Original Research

Association between *IL1A* and *IL1B* polymorphisms and primary open angle glaucoma in a Brazilian population

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Impact statement

This study is the first, according to our knowledge, to show the association between glaucoma and the functional –31C/T single nucleotide polymorphism. We provide evidence indicating that homozygotes CC at –31C/T and TT at –511 C/T of *IL1B* are at risk for glaucoma. We also demonstrated that these polymorphisms are in strong linkage disequilibrium (LD). Increasing evidence support the role of inflammation in glaucoma and this study is an important result that reinforces these findings. How IL-1 signaling influences the triggering and pathogenesis of glaucoma remains to be investigated. Greater understanding of the mechanisms leading to glaucoma will provide the development of new management strategies that target the primary disease lesion and maybe the discovery of new treatments.

Abstract

The aim of this study was to investigate the association of five polymorphisms in the *IL1A* and *IL1B* genes in Brazilian patients with primary open angle glaucoma (POAG). A case–control study, including 214 unrelated POAG patients and 187 healthy individuals, was conducted to evaluate the frequency of polymorphisms in the *IL1A* and *IL1B* genes. Ophthalmic evaluation was performed and genomic DNA was obtained from all participants. Five single nucleotide polymorphisms (SNPs): *IL1A* (–889C/T: rs1800587:C > T, +4845G/T:rs17561G>T) and *IL1B* (–31C/T:rs1143627:T > C, –511C/T:rs16944C>T and +3954C/T:rs1143634:C > T) were genotyped through direct sequencing. The association of individual SNPs was tested using logistic regression. There was an association between the –31C/T and –511 C/T polymorphisms in the *IL1B* gene with POAG ($p = 0.002$ and $p = 0.009$, respectively). High linkage disequilibrium was observed between the –31C/T and –511C/T polymorphisms. The statistical analysis showed that the T/C haplotype (–31/–511) in the *IL1B* gene is more frequent in controls ($p = 0.011$) and the C/T haplotype (–31/–511) is more common in POAG patients ($p = 0.018$). Among POAG cases, the genotypic distribution of the –31C/T and –511 C/T SNPs was significantly different in patients who underwent anti-glaucomatous surgery compared to patients without surgery ($p = 0.016$ and 0.023, respectively). There was no statistically significant difference for the remaining SNPs between POAG patients and controls. In conclusion, the C allele of the –31C/T and the T allele of the –511C/T polymorphisms in the *IL1B* gene may represent a "risk haplotype" for the development of POAG in Brazilian individuals. Further studies with larger cohorts of patients are necessary to substantiate these findings.

Keywords: *IL1A*, *IL1B*, glaucoma, inflammation, cytokines, optic disc, trabecular meshwork

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Introduction

Glaucoma is a progressive atrophy of the optic disc characterized by loss of retinal ganglion cells that leads to a corresponding visual field defect. It is the major cause of irreversible blindness worldwide.[1] Among several forms of glaucoma, the most prevalent is primary open angle glaucoma (POAG). POAG is a complex disease associated with risk factors such as high intraocular pressure (IOP) and positive family history. In glaucoma, IOP usually rises as a consequence of increased trabecular meshwork (TM) resistance to aqueous humor outflow, but the IOP-induced mechanical effects at the lamina cribrosa do not explain all the optic nerve damage that occurs during the course of the disease.[2–4] An interaction with other relevant factors including reduced neurotrophic supply, excitotoxicity, vascular[5–7] and immune system dysregulations,[8] and oxidative stress[7,9] may lead to neuronal and TM damage and hence contribute to the development of glaucoma.

How genetic and environmental factors interact leading to the development of POAG is not clearly understood. Actually, as expected based on its pathophysiology, the majority of cases of adult-onset POAG are considered complex traits, resulting from a large number of susceptibility variants in several genes, each one contributing with smaller effects.[10,11] In fact, researchers have found that disease-causing mutations in genes, which follow a Mendelian pattern of inheritance, are responsible for less than 10% of adult-onset POAG.[11] Following this approach, based on linkage studies, 17 loci were associated with POAG,[12–15] but four main genes were identified: Myocilin (*MYOC*, *GLC1A*), Optineurin (*OPTN*, *GLC1E*), WD repeat domain 36 (*WDR36*, *GLC1G*), and Neurotrophin-4 (*NTF4*, *GLC1O*). Therefore, GWAS approach as well as case–control studies involving candidate genes have been performed to identify other variants of small effect for POAG development. Among them, variations in genes that participate in the inflammation cascade have been suggested as glaucoma modifiers, including interleukin alpha (*IL1A*), interleukin beta (*IL1B*), and tumor necrosis factor alpha (*TNFA*).[16–18]

Interleukin 1 (IL-1) is a key mediator of the inflammatory process and stimulates the expression of other cytokines, metalloproteinases (MMPs), and adhesion molecules.[19,20] The superfamily of IL-1 includes IL-1α, IL-1β, and IL-ra proteins, which are encoded by *IL1A*, *IL1B*, and *IL1RN* genes, respectively. These genes form a cluster at 2q14.2 *locus* localized *in tandem* within a ~430 kb region. The regulatory regions of *IL1B* are distributed over thousands of base pairs upstream the transcription start point. Several single-nucleotide polymorphisms (SNPs) are important regulatory keys, distributed at different sites of the IL-1 genomic sequence, probably changing the affinity of ligands, creating new binding sites for transcription factors or interacting with each other to form haplotype structures.[21,22] There are several other ways of influencing activity and production of IL-1, particularly IL-1 β, including the inhibition by the IL-1β receptor antagonist (IL-1ra),[23] the control at mRNA stabilization level and by post-translational proteolytic processing, which involves cellular transport and secretion.[24,25] These multiple sites of regulation suggest that there is a tight control over the production and activity of IL-1β. Hence, an eventual dysregulation or even an interindividual variation may lead to increased susceptibility to a disease or to a disease per se.

Particularly in glaucoma, Wang et al. described a stress-induced response elicited in the TM cells, controlled by IL-1, through the transcription factor NF-κB.[26] They showed that the activation of this pathway might be protective to TM cells, inhibiting the apoptotic response caused by oxidative stress. This NF-κB activation can be progressively amplified by an autocrine loop and becomes self-sustaining, contributing to cell survival after damage by oxidative injury.

In this study, we aimed to compare the frequency of alleles and genotypes of the *IL1A* and *IL1B* genes between healthy and glaucomatous patients, as well as to investigate if haplotypes of *IL1A* and *IL1B* genes are risk or protection factors for glaucoma. The following polymorphisms were studied: *IL1A* –899C/T (rs1800587:C > T) and +4845G/T (rs17561G>T) and *IL1B* –31C/T rs1143627:T > C), –511C/T (rs16944:C > T) and +3954C/T (rs1143634:C > T).

Material and methods

Subjects

The studied population consisted of 214 randomly selected, unrelated patients with POAG and 187 healthy unrelated controls from the Clinical Hospital, University of Campinas (UNICAMP). Ophthalmic examination included IOP measurement by means of applanation tonometry; slit-lamp biomicroscopy and gonioscopy; evaluation of the optic disc with a 78-diopter lens; and automated perimetry (Humphrey System 24.2; Zeiss-Humphrey-Zeiss Systems, Dublin, CA, USA). Ocular history was obtained, including clinical and surgical data. POAG was defined by an open angle at gonioscopy and at least two of the following characteristics: (1) IOP above 21 mmHg (2) optic disc signs of glaucomatous damage (including localized neural rim defect, increased vertical cupping (>0.7), disc hemorrhages, and cup asymmetry greater than or equal to 0.2), and (3) corresponding visual field loss in achromatic perimetry (24–2, SITA Standard). The latter was characterized by the presence of three adjacent points with $p < 5\%$, one of them being with $p < 1\%$ at the "pattern deviation probability plot"; "pattern standard deviation" with $p < 5\%$ and the "glaucoma hemifield test" showing "borderline" or "outside normal limits." Control subjects with a positive familiar history of glaucoma, any sign of ocular alterations suggestive of secondary glaucoma, or other types of glaucoma were excluded. Normal control subjects had no signs of glaucomatous optic disc abnormalities and were aged 50 years or older with an IOP $\leq$ 16 mmHg.

This study was approved by the Ethics Committee of the University of Campinas (CEP no. 642/2009). The participating subjects signed an informed consent, in agreement with the principles enunciated in the Declaration of Helsinki.

DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood using a conventional phenol-chloroform procedure.[27] The polymorphisms were genotyped through polymerase chain reaction (PCR) and direct sequencing. Table 1 shows the sequences of sense and antisense primers for the five regions encompassing the polymorphisms *IL1A* (–899C/T and +4845G/T) and *IL1B* (–31C/T, –511C/T, and +3954C/T). The primers used for PCR and direct sequencing were chosen based on the sequences deposited in GenBank. The conditions of amplification were the same for all polymorphisms, except for the annealing temperature, as specified in Table 1: initial denaturation at 95°C for 5 min, followed by 35 cycles at 95°C for 1 min, annealing of the primers at a specific temperature for each region for 1 min and extension at 72°C for 1 min, ending with additional extension at 72°C for 7 min.

Direct sequencing was performed with BigDye Terminator Cycle Sequencing Kit v3.1® (Applied

Table 1. Primers, product sizes, and annealing temperatures for the amplification reactions.

Gene	Polymorphism	Sequences	Size	AT (°C)
IL1B	+3954 C/T	S 5′-TGTTCTTAGCCACCCCACTC-3′ AS 5′-AGCGTGCAGTTCAGTGATCG-3′	239 bp	63
	–511 C/T	S 5′-GAGCTTATCTCCAGGGTTGC-3′ AS 5′-TGGGACAAAGTGGAAGACAC-3′	309 bp	63
	–31 T/C	S 5′-GAGCTTATCTCCAGGGTTGC-3′ AS 5′-TGAAGAGAATCCCAGAGCAG-3′	410 bp	63
IL1A	+4845 G/T	S 5′-TCTGCTGACTGGGTGATTTC-3′ AS 5′-CATCCTCCCCTCCTTTCATT-3′	379 bp	63
	–889 C/T	S 5′-ATGGGGGCTTCACTATGTTG-3′ AS 5′-GACACACCTTGGGCATATCC-3′	418 bp	63

bp: base pair; S: sense; AS: antisense; AT: annealing temperature.

Table 2. Demographic and clinical features of the study subjects.

	POAG cases	Controls	p
Gender (M/F)%	(47.62/52.38)	(42.47/57.53)	0.314
Age (mean ± SD)	66.46 ± 12.80	67.41 ± 8.97	0.422
Intraocular pressure (worse eye)			
Range	12–60 mmHg	9–16 mmHg	
Mean ± SD	24.39 ± 8.17	12.19 ± 1.65	<0.001*
Vertical cup-disc ratio			
Range	0.5–1.0	0.1–0.6	
Mean ± SD	0.87 ± 0.12	0.31 ± 0.13	<0.001*

M: male; F: female; SD: standard deviation; POAG: primary open angle glaucoma.
*$p < 0.05$.

Biosystems Inc., Foster City, CA) and submitted to electrophoresis in the ABI Prism 3530 DNA Analyzer (Applied Biosystems Inc., Foster City, CA). Sequencing data were analyzed through the FinchTV program (Geospiza, Seattle, WA) and BLAST algorithm in the NCBI server.2.4 (www.ncbi.nlm.nih.gov/Blast).

Statistical analysis

The statistical analysis of demographic data was performed using the Open-Epi online calculator program (Open Source Epidemiologic Statistics for Public Health; http://www.openepi.com/OE2.3/Menu/OpenEpiMenu.htm, version 2.3.1). For non-parametric comparisons of continuous variables, the Wilcoxon-Mann-Whitney test was applied. For the analysis of categorical variables and odds ratios calculation, the chi-square test was used. For genotypic comparisons of polymorphisms, we used the SAS System for Windows (Statistical Analysis System), version 9.2. SAS Institute Inc., 2002–2008, Cary, NC, USA, employing the logistic regression model. P-values of less than 0.05 were considered statistically significant. The haplotype analysis was calculated by the Haploview program, version 4.2.[28] The Hardy-Weinberg equilibrium test was used for the analysis of the genotypes' distribution in this group of patients.

Results

A total of 214 POAG unrelated patients and 187 healthy subjects were recruited for the study. Demographic and clinical features for each group are exhibited in Table 2. We found no statistically significant differences in age and gender distribution between the groups. As expected, mean IOP and cup-to-disc ratio were significantly different. Alleles and genotypes distributions between cases and controls for the five polymorphisms (*IL1A* and *IL1B*) as well as POAG cases stratified according to the status of anti-glaucomatous surgery are shown in Table 3. The genotype distribution was consistent, with the Hardy-Weinberg equilibrium present in both groups for all polymorphisms.

The *IL1A* gene exhibited no significant difference between POAG cases and controls for the –889C/T and +4845G/T SNPs. Furthermore, the degree of LD ($D' = 0.86$) between –889C/T and +4845G/T SNPs was calculated, and there was no statistically significant difference between cases and controls, and no haplotype block was formed.

The C allele of the –31C/T *IL1B* SNP was a risk factor for glaucoma in this sample of the Brazilian population. The CC and CT genotypes presented, respectively, 2.445 and 1.815 times more chance (OR) of developing glaucoma than TT patients (Table 4) ($p = 0.002$ and 0.011, respectively). The significance was maintained when alleles were compared, with the C allele more frequent among cases than in controls (OR: 1.546; 95% CI: 1.168–2.05; $p = 0.002$). The post-hoc power calculation for this association was 84.3%, considering an alpha of 0.05%.

Regarding the –511C/T SNP at the *IL1B* gene, we observed that CT patients presented 2.189 times more chance (OR) of developing glaucoma when compared to TT patients ($p = 0.009$) (Table 4). The post-hoc power calculation for this association was 49.4%, considering an alpha

Table 3. Genotypic and allelic frequency of the *IL1A* and *IL1B* studied polymorphisms in a sample of the Brazilian population of glaucomatous (POAG) and healthy individuals and surgery frequency among POAG cases.

Gene polymorphism		POAG cases - n (%)	Controls - n (%)	Surgical POAG cases - n (%)	Non-surgical POAG cases - n (%)
IL1A	Genotypes				
–889 C/T	CC	58 (45.67)	72 (51.43)	29 (47.54)	26 (44.07)
	CT	58 (45.67)	58 (41.43)	29 (47.54)	27 (45.76)
	TT	11 (8.66)	10 (7.14)	3 (4.92)	6 (10.17)
	Alleles				
	C	174 (68.50)	202 (72.14)	87 (71.31)	79 (66.95)
	T	80 (31.50)	78 (27.86)	35 (28.69)	39 (33.05)
IL1A	Genotypes				
+4845 G/T	GG	70 (57.38)	64 (56.14)	34 (55.74)	36 (60.00)
	GT	49 (40.16)	45 (39.47)	27 (44.26)	22 (36.67)
	TT	3 (2.46)	5 (4.39)	0 (0.00)	2 (3.33)
	Alleles				
	C	189 (77.46)	173 (75.88)	95 (77.87)	94 (78.33)
	T	55 (22.54)	55 (24.12)	27 (22.13)	26 (21.67)
IL1B	Genotypes				
–31 T/C	TT	52 (24.53)	73 (39.25)	20 (19.61)	24 (33.80)
	CT	107 (50.00)	82 (44.08)	59 (57.84)	30 (42.25)
	CC	54 (25.47)	31 (16.67)	23 (22.55)	17 (23.95)
	Alleles				
	T	211 (49.53)	228 (61.29)	99 (48.53)	78 (54.93)
	C	215 (50.47)	144 (38.71)	105 (51.47)	64 (45.07)
IL1B	Genotypes				
–511 C/T	CC	59 (27.96)	73 (39.67)	22 (22.00)	27 (38.03)
	CT	106 (50.24)	85 (46.20)	59 (59.00)	30 (42.25)
	TT	46 (21.80)	26 (14.13)	19 (19.00)	14 (19.72)
	Alleles				
	C	224 (53.08)	231 (62.77)	103 (51.50)	84 (59.15)
	T	198 (46.92)	137 (37.23)	97 (48.50)	58 (40.85)
IL1B	Genotypes				
+3954 C/T	CC	83 (66.94)	93 (66.43)	36 (63.16)	38 (67.86)
	CT	37 (29.84)	41 (29.28)	20 (35.09)	15 (26.78)
	TT	4 (3.22)	6 (4.29)	1 (1.75)	3 (5.36)
	Alleles				
	C	203 (81.85)	227 (81.07)	92 (80.70)	91 (81.25)
	T	45 (18.15)	53 (18.93)	22 (19.30)	21 (18.75)

POAG: primary open angle glaucoma.

Table 4. Evaluation of polymorphisms in *IL1A* and *IL1B* genes and association with POAG through logistic regression analysis.

Gene polymorphism	Genotypes	OR	CI (95%)	p
IL1A–889 C/T	CT vs. CC	1.241	0.751–2.051	0.3986
	TT vs. CC	1.366	0.542–3.439	0.5085
IL1A+4845 G/A	GT vs. GG	0.996	0.587–1.688	0.9868
	TT vs. GG	0.126	0.126–2.388	0.4237
IL1B–31 T/C	CC vs. TT	2.445	1.387–4.311	0.0020*
	CT vs. TT	1.815	1.148–2.868	0.0107*
IL1B–511 C/T	CC vs. TT	1.543	0.988–2.411	0.0568
	CT vs. TT	2.189	1.213–3.952	0.0093*
IL1B+3954 C/T	CT vs. CC	1.011	0.593–1.725	0.9675
	TT vs. CC	0.747	0.204–2.739	0.6599

*$p < 0.05$; OR = odds ratio; CI = confidence interval.

of 0.05%. Similar to the –31C/T *IL1B* SNP, the alleles were compared and the significance was maintained (OR: 1.49; CI-95%: 1.121–1.983; $p = 0.006$). The comparison among the genotypes of the +3954C/T SNP did not reach, a statistically significant difference between POAG cases and controls. Among POAG cases, 174 patients were analyzed through the comparison of genotypes regarding the requirement of anti-glaucomatous surgery. Patients with the CT genotype of the –511 C/T *IL1B* SNP had 2.414 times more chance of undergoing surgery than TT patients.

Table 5. Evaluation of polymorphisms in *IL1A* and *IL1B* genes after stratification of the patients according to the requirement of anti-glaucomatous surgery through logistic regression analysis.

Gene polymorphism	Genotypes	OR	CI (95%)	p
IL1A–889 C/T	CT + TT vs. CC	0.869	0.424–1.784	0.7029
IL1A+4845 G/A	GT + TT vs. GG	1.191	0.578–2.453	0.6354
IL1B–31 T/C	CC vs. TT	1.666	0.683–4.059	0.2616
	CT vs. TT	2.414	1.182–4.930	0.0156*
IL1B–511 C/T	CC vs. TT	1.624	0.685–3.849	0.2712
	CT vs. TT	2.360	1.128–4.939	0.0227*
IL1B+3954 C/T	CT + TT vs. CC	1.231	0.566–2.679	0.5996

*$p < 0.05$; OR = odds ratio; CI = confidence interval.

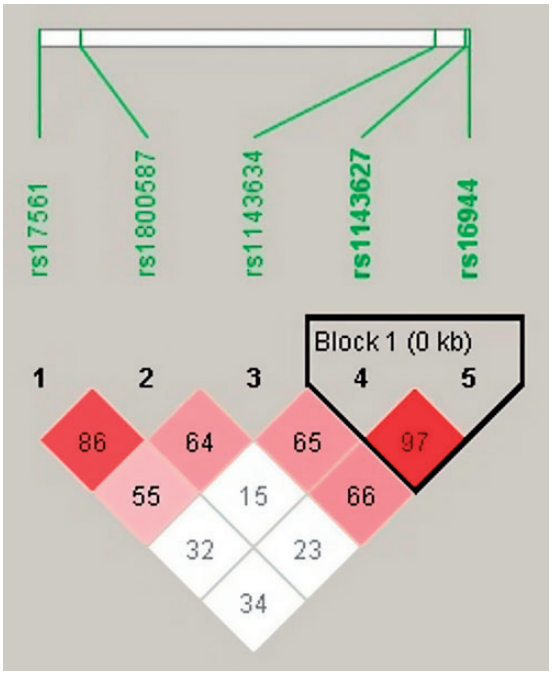

Figure 1. Haplotype structure of SNPs in the *IL1A* and *IL1B* genes. The linkage disequilibrium (LD) was estimated by calculating D′ and r^2, based in D′ between markers of 0.80 and $r^2 > 0.80$. The image was generated by Haploview 4.2. (A color version of this figure is available in the online journal.)

Similarly, for the –31C/T *IL1B* SNP, CT patients presented 2.360 times more chance (OR) of undergoing surgery than TT subjects ($p = 0.016$ and $p = 0.023$, respectively, Table 5).

In the *IL1B* haplotype analyses, high LD between the –511C/T and –31C/T SNPs was observed ($D' = 0.97$). Figure 1 was generated by the Haploview 4.2 program.[28] The LD was estimated by calculating D′ and r^2 based on D′ between markers of 0.80 and $r^2 > 0.80$. The T/C haplotype (–31/–511) was more frequent in controls, and the C/T haplotype (–31/–511) was more common in POAG cases. This association between haplotypes and glaucoma was statistically significant (p values = 0.011 and 0.018, respectively) (Table 6). There was also LD between the −511C/T and +3954C/T SNPs, as well as between the −31C/T and +3954C/T SNPs ($D' = 0.66$; $D' = 0.65$, respectively), but no haplotype block was observed.

Discussion

Aging is a complex phenomenon associated with a gradual decline in cellular and physiological function, diminished capacity to respond to stress and increased probability of developing degenerative diseases.[29] The age-related changes accelerate under the influence of oxidative stress and the production of reactive oxygen species (ROS), creating a pro-inflammatory microenvironment.[30] Indeed, it is known that chronic inflammation plays an important role in the initiation and/or progression of several age-related diseases, including atherosclerosis, Alzheimer's disease, osteoarthritis, and cancer.[23] The TM from glaucoma donors shows an increased expression of a well-established marker of cellular senescence, SA-beta-galactosidase (SA-β-gal),[31] as well as up-regulation of several proteins associated with inflammatory response, in agreement with the so-called "inflammaging" process.[26,31–33]

A complex phenotype termed senescence-associated secretory phenotype (SASP), characterized by the production of inflammatory cytokines by senescent cells, such as IL-6, IL-8, IL-1β and the surface-bound IL-1α, has been described.[34,35] The knowledge about the intracellular function of IL-1α is not clear, but there is a nuclear signal sequence that allows the binding to a DNA sequence, which enables the regulation of cell growth and proliferation, induction of senescence, and secretion of inflammatory cytokines.[36] The *IL1A* SNPs were positively correlated with several other chronic and neurodegenerative diseases such as Alzheimer's.[37,38] Previous studies with *IL1A* SNPs at the promoter region showed that the –889T allele may be a risk factor for glaucoma development[17] and that the T allele was associated with an increased production of IL-1α.[39] Other studies, including ours, did not confirm this finding and all the SNPs of IL1 cluster results are controversial.[40–44] We detected LD between −889C/T and +4845G/T SNPs at *IL1A*, as previously described, but no haplotype block was observed.[45] Moreover, we found no difference between POAG cases and controls, both in haplotype analysis and single-marker SNP analysis. This SNP has been positively correlated with several other chronic and neurodegenerative diseases such as Alzheimer's.[37,38]

Regarding *IL1B* SNPs, the –31C/T and –511C/T have been associated with clinical conditions such as gastric and lung cancer,[21] periodontitis,[46] and cardiovascular

Table 6. Frequency of –31 T/C and –511C/T haplotypes between cases and controls.

–31 T/C	–511C/T	Frequency (cases)	Frequency (controls)	Chi-square	p
T	C	0.493	0.600	6.47	0.011*
C	T	0.468	0.370	5.55	0.019*
C	C	0.035	0.023	0.80	0.371
T	T	0.004	0.008	0.39	0.534

diseases.[47] Previous studies showed controversial results for the association between –511C/T *IL1B* polymorphism and glaucoma.[48] Wang et al.[49] compared 245 healthy Chinese individuals to 231 normal-tension glaucoma (NTG) patients and found no association between –511C/T and +3954C/T SNPs and the disease. Similarly, in a different Chinese population of 58 POAG patients and 105 healthy controls, Lin et al. showed no association between –511C/T and glaucoma.[50] Another study by How et al.[41] confirmed these results and reported no association between –511 C/T, +3954C/T, and POAG. A recent study testing a cluster of SNPs in the IL-1 region showed no association with glaucoma, but the population included NTG patients.[51]

On the other hand, Markiwiecz et al. found an association between the –511 C/T variant and the risk of POAG, in accordance with our study. They studied 255 POAG patients and 256 controls in a Caucasian population.[48] The lack of association observed with the –511 C/T polymorphism in previous studies suggests that the number of subjects included in these studies may not be enough to establish a statistically significant association, or that the ethnicity may have an influence on this association. As previously described by Rogus et al.,[52] the CC homozygosity of –511 C/T is associated with increased IL-1β activity in tissue fluid and IL-1β production by peripheral blood mononuclear cells, while the TT homozygosity is not. The heterozygotes depend on other SNPs in a haplotype context to determine high levels of IL-1β. The genotype–phenotype correlation in this case is not well established.

The –31T/C SNP showed a statistically significant difference between glaucomatous phenotype and healthy individuals in this cohort. The variation of genetic characteristics regarding the promoter activity of *IL1B* may be complex. There is a typical TATA box as well as multiple transcription factors binding sites, including the CCAAT/enhancer-binding protein β, cAPM responsive element, nuclear factor kappa B, Spi/PU.1, and binding sites for NF-IL6 and AP1 proteins distributed at the regulatory region of the *IL1B* gene.[24,53] Also, within the region encompassed by nucleotides –570 to –552, a consensus sequence for a negative glucocorticoid response element and a transcription activator protein-2 binding site were documented.[54] None of the above elements seem to be compromised by the –511C/T SNP. However, the "C" allele of the –31C/T SNP at the –31 promoter position disrupts a "TATA" box, affecting the transcription activity of the *IL1B* promoter and leading to allele-specific binding of transcription factors.[22] This variation seems to affect IL-1β production. In fact, an increased production of IL-1β in the presence of the T allele of the –31C/T SNP has been previously demonstrated.[21,55,56] This is in accordance with the results observed for the –511C/T SNP, once there is a high degree of LD in a Caucasian population from the USA (ARIC—Atherosclerosis Risk in Communities study) between –31C/T and –511C/T.[52,57] However, isolated SNP analysis does not seem to better represent the genotype–phenotype correlation, and the biological activity of IL-1 seems to be based on haplotype structure rather than on isolated SNP variation.[22]

There is now increasing evidence that genes codifying inflammatory cytokines are polymorphic and various alleles may differ in their capability to produce the cytokine. There are 20 annotations of *IL1B*-validated genetic variations (SNPs), and several in the promoter region, making the comparison of haplotypes with different SNPs difficult.[22] Markiewicz et al.[48] analyzed only the –511C/T SNP in common with our study, so it was not possible to compare our haplotype results. How et al.[41] analyzed three SNPs (–899 *IL1A*, –511 *IL1B*, and +3954 *IL1B*) and the haplotype analyses were performed without the –31 T/C SNP. Lin et al.[50] included only two *IL1B* SNPs: –511 and an exon 5 SNP (with no identification), and no haplotype analysis was performed. The combination of these variations and how IL1 signal transduction is related with the pathogenesis of glaucoma remains to be investigated.

The +3954C/T is another SNP that has been reported to influence the production of IL-1β protein.[58] The *IL1B* +3954C/T SNP is a synonymous variant that does not change amino acid coding. It may, however, lead to inactivation of the original splicing site. The alternative splicing results in a premature stop codon or exon skipping, which can result in a truncated protein that is likely to be rapidly degraded or functionally inactive.[59] The global analysis in this study of +3954C/T showed no association between cases and controls, but considering that the −31TT/−511CC is a "protection haplotype," 51.3% of total subjects with the "protection haplotype" have the T allele of +3954C/T, compared to 13% of subjects with the "risk haplotype −31CC/−511TT," showing a tendency of protection for the T allele, although it does not reach statistical significance. Fini et al. showed a protection effect of the T allele of +3954C/T SNP in Caucasian POAG patients, but no association with –511C/T SNP.[16] One possible explanation is that protection can indirectly represent the −31T/C association, as there is some degree of LD between +3954C/T and –31C/T SNPs, findings that were confirmed by other authors,[47] although no haplotype block has been observed in this study. Further investigation is necessary to

understand how the SNPs are structurally correlated and how they could influence IL-1 expression.

Based on previous studies, this "protection haplotype" is correlated with increased IL-1β expression, and the augmented IL-1 production by TM cells may have beneficial effects on glaucoma. IL-1 is capable of increasing outflow facility, and, consequently, may lead to the reduction of IOP.[60,61] The production of local MMPs downstream of this IL-1-induced pathway may diminish the resistance to drainage, reducing IOP, and protecting against glaucoma.[62] In fact, when human anterior segment organ culture is perfused with MMP-3, an increased aqueous humor outflow facility is observed, whereas IL-1α blockage reduces outflow rates.[63] Consequently, extracellular matrix turnover, initiated by one or more MMPs, appears to be essential to the maintenance of IOP homeostasis. Birke et al. showed that IOP reduction by IL-1 is counteracted by TGF-β2, which is elevated in the aqueous humor of glaucoma patients.[61,64] TGF-β2 increases the synthesis and secretion of matrix proteins, fibronectin, elastin, and proteoglycans, while it decreases the synthesis of proteolytic enzymes degrading these proteins, causing reduced outflow facility.[65]

Another potential positive effect of IL-1 is that it could prevent cellular death by inhibition of the apoptotic response through the NF-kB pathway, induced by oxidative stress.[26] The stress-induced response, described by Wang et al., indicates that IL-1 has a central role in the production of endothelial leucocyte adhesion molecule-1 (ELAM-1 or E-selectin). It is present at the outflow pathway of the TM of diverse types of glaucoma, identified as a first molecular marker of human glaucoma.[26,66] Cumulative oxidative stress is capable of eliciting a sustained stress response, inducing the production of intracellular ROS in mitochondria: induction of IL-1α, IL-1β and ELAM-1 and activation of NF-κB pathway.[32] A recent study showed that *MYOC* mutations can stimulate the NF-kB/IL-1 pathway, and that wild-type *MYOC* inhibits the activation of this pathway acquiring an anti-inflammatory role.[67] What actually happens to change the balance towards the disease is unclear, and further studies are necessary to explain the role of IL-1 in glaucoma.

The need of surgical procedures (when topical drugs were not enough to control IOP) may indirectly represent higher severity of glaucoma. Indeed, the patients who did not need anti-glaucomatous surgery to control IOP had higher frequency of the TT genotype of the –31 SNP ($p = 0.016$), indicating a beneficial effect of IL-1 and a protective role of this genetic variation.

Conclusion

In conclusion, this study is the first, according to our knowledge, to show the association between glaucoma and the functional –31C/T SNP. We provide evidence indicating that homozygotes CC at –31C/T and TT at –511 C/T of *IL1B* gene are at risk for glaucoma. We also demonstrated that these polymorphisms are in quite strong LD. How IL-1 signaling influences the triggering and pathogenesis of glaucoma remains to be investigated.

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