 11.3% in adults, respectively); this was the only statistically significant difference noted and suggests a stronger association of DM comorbidity in the presence of a known disease-causing mutation.

F. Strengths, Limitations, and Implications for Future Studies

Our study has notable strengths and limitations. One strength is the use of a large, well-defined cohort of patients with primary mitochondrial disorders. The NAMDC registry is a national database of patients enrolled by specialized clinicians and researchers in mitochondrial disease in the United States and Canada. Patients in the registry undergo a standardized approach to medical record review developed by the consensus of experienced clinicians. As a result, assigned diagnostic groups are clinically relevant, despite the heterogeneity of these complex conditions. The NAMDC cohort also has the advantage of having a standardized, curated collection of clinical manifestations (including specific questions for several selected major endocrine manifestations). The utility of our analysis of endocrinopathies in mitochondrial disease is enhanced by having restricted the study population to patients with definite mitochondrial disease confirmed by molecular genetic testing.

This study also has inherent limitations. It was observational and cross-sectional, and some very rare subtypes were represented by necessity only by a small number of participants. Also, details of the extent and type of performed genetic testing and the level of heteroplasmy were not provided in our analyzed data set; interpretation of results and determination of pathogenicity of mutations was at the discretion of NAMDC physicians. The NAMDC registry has a preponderance of mtDNA molecular genetic diagnoses; with advances in molecular genetic testing approaches and improved uniformity of broad-scale, next-generation sequencing methodologies to all enrolled patients, we expect to identify additional pathologic nDNA defects. In addition, participants did not undergo systematic or identical screening or diagnostic evaluations for endocrine disorders; assignment of the presence or absence of these diagnoses was at the reporting clinician's discretion. Finally, participants were queried only about a specific subset of endocrine problems; no systematic information was available on hypoglycemia, obesity, polycystic ovary syndrome, types of hypogonadism, exocrine pancreatic insufficiency, adrenal insufficiency, GH deficiency, and dyslipidemia.

Despite these limitations, these estimates provide a much-needed initial evidence base for clinicians caring for these individuals. It also provides general estimates of prevalence that allow us to prioritize future efforts, guiding prospective studies on important gaps in the understanding of endocrinopathies associated with primary mitochondrial diseases. For example, additional phenotypic characterization regarding mitochondrial-associated DM is needed, including the basis for DM diagnosis (relative to the diagnostic criteria used), age at DM onset, body mass index at DM onset, presence/absence of diabetic ketoacidosis at DM onset (or during the disease course), presence/absence of pancreatic auto-antibodies at DM onset, insulin requirement at DM onset, time to insulin requirement (if at all needed), and other glucose-lowering medications used and their effects. In addition, more details on growth, including serial anthropometric measurements, the extent of any growth evaluation including provocative testing for GH deficiency, and/or testing for hypogonadism would be informative. Other endocrine conditions that pose a substantial clinical risk (hypoglycemia, adrenal insufficiency) could also be assessed. Our preliminary data do not suggest a significant excess of thyroid dysfunction, but usual screening practices likely vary; thus, this would be another potential future focus. Registries can provide a rich source for further analyses by developing standardized instruments for endocrine condition characterizations and laboratory/anthropometric measurement reporting and tracking.

G. Conclusion

Our findings support and extend previous studies highlighting the substantial burden of endocrine disorders in primary mitochondrial disease. In particular, we found that the age-specific prevalence of DM exceeded the expected background population prevalence and that rates of DM were substantially higher in some clinically defined mitochondrial disease syndromes, including MELAS and KSS/Pearson syndrome. There was a disproportionate burden of endocrine disorders in individuals with pathogenic mtDNA variants. We also identified a high prevalence of AGSM in primary mitochondrial disease that is likely complex and multifactorial. Taken together, these findings will inform future studies focused on estimating the burden of undiagnosed endocrine disease and improving testing approaches to screening in primary mitochondrial disease. With the more widespread availability of advanced molecular diagnostic techniques [35], the diagnosis of definite mitochondrial disease is likely to be made with increasing frequency [36]. Efforts to better understand its many endocrine manifestations will help optimize the clinical management of these complex diseases.

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