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Mitochondrial A3243G mutation results in corneal endothelial polymegathism

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Abstract

Purpose—The mitochondrial DNA point mutation A3243G leads to a spectrum of syndromes ranging from MIDD to MELAS. Ocular manifestations include pattern macular dystrophy and concentric perifoveal atrophy. Given the high metabolic demand of corneal endothelial cells, we performed specular biomicroscopy analysis in patients harboring the mitochondrial DNA point mutation A3243G to assess for the associated presence of corneal endothelial abnormalities.

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Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

Methods—We present a case series with participants from two institutions. Patients diagnosed with macular dystrophy associated with MIDD, or MELAS and the mitochondrial DNA point mutation A3243G were recruited. Exclusion criteria included a prior diagnosis, or a positive family history, of endothelial corneal dystrophy. Slit-lamp corneal examination and specular biomicroscopy were performed. Corneal endothelial cell count, cell size and polymegathism, and central corneal thickness were assessed. Patients diagnosed with MIDD or MELAS based on clinical history and examination were genetically tested for the mitochondrial DNA point mutation A3243G using pyrosequencing.

Results—Five patients (two male and three female participants), from five different families, and with different ethnic backgrounds met the inclusion criteria. Their ages ranged from 41 to 60 years. Corneal endothelial changes observed using slit-lamp examination were primarily mild to rare guttata. Specular biomicroscopy displayed mainly polymegathism associated with guttata. The average endothelial cell count was 2358 ± 456 cells per mm^2 , the average endothelial cell size was $442 \pm 103 \mu\text{m}^2$ and the average central corneal thickness (CCT) was $551 \pm 33 \mu\text{m}$. These values were similar to that of the average population. The average coefficient of variation (COV), an index of heterogeneity in cell size, was $42.0 \pm 4.1\%$. When compared to the average population, the average COV was significantly higher than predicted for the patients' age. None of the patients had signs of corneal edema. One patient had a pre-descemet's opacity.

Conclusions—In patients with the mitochondrial DNA point mutation A3243G, corneal endothelial polymegathism is present. This is mainly associated with mild guttata. The findings of corneal endothelial cell polymegathism may be a biomarker of mitochondrial disease, specifically in patients with the mitochondrial DNA A3243G mutation.

Keywords

mitochondrial diseases; endothelial corneal dystrophy; retinal dystrophy; specular microscopy; genetics

Introduction

A single point mutation in the mitochondrial DNA (mtDNA), A3243G, can lead to a spectrum of syndromes ranging from maternally inherited diabetes and deafness (MIDD) to mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS).[1, 2] Ocular manifestations include pattern macular dystrophy and concentric perifoveal atrophy.[3] The A3243G point mutation occurs in the coding region of the tRNA^{Leu(UUR)} gene. While the exact effect of the mutation is unclear, a cybrid (cytoplasmic hybrid), containing the mutant mtDNA, has indicated that the A3243G point mutation resulted in severe respiratory chain deficiency and an overall reduction in protein synthesis.[4]

Mitochondria play an important role in metabolically active cells such as the retinal pigment epithelium (RPE) and the corneal endothelium. Downregulation of mitochondrial genes has been associated with Fuchs' endothelial corneal dystrophy (FECD).[5] Thus, we studied patients harboring the A3243G mtDNA point mutation to assess morphological changes of the corneal endothelium.

Methods

Patients diagnosed with mtDNA A3243G macular dystrophy associated with MIDD or MELAS at Columbia University Medical Center, NY or UCLA, CA, were enrolled. Exclusion criteria included a prior diagnosis, or a positive family history, of endothelial corneal dystrophy. Three patients were previously diagnosed based on history and clinical examination, but did not have genetic confirmation at the time of presentation. These patients underwent confirmatory genetic testing using pyrosequencing, a superior method of detecting low levels (>1%) of heteroplasmy in mitochondrial point mutations.[6] Briefly, blood was obtained and mitochondrial DNA was extracted using Qiagen FlexiGene DNA Kit (Hilden, Germany). PCR amplification of the desired DNA was performed with the following primers: forward primer labeled 5'-AGAACAGGGTTTGTAAAGATGGC-3' and reverse primer labeled with biotin, 5'-GTTTAAAGTTTATGCGATTACCG-3'. The amplified DNA was analyzed with the sequencing primer 5'-GGTTTGTAAAGATGGCAG-3', and pyrosequencing was conducted as described by Yan *et al.*, at the Genetic Resources Core Facility, McKusick-Nathans Institute of Genetic Medicine, Baltimore, MD.[6] Clinical examination using slit-lamp microscopy and a detailed chart review was performed in all cases. Specular microscopy and fundus blue autofluorescence (FAF) images were obtained. Automated analysis of endothelial cell count, size and coefficient of variation was performed using the Tomey EM-3000 (Nürnberg, Germany) or Konan Cellchek XL (Konan Medical, USA) specular microscope software. All steps were HIPAA-compliant.

Results

All patients exhibited macular dystrophy consistent with the clinical presentation of mtDNA point mutation A3243G, as illustrated by the presence of perimacular RPE mottling and a remarkable ring-like perifoveal distribution of nummular RPE atrophy with fundus examination and FAF imaging (Fig. 1). Three patients (Patients 1,2 and 3), underwent genetic analysis using the pyrosequencing method which confirmed the A3243G mitochondrial mutation in each case. The fourth and fifth cases presented with A3243G confirmation. For Patients 1–3, the sequence GGAG was input in the mutation position in order to increase the detection accuracy (Supplementary Fig. 1).

Slit lamp biomicroscopy displayed rare to mild guttata, noted peripherally in four patients, and centrally in two patients. One patient also displayed a retro-corneal membrane (Fig. 2). Anterior segment optical coherence tomography showed that the opacity was at the level of the pre-descemet's layer (Fig. 2). Specular biomicroscopy illustrated significant polymegathism (Fig. 2) in all 5 cases. Detailed measurements of endothelial cell morphology are indicated in Table 1. Since heteroplasmy can lead to different mutational load, each eye was analyzed separately. The average endothelial cell density was 2358 ± 456 cells per mm^2 (ranging from 1550 to 2941 cells per mm^2 , $n=10$ eyes). With the exception of one patient (P5), who had a history of cataract surgery extraction, the average endothelial cell density was not significantly different from that of the average population[7, 8]. The average endothelial cell size was $442 \pm 103 \mu\text{m}^2$ (ranging from 340 to $645 \mu\text{m}^2$, $n=10$ eyes). Similarly, with the exception of one patient (P5), the average endothelial cell size was not

significantly different from that of the average population.[7, 8]. Qualitative analysis revealed the presence of very large endothelial cells in all cases (Fig. 2). These cells were accompanied by the presence of small neighboring cells. This heterogeneity in cell size is best quantified by the coefficient of variation (COV), an index of polymegathism.[9] The average COV was $42.0 \pm 4.1\%$ (38 to 49%, n=10 eyes). When compared to the average American population, the average COV was significantly higher than predicted for the patients' age (see Table 1).[7, 8] Corneal edema was not observed in any patient. The average central corneal thickness (CCT) was $551 \pm 33 \mu\text{m}$ (ranging from 502 to 597 μm , n=8 eyes). CCT obtained from Patient 5 was excluded from this analysis since the patient had a history of refractive LASIK surgery. CCT values were similar to that of the average population.

Discussion

In this series of 5 cases of genetically confirmed A3243G associated macular dystrophy, remarkable corneal endothelial abnormalities were identified in each case. While absolute quantitative endothelial cell count as well as the average endothelial cell size were not significantly different in patients 1 – 4, there was remarkable heterogeneity in the cell size in all patients. COV is a reliable indicator of polymegathism.[9] When compared with COV from 3 prior studies of normal patients, the average COV in our 5 cases was more than two to six standard deviations above the mean.[7, 8, 10] Moreover, with qualitative analysis, each case illustrated endothelial cell polymegathism which was a distinctive feature.

Polymegathism of corneal endothelium has been identified in patients with diabetes mellitus.[11] A large population-based study demonstrated that higher COV in diabetic patients compared to controls was statistically significant only in subjects in their sixth and seventh decade.[12] In our series, only two patients (Patients 1 and 5, who were 41 and 60 years of age, respectively) had diabetes. Anterior uveitis has also been associated with morphological changes in corneal endothelium[13] but none of the patients in this series reported a history of anterior uveitis.

The corneal endothelial disorder associated with the A3243G mutation harbored features similar to Fuchs' endothelial corneal dystrophy. However, in our series, neither severe guttata nor corneal edema were observed. One patient displayed a pre-descemet's opacity that was clinically reminiscent of posterior polymorphous corneal dystrophy. Gonioscopic examination was unremarkable. Post-mortem ultrastructural analysis of corneal endothelium revealed cellular distention with enlarged mitochondria in two patients with the mtDNA point mutation A3243G.[14] Similarly, post-mortem histopathologic assessment of one patient with MELAS did identify large corneal endothelial cells in the periphery but not at the center.[15] In our series, the distinctive feature was increased polymegathism of the central corneal endothelium. Our observations are also consistent with few case reports of endothelial abnormalities in patients with other mitochondrial disorders.[16–21]

Conclusion

The mtDNA point mutation A3243G results in corneal endothelial polymegathism. The prevalence of MIDD in the diabetic population is approximately 1.5%, [22] and the prevalence of the mtDNA point mutation A3243G in the Caucasian population is about 0.24%. [23] We anticipate that a large subset of patients harboring the A3243G mutation may have a subclinical presentation of corneal dystrophy and may benefit from corneal consultation. Further, the findings of corneal endothelial cell polymegathism may be a biomarker of mitochondrial disease, specifically in patients with A3243G mutation. While none of the patients in our series demonstrated clinical signs of corneal decompensation, a larger prospective study is needed to determine whether endothelial cell loss occurs at a greater rate than in general population. Our findings also underscore the importance of mitochondrial function in the viability of the corneal endothelium.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments / Disclosure

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Fig. 1. Color fundus photograph (Top) and fundus autofluorescence (FAF, bottom) images of Patient 1 illustrate areas of diffuse perimacular RPE mottling associated with a ring-like distribution of nummular RPE atrophy, best appreciated with FAF and characteristic of A3243G mutation. Note the variable sized flecks with peripapillary involvement and relative foveal sparing.

Figure 2.

Top left) High magnification slit-lamp biomicroscopic photography showing a retrocorneal membrane. Top right) High resolution anterior segment optical coherence tomography localizing the opacity to the pre-Descemet's layer. Bottom) Specular biomicroscopy of a control subject and three eyes of patients with mtDNA point mutation A3243G. Note the remarkable presence of endothelial cell polymegathism in the A3243G affected cases.

Patient demographics and specular microscopy analysis. COV, coefficient of variation; CCT, central corneal thickness. Age-adjusted values from population-based studies are highlighted in gray. Note that Patient 5 had cataract extraction with intraocular lens implant and LASIK refractive surgery, hence the reduced endothelial cell count and thin CCT (*). Average CCT did not include CCT values from Patient 5.

Table 1.

Patient	Sex	Age	Race	Laterality	Cell density (Cells per mm ²)	Age-predicted cell density (cells per mm ²) ±SD[8]	Average cell size (μm ²)	Age-predicted cell size (μm ²) ±SD [7], [8]	COV (%)	Age-predicted COV (%) [7],[8]	CCT (μm)
1	M	41	African American	Right	2941	2619 ± 321	340	387.3 ± 50.4	39	27.0 ± 3.4	549
				Left	2079		481		41		542
2	F	54	Caucasian	Right	2386	2625 ± 172	419	382.4 ± 25.0	43	29.0 ± 3.2	518
				Left	2429		412		38		502
3	M	43	Caucasian	Right	2625	2619 ± 321	381	387.3 ± 50.4	38	27.0 ± 3.4	562
				Left	2654		377		38		547
4	F	48	Caucasian	Right	2695	2619 ± 321	371	387.3 ± 50.4	49	27.0 ± 3.4	597
				Left	2552		392		47		592
5	F	60	Hispanic	Right	1550*	2625 ± 172	645	382.4 ± 25.0	41	29.0 ± 3.2	(471)*
				Left	1664*		601		46		(480)*
Average ± SD					2358 ± 456		442 ± 103		42.0 ± 4.1		551 ± 33