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Evaluation of *CNTNAP2* gene polymorphisms for exfoliation syndrome in Japanese

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Purpose: To investigate the contactin-associated protein-like 2 (*CNTNAP2*) gene for single-nucleotide polymorphisms (SNPs) in Japanese patients with the exfoliation syndrome (XFS).

Methods: One hundred and eight unrelated Japanese patients with the XFS, and 199 normal controls were studied. Genomic DNA was extracted from the leukocytes of the peripheral blood, and 8 SNPs, rs826802, rs1404699, rs7803992, rs700308, rs4725736, rs2107856, rs2141388, and rs6970064, were amplified by polymerase chain reaction (PCR), directly sequenced, and genotyped.

Results: The allele frequencies of rs1404699 (p=8.57XE-3, odds ratio (OR)=1.59, 95% confidential intervals (CI); 1,12–2.24) and rs7803992 (p=5.43XE-4, OR=1.86, 95% CI; 1.31–2.65) were statistically significantly different between XFS and controls. In addition, there were significant differences in these genotype frequencies (p=0.0197 and 1.75XE-3). The allele and the genotype frequencies of rs2107856 and rs2141388, which were statistically significant SNPs in an earlier study, were not significantly different.

Conclusions: The variants, rs1404699 and rs7803992, of *CNTNAP2* should be associated with XFS in the Japanese population.

The exfoliation syndrome (XFS; OMIM 177650) is a generalized disorder of the extracellular matrix and is characterized clinically by the pathological accumulation of abnormal fibrillar material in the anterior segment of the eye [1-3]. This predisposes the eye to glaucomatous optic neuropathy. The XFS has also been associated with lens zonule weakness, severe chronic secondary open-angle glaucoma, cataract formation, and also a spectrum of other serious spontaneous and surgical intraocular complications.

The prevalence of XFS varies markedly between populations being highest in Scandinavian countries, while the Anglo-Celtic Caucasians have a markedly lower prevalence [4-7]. The incidence increases with age and is highest in the age group between 70 and 80 years [5]. The prevalence of XFS in Japan was reported to be 1.1% in one study [8] and 4.8% in another study [9].

Thorleifsson et al. [10] found a strong association between single-nucleotide polymorphisms (SNPs) in the lysyl oxidase–like 1 (*LOXL1*) gene and XFS in the Swedish and Icelandic populations using a genome-wide association study (GWAS). This association was replicated in the United States of America [11-13] and also in other populations [14-23].

LOXL1 is a member of the lysyl oxidase family of proteins that catalyzes the oxidative deamination of lysine residues of tropoelastin [24]. The homeostasis of elastic fibers requires the lysyl oxidase-like 1 protein [25], and *LOXL1* plays an important role in elastogenesis. Thus, it is quite possible that defects in *LOXL1* can cause features of XFS that result from an aberrant production of elastin and accumulation of fibrillar materials in the anterior segment of the eye.

A GWAS was recently performed using a DNA-pooling approach, and a single genotype at the contactin-associated protein-like 2 (*CNTNAP2*) locus had significant associations between XFS and exfoliation glaucoma and two SNPs (rs2107856 and rs2141388). These findings were confirmed in an independent German cohort but not in an Italian cohort [26]. *CNTNAP2* is a large gene spanning 2.3 mb of DNA on chromosome 7 and has 24 exons, and codes for the contactin-associated protein-like 2 (*CNTNAP2*, also called Caspr2). CNTNAP2 is member of the neurexin superfamily [27,28] and is possibly involved in stabilizing the location of the potassium channels in the juxtaparanodal region of the neuron [27]. It has been suggested to be a candidate gene for various neuropsychiatric disorders, e.g., the cortical dysplasia-focal epilepsy syndrome [29] and Pitt-Hopkins-like mental retardation [30]. However, its exact function and regulation are not known.

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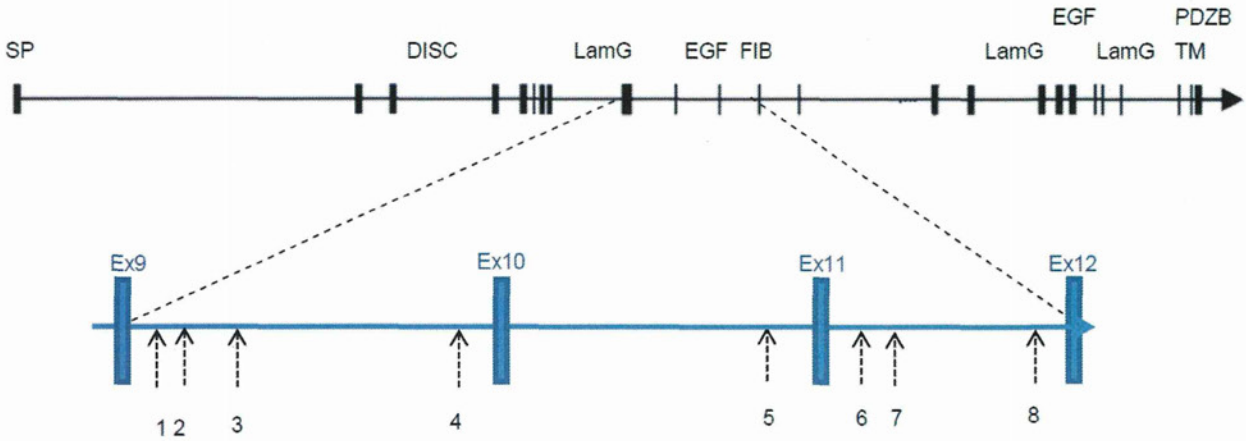

Figure 1. *CNTNAP2* gene structure. The 8 SNPs studied were; 1. rs826802, 2. rs144699, 3. rs7803992, 4. rs700308, 5. rs4725736, 6. rs2107856, 7. rs2141388, and 8. rs6970064. SP, signal peptide; DISC, discoidin-like domain; LamG, laminin-G domain; EGF, epidermal growth factor like domain; FIB, fibrinogen-like domain;TM, transmembrane region; PDZBD, PDZ-domain binding site.

The purpose of this study was to investigate 8 SNPs variations in *CNTNAP2* in Japanese patients with the XFS.

METHODS

One hundred and eight unrelated Japanese patients with XFS (mean age 73.61±6.75 years; 57 men, 51 women) and 199 controls (mean age 69.7±11.3 years; 101 men, 98 women) were studied. The controls were matched by age and gender. The XFS group included 85 exfoliation glaucoma (XFG) patients. They were examined at the ophthalmic clinic of the Tohoku University Hospital, Sendai, Japan, and the Ehime University Hospital, Ehime, Japan. The purpose and procedures were explained to all patients, and an informed consent was obtained. This study was approved by the Institutional Review Boards of the Tohoku University and Ehime University, and the procedures used conformed to the tenets of the Declaration of Helsinki.

Routine ophthalmic examinations were performed on all patients. The criteria used to classify a patient as having XFS was an open anterior chamber angle with accumulation of abnormal fibrillar material in the anterior segment of the eye. In addition, three other criteria for XFG had to be met: 1) applanation intraocular pressure (IOP) >22 mmHg in each eye; 2) glaucomatous cupping in each eye including a cup-to-disc ratio >0.7; and 3) visual field defects determined by Goldmann perimetry and/or Humphrey field analyzer consistent with the glaucomatous cupping in at least one eye. The control subjects had the following characteristics: 1) IOP less than 22 mmHg; 2) normal optic discs; and 3) no family history of glaucoma.

Genomic DNA was extracted from the leukocytes of peripheral blood and purified with the Qiagen QIAamp Blood Kit (Qiagen, Valencia, CA). Genomic DNA was extracted from the leukocytes of the peripheral blood, and the 6 SNPs, rs1404699, rs700308, rs4725736, rs2107856, rs2141388, and rs6970064, were chosen from the earlier studies. Two newly identified SNPs, rs826802 and rs7803992, were designed around intron 9 of the gene. The *CNTNAP2* gene structure with the location of the 8 SNPs is shown in Figure 1. They were amplified by polymerase chain reaction (PCR), directly sequenced, and genotyped. The amplifications were performed at 60 °C annealing temperature. The PCR fragments were purified with ExoSAP-IT (USB, Cleveland, OH), sequenced by the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Perkin-Elmer, Foster City, CA) by an automated DNA sequencer (ABI PRISM™ 3100 Genetic Analyzer, Perkin-Elmer). The allele frequencies, genotypes, and haplotypes of the *CNTNAP2* SNPs were determined.

Statistical analysis: The significance of associations between the phenotype and SNPs were determined by contingency table analysis using chi-square or Fisher's exact test. The odds ratios, approximating to relative risks, were calculated as a measure of the association between the *CNTNAP2* allele frequency and the phenotype. For each odds ratio, the 95% confidence intervals were calculated. The inferred haplotypes, quantified between all pairs of biallelic loci, were estimated using the SNPAlyze program version 7.0 (Dynacom, Yokohama, Japan). Additionally, a permutation test was performed to test the deviations of allelic frequencies of the SNPs and haplotypes. The Hardy–Weinberg equilibrium was analyzed using gene frequencies obtained by simple gene counting and the chi-square test with Yates' correction for comparing observed and expected values.

RESULTS

The allele frequencies and genotypes of the 8 *CNTNAP2* SNPs, rs826802, rs1404699, rs7803992, rs700308, rs4725736, rs2107856, rs2141388, and rs6970064, were determined in the XFS patients.

TABLE 1. *CNTNAP2* ALLELE FREQUENCIES IN PATIENTS WITH EXFOLIATION SYNDROME AND IN CONTROLS IN JAPANESE.

		MAF in this study			MAF in previous study*		
dbSNP	Allele	XFS (n=108) XFG (n=85)	Control (n=199)	p-value	XFS (n=770)	Control (n=444)	p-value
rs826802	T	0.435	0.372	0.0884	N/A	N/A	N/A
		0.429		0.0198			
rs1404699	T	0.412	0.307	8.57XE-3	0.445	0.397	0.0225
		0.388		0.0581			
rs7803992	G	0.394	0.259	5.43XE-4	N/A	N/A	N/A
		0.359		0.016			
rs700308	A	0.407	0.432	0.553	0.138	0.103	0.0117
		0.412		0.653			
rs4725736	A[1]	0.472	0.402	0.093	0.585	0.637	0.0121
		0.441		0.385			
rs2107856	G[2]	0.450	0.432	0.687	0.709	0.776	0.0003
		0.441		0.843			
rs2141388	C[3]	0.444	0.437	0.863	0.709	0.777	0.0002
		0.441		0.930			
rs6970064	A[4]	0.181	0.123	0.0524	0.418	0.463	0.0306
		0.182		0.0631			

*reported by Krumbiegel et al. [26]. MAF; minor allele frequency, XFS; Exfoliation Syndrome, XFG; Exfoliation Glaucoma. The significance of the association was determined by a contingency table analysis using the χ^2 test. Upper columns show XFS data, and lower columns show XFG data. 1. There was a difference between the Caucasian and Japanese. Minor allele in previous study was C. 2. Minor Allele in previous study was T. 3. Minor Allele in previous study was T. 4. Minor Allele in previous study was G.

Distribution of CNTNAP2 variants in XFS patients and control subjects: The allele frequencies of rs1404699 (p=8.57XE-3, odd ratio (OR)=1.59, 95% confidential intervals (CI); 1,12–2.24) and rs7803992 (p=5.43XE-4, OR=1.86, 95% CI; 1.31–2.65) were statistically significant between the XFS group and the control group (Table 1). There were also significant differences in these genotype frequencies (p=0.0197 and 1.75XE-3; Table 2). Only the rs7803992 was significantly different between the XFG group and the control group (p=0.016; Table 1). Compared with the allele frequencies of rs2107856 and rs2141388 statistically significant SNPs in a previous study [26], our results showed no significantly difference between the XFS group and the control group (Table 1). Also, the genotype frequencies of those in *CNTNAP2* were not significantly higher in the two groups than in the control group (Table 2).

The genotype frequencies of rs700308 and rs6970064 were statistically significant (p=0.0402 and 0.0315), but the allele frequencies were not significantly different (p=0.553 and 0.0524) between the XFS group and the control group. All SNPs adhered to the Hardy–Weinberg expectations (p>0.05).

Haplotype analyses at CNTNAP2 LD block in the Japanese population: The inferred haplotypes between all pairs of biallelic loci on rs1404699 and rs7803992 were estimated (Table 3). The haplotype-based associations were tested with a 1,000 iterated permutation test. Four major haplotypes; C-A, T-G, T-A, C-G (each frequency >5%) were found in the XFS subjects and normal controls. T-G was over-represented in the XFS subjects with a highly significant difference in frequency compared to the control group (0.327 versus 0.202; p=0.003). In addition, the C-A haplotype was significantly less represented in the XFS subjects (0.522 versus 0.637; p=0.003).

Two locus analyses: A strong correlation between variants in *LOXL1* and XFS has been reported [10], *LOXL1* common risk haplotype is T-G (the major alleles T of the coding SNPs rs1048661 and major alleles G of the coding SNPs rs3825942) in Japan, instead of G-G in Europeans. We investigated how the variants in *LOXL1* gene were related to *CNTNAP2*. We sorted our subjects for carriers and non-carriers of the risk haplotype T-G (Table 4). The numbers in the subgroup of non-T-G carriers was quite small, and there was no association of *CNTNAP2* SNPs with the *LOXL1* non-risk haplotype (Table 4; p=0.53 and 0.69, respectively). Besides the subgroups risk of T-G carriers, there was no significant association (Table 4; p=0.072 and 0.084, respectively).

DISCUSSION

Association between CNTNAP2 and XFS: We compared the findings of Krumbiegel and colleagues [26] to that obtained

Table 2. Frequency of genotypes *CNTNAP2* gene in patients with exfoliation syndrome and in controls in Japanese.

dbSNP	Allele	XFG (n=108)	p value*	XFG (n=85)	p value*	Control (n=199)
rs826802	G/G	36 (33.3)	0.224	27 (31.8)	0.425	77 (38.7)
	G/T	50 (46.3)		43 (50.6)		96 (48.2)
	T/T	22 (20.4)		15 (17.6)		26 (13.1)
rs1404699	C/C	38 (35.2)	0.0197	32 (37.6)	0.121	93 (46.7)
	C/T	51 (47.2)		40 (47.1)		90 (45.2)
	T/T	19 (17.6)		13 (15.3)		16 (8.1)
rs7803992	A/A	38 (35.2)	1.75XE-3	31 (36.5)	6.22XE-3	112 (56.3)
	A/G	55 (50.9)		47 (55.3)		71 (35.7)
	G/G	15 (13.9)		7 (8.2)		16 (8.0)
rs700308	G/G	45 (41.7)	0.0402	33 (38.8)	0.282	63 (31.7)
	G/A	38 (35.2)		34 (40.0)		100 (50.3)
	A/A	25 (23.1)		18 (21.2)		36 (18.1)
rs4725736	C/C	34 (31.5)	0.0659	27 (31.8)	0.385	69 (34.7)
	C/A	46 (42.6)		41 (48.2)		100 (50.3)
	A/A	28 (25.9)		17 (20.0)		30 (15.1)
rs2107856	T/T	39 (36.1)	0.091	29 (34.1)	0.541	63 (31.7)
	T/G	41 (38.0)		37 (43.5)		100 (50.3)
	G/G	28 (25.9)		19 (22.4)		36 (18.1)
rs2141388	T/T	39 (36.1)	0.100	29 (34.1)	0.470	61 (30.7)
	T/C	42 (38.9)		37 (43.5)		106 (53.3)
	C/C	27 (25.0)		19 (22.4)		32 (16.1)
rs6970064	G/G	74 (68.5)	0.0315	58 (68.2)	0.0345	151 (75.9)
	G/A	29 (26.9)		23 (27.1)		47 (23.6)
	A/A	5 (4.6)		4 (4.7)		1 (0.5)

XFS; Exfoliation Syndrome, XFG; Exfoliation Glaucoma. Data presented are number of patients, unless otherwise indicated. The significance of the association was determined by a contingency table analysis using the χ^2 test.

Table 3. Haplotype analysis with rs1404699 and rs7803992 in patients with exfoliation syndrome and in controls in Japanese.

Haplotype	Overall	XFS	Control	p-value
C-A	0.5966	0.5217	0.637	0.003
T-G	0.2464	0.3273	0.2024	0.003
T-A	0.0972	0.0847	0.1041	0.489
C-G	0.0597	0.0662	0.0564	0.708

XFS; Exfoliation Syndrome. The significance of the association was determined by a contingency table analysis using the χ^2 test.

from our Japanese cohorts. We found that two SNPs in *CNTNAP2* were strongly associated with XFS. In an earlier study [26], the frequencies of rs2107856 and rs2141388 SNPs in *CNTNAP2* were confirmed in an independent German cohort but not in the Italian cohort. Although neither the rs2107856 or rs2141388 SNPs was significant in our study, rs1404699 and nearby rs7803992 were statistically significant between the XFS group and the control group. Thus, it is possible that *CNTNAP2* could be associated with XFS. Like other susceptible variants of a complex disease, the OR in the earlier study was modest at about 1.4. In our study, the highest OR was 1.86 for rs7803992. This difference can be explained by racial differences and heterogeneities. Because the number of XFG patients was small, it seemed that the statistical power was weak.

No association between CNTNAP2 and LOXL1 in Japanese: Because a strong association of variants in *LOXL1* in XFS has been reported [10], we compared the allele frequencies at *CNTNAP2* locus based on the presence of the identified Japanese *LOXL1* common risk haplotype T-G. We found no significant association to allele T of the rs1404699 and rs7803992 SNPs of *CNTNAP2* in carriers of *LOXL1* the risk T-G haplotype (Table 4), and also in non-risk haplotypes. These findings suggest that there is no association between

Table 4. Association of *LOXL1* common-risk haplotype T-G, composed of rs1048661 and rs3825942, with *CNTNAP2* SNPs rs1404699 and rs7803992.

LOXL1 haplotype	Cases	Control	*CNTNAP2* SNP	Cases MAF	Control MAF	p-value
T-G carriers	103	52	rs1404699	0.413	0.308	0.072
			rs7803992	0.398	0.298	0.084
Non T-G carriers	5	147	rs1404699	0.400	0.306	0.53
			rs7803992	0.300	0.245	0.69

LOXL1; lysyl oxidase–like 1, MAF; minor allele frequency.

CNTNAP2 and *LOXL1* in the Japanese. This would then mean that a *LOXL1*-independent mechanism is involved in *CNTNAP2* function.

In a molecular genetic study, the most promising loci at 18q12.1–21.33 and 2q, 17p, and 19q have been proposed to be the susceptible loci in a Finnish population in an autosomal dominant mode of inheritance [31]. In a microarray study, 23 genes with different expression patterns in the anterior segment tissues of eyes with XFS have recently been reported [32]. This strongly suggests that an unidentified gene or environmental factors independent of the *LOXL1* gene strongly influence the phenotypic expression of the XFS.

CNTNAP2 function and molecular genetics: CNTNAP2 is a single-pass transmembrane protein with multiple protein-interaction motifs typical of the neurexins, e.g., epidermal growth factor repeats, laminin globular domains, and F5/8-type C domain, and a putative PDZ-binding site. Poliak et al. [33] reported that CNTNAP2 is necessary to maintain the potassium channels at the juxtaparanodal region in myelinated axons. The SNPs we selected were located in introns 9, 10, and 11 (Figure 1), while several SNPs related to autism were located in intron 2 [34] and intron 13 [35]. The cortical dysplasia-focal epilepsy syndrome is caused by a single nucleotide deletion in Exon 22. Therefore, it seems that our SNPs have nearly no correlation with neuropsychiatric disorders. The rs1404699 and rs7803992 SNPs are located in intron 9 of the *CNTNAP2* gene. Exon 9, nearby to intron 9, codes for the laminin globular domain, which contains proteins that play a wide variety of roles in cell adhesion, signaling, migration, assembly, and differentiation of cells. We suggest that alterations in membrane stabilization may contribute to the abnormal exfoliation matrix processes, which are associated with cell-surface irregularities, basement membrane destruction and degenerative alterations.

Conclusions: Identification of XFS-associated SNPs that will allow early detection of an increase in the IOP, or even before an elevation of IOP, would be desirable. Our findings showed that variants of *CNTNAP2* rs1404699 and rs7803992 are significantly associated with XFS in the Japanese population. More studies of the functions and genotype-phenotype correlation of *CNTNAP2* are required to determine the pathophysiology of XFS. In addition, further studies searching for secondary genetic and environmental factors that contribute to XFS is required to gain better understanding of the complex etiology of XFS.

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Association of Toll-like Receptor 4 Gene Polymorphisms in Japanese Subjects With Primary Open-Angle, Normal-Tension, and Exfoliation Glaucoma

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• PURPOSE: To determine whether polymorphisms in the Toll-like receptor 4 (*TLR4*) gene are associated with primary open-angle glaucoma (POAG), normal-tension glaucoma (NTG), and exfoliation glaucoma (XFG) in Japanese individuals.

• DESIGN: Genetic association study.

• METHODS: SETTING: Multicenter study. STUDY POPULATION: One hundred eighty-four unrelated Japanese patients with POAG, 365 unrelated patients with NTG, and 109 unrelated patients with XFG from 5 hospitals. PROCEDURES: Genomic DNA was extracted from leukocytes of the peripheral blood, and 8 polymorphisms in the *TLR4* genes were amplified by polymerase chain reaction (PCR) and directly sequenced. Allele and genotype frequencies and the inferred haplotypes were estimated. MAIN OUTCOME MEASURES: Differences in allele and genotype frequencies and haplotypes between subjects with POAG, NTG, and XFG.

• RESULTS: The allele frequency of rs2149356 of the *TLR4* gene in the POAG, NTG, and XFG groups was the most significantly different from that of the control group (minor allele frequency 0.446, 0.395, 0.404, vs 0.308; $P = .000058$, $P = .0030$, and $P = .015$). The allele frequencies of the 5 *TLR4* SNPs were higher in all of the glaucoma groups than that in the control group. The statistics of genotypes of *TLR4* were approximately the same for all allele frequencies. The haplotypic frequencies with Tag SNPs studied earlier showed that only POAG was statistically significant. Other haplotypes, such as rs10759930, rs1927914, rs1927911, and rs2149356, had higher statistical significance (overall $P = .00078$ in POAG, overall $P = .018$ in NTG, and overall $P = .014$ in XFG).

• CONCLUSIONS: This study demonstrated that TLR4 polymorphisms are associated with NTG in the Japanese, and they also play a role in the pathogenesis of POAG and XFG. (Am J Ophthalmol 2012;154:825–832. © 2012 by Elsevier Inc. All rights reserved.)

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GLAUCOMA IS A COMPLEX, HETEROGENEOUS DISease characterized by a progressive degeneration of the axons of the retinal ganglion cells (RGCs). It is the second-highest cause of blindness worldwide, affecting approximately 70 million people.[1] Primary open-angle glaucoma (POAG), the most common type of glaucoma, is associated with an elevated intraocular pressure (IOP). However, there are some POAG patients who have normal IOPs of <22 mm Hg, and they are classified as having normal-tension glaucoma (NTG).[2] The prevalence of NTG is higher among the Japanese than among whites.[3,4] POAG and NTG appear to be a continuum of glaucoma with overlapping causative factors in addition to the IOPs. It is believed that the mechanism shifts from predominantly elevated IOP in POAG to that of independent factors in eyes with NTG. Although the precise molecular basis of POAG and NTG has not been determined, the glaucoma in patients with POAG and NTG is probably heterogeneous and is caused by the interaction of multiple genes and environmental factors.

Several genetic loci that contribute to the susceptibility of eyes to POAG/NTG have been identified, and at least 15 loci, from GLC1A to GLC1O, have been linked to POAG.[5] Three genes have been identified worldwide: the myocilin (*MYOC*) gene,[6] the optineurin (*OPTN*) gene,[7] and the WD repeat domain 36 (*WDR36*) gene,[8] with a

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TABLE 1. Toll-like Receptor 4 Single-Nucleotide Polymorphisms Allele Frequencies in Patients With Primary Open-Angle, Normal-Tension, and Exfoliation Glaucoma and in Controls in Japanese

	This Study							Previous Study		
SNP	POAG (n = 184)	*P* Value	NTG (n = 365)	*P* Value	XFG (n = 109)	*P* Value	Control (n = 216)	NTG (n = 250)	Control (n = 318)	*P* Value
rs10759930	0.454	.00022	0.396	.018	0.404	.052	0.326	0.422	0.347	.010
rs1927914	0.457	.00012	0.400	.0096	0.417	.019	0.324	0.420	0.347	.012
rs1927911	0.448	.000076	0.392	.0066	0.404	.021	0.313	0.408	0.344	.028
rs12377632	0.467	.000014	0.364	.10	0.399	.038	0.317	0.412	0.343	.017
rs2149356	0.446	.000058	0.395	.0030	0.404	.015	0.308	0.408	0.343	.024
rs11536889	0.258	.471	0.264	.28	0.248	.74	0.236	0.232	0.256	.35
rs7037117	0.223	.045	0.221	.027	0.225	.072	0.167	0.252	0.182	.0044
rs7045953	0.090	.112	0.074	.37	0.078	.39	0.060	0.098	0.078	.21

NTG = normal-tension glaucoma; POAG = primary open-angle glaucoma; SNP = single nucleotide polymorphisms; XFG = exfoliation glaucoma.

The significance of the association was determined the χ^2 test.

diverse mutation spectrum. Other studies have reported that the *OPTN* and *WDR36* variants do not predispose individuals to POAG and NTG.[9,10] The pseudoexfoliation syndrome (XFS; OMIM:177650) is a generalized disorder of the extracellular matrix and is characterized by the pathologic accumulation of abnormal fibrillar material in the anterior segment of the eye.[11] A recent genome-wide association study (GWAS) showed a strong association between single nucleotide polymorphisms (SNPs) in the *lysyl oxidase–like 1* (*LOXL1*) gene and XFS in the Swedish and Icelandic populations.[12] The association between the *LOXL1* gene and XFS and exfoliation glaucoma (XFG) has also been found in the Japanese population.[13,14] XFG is a common identifiable cause of open-angle glaucoma worldwide, affecting an estimated 60 to 70 million people.[15] Inflammation and oxidative stress may be a modifiable risk factor in the management of patients with XFS and XFG.

An IOP elevation is considered a major risk factor for glaucoma, but an elevated IOP is not associated with glaucomatous characteristics in all glaucoma patients. Other possible pathogenetic factors, such as autoimmune mechanisms including apoptosis, may be involved in some patients with glaucoma.[16] Wax and associates were the first to report an elevation of antibody titers in patients with NTG (eg, an increase in the level of heat shock protein 60 [HSP60] antibodies)[17] and also higher levels of antibodies against small HSPs (eg, [alpha] A-crystalline, [alpha] B-crystalline, and HSP27) in NTG patients.[18] A number of other autoantibodies against retinal or optic nerve proteins have been identified in many NTG patients. Because some glaucoma patients have increased titers of serum antibodies against these proteins, the degeneration of the RGCs in glaucoma may be attributable to a failure of immune regulation of both pro-apoptotic and protective pathways.

The Toll-like receptor (*TLR*) family, an anchor of innate immunity system, recognizes external ligands and differentiates self from nonself proteins. The ability of a tissue to recognize pathogens is mediated by a set of receptors that are referred to as pattern-recognition receptors (PRRs). To date, 13 members of the *TLR* family have been identified in mammals. *TLR4* is a transmembrane receptor that mediates immune responses to exogenous and endogenous ligands, and not only recognizes bacterial lipopolysaccharides (LPSs) but is also activated by endogenous ligands such as heat shock proteins (HSPs).[19] Toll-like receptors (TLRs) can also recognize endogenous ligands that are induced during inflammatory responses.[20] Recently, the *TLR4* (OMIM 603030) gene was implicated in NTG in the Japanese population,[21] but not in the South Korean population.[22]

Glaucoma is a neurodegenerative disease, but the mechanisms causing the RGC loss are still undetermined. Several studies have pointed to a possible involvement of autoimmune mechanisms in the pathogenesis of glaucoma, especially NTG. On the other hand, it is believed that the mechanisms shift from predominantly elevated IOP in the POAG and XFG to other factors such as autoimmune reactions in NTG.

Thus, the purpose of this study was to determine whether mutations in the *TLR4* gene contributed to POAG, NTG, and XFG in unrelated Japanese patients.

PATIENTS AND METHODS

• **PATIENTS:** One hundred eighty-four unrelated Japanese patients with POAG (119 men and 65 women; mean age 64.6 ± 14.3 years), 365 unrelated Japanese patients with NTG (171 men and 194 women; mean age 58.6 ± 13.1 years), and 109 unrelated Japanese patients with XFG (57 men and 52 women; mean age 77.6 ± 6.2 years) were studied. They were diagnosed with glaucoma in the ophthalmological clinic of the Tohoku University Hospital, Sendai; Niigata University Hospital, Niigata; Tokyo Met-

TABLE 2. Frequency of Genotypes of Toll-like Receptor 4 Gene in Patients with Primary Open-Angle, Normal-Tension, and Exfoliation Glaucoma and in Controls in Japanese[a]

	This Study				Previous Study	
	POAG (n = 184)	NTG (n = 365)	XFG (n = 109)	Control (n = 216)	NTG (n = 250)	Control (n = 318)
rs10759930 T/C						
T/T	49 (26.6%)	141 (38.6%)	40 (36.7%)	103 (47.7%)	81 (32.4%)	137 (43.1)
T/C	103 (56.0%)	159 (43.6%)	50 (45.9%)	85 (39.4%)	127 (50.8%)	141 (44.3%)
C/C	32 (17.4%)	65 (17.8%)	19 (17.4%)	28 (12.9%)	42 (16.8%)	40 (12.6%)
P value[b]	.000085	.074	.16		.028	
P value[c] (dominant)	.000015	.032	.060			
rs1927914 A/G						
A/A	47 (25.5%)	137 (37.5%)	38 (34.9%)	105 (48.6%)	82 (32.8%)	137 (43.1%)
A/G	106 (57.6%)	164 (44.9%)	51 (46.8%)	82 (38.0%)	126 (50.4%)	141 (44.3%)
G/G	31 (16.9%)	64 (17.5%)	20 (18.3%)	29 (13.4%)	42 (16.8%)	40 (12.6%)
P value[b]	.000011	.030	.059		.036	
P value[c] (dominant)	.0000022	.0089	.018			
rs1927911 G/A						
G/G	51 (27.7%)	139 (38.1%)	40 (36.7%)	106 (49.1%)	87 (34.8%)	141 (44.3%)
G/A	101 (54.9%)	166 (45.5%)	50 (45.9%)	85 (39.4%)	122 (48.8%)	135 (42.5%)
A/A	32 (17.4%)	60 (16.4%)	19 (17.4%)	25 (11.5%)	41 (16.4%)	42 (13.2%)
P value[b]	.000072	.027	.080		.067	
P value[c] (dominant)	.000013	.0095	.034			
rs12377632 C/T						
C/C	53 (28.8%)	137 (37.5%)	41 (37.6%)	104 (48.1%)	86 (34.4%)	140 (44.0%)
C/T	90 (48.9%)	190 (52.1%)	49 (45.0%)	87 (40.3%)	122 (48.8%)	138 (43.4%)
T/T	41 (22.3%)	38 (10.4%)	19 (17.4%)	25 (11.6%)	42 (16.8%)	40 (12.6%)
P value[b]	.00012	.020	.13		.053	
P value[c] (dominant)	.000079	.012	.071			
rs2149356 G/T						
G/G	53 (28.8%)	139 (38.1%)	40 (36.7%)	107 (49.5%)	87 (34.8%)	140 (44.0%)
G/T	98 (53.3%)	164 (44.9%)	50 (45.9%)	85 (39.4%)	122 (48.8%)	138 (43.4%)
T/T	33 (17.9%)	62 (17.0%)	19 (17.4%)	24 (11.1%)	41 (16.4%)	40 (12.6%)
P value[b]	.00012	.015	.062		.070	
P value[c] (dominant)	.000025	.0069	.028			
rs11536889 G/C						
G/G	95 (51.6%)	196 (53.7%)	62 (56.9%)	127 (58.8%)	146 (58.4%)	177 (55.6%)
G/C	83 (45.1%)	145 (39.7%)	40 (36.7%)	76 (35.2%)	93 (37.2%)	119 (37.4%)
C/C	6 (3.3%)	24 (6.6%)	7 (6.4%)	13 (6.0%)	11 (4.4%)	22 (6.9%)
P value[b]	.083	.49	.95		.42	
P value[c] (dominant)	.15	.23	.74			
rs7037117 A/G						
A/A	111 (60.3%)	222 (60.8%)	65 (59.6%)	153 (70.8%)	138 (55.2%)	213 (67.0%)
A/G	64 (34.8%)	125 (34.2%)	39 (35.8%)	54 (25.0%)	98 (39.2%)	94 (29.6%)
G/G	9 (4.9%)	18 (4.9%)	5 (4.6%)	9 (4.2%)	14 (5.6%)	11 (3.5%)
P value[b]	.082	.049	.12		.015	
P value[c] (dominant)	.027	.015	.043			
rs7045953 A/G						
A/A	152 (82.6%)	313 (85.8%)	93 (85.3%)	191 (88.4%)	203 (81.2%)	269 (84.6%)
A/G	31 (16.8%)	50 (13.7%)	15 (13.8%)	24 (11.1%)	45 (18.0%)	49 (15.4%)
G/G	1 (0.6%)	2 (0.5%)	1 (0.9%)	1 (0.5%)	2 (0.8%)	0 (0.0%)
P value[b]	.25	.66	.69		.19	
P value[c] (dominant)	.097	.36	.43			

NTG = normal-tension glaucoma; POAG = primary open-angle glaucoma; XFG = exfoliation glaucoma.

[a]Data presented are number of patients, unless otherwise indicated.

[b]Significance of the association determined by a contingency table analysis using the χ^2 test.

[c]Significance by a dominant model.

ropolitan Police Hospital, Tokyo; Ideta Eye Hospital, Kumamoto; and Ehime University Hospital, Ehime, Japan. All of the subjects were enrolled from 2004 through 2010.

Routine ophthalmic examinations were performed on all patients. The criteria for classifying a patient as having POAG were: applanation IOP >22 mm Hg in each eye; glaucomatous cupping including cup-to-disc ratio >0.7 in each eye; visual field defects determined by Goldmann perimetry and/or Humphrey visual field analysis consistent with the glaucomatous cupping in at least 1 eye; and an open anterior chamber angle. Patients with glaucoma of secondary causes (eg, trauma-, uveitis-, or steroid-induced) were excluded. The criteria for NTG were applanation IOP <22 mm Hg in both eyes at each examination and the same characteristics as that of the POAG group. The IOP used for the statistical analyses was the clinic-based value. We checked the IOP in at least 3 visits and the measurements were made during the daylight hours. Patients were excluded if the IOP was 22 mm Hg or more for any of the measurements. The criteria for XFG were an open anterior chamber angle with accumulation of abnormal fibrillar material in the anterior segment of the eye and the same characteristics as the POAG group.

The control subjects (116 men and 100 women; age, 69.7 ± 11.3 years) had the following characteristics: IOP <22 mm Hg, normal optic discs, and no family history of glaucoma. To decrease the chance of studying individuals with presymptomatic glaucoma, we studied individuals who were older than 60 years in this group.

• **SAMPLE PREPARATION AND MUTATION SCREENING:** Genomic DNA was extracted from leukocytes of peripheral blood and purified with the Qiagen QIAamp DNA Blood Kit (Qiagen, Valencia, California, USA). Eight SNPs were amplified by polymerase chain reaction (PCR) using 0.5 μM intronic primers, 0.2 mM dNTPs, and 0.5 U Ex Taq polymerase (Takara, Shiga, Japan) with 30 ng template DNA in the amplification mixture (25 μL). The annealing temperature and sequence of primer set are given in the Supplemental Table (available at AJO.com).

Oligonucleotides for the amplification and sequencing were selected using Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi/, provided in the public domain by the Massachusetts Institute of Technology, Cambridge, Massachusetts, USA). The PCR fragments were purified with ExoSAP-IT (USB, Cleveland, Ohio, USA), sequenced by the BigDye Terminator v3.1 Cycle Sequencing Kit (Perkin-Elmer, Foster City, California, USA) on an automated DNA sequencer (ABI PRISM 3100 Genetic Analyzer, Perkin-Elmer).

• **STATISTICAL ANALYSES:** Differences in the genotype frequencies among the cases and controls were tested by Fisher exact test or χ^2 depending on the cell counts. The inferred haplotypes and LD (linkage dysequilibrium), expressed as D',[23] quantified between all pairs of biallelic loci, were estimated using the SNPAlyze program version 5.0.3 (Dynacom, Yokohama, Japan). The significance of an association was determined by contingency table analysis using χ^2 or Fisher exact tests. The Hardy-Weinberg equilibrium was analyzed using gene frequencies obtained by simple gene counting and the χ^2 test with Yates' correction for comparing observed and expected values.

RESULTS

• **HAPLOTYPE BLOCK:** All of the 8 SNPs in the *TLR4* gene were genotyped, and all were in Hardy-Weinberg equilibrium in the glaucoma cases and control subjects. All SNPs were located in 1 haplotype block, and the magnitude of the LD between each SNP was very high, with a pairwise D' of more than 0.90. However, rs11536889 had a pairwise D' less than 0.80.

• **ALLELE AND GENOTYPE FREQUENCIES IN *TLR4* VARIANTS DETECTED IN SUBJECTS:** The allele frequencies of the 8 SNPs in the glaucoma cases and control subjects are shown in Table 1. The frequencies of the minor alleles of all SNPs were higher in the glaucoma cases than in control subjects. In the POAG subjects, the allele frequencies of 6 SNPs (rs10759930, rs1927914, rs1927911, rs12377632, rs2149356, and rs7037117) were significantly different from the control group ($P < .05$). In addition, 5 SNPs (rs10759930, rs1927914, rs1927911, rs2149356, and rs7037117) in NTG subjects and 4 SNPs (rs1927914, rs1927911, rs12377632, and rs2149356) in XFG subjects were significantly different from that in the control group ($P < .05$; Table 1). Three SNPs, rs1927914, rs1927911, and rs2149356, were identical for the POAG, NTG, and XFG groups. Among these 3 SNPs, the minor allele of rs2149356, located in intron 2 of *TLR4*, conferred the highest increased risk of POAG ($P = .000058$, OR = 1.77, 95% CI = 1.31–2.39), NTG ($P = .0030$, OR = 1.51, 95% CI = 1.17–1.95), and XFG ($P = .015$, OR = 1.56, 95% CI = 1.11–2.20).

The genotype frequencies of 8 SNPs are shown in Table 2. The genotype frequency of 5 SNPs was significantly higher in the POAG and NTG subjects than in the controls, and none of the SNPs was significantly higher in the XFG subjects than in the control group ($P = .16$, $P = .059$, $P = .080$, $P = .13$, $P = .062$, $P = .95$, $P = .12$, $P = .69$, respectively; χ^2 test). Considering the dominant model, 4 SNPs in the XFG group were significant compared with the genotype frequencies of the control group. In POAG, NTG and XFG individuals bearing the minor allele of rs2149356 had the most significantly increased risk for glaucoma over that of control subjects ($P = .00014$, $P = .015$, $P = .062$, respectively).

• **HAPLOTYPE ANALYSIS:** The haplotype frequencies of the Tag SNPs (rs10759930, rs11536889, rs7037117, and

TABLE 3. Haplotype Frequencies of Tag Single Nucleotide Polymorphisms of the Toll-like Receptor 4 Gene Compared with Previous Study

	This Study										Previous Study			
Tag SNPs rs10759930, rs11536889, rs7037117, and rs7045953	POAG (n = 184)	*P* Value	Overall *P* Value (POAG)	NTG (n = 365)	*P* Value	Overall *P* Value (NTG)	XFG (n = 109)	*P* Value	Overall *P* Value (XFG)	Control (n = 216)	NTG (n = 250)	Control (n=318)	*P* Value	Overall *P* Value (NTG)
TGAA	0.311	.000072	.00097	0.360	.003	.057	0.362	.036	0.134	0.448	0.350	0.402	.070	.044
TCAA	0.228	.882		0.242	.465		0.229	.863		0.223	0.226	0.247	.41	
CGAA	0.208	.030		0.164	.519		0.173	.439		0.150	0.166	0.159	.75	
CGGA	0.125	.406		0.137	.126		0.146	.141		0.107	0.154	0.102	.0090	
CGGG	0.090	.080		0.074	.280		0.067	.664		0.058	0.096	0.077	.26	
Tag SNPs rs10759930 and rs7037117														
TA	0.539	.00014	.0017	0.603	.020	.085	0.591	.044	0.201	0.674	0.575	0.649	.0044	.010
CG	0.216	.063		0.219	.023		0.219	.086		0.164	0.249	0.179	.21	
CA	0.238	.0073		0.178	.524		0.185	.479		0.162	0.173	0.169		

NTG = normal-tension glaucoma; POAG = primary open-angle glaucoma; SNP = single nucleotide polymorphism; XFG = exfoliation glaucoma.

TABLE 4. Haplotype Frequencies of Tag Single Nucleotide Polymorphisms of the Toll-like Receptor 4 Gene Between Primary Open-Angle, Normal-Tension, and Exfoliation Glaucoma and Control Subjects

Tag SNPs rs10759930, rs1927914, rs1927911, and rs2149356	POAG (n = 184)	*P* Value	Overall *P* (POAG)	NTG (n = 365)	*P* Value	Overall *P* (NTG)	XFG (n = 109)	*P* Value	Overall *P* (XFG)	Control (n = 216)
TTCC	0.516	.000014	.00078	0.589	.009	0.018	0.573	.020	.014	.667
CCTA	0.418	.00033		0.384	.003		0.395	.012		.296
CCCC	0.005	.138		0.008	.211		NA	NA		.016

NTG = normal-tension glaucoma; POAG = primary open-angle glaucoma; XFG = exfoliation glaucoma.

rs7045953) were studied earlier.[21] The results showed that NTG and XFG were not statistically significant (overall $P = .057$, $P = .134$), but POAG was statistically significant (overall $P = .00097$; Table 3). The tag SNPs, rs10759930 and rs7037117, used in that study were similar with these haplotypes (Table 3).

Other haplotypes, rs10759930, rs1927914, rs1927911, and rs2149356, had higher statistical significance (overall $P = .00078$ in POAG; overall $P = .018$ in NTG, and overall $P = .014$ in XFG; Table 4).

DISCUSSION

• **TOLL-LIKE RECEPTOR 4 POLYMORPHISMS IN PRIMARY OPEN-ANGLE GLAUCOMA, NORMAL-TENSION GLAUCOMA, AND EXFOLIATION GLAUCOMA SUBJECTS:** Shibuya and associates showed that rs7037117, located in the 3′-untranslated region of *TLR4*, was most strongly associated with NTG.[21] Compared to earlier reports, the intragenic SNP rs2149356 could be more associated with NTG and also with POAG and XFG in this study. The statistics of all 8 genotypes showed that *TLR4* had approximately the same tendency for all corresponded allele frequencies (Table 2). The haplotypes rs10759930, rs1927914, rs1927911, and rs2149356 had the higher statistically significant values in both groups (overall $P = .00078$, $P = .018$, and $P = .014$, respectively; Table 4). On the other hand, this haplotype was shown to be not significant, and even in the original study,[21] it was statistically marginal ($P = .044$ for 4 SNPs and $P = .010$ for 2 SNPs). Thus, these haplotypes and/or SNPs are valuable for screening for glaucoma in the Japanese.

Subjects enrolled in this study and those reported by Shibuya and associates[21] were from across Japan; however, the subjects from our study were predominantly from northern Japan. The difference in the heterogeneity may explain the slight differences between the 2 studies. An association between the SNPs and POAG and XFG was not expected before this study because the IOP has a predominant effect on these diseases. So it is interesting that *TLR4* would be associated with those phenotypes of POAG and XFG, and the risk associations were stronger in POAG than in NTG. Recently, Suh, and associates showed that *TLR4* gene polymorphisms do not associate significantly with NTG in a Korean population,[22] but they did not examine it in POAG and XFG subjects. It should be evaluated in various types of glaucoma in different populations.

• **FUNCTION OF *TLR4* GENE:** Innate immunity produces antimicrobial peptides against many kinds of pathogens in the host defense system, and these induce adaptive immunity secondarily. Together, they play important roles in the total immune system.[24] Targeting TLR signaling has implications in the control of infection, vaccine design, desensitization to allergens, and downregulation of inflammation. *TLR4*-deficient mice were reported to have an upregulation of NADPH oxidase (Nox3), which increased the oxidative stress.[25] Although the function of the 8 SNPs on the *TLR4* gene was not examined, rs10759930 and rs1927914 exist within the 5′ untranslated region, rs1927911, rs12377632, and rs2149356 exist within introns, and rs11536889, rs7037117, and rs7045953 exist within the 3′ untranslated region. There is a possibility that these SNPs influence the stability of the mRNA and expression of the *TLR4* gene because rs11536889 exists near exon3.

TLR4 is expressed in the conjunctiva, cornea, iris, ciliary body, choroid, retina, and retinal pigment epithelium. In the retina, changes in the glial cells may be associated with glaucoma, especially NTG, which is not so dependent on the IOP. Widespread chronic stress is evident in the retina and optic nerve head by the strong upregulation of the HSPs in glaucomatous eyes.[26] Recently, an upregulation of toll-like receptors TLR2, TLR3, and TLR4 was found in human glaucoma donor eyes, which is consistent with the strongly increased level of expression of HSPs.[27] Immunohistochemical analyses supported an upregulated expression of TLRs in both microglia and astrocytes in glaucomatous retinas. It has been postulated that changes in the microenvironment of injured axons will alter the glycosaminoglycan composition in the lamina cribrosa, and this may account for the increased vulnerability of the remaining axons to sustain further damage independent of the IOP.

The significance of these findings in POAG more than NTG raises further speculation. Chronic stress could influence the aqueous humor and may adversely affect the outflow structure to increase resistance to outflow, with alterations of the trabecular meshwork and intrascleral channels and collapse of the Schlemm canal. There is a possibility that *TLR4* might have an effect on the alterations of the aqueous humor dynamics and injury to the glaucomatous retina in eyes with POAG and XFG.

• **AUTOIMMUNE DISEASES, CHRONIC INFLAMMATION, AND GLAUCOMA:** To date, the genes of the TLR family have not been candidates as genetic modifiers of glaucoma susceptibility, but they have been implicated in other autoimmune diseases and allergic diseases, including rheumatoid arthritis[28,29] and bronchical asthma.[30,31] It is interesting that the net *TLR4* sequence variants and the *TLR4* signaling network would affect not only the development of NTG but also POAG and XFG. Chronic infection by certain bacteria and viruses may play a role in inflammation.[32] More specifically, chronic infection by *Helicobater pyori* may induce a persistent systemic and vascular inflammation and endothelial dysfunction.[33] The results of one study showed that the specific IgG antibody levels of *H. pylori* were significantly increased in the aqueous humor and serum of patients with POAG and

XFG.[34] In addition, the titer of *H. pylori* antibody in the aqueous humor might reflect the severity of glaucomatous damage in POAG patients. We hypothesized that some types of chronic infection and/or inflammation can lead to the development of glaucoma especially POAG.

In conclusion, we have identified *TLR4* SNPs as genetic susceptibility alleles for POAG, NTG, and XFG in the Japanese population. Our findings would support the idea that changes in the regulation of TLR signaling in human glaucoma may be associated with innate and adaptive immune responses. Further investigations on different ethnic populations, and on the structure and function of the *TLR4* protein, would be helpful in understanding the pathogenesis of POAG, NTG, and XFG.

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Y. Takano and D. Shi contributed equally to this work.

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SUPPLEMENTAL TABLE. Primer Sequences for Toll-like Receptor 4 Gene Amplification Used in This Study

	Forward Primer	Reverse Primer	Annealing Temperature(C)
rs10759930	gtacagggtgtttgggagga	catggaccaatgctcttgtg	63
rs1927914	tgatgaggattgaaaatgtgga	acaaaatggtccctcacagc	60
rs1927911	ttaaatactccatatcattggggagac	gagagcattcagaaattagatgg	62
rs12377632	tggtatttggctttctgttcc	aaggtttctggggcaagttt	56
rs2149356	ccttggatcaagtttagccatt	tttccacaaaactcgctcct	60
rs11536889	ccctgtacccttctcactgc	gtttctgaggaggctggatg	62
rs7037117	ttaacccctttcccacctttc	agagtttgggacctgctcaa	60
rs7045953	ttcccatgttccctcatttc	ggggcaaaagagaaactcct	59

Combined 25-Gauge Microincision Vitrectomy and Toric Intraocular Lens Implantation With Posterior Capsulotomy

Hiroshi Kunikata, MD, PhD; Naoko Aizawa, MD; Yasuhiko Meguro, MD; Toshiaki Abe, MD, PhD; Toru Nakazawa, MD, PhD

PURPOSE: To evaluate the efficacy of combined 25-gauge microincision vitrectomy surgery (MIVS) and toric intraocular lens (IOL) implantation with posterior capsulotomy.

METHODS: Noncomparative, interventional case series performed at a single center. Twelve patients with vitreoretinal disease and cataracts, with pre-existing regular corneal astigmatism greater than 1 diopter, underwent 25-gauge MIVS and toric IOL implantation with posterior capsulotomy.

RESULTS: The toric IOL was successfully implanted in each case. At 6 months postoperatively, mean axis rotation was 5.7° ± 3.1°. At 1 month postoperatively, mean uncorrected and best corrected visual acuity improved; the improvement was maintained after 6 months. The absolute residual refractive cylinder was significantly lower postoperatively than the pre-existing regular corneal cylinder (P = .003). There were no surgical complications except a temporary posterior irissynechia in one case.

CONCLUSIONS: Combined 25-gauge MIVS and toric IOL implantation with posterior capsulotomy is a practical and safe method to treat vitreoretinal disease and cataracts with pre-existing corneal astigmatism.

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INTRODUCTION

Recent advanced sutureless vitrectomy techniques have hastened visual recovery, with reduction in postoperative astigmatism, conjunctival injection, pain, and discomfort.[1-7] The correction of refractive errors, including corneal astigmatism, has thus become a consideration in vitrectomy combined with cataract surgery. Toric intraocular lenses (IOLs) have been implanted in patients worldwide, and their feasibility has been demonstrated.[8-10] Over 30% of eyes indicated for cataract surgery have corneal astigmatism of at least 1.00 diopter (D).[11] Nevertheless, because of the technical difficulty of vitreous surgery and the emphasis on retinal disease control, toric IOLs have not been combined with vitrectomy surgery.

Twenty-five-gauge microincision vitrectomy surgery (25G MIVS) was first reported in 2002, and this technique is commonly used throughout the world.[12,13] Some patients (fewer than 1%) should forego MIVS or only undergo it with caution;[14] however, the indications for 25G MIVS have expanded to diseases including proliferative diabetic retinopathy (PDR), rhegmatogenous retinal detachment, giant retinal tear, intraocular foreign body, and IOL dislocation.[15-25] The increase in popularity of 25G MIVS has been enhanced by studies that have demonstrated its advantages for postoperative quality of vision. This is because intraoperative suturing is not required.[1-7] Recently, to prevent postoperative posterior capsule opacification (PCO) in patients with vitreoretinal disease who must have a vitrectomy combined with cataract surgery, a primary posterior capsulotomy technique using a 25-gauge vitreous cutter has been

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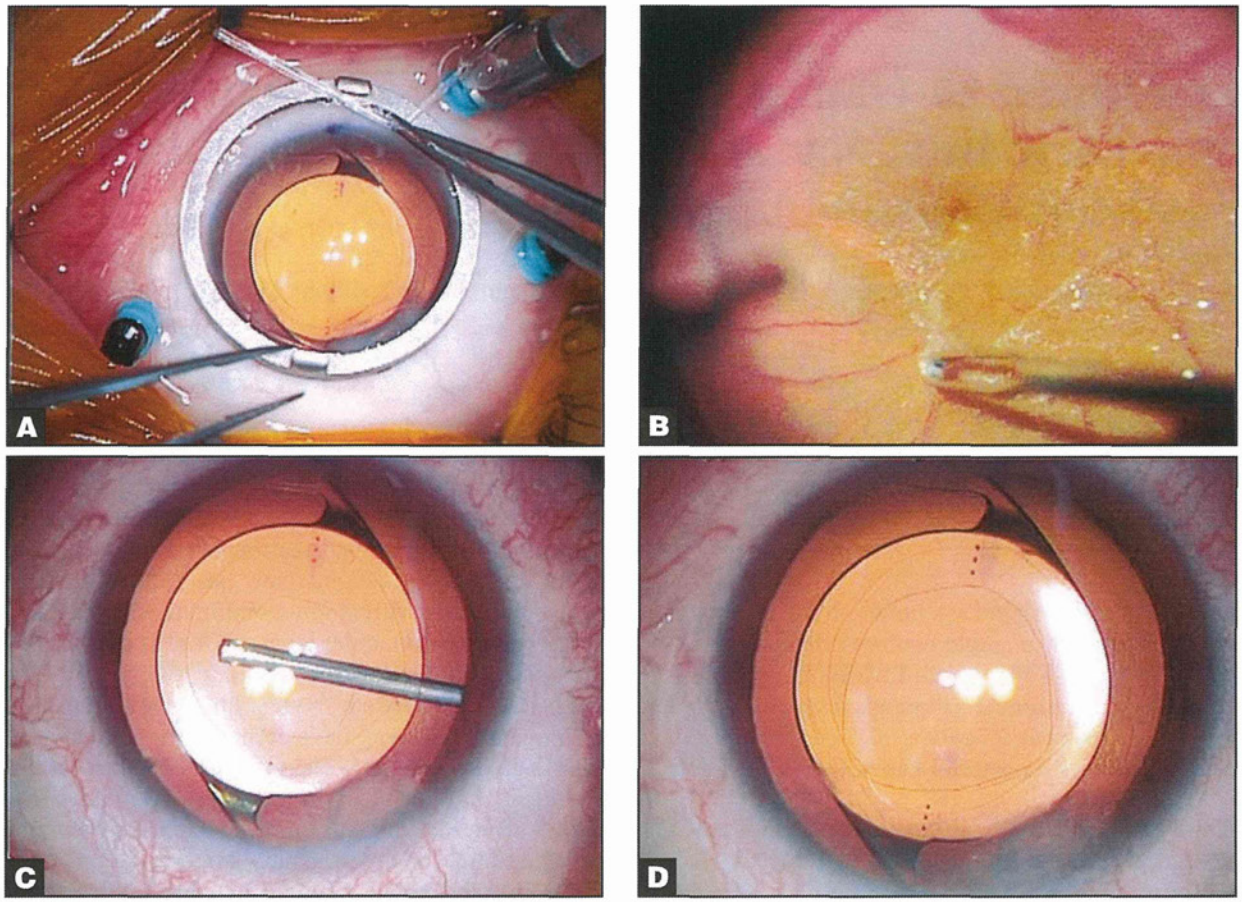

Figure 1. Representative eye with an epiretinal membrane (ERM) (Patient 2 in Table 1). Fundus and intraoperative photographs of the eye of a 60-year-old woman with an ERM. The eye had undergone combined 25-gauge microincision vitrectomy surgery (25G MIVS) and toric IOL implantation with posterior capsulotomy. (A) Intraoperative photograph of the anterior segment indicating a toric IOL implanted before 25G MIVS. (B) Intraoperative photograph of the fundus showing peeling of the epiretinal membrane, which was clearly visible thorough a toric IOL. (C) Intraoperative photograph of the anterior segment showing the center of the posterior capsule removed using a 25-gauge vitreous cutter. (D) The posterior capsule has been removed circularly and completely.

reported.[26,27] However, to the best of our knowledge, a procedure combining 25G MIVS and toric IOL implantation with posterior capsulotomy has not previously been reported, despite the large number of patients with concomitant vitreoretinal disease, cataracts, and pre-existing regular corneal astigmatism.

This report describes a technique combining 25G MIVS and toric IOL implantation with posterior capsulotomy.

PATIENTS AND METHODS

We reviewed the medical records of six consecutive eyes with retinal disease, cataracts, and corneal astigmatism that had undergone a combined surgery of phacoemulsification, toric IOL implantation with posterior capsulotomy, and 25G MIVS. The retinal diseases included epiretinal membrane (ERM) (eight eyes), macular hole (MH) (three eyes), and PDR (one eye). Preoperative demographics and surgical outcomes are shown in Table 1. Only eyes that had undergone a combined surgery of phacoemulsification, toric IOL implantation with posterior capsulotomy, and 25G MIVS by a single surgeon (HK) at the Surgical Retina Service at Tohoku University Hospital in Sendai, Japan, from February 2011 through March 2012 were included. Eyes with no corneal astigmatism, zonular weakness, or history of 23-gauge MIVS were excluded.

After explanation of the procedure, its purpose, risks and benefits, informed consent was obtained. The study conformed to the tenets of the Declaration of Helsinki and was approved by the institutional review board of the Tohoku University School of Medicine.

PREOPERATIVE ASSESSMENT AND INTRAOCULAR LENS

All patients were examined by slit lamp with pupils completely dilated. Axial length was measured

TABLE 1

Patient Demographics and Visual Improvement After Implantation of the Toric IOL and Axis Rotation of the Toric IOL Between Follow-up Visits in 12 Eyes

Patient No.	Age (Y)	Sex	Diagnosis	Preop Absolute Corneal Cylinder (D)	Postop Absolute Refractive Cylinder (D)	Visual Acuity (logMAR)				Intraop F/G Exchange	Axis of Toric IOL (Degrees)				
						UCVA		BCVA			Intended	1 Week Postop	1 Mos Postop	6 Mos Postop	Final Rotation
						Preop	Postop	Preop	Postop						
1	62	F	ERM	2.00	1.00	1.40	0.05	0.00	−0.08	N	101	91	93	95	6
2	60	F	ERM	2.00	0.25	1.15	0.00	0.05	−0.08	N	83	74	71	74	9
3	61	M	ERM	2.25	2.00	1.40	1.22	0.10	−0.08	N	86	89	87	89	3
4	70	F	ERM	1.50	0.25	0.22	0.05	0.22	−0.18	N	179	166	166	167	12
5	63	F	ERM	2.50	1.00	1.00	0.40	−0.08	0.30	N	96	93	91	92	4
6	53	M	ERM	1.50	0.50	1.05	0.05	0.00	−0.08	N	88	95	96	95	7
7	77	F	ERM	1.50	1.50	1.00	0.00	0.30	0.00	N	18	26	28	21	3
8	64	F	ERM	1.50	0.50	1.52	1.00	0.30	0.10	N	84	86	89	86	2
9	68	M	MH	2.75	0.75	0.70	0.52	0.70	0.10	Y	169	166	171	175	6
10	63	F	MH	2.00	0.75	0.70	−0.08	0.70	−0.08	Y	93	84	85	84	9
11	66	F	MH	1.25	0.75	0.82	0.40	0.70	−0.08	Y	53	52	51	50	3
12	66	F	PDR	1.75	0.25	1.40	−0.08	1.15	−0.08	N	169	168	166	173	4
Mean	64.4			1.88	0.79	1.03	0.29	0.34	−0.02						5.7

Preoperative corneal cylinder versus postoperative refractive cylinder, Wilcoxon signed-rank test: P = .003. Preoperative UCVA versus postoperative UCVA, Wilcoxon signed-rank test: P = .002. Preoperative BCVA versus postoperative BCVA, Wilcoxon signed-rank test: P = .015.

IOL = intraocular lens; UCVA = uncorrected visual acuity; BCVA = best corrected visual acuity; logMAR = logarithm of the minimum angle of resolution; F/G = fluid-air; ERM = epiretinal membrane; MH = macular hole; PDR = proliferative diabetic retinopathy.

using A-scan biometry (UD-7000; Tomey, Nagoya, Japan). Corneal astigmatism was determined by manual keratometry (TONOREF RKT-7700; Nidek, Aichi, Japan). The target postoperative spherical equivalent was aimed at emmetropia or –0.5 using the SRK/2 formula. IOL cylinder power and alignment axis were calculated using a Web-based toric IOL calculator program (http://www.acrysoftoriccalculator.com). This was done in patients with pre-existing regular corneal astigmatism greater than 1 D, taking into account the keratometry readings and mandatory data input on the position of the incision and surgery-induced astigmatism at an 11 o'clock corneal incision (0.50 D). The toric IOL design was based on the one-piece AcrySof platform (Alcon Laboratories, Fort Worth, TX). The overall haptic length was 13.0 mm, and the optic diameter was 6.0 mm. Three IOL variations (AcrySof SN6AT3, SN6AT4, and SN6AT5) treat different levels of pre-existing corneal astigmatism.

Corneal, internal, and ocular aberration were assessed preoperatively and 1 week, 1 month, and 6 months postoperatively with a wavefront analyzer (KR-9000PW; Topcon, Tokyo, Japan).

With the patient seated and under slit lamp to avoid ocular torsion, the 6 o'clock position was marked at the corneal limbus using a 27-gauge needle.

SURGICAL PROCEDURE

Under retrobulbar anesthesia, first the actual implantation axis was marked using a two-blade DK axis marker 9-729 and Mendez degree gauge 9-707R-1 (both Duckworth & Kent, Hertfordshire, England). Then three ports for 25G MIVS were created using the oblique sclerotomy technique with the Accurus vitrectomy system (Alcon Laboratories, Fort Worth, TX).[28] An infusion cannula was inserted through the inferotemporal sclera, followed by the insertion of two cannulas through the superotemporal and the superonasal regions.

A 2.4-mm corneal incision at the 11 o'clock position, continuous curvilinear capsulorrhexis, phacoemulsification using the divide-and-conquer technique, and irrigation/aspiration were performed. The toric IOL was implanted into the capsular bag with a Monarch 3 injector (Alcon Laboratories, Fort Worth, TX) and a D cartridge. Subsequently, the IOL was rotated with a lens hook so that the cylindrical axis of the lens was aligned with the corneal marks of the corneal astigmatism. Then viscosurgical material was removed gently by irrigation/aspiration.

After completion of IOL implantation, a sutureless contact lens was placed on the cornea to view the vitreal cavity. 25G MIVS was performed, comprising core vitrectomy, creation of a posterior vitreal detachment, peripheral vitrectomy, and ERM and internal limiting membrane (ILM) peeling. Endolaser photocoagulation was performed for the case with PDR. Fluid/air exchange was performed in the eyes with MH. Finally, just after the procedure was complete, the axis alignment of the implanted toric IOL was rechecked.

Antibiotics and corticosteroids were injected subconjunctivally at the end of surgery in all cases. All patients were prescribed a combination of antibiotic and corticosteroid eye drops four times daily and nonsteroid anti-inflammatory eye drops two times daily for 4 weeks.

POSTOPERATIVE MEASUREMENTS

One month postoperatively, uncorrected visual acuity (UCVA), best corrected visual acuity (BCVA), subjective refraction, and slit lamp examination were recorded, and postoperative corneal astigmatism was assessed by A-scan biometry. When the axis of the toric lens was measured, the pupil was dilated to enable visualization of the three dots on the optic periphery. The anterior segment was imaged with slit photography. A black measurement line and a circular scale in the periphery with single degree steps were then overlaid on the photograph so that the angle could be read. The axis of the toric IOL was evaluated at 1 week, 1 month, and 6 months postoperatively.

The patients were evaluated for intraoperative and postoperative complications associated with the surgery.

STATISTICAL ANALYSES

Data are presented as the mean ± standard deviation. The significance of the difference between the pre- and postoperative data was determined by Wilcoxon signed-rank tests or the Friedman test. The decimal BCVA was converted to logarithm of the minimal angle resolution (logMAR) units for statistical analysis. A P value of less than 0.05 was considered to be statistically significant.

RESULTS

As summarized in Table 1, there were three men and nine women, with a mean age at the time of surgery of 64.4 ± 5.9 years. All patients underwent 25-gauge MIVS and implantation of the toric IOL with posterior capsulotomy. All of the IOLs were successfully fixed in the capsular bag. The ERMs and ILMs were successfully removed in all eight eyes with ERM, and the ILMs were removed and fluid/air exchange was performed after insertion of the toric IOL in the three eyes with MH. The implanted toric IOL was stable during the fluid/air exchange procedure,