Protean Phenotypic Features of the A3243G Mitochondrial DNA Mutation

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Objective: To describe the spectrum of clinical symptoms, signs, and laboratory features associated with A3243G, a mitochondrial DNA point mutation that affects multiple organs with varying severity, making the diagnosis and treatment of these patients complex.

Design: Cohort study.

Setting: Columbia University Medical Center.

Participants: A cohort of 123 matrilineal relatives from 45 families, including 45 fully symptomatic patients with mitochondrial myopathy, encephalomyopathy, lactic acidosis, and stroke-like episodes (syndrome), 78 carrier relatives, and 30 controls.

Main Outcome Measures: Data gathered from standardized medical history questionnaires, neurological and ophthalmological examination forms, and laboratory tests. We compared data between 3 groups.

Results: Mutation carriers’ clinical and laboratory results frequently had many abnormalities. In addition to neurological symptoms, they often had cardiac, endocrine, gastrointestinal, and psychiatric symptoms.

Conclusions: The A3243G mutation carriers have multiple medical problems, suggesting that the A3243G mutation should be considered as an etiological factor in patients with multisystem clinical presentations or a family history compatible with matrilineal inheritance. Because some medical problems affecting A3243G mutation carriers are treatable, early detection and proactive management may mitigate the burden of morbidity.


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MITOCHONDRIAL MYOPATHY, encephalomyopathy, lactic acidosis, and stroke-like episodes (syndrome) (MELAS) is a devastating multisystem syndrome characterized by progressive encephalopathy and stroke-like episodes leading to disability and early death. It is most commonly associated with the mitochondrial DNA (mtDNA) A-to-G point mutation at nucleotide 3243. However, many carriers of the A3243G mutation do not develop the full MELAS phenotype. Instead, they may be asymptomatic or express a wide spectrum of clinical symptoms that suggest multiorgan involvement but vary in clinical severity from mild to severe involvement, although they do not develop the stroke-like episodes typical of MELAS. The clinical manifestations of the A3243G mutation are probably underrecognized and the mutation appears to be more prevalent than previously thought. Although this phenotypic variability is incompletely understood, it is certainly due, at least in part, to heteroplasmy, with varying proportions of mutant and wild-type mtDNA molecules in different tissues. The clinical features of the A3243G mutation have thus far been described in small series or in retrospective studies based on clinical databases. We hypothesize that multiple organ systems are affected in A3243G carriers to a greater degree than previously reported. Here, we describe the phenotypic spectrum associated with the A3243G mutation based on a cohort of 123 carriers of the mutation from 45 families using standardized questionnaires and examination scores. This information will be useful to clinicians in diagnosing and treating patients with multisystem manifestations due to the A3243G mutation.
STUDY POPULATION

We included all A3243G mutation carriers and their matrilineal relatives who were followed up in an observational study at Columbia University Medical Center. Patrilineal relatives or relatives by marriage were used as controls. Based on their clinical phenotype, subjects (n = 153) were divided into 3 groups. Individuals harboring the A3243G mutation were classified as patients with fully symptomatic MELAS when there was clear evidence of focal brain involvement in addition to lactic acidosis (ie, all subjects in this group have, by definition, a history of stroke-like episodes, focal seizures, or both). Matrilineal carrier relatives without focal seizures or strokes were grouped as carrier relatives. Family members by marriage or friends were recruited as control subjects. The 3 groups are therefore: (1) fully symptomatic patients with MELAS (n = 45; patients), (2) asymptomatic or symptomatic relatives who represent obligate carriers by pedigree analysis (n = 78; carrier relatives), and (3) noncarrier controls, ie, patrilineal relatives or relatives by marriage (n = 30; controls).

Subjects were self-referred or referred by their physicians. To recruit subjects, we disseminated information on relevant Web sites and at meetings of patient voluntary organizations. We recruited subjects from 45 families, 44 residing in the United States and 1 residing in South America.

STUDY DESIGN

In a cross-sectional design, we conducted a comprehensive medical evaluation that included the following elements: neurological examination as semiquantitatively rated with the Columbia Neurological Score, a medical history questionnaire, a neuropsychological screening, a Karnofsky score (assessing daily living functional abilities), and laboratory measures including venous lactate, venous pyruvate, blood glucose, hemoglobin A1c, a lipid panel, a thyroid function panel, a liver function panel, aspartate transaminase, alanine transaminase, uric acid, docosahexaenoic acid (DHA), D-2-hydroxybutyrate, and urine organic acids.

DETAILED DESCRIPTION OF STUDY PROCEDURES

Clinical Outcomes

Columbia Neurological Examination Score. A neurologist used a semiquantitative tool to score the following physical examination domains: height, weight, and head circumference; general medical examination; funduscopic examination; cranial nerves; stance and gait; involuntary movements; sensation; cerebellar function; muscle bulk, tone, and strength; tendon reflexes; Babinski sign; and other findings. Results of these domains were scored as normal or abnormal and summarized with the Columbia Neurological Score, ranging from 0 to 76, with 76 considered normal. We have previously shown that the instrument has good interrater reliability and correlates with other measures of disease severity.

Medical History. A trained interviewer (M.S. or K.E.) conducted structured in-person interviews to obtain information on subjects’ developmental, medical, familial, social, educational, and behavioral histories. This questionnaire was developed by the investigators based on their clinical observations in patients with mitochondrial disorders and was designed to provide a comprehensive survey of the medical and neuropsychiatric problems associated with the A3243G mutation. For subjects with cognitive impairment (determined by neuropsychological testing), a caregiver was included in the interview to provide information. If the subject was severely impaired or unable to participate in the interview, the caregiver was interviewed as a proxy. A series of questions was asked in simple terms to obtain yes or no answers. Information on the following areas was obtained: (1) health issues during the newborn period, 12 questions; (2) developmental milestone accomplishments in speech and motor skills, 19 questions; (3) educational milestones, 11 questions; (4) basic medical history including a review of all major body systems, 21 questions; (5) surgery and hospitalizations, 4 questions; (6) alcohol and other drug use, 8 questions; (7) psychiatric history, including depression, hallucinations, delusions, suicide attempts, other manifestations, and hospitalization or treatment for these disorders, 22 questions; (8) exercise intolerance, 3 questions; (9) neurological symptoms, including headaches, migraines, strokes, seizures, loss of consciousness, clumsiness, memory problems, and myoclonus, 22 questions; (10) women’s health, including onset and cessation of menstruation, pregnancy-related issues, and gynecological problems, 11 questions; and (11) behavioral issues, 12 questions.

Neuropsychological Testing. Cognitive function was assessed using a brief global mental status examination as a screen. This modified Mini-Mental State examination was used for subjects aged 18 years and older only. Depending on the total score (maximum, 57), a categorical score was assigned (0, highest possible score; total score 50–57; 1, total score 30–49; 2, total score <30). We used the Karnofsky score, an established semiquantitative scale, to evaluate daily living functional abilities.

Laboratory Outcomes

All laboratory tests were performed in the hospital laboratory according to standard procedures and evaluated against reference ranges derived from a control population. The presence of the mtDNA A3243G mutation was confirmed in DNA extracted from leukocytes according to standard procedures, as previously described.

Multislice Proton Magnetic Resonance Spectroscopic Imaging

These data, including lateral ventricular lactate levels, were recorded using previously described methods.

SETTING

All evaluations took place at a single site, the Columbia University Medical Center in New York, New York. Participants traveled to the study site from locations across the United States, with the exception of one family who came from South America.

DATA ANALYSIS

For categorical measurements, we used the Mantel-Haenszel χ² test to determine the statistical significance of differences between groups. For continuous measurements, we used the Kruskal-Wallis test. Comparisons between individual groups were performed using Bonferroni correction to adjust for multiple comparisons.
The 45 patients with MELAS had a mean (SD) age of 29 (14) years (range, 4-60 years) and 51% were men. The 78 carrier relatives had a mean (SD) age of 38 (17) years and 29% were men. The control subjects had a mean (SD) age of 49 (14) years and 51% were men. The mean (SD) proportion of mutant mitochondrial DNA in the blood was 27% (22%) (range, 1%-88%) in the patients with MELAS and 11% (15%) (range, 0%-62%) in the carrier relatives.

CLINICAL FEATURES

Neurological Symptoms

Patients with MELAS invariably had multiple and severe neurological symptoms including strokes, seizures, and cognitive impairment. However, carrier relatives also frequently had neurological symptoms, and they had migraine headaches more commonly than control subjects. Other common neurological symptoms reported by carriers included limb weakness, loss of sensation, difficulty with balance, clumsiness, and myoclonic jerks (Table 1).

Nonneurological Symptoms

Carriers of the A3243G mitochondrial mutation experienced a wide spectrum of symptoms related to multiple organs and varying in severity. These same symptoms were often severe in the patients (Table 2).

Birth weights were not different between groups; however, perinatal difficulties, motor and speech delays, and difficulty chewing or swallowing were more frequently reported by patients with MELAS and carrier relatives than by control subjects. Similarly, both patients with MELAS and carrier relatives were more likely to have attended special education classes. Statistically significant differences between patients and carrier relatives were seen for perinatal difficulties and motor delay (Table 2) compared with control subjects.

Exercise intolerance was the most common symptom in patients (93%) and the second most common concern in carrier relatives (38%); in contrast, it was reported by only 20% of controls.

Hearing impairment was common in patients (77%), with 49% using hearing aids and 4% using cochlear implants. Hearing loss in carrier relatives (35%) was more common than in controls (28%). Carrier relatives used hearing aids more frequently than control subjects (14% vs 4%). Gastrointestinal disturbance was the most common symptom in patient carriers (43%), whereas it was the third most common symptom in patients with MELAS (64%). A plethora of gastrointestinal tract problems were reported including food allergies, intolerance to certain foods, nausea, vomiting, constipation, bloating, diarrhea, cramping, swallowing difficulties, and bowel obstruction. Diabetes was significantly more common in patients with MELAS and carriers than in control subjects. Heart disease, frequent infections, breathing difficulty, and delayed puberty were more common in patients with MELAS and carriers than in controls, but the difference did not reach statistical significance. Daily living functional abilities, as measured with the Karnofsky score, were significantly lower not only in patients with MELAS, but also in carrier relatives compared with control subjects (P <.001).

Neurobehavioral Features

Cognitive problems were frequent, with relatively early onset in patients with MELAS while carrier relatives experienced very mild and less frequent difficulties (Table 3). Patients performed poorly on the Modified Mini-Mental Status examination (mean [SD], 1.32 [0.76]) and carriers showed mildly reduced performance (mean [SD], 0.24 [0.47]) compared with controls (mean [SD], 0.14 [0.35]). Patients with MELAS have a high prevalence of psychiatric symptoms including hallucinations, depression, and delusions. The burden of neurobehavioral symptoms was increased not only in patients with MELAS, but also in carrier relatives (Table 3). The most common behavioral symptoms reported by carrier relatives were low frustration threshold and distractibility. Depression was reported by approximately one-third of all carriers, compared with 17% in the control group.

Table 1. Proportion of Subjects by Group Who Responded Affirmatively to a Structured Medical History Questionnaire

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Patients With MELAS</th>
<th>Carrier Relatives</th>
<th>Controls</th>
<th>P Valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>91</td>
<td>58</td>
<td>52</td>
<td>&lt;.001b,c</td>
</tr>
<tr>
<td>Balance difficulty</td>
<td>81</td>
<td>20</td>
<td>4</td>
<td>&lt;.001b,c</td>
</tr>
<tr>
<td>Age at onset of migraines, y</td>
<td>77 (19.3 [10.0])</td>
<td>32 (21.9 [12.3])</td>
<td>16 (21.3 [10.4])</td>
<td>&lt;.001b,d</td>
</tr>
<tr>
<td>Limb weakness</td>
<td>71</td>
<td>22</td>
<td>4</td>
<td>&lt;.001b,d</td>
</tr>
<tr>
<td>Age at onset of clumsiness, y</td>
<td>62 (19 [14])</td>
<td>23 (37 [17])</td>
<td>4</td>
<td>&lt;.001b,d</td>
</tr>
<tr>
<td>Age at onset of myoclonus, y</td>
<td>50 (24 [17])</td>
<td>15 (33 [15])</td>
<td>0</td>
<td>&lt;.001b,d</td>
</tr>
<tr>
<td>Loss of sensation</td>
<td>48</td>
<td>19</td>
<td>4</td>
<td>&lt;.001b,d</td>
</tr>
</tbody>
</table>

Abbreviation: MELAS, mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (syndrome).

a The x² test was used to evaluate the heterogeneity across the 3 groups.
b Significant difference between patients and controls.
c Significant difference between carrier relatives and controls.
d Significant difference between patients and controls.
Physical Examination Features

Physical examination showed that both patients with MELAS and their carrier relatives differed from controls in their anthropometric characteristics. Patients with MELAS and carrier relatives were more likely than controls to be underweight, of short stature, and have a small head circumference (Table 4). Patients with MELAS and carrier relatives had significantly lower overall Colombia Neurological Scores than carrier relatives (mean [SD], 57.1[9.7] and 69.6[6.7] compared with control scores of 72.6[3.2]). While patients were significantly different from control subjects in all domains, carrier relatives had significantly more abnormal results than controls in 4 domains: (1) general physical examination, (2) funduscopy, (3) cranial nerve examination, and (4) cerebellar testing.

NEUROIMAGING AND LABORATORY FEATURES

The magnetic resonance spectroscopy ventricular lactate
carrier relatives when compared with control subjects (Table 5). Hemoglobin, urine creatinine, and triiodothyronine (T₃) were significantly different in patients with MELAS and carriers when compared with control subjects. Serum creatinine, aspartate aminotransferase, alanine aminotransferase, triglycerides, and thyroid hormone (T₄) were significantly different in patients with MELAS but also in carrier relatives, as shown in Table 5.

### Table 4. Physical Examination Features

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients With MELAS</th>
<th>Carrier Relatives</th>
<th>Controls</th>
<th>P Value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropomorphic measurements</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underweight&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42</td>
<td>5</td>
<td>0</td>
<td>&lt;.001&lt;sup&gt;b,c,e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Short stature&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33</td>
<td>14</td>
<td>0</td>
<td>&lt;.001&lt;sup&gt;b,d,e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Small head circumference&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37</td>
<td>14</td>
<td>7</td>
<td>.003&lt;sup&gt;b,e&lt;/sup&gt;</td>
</tr>
<tr>
<td>BMI percentile&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20</td>
<td>1.4</td>
<td>0</td>
<td>&lt;.001&lt;sup&gt;b,e&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Physical examination and neurological parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tendon reflexes (max, 10)</td>
<td>48</td>
<td>4.8 (2.7)</td>
<td>7.0 (2.8)</td>
<td>7.1 (2.9)</td>
</tr>
<tr>
<td>Stance and gait (max, 7)</td>
<td>49</td>
<td>3.4 (2.7)</td>
<td>6.3 (1.7)</td>
<td>7.0 (0.2)</td>
</tr>
<tr>
<td>Sensation (max, 3)</td>
<td>70</td>
<td>2.1 (1.0)</td>
<td>2.8 (0.6)</td>
<td>3.0 (0.2)</td>
</tr>
<tr>
<td>Muscle tone, bulk, strength (max, 6)</td>
<td>77</td>
<td>4.6 (2.0)</td>
<td>5.7 (1.0)</td>
<td>6.0 (1.0)</td>
</tr>
<tr>
<td>Funduscopic examination (max, 4)</td>
<td>78</td>
<td>3.1 (1.0)</td>
<td>3.8 (0.5)</td>
<td>4.0 (0.0)</td>
</tr>
<tr>
<td>Cerebellar (max, 8)</td>
<td>80</td>
<td>6.4 (2.0)</td>
<td>7.7 (0.7)</td>
<td>8.0 (0.0)</td>
</tr>
<tr>
<td>Babinski sign (max, 2)</td>
<td>80</td>
<td>1.6 (0.9)</td>
<td>1.9 (0.4)</td>
<td>2.0 (0.0)</td>
</tr>
<tr>
<td>Cranial nerves (max, 12)</td>
<td>88</td>
<td>10.6 (1.1)</td>
<td>11.6 (0.8)</td>
<td>11.9 (0.2)</td>
</tr>
<tr>
<td>General physical examination (max, 13)</td>
<td>90</td>
<td>11.7 (1.1)</td>
<td>12.4 (0.9)</td>
<td>12.8 (0.4)</td>
</tr>
<tr>
<td>Involuntary movements (max, 7)</td>
<td>92</td>
<td>6.5 (1.1)</td>
<td>6.9 (0.4)</td>
<td>6.9 (0.3)</td>
</tr>
<tr>
<td>Summary score (max, 76)</td>
<td></td>
<td>57.1 (9.7)</td>
<td>69.6 (7.3)</td>
<td>72.6 (3.2)</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); max, maximum; MELAS, mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (syndrome).

<sup>a</sup> The χ² test was used to evaluate the heterogeneity in anthropomorphic measurements across the 3 groups. The Kruskal-Wallis test was used to evaluate the overall heterogeneity in physical and neurological parameters.

<sup>b</sup> Defined as less than the third percentile.

<sup>c</sup> Significant difference between patients and controls.

<sup>d</sup> Significant difference between patient relatives and controls.

<sup>e</sup> Significant difference between patients and controls.

### COMMENT

We studied 123 subjects with the A3243G mtDNA mutation and 30 controls using a comprehensive battery of prespecified clinical and laboratory measures.

Recent epidemiological evidence suggests a population prevalence for the A3243G mutation of 236 to 100,000 in whites, indicating that this genetic defect is underrecognized.<sup>8</sup> Identifying which patients to screen for the A3243G mutation can be challenging, and our study provides comprehensive data on the frequency and identifies a previously underestimated high prevalence of diabetes mellitus, gastrointestinal problems, and neurobehavioral issues.

It is important to recognize that not only patients with MELAS but also their maternal relatives carrying the A3243G mutation without the full MELAS phenotype have a high prevalence of neurological and medical problems including hearing loss, diabetes, exercise intolerance, gastrointestinal disorders, depression, short stature, and a history of educational difficulties. In our cohort, more than 60% of carrier relatives had symptoms suggestive of mitochondrial disease in 2 or more organ systems. Many of these features had been recognized in A3243G carriers before, but our study better defines their frequency and identifies a previously underestimated high prevalence of diabetes mellitus, gastrointestinal problems, and neurobehavioral issues.

A limitation of our study is that we recruited controls who were patrilineal relatives or relatives by marriage. As a result, they are older than the patients with MELAS. However, an older group would be expected to have a higher prevalence of medical problems, so that this limitation would not have resulted in false-positive conclusions.

In summary, our results suggest that mitochondrial dysfunction due to the A3243G mutation should be suspected in patients with multisystem clinical presentations that include subtle signs often associated with the A3243G mutation, especially exercise intolerance, hearing loss, gastrointestinal problems, and diabetes. These results are important because the A3243G mutation is probably underrecognized in the community. Better knowledge of the frequency of the clinical features associated with this mutation should lead to enhanced genetic screening, which can in turn accelerate diagnosis and avoid unnecessary tests. These results also have important implications for known
A3243G carriers by improving our ability to proactively care for these patients and to recognize medical problems in a proactive fashion.

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2. Hirano M, Pavlakis SG. Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (syndrome); MRS, magnetic resonance spectroscopy; NA, not available; T3, triiodothyronine; T4, thyroxine.