Mitochondrial Damage in the Trabecular Meshwork of Patients With Glaucoma

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Objectives: To analyze the frequency of mitochondrial DNA (mtDNA) damage in patients with primary open-angle glaucoma. Oxidative damage plays a major role in glaucoma pathogenesis. Since no environmental risk factor for glaucoma is recognized, we focused our attention on mitochondria, the main endogenous source of reactive oxygen species.

Methods: Mitochondrial damage was evaluated analyzing a common mtDNA deletion by real-time polymerase chain reaction in trabecular meshwork collected at surgery from 79 patients with primary open-angle glaucoma and 156 unaffected matched controls. In the same samples, polymorphisms of genes encoding for antioxidant defenses (*GSTM1*), repair of oxidative DNA damage (*OGG1*), and apoptosis (*FAS*) were tested.

Results: Mitochondrial DNA deletion was dramatically increased (5.32-fold; $P=.01$) in trabecular meshwork of patients with glaucoma vs controls. This finding was paralleled by a decrease in the number of mitochondria per cell (4.83-fold; $P<.001$) and by cell loss (16.36-fold; $P<.01$). Patients with glaucoma bearing the *GSTM1*-null genotype showed increased amounts of mtDNA deletion and a decreased number of mitochondria per cell as compared with *GSTM1*-positive subjects. Patients bearing a *FAS* homozygous mutation showed only a decreased number of mitochondria per cell.

Conclusions: Obtained results indicate that mitochondrion is targeted by the glaucomatous pathogenic processes. Some subjects bearing adverse genetic assets are more susceptible to this event.

Clinical Relevance: Oxidative damage to the trabecular meshwork exerts a pathogenic role in glaucoma inducing mitochondrial damage and triggering apoptosis and cell loss. This issue may be useful to develop new glaucoma molecular biomarkers and to identify high-risk subjects.

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THE TRABECULAR MESHWORK (TM) is a key region for the initiation of glaucoma, the main cause of irreversible blindness worldwide. This tissue provides the conventional aqueous humor outflow from the anterior chamber. The functional impairment of TM leads to dysfunction of the outflow pathway[1] and triggers glaucoma pathogenesis. The progressive loss of TM cells observed in patients with glaucoma has been attributed to the long-term effect of oxidative free radicals.[2,3] Reactive oxygen species are able to alter TM function.[4,5] Accordingly, the intraocular pressure (IOP) increase, characterizing most glaucomas, is related to oxidatively induced degenerative processes affecting TM. Oxidative stress plays a role in glaucoma development initially by damaging TM cells, then altering nitric oxide and endothelin homeostasis, and finally contributing to ganglional cell death.[6]

Although much experimental evidence indicates that oxidative damage is implicated in the pathogenesis of primary open-angle glaucoma (POAG), the source of such damage remains to be identified. No environmental sources of oxidative stress, such as cigarette smoke or radiation, have been involved in glaucoma pathogenesis.[7] Mitochondria are the most important endogenous source of reactive oxygen species in cells.[8] Mitochondria possess their own genetic material, the mitochondrial DNA (mtDNA), a circular double-strand DNA lacking nucleosomal structure and DNA repair systems. These features make mtDNA particularly susceptible to damage induced by reactive oxygen species, which causes mitochondrial failure and increased endogenous production of oxidative damaging species, thus establishing a "vicious cycle."[9]

Mitochondrial damage is involved in the pathogenesis of many chronic degenerative diseases. Some experimental findings suggest a possible role of mitochondrial damage in POAG, which, however, still remains to be demonstrated.[10,11] A common marker of mtDNA damage associated with oxidative stress is the 4977–base pair (bp) common deletion ($mtDNA^{4977}$),[9] involving the loss of more than one-fourth of the whole mitochondrial genome, disrupting 3 complexes of genes coding for proteins involved in oxidative phosphorylation.[12]

The aim of our study was to analyze the frequency of mtDNA^{4977} in patients with POAG vs controls to evaluate the role of mitochondrial dysfunction in the glaucomatous TM. This end point was analyzed in the iris of the same patients to evaluate the peculiar susceptibility of TM to oxidative stress as compared with other anterior chamber tissues. This issue is relevant because we recently demonstrated that TM is the most sensitive tissue to in vitro oxidative stress in the anterior chamber.[13]

The quantification of mtDNA^{4977} was carried out by quantitative polymerase chain reaction (QPCR), which is highly sensitive and reliable as confirmed by the increasing number of articles using this method.[14,15]

Insofar, evidence demonstrating the purported role of mitochondrial damage in POAG pathogenesis is overwhelming, but most results were not obtained using POAG target tissue, ie, the TM. Our study provides for the first time, to our knowledge, evidence that mitochondrial damage really occurs in the TM of patients with glaucoma, thus providing evidence for its pathogenic role in glaucoma.

Genetic factors predispose to POAG development. However, glaucomas caused by single gene mutations are extremely rare. The most relevant POAG-related mutations are those occurring in *MYOC* and *OPTN*, which occur in less than 3% of POAG.[16] *MYOC* was demonstrated to be a structural component of the mitochondrial wall, further suggesting an important role of mitochondrial damage in POAG-related TM degeneration.[17]

Accordingly, the analysis of genetic risk factors should be also focused on genetic polymorphisms conferring a moderate risk but widely spread in the population. On this basis, we decided to analyze the role of frequent genetic polymorphisms as possible contributors to the mtDNA damage occurring in glaucomatous TM. Selected genes included (1) glutathione *S*-transferase 1 (*GSTM1*), coding a protein involved in the scavenging of reactive oxygen radicals[18]; (2) 8-oxo-guanine glycosylase 1 (*OGG1*), coding a protein repairing 8-oxo-2′-deoxyguanosine, the main oxidative DNA lesions increased in the TM of patients with POAG[19]; and (3) *FAS*, coding a protein involved in the activation of the apoptotic cascade.[20]

GSTM1 represents a pivotal intracellular defense against oxidative stress and its homozygous deletion has been associated with an increased level of oxidative DNA damage in TM.[19] Accordingly, a *GSTM1* polymorphism has been proposed as a possible risk factor for glaucoma, although other studies did not confirm this finding.[21,22] *OGG1* adverse polymorphisms inducing failure in its repairing ability have been related to increased sensitivity to oxidative damage, resulting in cell death.[23] *FAS* polymorphisms modulate cell attitude to undergo apoptosis as a consequence of oxidative stress.[20] The goal of our study was to analyze the interaction between mtDNA damage, genetic polymorphisms, and POAG.

METHODS

SUBJECT RECRUITMENT AND SAMPLE COLLECTION

The study had a case-control design. Recruited cases had POAG requiring surgical intervention. Inclusion criterion for cases was the presence of POAG with no tonometric compensation established by clinical and instrumental examinations, as elsewhere reported.[19] Main elements for POAG diagnosis were papilla morphology, IOP values, and visual field analysis.

Exclusion criterion was the presence of any other ocular, systemic, or neurological diseases other than POAG-related optic nerve damage. An additional exclusion criterion was the presence of any glaucoma types other than POAG. The TM samples were collected by standard surgical trabeculectomy, as previously reported.[2,19] All the enrolled patients furnished an informed written consent and were treated in accordance with the Declaration of Helsinki.

Trabeculectomy specimens were collected from 79 patients with glaucoma, including 49 women and 30 men, mean (SE) age, 66.4 (2.19) years. All patients had an elevated IOP (minimum, 23 mm Hg; maximum, 36 mm Hg; mean [SE], 27.8 [3.69] mm Hg). Specificity of TM alterations was examined by analyzing the iris in a subgroup of 19 patients.

For control samples, we used trabeculectomy specimens collected from 156 subjects, mean (SE) age, 65.1 (1.17) years and sex matched with cases. Samples were collected from glaucoma-free cornea donors by the Melvin Jones Eye Bank, as previously reported.[19]

Sample collection from controls was performed no later than 1 hour after death, thus ensuring cell viability, as necessary for the corneal transplant. Samples were immersed in stabilizing buffers containing antioxidants and stored in a deep freezer (–80°C). DNA purification was performed by incubating samples with proteinase K followed by solvent extraction in an oxygen-free atmosphere.[19]

DETECTION OF mtDNA^{4977} DELETION AND mtDNA COPY NUMBER

Quantification of mtDNA^{4977} deletion and total mtDNA were performed by real-time PCR (QPCR) using fluorescent probes (**Figure 1**). Two QPCR reactions were performed in parallel for each sample, the first to detect the amount of total mtDNA, the second to quantify mtDNA^{4977} deletion. The total mtDNA reaction was carried out using 2 primers (total mtDNA sense: CCATCTTTGCAGGCACACTCATC and total mtDNA antisense: ATCCACCTCAACTGCCTGCTATG) flanking a sequence of mtDNA (474 bp) known not to be susceptible to the deletion[24] and an ad hoc–designed FAM-labeled molecular beacon (total mtDNA: 5′-FAM-CGCGATCTCACGCAAGCAACCGCATCCATGATCGCG-BHQ1-3′). The mtDNA^{4977} deletion reaction was performed using 2 primers (deleted mtDNA sense: GGCCCGTATTTACCCTATAG and deleted mtDNA antisense: GGTGAGAAGAATTATTCGAGTG) recognizing mtDNA regions flanking the deletion site plus a molecular FAM-labeled beacon (deleted mtDNA: 5′-FAM-CGCGATCCAGCCTAGCATTAGCAGGAATACCTTGATCGCG-BHQ1-3′) targeting a DNA sequence between the 2 primers and ahead of the deletion site. Because of the short elongation time, only deleted mtDNA can efficiently produce amplicons (ie, double-strand DNA 400 bp) containing the target sequence for the deleted mtDNA molecular beacon.

A Basic Local Alignment Search Tool search for the deleted mtDNA amplicon and probe were performed with high specificity. The stringent conditions of the reaction are a further warrant for specificity. The incidental cross-reaction of the probe with genomic DNA was further assumed as negligible because mtDNA presents in each cell in multiple copy as compared with nuclear DNA (nDNA).

The reaction conditions were 5 µL of 10× PCR buffer, 0.4 µL of 100mM dNTP mix, 2 µL of 50mM magnesium chloride, 0.5 µL of platinum Taq polymerase (Invitrogen Corp, Carls-

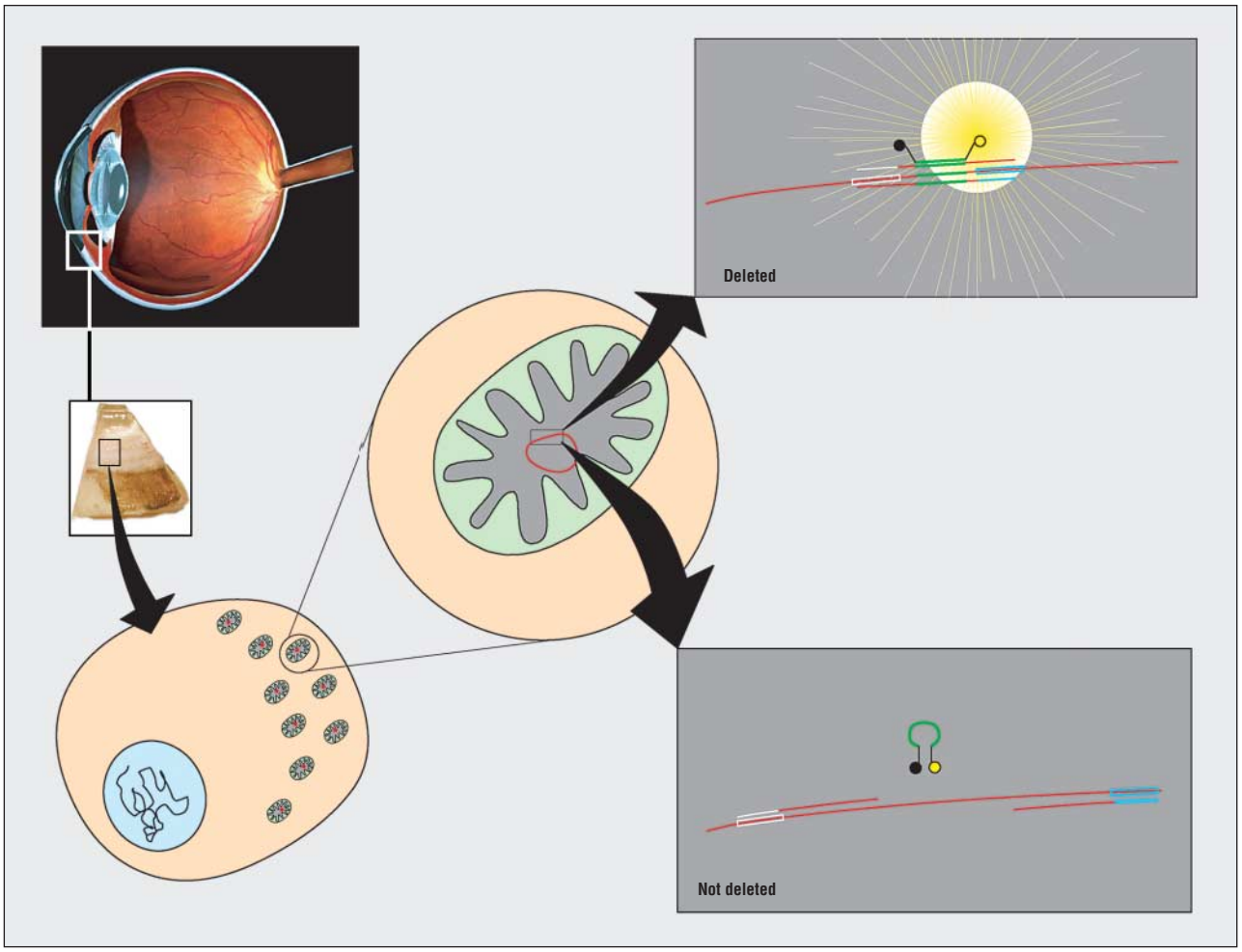

Figure 1. Evaluation of the 4977–base pair mitochondrial DNA (mtDNA^{4977}) deletion and mtDNA copy number by real-time quantitative polymerase chain reaction in the trabecular meshwork. Molecular beacon probes emit fluorescent light only when the deletion of mtDNA is present.

bad, California), 37.1 µL of sterile water, 1 µL of 10µM sense primer, 1 µL of 10µM antisense primer, 2 µL of molecular beacon, and 1 µL of DNA (25 ng).

The QPCR reactions were performed in a rotating real-time thermocycler (Rotorgene3000; Corbett Life Science, Sydney, Australia), using the following temperature ramp: 94°C for 2 minutes followed by 45 cycles at 94° for 30 seconds, annealing temperature of 54°C for total mtDNA and 51°C for deleted mtDNA for 30 seconds, and 72°C for 30 seconds. Fluorescence was acquired at the end of each annealing step. Each sample was tested in duplicate.

The reaction efficacies for the total mtDNA and mtDNA^{4977} deletion quantifications were assayed and the results were 0.73 and 0.78, respectively. An internal control was tested in each reaction and the results were expressed as relative quantity (sample/internal control). The specificity of the amplification product and the results were confirmed by capillary electrophoresis using the Agilent 2100 Bioanalyzer with a DNA 1000 Series II kit (Agilent Technologies, Waldbronn, Germany), thereby confirming QPCR results by capillary electrophoresis of amplified mtDNA sequences.

The relative ratio of deleted mtDNA to total mtDNA was assessed for each sample and normalized for cell number assayed quantifying by QPCR the housekeeping gene *GAPDH*. The amount of mtDNA^{4977} deletion was expressed as percentage of total mtDNA.

EVALUATION OF THE nDNA:mtDNA RATIO

The relative amount of total nDNA in each sample was evaluated by quantifying the copies of *GAPDH* by QPCR. This parameter was related both to mtDNA copy number (nDNA:mtDNA ratio) and to the amount of wet tissue processed (DNA per milligram of wet tissue). This last parameter was assumed as an indicator of cell number in TM.

GENETIC POLYMORPHISM ANALYSES

Genetic polymorphisms were analyzed in an aliquot (0.1 µg) of nDNA as purified from TM samples. The *OGG1* Ser326Cys polymorphism, resulting from a C>G transversion in exon 7, was evaluated as previously described by QPCR using a pair of gene-specific molecular beacons to discriminate between the occurrence of this genetic variant on both alleles (homozygous mutant), 1 allele only (heterozygous), or no allele (wild type).[25] The *FAS* promoter 670 polymorphism, resulting from an A/G substitution at the 670 nucleotide position in the enhancer region of the gene, was evaluated by QPCR using a set of gene-specific molecular beacons to distinguish the occurrence of this genetic variant on both alleles (homozygous mutant), 1 allele only (heterozygous), or no allele (wild type).

Table 1. mtDNA Damage in TM as Detected in 79 Patients With POAG and 156 Controls

Disease Status	Mean (SE)			
	mtDNA[4977] Deletion:mtDNA Ratio, %[a]	Total mtDNA[b]	mtDNA:nDNA Ratio[c]	Total nDNA per Milligram of Wet Tissue Ratio[d]
Controls	6.52 (2.31)	17.96 (1.63)	8.47 (1.00)	9.49 (1.69)
POAG	34.68 (23.57)[e]	3.72 (1.29)[f]	51.6 (0.48)[g]	0.58 (0.14)[h]

Abbreviations: mtDNA, mitochondrial DNA; mtDNA[4977], 4977–base pair mtDNA; nDNA, nuclear DNA; POAG, primary open-angle glaucoma; TM, trabecular meshwork.

[a] This molecular end point reflects the amount of mtDNA[4977] deletion as an indicator of mitochondrial damage.

[b] This molecular end point reflects the total mtDNA copy number, its decrease expressing the mitochondrial loss in the examined tissue (TM).

[c] This molecular end point reflects the total mtDNA copy number per cell; its decrease is expression of mitochondrial loss normalized for the number of cells composing the examined tissue (TM).

[d] This molecular end point reflects the number of cells composing the examined tissue (TM), its decrease expressing the occurrence of cell loss.

[e] Significantly higher than controls at $P<.05$.

[f] Significantly lower than controls at $P<.001$.

[g] Significantly lower than controls at $P<.05$.

[h] Significantly lower than controls at $P<.01$.

Molecular beacon sequences were human *FAS* wild type: 5′-FAS-CGCGATCTGTCCATTCCAGAAACGTCT-GTGAGCGATCGCG-BHQ1-3′ and human *FAS* mtDNA: 5′-HEX-CGCGATCTGTCCATTCCAGGAACGTCT-GTGAGCGATCGCG-BHQ-3′. The PCR primer sequences were human *FAS* sense: 5′-CCCTTTTCAGAGCCCTATGG-3′ and human *FAS* antisense: 5′-TGCTGGAGTCACTCAGAGAAAG-3′. The reaction was performed at 94°C for 2 minutes, followed by 45 cycles at 94°C for 30 seconds, 66°C for 15 seconds, 64°C for 15 seconds, 53°C for 30 seconds, and 72°C for 30 seconds. The HEX signal was acquired at the end of the 66°C step and FAM signal, at the end of the 64°C step.

The *GSTM1* polymorphism, including the gene presence on 1 or both alleles (positive) or its homozygous deletion (null), was investigated by QPCR as previously described.[25] All primer sequences and PCR conditions were determined using Beacon Designer software (Premier Biosoft International, Palo Alto, California) purchased from TIB Molbiol (Berlin, Germany).

STATISTICAL ANALYSES

Comparisons among quantitative variables in different groups of patients were executed by analysis of variance and *t* test for unpaired data after checking the normality of the distribution by skew kurtosis analysis. The accepted level of significance in all cases was $P<.05$. In situations without a normal distribution of data, a nonparametric test was used (Mann-Whitney *U* test and Kruskal-Wallis test). Correlations between continuous variables were tested by linear regression analysis. Differences of frequency distributions among nominal variables were tested by χ^2 test. All statistical analyses were performed using Statview software (Abacus Concept, Berkeley, California).

RESULTS

mtDNA COPY NUMBER AND mtDNA[4977] DELETION IN TM OF PATIENTS WITH POAG VS CONTROLS

A remarkably high level of molecular damage was detected in mtDNA in the TM of patients with POAG as compared with unaffected controls. In particular, there was a statistically significant 5.32-fold increase in the level of mtDNA[4977] deletion in the TM of patients with glaucoma as compared with controls (**Table 1**). These data were confirmed by capillary electrophoresis, resulting a 1.7-fold increase in the level of mtDNA[4977] deletion in the TM of patients with glaucoma as compared with controls (**Figure 2**). Furthermore, the results were validated performing the same analysis on mtDNA as purified from isolated mitochondria (Mitochondrial Isolation Kit; Pierce, Rockford, Illinois) collected from the same tissue.

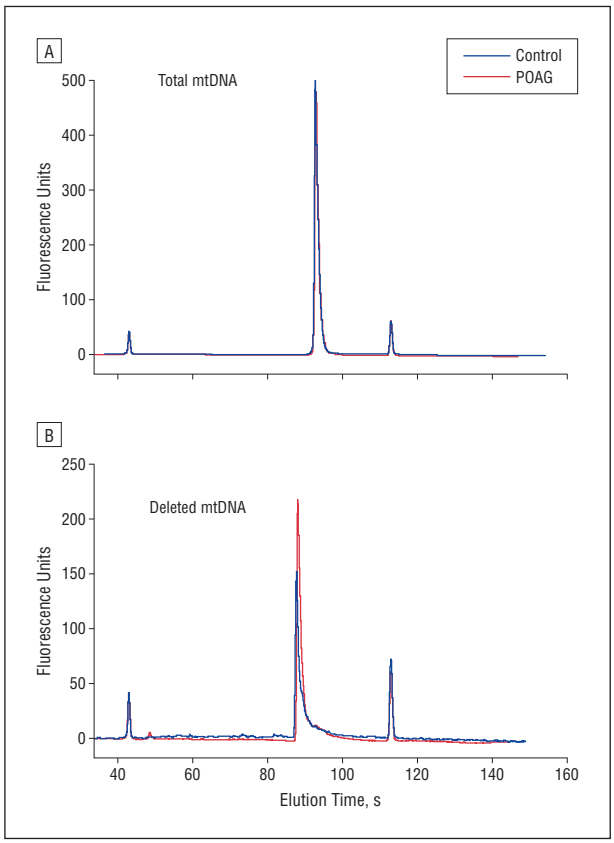

Figure 2. Electropherogram of the amplification products. A, The peak at elution time 95 seconds corresponds to the 474–base pair (bp) tract of mitochondrial DNA (mtDNA) not susceptible to deletion. B, The peak at elution time 90 seconds corresponds to the 400-bp amplicon flanking the deletion site. The blue line corresponds to a control sample and the red line, to a primary open-angle glaucoma (POAG) sample. Part A shows that the amount of mtDNA in both samples was the same (the curves overlap perfectly). Part B shows that the deletion amount is higher in the POAG sample than in the control sample (red peak higher than the blue one).

Table 2. mtDNA Damage as Evaluated in Parallel in the TM and Iris of the Same 19 Patients With POAG

Disease Status	Mean (SE)			
	mtDNA^{4977} Deletion:mtDNA Ratio, %[a]	Total mtDNA[b]	mtDNA:nDNA Ratio[c]	Total nDNA per Milligram of Wet Tissue Ratio[d]
TM	49.23 (18.10)[e]	1.09 (0.34)[f]	3.81 (0.36)[f]	0.38 (0.08)[e]
Iris	15.28 (10.04)	2.17 (0.37)	7.68 (1.51)	0.12 (0.01)

Abbreviations: mtDNA, mitochondrial DNA; mtDNA^{4977}, 4977–base pair mtDNA; nDNA, nuclear DNA; POAG, primary open-angle glaucoma; TM, trabecular meshwork.
[a] This molecular end point reflects the amount of mtDNA^{4977} deletion as an indicator of mitochondrial damage.
[b] This molecular end point reflects the total mtDNA copy number, its decrease expressing the mitochondrial loss in the examined tissue (TM).
[c] This molecular end point reflects the total mtDNA copy number per cell; its decrease is expression of mitochondrial loss normalized for the number of cells composing the examined tissue (TM).
[d] This molecular end point reflects the number of cells composing the examined tissue (TM), its decrease expressing the occurrence of cell loss.
[e] Significantly higher than the iris at $P<.05$.
[f] Significantly lower than the iris at $P<.01$.

Table 3. Frequency of Analyzed Genetic Polymorphisms in Patients With Glaucoma, Controls, and the General Population as Inferred From Available Literature

	%			
	Patients With POAG	Controls	General Population	OR (95% CI)
GSTM1				
Wild type	32	46	60[25]	
Null polymorphism	68[a]	54	40	5.68 (3.15-10.24)
OGG1				
Wild type	50	42	61[25]	
Heterozygous mutant	46	53	34	0.74 (0.43-1.27)
Homozygous mutant	4	5	5	0.74 (0.19-2.83)
FAS				
Wild type	39	31	38[20]	
Heterozygous mutant	49	54	50	0.84 (0.49-1.44)
Homozygous mutant	12	15	13	0.83 (0.38-1.86)

Abbreviations: CI, confidence interval; OR, odds ratio; POAG, primary open-angle glaucoma.
[a] Statistically significant difference in patients with POAG vs controls ($P<.05$).

The variability of the results obtained in cases was remarkable and cannot be attributed to interexperimental variability, which fell lower than 30% when replicate samples were analyzed. Nevertheless, the difference in mtDNA^{4977} levels as detected in controls and cases was remarkable and statistically significant ($P=.01$).

Furthermore, both the amount of mtDNA (4.83-fold) and the nDNA per milligram of wet tissue ratio (16.36-fold) were reduced in glaucomatous TM (Table 1). This decrease of the nDNA per milligram of wet tissue ratio indicates the occurrence of a dramatic drop in cell number in the TM. This finding was paralleled by a rising incidence of mtDNA damage, as indicated by the increase in mtDNA deletion and the decrease in mtDNA copy number per cell.

A negative correlation between age and mtDNA:nDNA ratio was observed in controls ($r=-0.192$; $P<.05$) but not in patients with POAG ($r=-0.069$; $P=.27$).

TM VS IRIS COMPARISON IN PATIENTS WITH POAG

Mitochondrial loss and damage observed in patients with POAG selectively occurred only in the TM and not in the iris. In fact, mtDNA deletions were significantly higher in the TM than in the iris of the same patients, while mtDNA copy number and mtDNA:nDNA ratio were significantly decreased in the TM as compared with the iris. Finally, the nDNA per milligram of wet tissue ratio was lower in the TM than in the iris, a finding amenable to the different cellularity of these tissues (**Table 2**).

FREQUENCIES OF GENETIC POLYMORPHISMS

The percentages of various polymorphisms as analyzed in patients with POAG and controls are reported in **Table 3**. None of these differences was statistically significant except an increased frequency of the *GSTM1*-null genotype in patients with POAG as compared with controls. By comparison, the frequency of the various genotypes as detected for each gene in the general population is reported (Table 3).

INFLUENCES OF GENETIC POLYMORPHISMS ON MITOCHONDRIAL DAMAGE

OGG1 polymorphisms did not exert any significant influence on any of the molecular end points tested (Table 3). The *GSTM1* homozygous deletion was associated with an increased level of mtDNA deletion (1.7-fold) and a decreased amount of total mtDNA (2.5-fold) and mtDNA:nDNA ratio (2.3-fold). Conversely, the amount of nDNA per milligram of wet tissue was not affected (Table 3). The *FAS* homozygous mutation was significantly correlated both with a decreased amount of mtDNA (2.3-fold) and mtDNA:nDNA ratio (3.2-fold). Conversely, no effect of this mutation was detected on the amounts of deleted mtDNA and nDNA per milligram of wet tissue (Table 3).

RELATIONSHIPS AMONG MOLECULAR END POINTS

The correlation between the mtDNA deletion and the mtDNA:nDNA ratio was not significant in controls ($r=0.090$; $P=.25$) but was of borderline statistical significance in patients with glaucoma ($r=-0.277$; $P=.06$). These results indicate that mtDNA deletion is inversely related to the decrease of mtDNA copy number in patients with glaucoma but not in controls. No correlation

was observed among the other molecular end points tested in controls or patients with glaucoma.

COMMENT

Though many studies in the literature analyze mitochondrial dysfunction in POAG, most of them were carried out on surrogate tissue (**Table 4**). To the best of our knowledge, our study is the largest study performed analyzing genetic polymorphisms and molecular alterations directly in TM.

Our study provides evidence that mtDNA damage occurs in the target tissue of POAG, the TM. Such damage is detectable only in the TM and not in other anterior chamber districts, eg, the iris. Genetic polymorphisms affect the amount of mtDNA damage in TM.

The level of mtDNA[4977] detected in glaucomatous TM is remarkably high. By comparison, the level of mtDNA[4977] as detected by our group using the herein-reported QPCR method in stemlike mammary cells spanned from 0.9% to 2.1% only.[35] Markaryan et al[15] detected a similar range of molecular lesions in different tissues of cochlear structures using the same method.

The high interindividual variation in the level of mtDNA[4977], especially in patients with POAG, could be due to some still unidentified genetic polymorphisms or to the variability in the dietary intake of antioxidants. It is unlikely that such variability could be due to differences in diseases status because all patients were carriers of advanced unbalanced POAG requiring surgical trabeculectomy. Other variables possibly contributing to this variability might be related to mitochondrial haplotypes, which are characterized by different sensitivity to oxidative damage and undergo interindividual variations.[36] However, to our knowledge, no direct relationship between mtDNA haplotypes and POAG prevalence has been reported.[37]

A further explanation for the high variability of mtDNA deletion observed in POAG may be due to the low amount of mtDNA detected that affects PCR amplification conditions requiring many amplification cycles.

Our findings shed light on mechanisms contributing to TM degeneration in POAG. In cases of oxidative stress, mitochondrial damage triggers apoptosis.[38,39] These mechanisms result in degenerative phenomena in tissues composed of perennial cells, such as TM.

Our results are in agreement with previous studies performed in vitro in TM primary cultures collected from patients with POAG and donor eyes, indicating that in POAG mitochondrial dysfunction results in intracellular calcium and oxidative overload.[30] Primary open-angle glaucoma–related mitochondrial alterations detected in vitro include lower adenosine triphosphate production and decreased transmembrane potential resulting in mitochondrial complex I defect associated with the degeneration of TM cells.[11] The occurrence of mitochondrial functional defects in glaucomatous TM cells resulting in abnormal vulnerability to intracellular calcium increase has been reported.[38]

Mitochondrial haplogroups have been examined as a possible genetic factor for glaucoma. No association between mitochondrial haplogroups and POAG has been reported, although a possible association with primary angle-closure glaucoma has been reported in a small study.[37,40]

Table 4. Mitochondrial Dysfunction in TM and Surrogate Tissues of Patients With Glaucoma[a]

Substratus	Mitochondrial Defect	Source
Human TM cultures	Pro370Leu mutant myocilin	26,27
	Truncated myocilin	28
TM of the adult rhesus monkey	Swollen mitochondria in endothelial cells	29
Primary cultures of TM cells	Dysfunction in calcium regulation	30
	Myocilin partially released from mitochondrial compartments	31
TM tissue	Defect in the mitochondrial complex I	11
Porcine TM cells	Increase in iROS and expression of inflammatory mediators.	32
Blood samples	*CYP1B1* mutations	33
	mtDNA changes	10
Human corneal fibroblasts	Myocilin mutations in human TM cells	34
Human TM biopsies	mtDNA deletion	Current study

Abbreviations: iROS, intracellular reactive oxygen species; mtDNA, mitochondrial DNA; TM, trabecular meshwork.
[a] All cultured cells, nontarget; only 1 on TM from living patients.

Mitochondrial damage and loss occurring in TM trigger both degenerative and apoptotic phenomena resulting in cell loss. This cell loss clinically manifests as IOP increase. Decrease of TM cellularity during glaucoma course has been previously reported, although to a lesser extent than those indicated by our results.[41] Changes in TM composition during glaucoma course could contribute to the detected decrease of the DNA per millgram of wet tissue ratio.

The TM specificity of mtDNA damage in POAG is supported by the comparison with the iris of the same patients with POAG in whom such damage was not detected. This comparison was performed between 2 neighbor tissues of the anterior chamber, thus being highly specific. The lack of mtDNA damage in tissues different from TM is in agreement with the negative findings reported by other studies in blood lymphocytes of patients with POAG.[42]

A comparison of mitochondrial damage and genetic polymorphisms does not support a major role for *OGG1* in POAG. Conversely, both *GSTM1* and *FAS* appear to affect molecular damage occurring in TM of patients with POAG. Patients devoid of *GSTM1* activity have increased mtDNA deletion. This finding may be interpreted as resulting from the decreased antioxidant defenses observed in subjects with *GSTM1* deletion, demonstrated by the increased TM oxidative DNA damage in the *GSTM1*-null vs *GSTM1*-positive patients with POAG.[19]

FAS homozygous mutations did not exert an influence on the level of mtDNA deletion while decreasing the mtDNA copy number and the mtDNA:nDNA ratio. In the anterior chamber of the eye, *FAS* has been demonstrated to provoke apoptosis increasing myocilin release from mitochondria to cytosolic compartments of TM cells.[31] Further studies are required to clarify in which glaucoma types, other than POAG, high levels of mtDNA deletion occur in TM.

In conclusion, our results indicate that patients with POAG bear genetic predisposition to oxidative stress contributing to mtDNA damage and apoptosis. Mitochondrial DNA deletion is a severe damage transmitted during mitochondrial mitosis to new mitochondrial progeny. Accordingly, mitochondrial damage progressively increases with age. On this basis, POAG may be interpreted as a mitochondriopathy affecting TM cells, thus hampering aqueous humor outflow.

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