Macular Dystrophy Associated With the A3243G Mitochondrial DNA Mutation

Distinct Retinal and Associated Features, Disease Variability, and Characterization of Asymptomatic Family Members

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Objectives: To determine (1) detailed retinal and audiological features of probands harboring the A3243G mitochondrial DNA mutation (m.3243A>G) and their asymptomatic maternal relatives, (2) intrafamilial and interfamilial phenotypic variability, and (3) the presence of other systemic features.

Methods: Seven probands harboring the A3243G mitochondrial DNA mutation and 36 asymptomatic maternal relatives were ascertained. Participants underwent ophthalmologic examination, fundus photography, autofluorescence imaging, and audiological evaluation and completed a questionnaire. Blood samples were taken to test for diabetes, determine renal function, and screen relatives for the A3243G mutation.

Results: The A3243G mutation was associated with both intrafamilial and interfamilial variable expressivity regarding retinal appearance, hearing loss, diabetes, and other systemic features. The most common macular appearance in maternal relatives (one-third of those positive for the mutation) was mild abnormalities of the retinal pigment epithelium (more clearly identified using autofluorescence), which may therefore be a useful clinical indicator suggesting positive mutation status. Four probands and 13 mutation-positive relatives were found to have evidence of significant bilateral, cochlear, symmetrical age-adjusted hearing loss, predominantly affecting high frequencies.

Conclusions: Hearing loss and macular disturbance were the most frequent findings in mutation-positive participants, with 95% of mutation-positive relatives having hearing loss. Diabetes was the least frequent finding. Patients with progressive hearing loss may merit ophthalmologic assessment to detect retinal abnormalities consistent with the A3243G mutation. Conversely, patients with macular features in keeping with the A3243G mutation should have audiological testing, even in the absence of diabetes or a positive family history.

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MITOCHONDRIAL DISEASES are a clinically heterogeneous group of disorders that arise as a result of dysfunction of the mitochondrial respiratory chain. They can be caused by mutations of nuclear or mitochondrial DNA (mtDNA). Maternally inherited diabetes and deafness (MIDD; OMIM 520000) is a recently described subtype of diabetes mellitus that most commonly cosegregates with an adenine to guanine transition at position 3243 of mtDNA (A3243G) in a transfer RNA leucine (tRNA-Leu(UUR)) encoding region. Maternally inherited diabetes and deafness is most commonly characterized by a normal or low body mass index, sensorineural deafness, and diabetes, which often requires insulin therapy, with onset usually during the third or fourth decade of life. Maternal inheritance is the usual pattern of inheritance. The A3243G mtDNA mutation can also be associated with severe encephalopathy, with death at a young age (mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes [MELAS]).

The A3243G point mutation has been previously associated with a wide range of clinical manifestations, including retinal abnormalities, cardiomyopathy, chronic progressive external ophthalmoplegia, myopathy, gastrointestinal dysmotility, and renal failure. It has been proposed that the load of mutant mtDNA present in an individual determines disease severity, with the amount of mutant mtDNA varying not only between patients but also from tissue to tissue within an individual, thereby partly accounting for the observed phenotypic heterogeneity. Other factors may also modify disease severity and manifestations, including environmental or nuclear genetic influences.

Macular pattern dystrophy has been described in association with MIDD.
In a multicenter study, 86% of patients with MIDD had bilateral macular pattern dystrophy characterized by retinal pigment epithelium (RPE) hyperpigmentation that surrounds the macula or that is more extensive and also encompasses the optic disc. However, prognosis is generally good, with 80% of patients in the multicenter study having a visual acuity of 6/7.5 or better OU. In a previous study, we observed that most of our patients with the A3243G point mutation presented with either a very limited or absent family history that would support the diagnosis of this disorder. We have therefore undertaken an investigation to evaluate clinical or subclinical evidence of disease among maternal relatives of probands with the A3243G mutation associated with MIDD and thereby to also determine the intrafamilial and interfamilial phenotypic variability. To the best of our knowledge, neither an extensive retinal nor auditory survey of asymptomatic maternal family members of a mutation-positive proband has been previously performed. These data may help improve advice on prognosis and genetic counseling.

All patients with a diagnosis of macular dystrophy associated with the A3243G mtDNA mutation in the Medical Retina Clinic at Moorfields Eye Hospital between 1995 and 2002 were ascertained. Maternal relatives of these probands were invited to participate in the study.

Participants

Twelve probands were ascertained, and in 7 of these families, maternal relatives agreed to take part in the study. None of the maternal relatives enrolled had a previous diagnosis of being affected. During the course of the study, a total of 43 individuals were evaluated (including the 7 index cases). All participants completed a questionnaire (eAppendix, available at http://www.archophthalmol.com) requesting information on demographics, presence or absence of visual symptoms, and medical history, including presence or absence of diabetes, hearing loss, cardiac or neurological disease, and maternal history of diabetes and hearing loss. Forty participants underwent ophthalmologic assessment and 23 had extensive audiological testing.

Ophthalmologic and Audioligical Examinations

All assessed patients had a detailed ophthalmologic examination and underwent fundus photography and fluorescein imaging. Fundus autofluorescence imaging was performed with a confocal scanning laser ophthalmoscope (either the Zeiss Prototype; Carl Zeiss Inc, Oberkochen, Germany; or the Heidelberg Retina Angiograph; Heidelberg Engineering, Heidelberg, Germany), using previously published techniques. Only 3 relatives were not available for ophthalmologic assessment. Detailed audiological examination methods are available at http://www.archophthalmol.com.

Laboratory Testing

The A3243G mtDNA mutation was screened for in blood using previously described techniques that detect mutant mtDNA at the 5% level. Because lower mutation loads in blood (<5%) may fail to be detected, in participants in whom the A3243G mutation was not identified, we designated these as not detected rather than as negative cases.

Patients who did not have a history of diabetes were investigated with fasting blood glucose levels according to the recommendations of the American Diabetic Association. Renal function tests were also performed owing to the association of renal dysfunction with the A3243G mutation in MIDD.

Results

Forty-three participants from 7 families were included in the study, consisting of 7 probands and 36 maternal relatives (Figure 1). Probands were aged from 46 to 73 years, with family members ranging in age from 19 to 92 years. Two of the family members included in the study completed a questionnaire but were not available for ophthalmologic examination, and a third maternal relative had recently died but clinical notes were available for review. Of the 43 participants in the study, 28 (65%) were women and 15 (35%) were men, with 6 of the 7 probands being women. Clinical findings are summarized in Table 1 and Table 2 and in 3 eTables (available at http://www.archophthalmol.com).

Mutation Analysis

Forty patients were available for mtDNA mutation analysis of peripheral blood (Table 2 and eTables 2-3, available at http://www.archophthalmol.com). Several maternal relatives, including members of family 1 (II-3, II-12, III-4, and IV-4) and family 2 (IV-3 and IV-9) in whom the A3243G mutation was not detected, had ophthalmologic, audiologic, or systemic features consistent with affected disease status. These individuals demonstrate the importance of detailed clinical assessment and subsequent genetic testing, if indicated, of other tissues, including skeletal muscle, before designating unaffected or negative status; this has critical implications for genetic counseling and risk assessment. It is likely that these individuals have a low mutation load in their blood (<5%) that has failed to be detected. Furthermore, it is possible that they may also have a lower mutation load compared with probands in target tissues such as in the retina, partly explaining their milder asymptomatic phenotype.

Ophthalmologic Assessment

Visual acuity was better than or equal to 6/12 in most individuals, with only 5 participants having worse vision in 1 or both eyes, consistent with previous reports suggesting a generally good visual prognosis. Six of the 7 probands had visual symptoms, with main complaints of blurred vision and difficulty adapting to bright conditions. All 7 probands had evidence of maculopathy (Table 1). Two distinct macular dystrophy phenotypes were seen, the most common being discontinuous circumferentially distributed and oriented perifoveal atrophy (type 1) (Figure 2). This characteristic appearance was seen in 6 of the 7 probands. The remaining
Figure 1. Pedigrees of each of the 7 families included in the study, showing evidence of matrilineal inheritance. Denoted abnormalities are based on clinical examinations undertaken in this study or previous formally obtained data. * indicates positive A3243G mutation status; †, mutation not detected.
The proband had features consistent with a pattern dystrophy (type 2) (Figure 3). Of the 34 maternal relatives assessed, none had been ascertained as having eye disease. A normal fundus was found in 11 participants, 10 had mild abnormalities of the RPE with macular mottling, 8 had macular drusen, 4 had a maculopathy consistent with the characteristic retinal phenotype described previously (type 1), and 1 patient had myopic changes (Table 2 and eFigure, available at http://www.archophthalmol.com). Family members with drusen ranged in age from 23 to 82 years, with a median age of 55 years. Only 2 patients of the 8 with drusen were found to harbor the mutation. In direct contrast, all 10 patients with mild RPE abnormalities were positive for the mutation (Table 2), suggesting that this retinal appearance may be a reliable clinical indicator of positive mutation status in a significant proportion of maternal relatives (approximately one-third of participants in this study). The age range of patients with RPE mottling was 25 to 65 years, with a median age of 48 years. Only 1 of these patients had visual symptoms, reporting difficulties with vision at night (family 2, III-8; aged 58 years). Three participants from family 2 and 1 from family 3 had a maculopathy consistent with the characteristic MIDD (A3243G) phenotype (Table 2). Three of these 4 patients tested positive for the mitochondrial mutation. The fourth patient (family 2, II-8) died before testing could be performed, though, because she was the mother of the proband, it is highly likely that she also harbored the A3243G mutation. The age range in these patients with MIDD maculopathy was 48 to 92 years. Three of these 4 patients had visual symptoms (family 2, II-4 and II-8; family 3, IV-1); family member IV-1 complained of blurred central vision and all 3 patients reported difficulty with night vision.

Fundus autofluorescence imaging was performed on all examined family members. In those participants with characteristic MIDD maculopathy, autofluorescence imaging revealed a recognizable phenotype that in most cases was different from that seen in other macular dystrophies (Figure 2). Normal autofluorescence images were obtained in participants who were clearly defined as unaffected and were not found to harbor the mutation. Mild abnormalities in autofluorescence imaging, including a speckled pattern of increased and decreased autofluorescence (especially in individuals with RPE macular mottling) (eFigure, available at http://www.archophthalmol.com), were present in participants who were defined as being affected on the basis of macular disturbance and/or objective hearing loss but lacked the typical MIDD-associated maculopathy. In some individuals, macular RPE disturbance was more clearly seen on autofluorescence imaging than ophthalmoscopy, including participant IV-1 in family 5, suggesting that autofluorescence imaging may be helpful in indicating positive mutation status.

**Audiological Assessment**

Four female probands with the A3243G point mutation and 19 maternal relatives (5 men) from 6 different families were available for audiological evaluation. All 4 probands and 16 maternal relatives (13 mutation-positive relatives) had evidence of significant bilateral, cochlear, symmetrical age-adjusted hearing loss, predominantly affecting the high frequencies. Only 1 individual harboring the mutation (family 5, V-3) had normal hearing, while all other mutation-positive participants had evidence of objective hearing loss, suggesting that audiological testing is a sensitive method of predicting positive mutation status. Audiological findings are summarized in eTables 1 and 2 (available at http://www.archophthalmol.com).

In regression analysis, there was a significant relationship for both age-adjusted low- to mid-frequency hearing loss and age-adjusted high-frequency hearing loss with age in family 2, the largest pedigree with audiometric data ($R^2 = 0.589$, $P = .006$, for both regression analyses). This analysis confirms that the severity of hearing loss across all frequencies in participants with the A3243G mutation was associated with increasing age and was also greater than what would be expected with age-related hearing loss alone. The maternal relatives who tested positive for the A3243G mutation had significantly greater age-related hearing loss across both low to middle frequencies ($P = .02$) and high frequencies ($P = .01$) than those in whom the mutation was not detected.

The severity of hearing loss was found to increase with greater retinal abnormality in the mutation-positive individuals (18 of 23), reaching statistical significance at high frequencies. Participants with a normal macular appearance or drusen (5 individuals) had the least severe

### Table 1. Characteristics of Probands With Maternally Inherited Diabetes Mellitus (DM) and Deafness at Initial Examination

<table>
<thead>
<tr>
<th>Family</th>
<th>Sex/Age, y</th>
<th>Visual Symptoms</th>
<th>Self-Reported Hearing Loss</th>
<th>Macular Phenotype&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Cochlear Hearing Loss</th>
<th>DM</th>
<th>Maternal FH of DM</th>
<th>Maternal FH of Hearing Loss</th>
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<td>1</td>
<td>F/46</td>
<td>No</td>
<td>Yes</td>
<td>Type 1</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No b</td>
</tr>
<tr>
<td>2</td>
<td>F/54</td>
<td>Yes</td>
<td>No</td>
<td>Type 1</td>
<td>Yes</td>
<td>No</td>
<td>Yes&lt;sup&gt;c&lt;/sup&gt;</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>F/73</td>
<td>Yes</td>
<td>Yes</td>
<td>Type 1</td>
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<td>No</td>
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<tr>
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<tr>
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<td>Yes</td>
<td>No</td>
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<tr>
<td>7</td>
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<td>Yes</td>
<td>Type 1</td>
<td>Yes</td>
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</table>

<sup>a</sup> Type 1, discontinuous circumferentially distributed and oriented perifoveal atrophy; type 2, pattern dystrophy.

<sup>b</sup>Hearing loss diagnosed after the age of 75 years. Probands 1 and 4 had previously clinically diagnosed hearing loss.

<sup>c</sup>Adult-onset DM diagnosed at age 60 years.

Abbreviation: FH, family history.
age-adjusted hearing loss across both low to middle frequencies (mean [SD], 5.9 [9.1] dB) and high frequencies (mean [SD], 9.6 [18.0] dB). Participants with macular RPE disturbance (8 individuals) had an intermediate severity of age-adjusted hearing loss across both low to middle frequencies (mean [SD], 10.2 [11.1] dB) and high frequencies (mean [SD], 25.5 [12.5] dB); whereas individuals with maculopathy (type 1 and 2, 5 participants)
had the most marked age-adjusted hearing loss across both low to middle frequencies (mean [SD], 25.7 [16.8] dB) and high frequencies (mean [SD], 36.0 [9.3] dB). These observed differences across the 3 macular phenotypic groups did not reach statistical significance for low to middle frequencies ($P=0.054$, one-way analysis of variance), but were significant for the high frequencies ($P=0.02$, one-way analysis of variance).

**NONOCULAR FEATURES**

The index cases and family members were all given a survey to ascertain any additional symptoms. Hearing loss was the most commonly reported abnormality, with neurological and then cardiac disorders the next most frequently documented (eTable 3; available at http://www.archophthalmol.com). No participants reported ophthalmoplegia.

**Probands**

Of the 7 probands (Table 1), 2 had diabetes and 5 had self-reported hearing loss (the remaining 2 probands were found to have hearing loss on objective testing) (eTables 1 and 2, available at http://www.archophthalmol.com). All probands had additional systemic features (eTable 3, available at http://www.archophthalmol.com). Five had cardiac disease (including ischemic heart disease, cardiomyopathy, arrhythmia, and conduction abnormalities). Six had neurological disorders (including ataxia, migraine, ptosis, seizures, stroke, and peripheral neuropathy). Myopathy was recorded in 4 probands as self-reported muscle weakness. Only proband 4 had renal disease, believed to be caused by hypertension. Only probands 2 and 6 reported psychiatric symptoms (severe depression).

Two probands reported a positive maternal history of diabetes (Table 1), 1 of them with type II diabetes diagnosed after the age of 60 years. Three index cases reported a maternal history of hearing loss; while in 2 additional cases, age-related hearing loss was reported after the age of 75 years.

**Maternal Family Members**

When questioned directly, maternal family members were found to have significantly more nonocular features than their respective probands had reported on their behalf. A definite history of hearing loss was found in 2 additional families, resulting in a total of 5 families with a maternal history of hearing loss. One additional family was found to have a positive history of diabetes; therefore, a total of 3 families in the study had a maternal history of diabetes. Systemic clinical features were found to be in-

![Figure 2. Fundus photographs (A) and autofluorescence images (B) of the proband from family 1 (III-3), showing a bilateral symmetrical typical maternally inherited diabetes and deafness–associated maculopathy (type 1), characterized by discontinuous circumferentially distributed and oriented perifoveal atrophy.](http://www.archophthalmol.com)
creasingly common with advancing age (eTable 3, available at http://www.archophthalmol.com).

A total of 16 (43%) maternal relatives from all 7 families self-reported (10 relatives) or had previously clinically diagnosed hearing loss (6 relatives); in only 2 of these patients, both from family 1, aged 82 and 46 years, was the A3243G mutation not detected. In contrast, only 5 relatives were identified as having diabetes (14%), with 3 harboring the A3243G mutation and the remaining 2 participants not having been screened for the mtDNA mutation. Four of these participants had a preexisting diagnosis of diabetes; the remaining participant had diabetes diagnosed via fasting glucose testing undertaken as part of the study.

Neurological abnormalities, including ataxia or balance problems, seizures, migraine, and stroke, were reported in 14 (38%) maternal relatives from 5 families. Ataxia or balance problems were reported in 3 families; if probands are included, ataxia was documented in 5 families. Seizures were reported by 2 members of family 2, 1 of whom, on further questioning, admitted to having had febrile convulsions; whereas migraine was reported in all families except family 4, with 6 of these 10 individuals being mutation positive. A history of stroke was reported in 2 relatives: a member of family 1 in whom the A3243G mutation was not detected and a mutation-positive member of family 3.

Five maternal relatives reported renal abnormalities unlikely to be related to the A3243G mutation (2 participants were mutation positive). Cardiac disease was reported in 9 maternal relatives from 5 families, with the A3243G mutation detected in 4 participants. Self-reported muscle weakness or easy fatigability was reported in 3 maternal relatives (all mutation positive) from 3 families.

RENAL FUNCTION AND FASTING BLOOD GLUCOSE

Renal function was normal in all probands and the 30 family members tested. Two probands and 3 family members had a previous diagnosis of diabetes and repeat testing was not performed (Table 2 and eTable 3, available at http://www.archophthalmol.com). Fasting blood glucose analysis was performed in the 5 probands who did not have a previous diagnosis of diabetes and in 27 relatives (Table 2). Two relatives were found to have diabetes (1 harboring the A3243G mutation [family 6, III-4] and 1 not [family 2, IV-9]); an additional relative had impaired fasting glucose levels (mutation positive) (Table 2). In total, 19% of the study population had abnormal glucose metabolism; 7 participants (6 mutation positive) had diabetes and 1 patient with impaired fasting glucose was identified.
To the best of our knowledge, an extensive retinal and auditory survey to evaluate evidence of disease among asymptomatic maternal relatives of A3243G mutation-positive probands and thereby also determine the degree of intrafamilial and interfamilial phenotypic variability has not been previously performed. Such natural history data are invaluable in counseling affected individuals and will also be important in the event of future therapy. Furthermore, if clinical signs are found to be specific to the disorder, this would help the clinician to identify additional cases on clinical grounds alone.

Our study demonstrates that patients with retinal features associated with the A3243G mutation should be carefully questioned about hearing loss and diabetes, both personally and within their extended family. Audiological testing and a fasting blood glucose examination may be warranted because many of these patients report no hearing or metabolic abnormalities. The data also illustrate that every individual with overt maculopathy who underwent assessment had demonstrable hearing loss, suggesting that audiological testing can assist in confirming the diagnosis.

The estimated prevalence of an underlying mitochondrial mutation in patients with diabetes ranges from 0.13% to 2.8%.21-29 The systemic manifestations of diabetic patients with the A3243G point mutation were reported in a multicenter study to identify clinical characteristics that may help select diabetic patients in whom screening for mitochondrial mutations would be worthwhile.20 All patients included in the study had diabetes, 98% had bilateral sensorineural hearing loss, and 86% of participants who received an ophthalmologic examination had demonstrable hearing loss, suggesting that audiological testing can assist in confirming the diagnosis.

The percentage of patients with diabetes and hearing loss in this group was very high, as would be expected, given that patients were enrolled in the study based on the presence of both of these traits. In general, patients with the A3243G mtDNA mutation have a wide variety of disease manifestations, including MIDD.3,4,9,30-33 Patients with macular dysrophy associated with the A3243G mutation that was diagnosed in an ophthalmology clinic may have more variable systemic manifestations than patients who have been ascertained based on the presence of diabetes and hearing loss. In the small cohort of probands first seen with macular dysrophy who were included in our study, 71% (5 of 7) had symptomatic hearing loss (100% following detailed audiological testing), while only 29% (2 of 7) had diabetes. A potential confounding factor that should be borne in mind in studies evaluating rare genetic subtypes of common conditions, such as diabetes, is that some individuals in these families will have diabetes that is unrelated to a rare genetic variation.

Age-adjusted hearing loss at high frequencies was seen to increase as the documented ophthalmoscopic macular disturbance became more marked. Age accounted for more than 50% of the variability of the age-adjusted hearing thresholds in family 2. In addition to mtDNA mutation load, it is possible that other genetic and/or environmental influences could account for the remainder of the observed intrafamilial and interfamilial variability, with these factors also being involved in determining the variable expressivity of retinal, endocrine, and systemic features seen in MIDD. It has previously been suggested that the mitochondrial haplotypic background does not significantly modulate the phenotypic expression of the A3243G mutation,34 whereas there has been increasing evidence that mutations in nuclear-encoded mitochondrial proteins may play a role in modifying the effect of mitochondrial mutations.35-37

Clinical or subclinical hearing loss is reportedly the most common feature associated with the A3243G mitochondrial mutation in individuals with a wide range of mitochondrial encephalomyopathies, including MELAS or MELAS/myoclonic epilepsy with ragged-red fibers overlap syndrome; however, none of the patients were screened for macular dysrophy.38 It has been postulated that mitochondrial dysfunction causes a reduction in adenosine triphosphate, which in turn leads to an ion imbalance that results in hair cell and stria vascularis cell death.39 Postmortem histopathologic examination of the temporal bone of a woman with MIDD due to the A3243G mutation has indeed identified diffuse outer hair cell loss, severe degeneration of the stria vascularis, as well as a reduction of spiral ganglion cells.40 Similar histopathologic findings have been reported in association with the A3243G mutation in MELAS, though marked loss of neurons and gliosis in the ventral cochlear nuclei were also noted.41

We have identified that maternal relatives of patients with the A3243G mutation associated with MIDD may have funduscopic abnormalities and/or clinical or subclinical hearing loss, with evidence of progression with age. We conclude that patients/relatives with progressive hearing loss merit ophthalmologic assessment to detect retinal abnormalities consistent with the A3243G point mutation. Conversely, patients with A3243G-associated macular features should have audiological testing, even in the absence of either diabetes or self-reported hearing loss. Furthermore, a negative screen for the A3243G mtDNA mutation does not exclude carrier status, because the A3243G mutation load in blood significantly reduces over time;42 therefore even “mutation-negative” participants may warrant ophthalmologic and audiological assessment, with subsequent genetic screening of other tissues with a higher mutation load than blood, including skeletal muscle testing if indicated.

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