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Polymorphism of β-adrenergic receptors and susceptibility to open-angle glaucoma

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Purpose: The human trabecular meshwork and ciliary body, which express β-adrenergic receptors (ADRB1 and ADRB2), control aqueous humor dynamics. We investigated associations of *ADRB* polymorphisms with open-angle glaucoma (OAG), because *ADRB* gene polymorphisms alter receptor function.

Methods: We studied 240 Japanese controls and 505 Japanese OAG patients including 211 with primary open-angle glaucoma (POAG), and 294 with normal-tension glaucoma (NTG). Associations of four polymorphisms (Ser49Gly and Arg389Gly in the *ADRB1* gene; Arg16Gly and Gln27Glu in the *ADRB2* gene) were compared between patients and controls. Age, intraocular pressure (IOP), and visual field defects at diagnosis were examined for associations with polymorphisms.

Results: The Arg389Gly polymorphism in the *ADRB1* gene showed significantly different allele and genotype frequencies in patients with NTG than in controls (p=0.004 and 0.006, respectively). Other polymorphisms did not show a significant frequency difference. In POAG patients, carriers of Gly16 in the *ADRB2* gene were significantly younger at diagnosis than noncarriers (p<0.001). The IOP at diagnosis was significantly higher in OAG patients carrying 27Glu in the *ADRB2* gene than in patients without this allele (p<0.001). Clinical characteristics of OAG patients did not differ significantly in relation to other polymorphisms.

Conclusions: Certain polymorphisms of the *ADRB1* and *ADRB2* genes influence the pathophysiology of OAG in Japanese patients.

Open-angle glaucoma (OAG), a neurodegenerative disease with significant multifactorial components, is characterized by optic nerve head cupping and visual field loss often related to elevated intraocular pressure (IOP). OAG affects over 45 million people, nearly 2% of the world population over the age of 40 years [1]. The second most common cause of bilateral blindness worldwide [2], OAG is divided into two subtypes: primary open-angle glaucoma (POAG) with an IOP >21 mmHg; and normal-tension glaucoma (NTG) with an IOP less than or equal to 21 mm Hg at any time. In Japan, NTG accounts for 92% of eyes with OAG, a higher proportion than Caucasians [3]. Although the best-known risk factor for OAG is increased IOP [4], OAG is a complex condition caused by multiple genes together with environmental factors that contribute to the phenotype [5,6].

Some studies have shown that interindividual variations of ocular parameters, such as IOP and optic nerve head cupping, may have a genetic background [7-9]. Additionally, at least seven chromosomal loci have been shown to be linked to OAG [10,11], and several polymorphisms of the genes have shown association with OAG [12-14]. However, the underlying molecular mechanisms linking polymorphisms to characteristics of OAG are not well understood.

The β-adrenergic receptor (ADRB) is expressed in the human ciliary body and trabecular meshwork, structures involved in aqueous humor production and outflow, respectively [15-17]. Wax et al. [15] reported that ADRBs in membrane homogenates of human iris-ciliary body are predominantly of the ADRB2 subtype, with ADRB1 comprising about 10% of the total number of ADRBs in the entire iris-ciliary body. Antagonists of ADRBs, which include nonselective β1, β2-antagonists, and relatively selective β1-antagonists, are used widely as topical preparations to lower IOP to treat ocular hypertension and glaucoma [18,19]. Topically applied beta-blockers reduce IOP by decreasing aqueous humor formation by the ciliary body [18]. Aqueous humor is formed primarily in the nonpigmented ciliary epithelial cells of the ciliary process [20], where the rate of aqueous humor formed appears to be under adrenergic control.

Trabecular meshwork cells form the primary outflow pathway for aqueous humor. Aqueous humor outflow appears to be increased by adrenergic agonists. For instance, epinephrine and isoproterenol enhance aqueous humor outflow via a paracellular pathway related to trabecular meshwork cells and Schlemm's canal endothelial cells through a β-adrenergic-

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mediated response that widens the intercellular space and reduces the cell area [21].

Trabecular meshwork cells in culture express β-adrenergic receptors that have been characterized as the β2 subtype by ligand competition studies [22]. Decreases in resistance to outflow in response to epinephrine and isoproterenol appear to be mediated by β2-adrenergic receptors on trabecular endothelial cells.

In the human optic nerve, ADRB2s were observed [23] specifically in astrocytes [24] or the wall of the microvessels of the optic nerve [25]. When an optic nerve was crushed or transected in rabbits and rats, ADRB2 was significantly increased [24]. Thus, ADRB plays an important role in the pathogenesis of glaucoma.

Several polymorphisms have been found in the coding region of the human β1-adrenergic receptor (*ADRB1*) [26,27] and β2-adrenergic receptor (*ADRB2*) genes [28-31] that alter receptor function in patients with heart failure [26,27], nocturnal asthma [28-30], and essential hypertension [31]. A previous association study in a Turkish population that examined whether the *ADRB2* gene is involved in glaucoma found no association of glaucoma with the Arg16Gly, Gln27Glu, or Thr164Ile polymorphisms of the gene [32].

Nonetheless, because functional differences of the *ADRB1* or *ADRB2* gene could have an impact on the aqueous humor dynamics or optic nerve degeneration, we hypothesized that variants of *ADRB* genes could be genetic risk factors for glaucoma or could modify glaucoma. We sought to determine whether single-nucleotide polymorphisms (SNPs) in *ADRB1* and *ADRB2* genes were associated with OAG in a Japanese population.

METHODS

Patient and control subjects: Blood samples were collected at eight ophthalmology centers located throughout Japan. OAG patients whose age at diagnosis was younger than 39 years and patients with over -5.5 D of myopia were excluded. OAG patients with known mutations in myocilin [33] or optineurin [34] were also excluded. The 745 subjects, none of whom were related, included 211 POAG patients, 294 NTG patients, and 240 controls with no eye disease.

This human research followed the provisions of the Declaration of Helsinki. Written informed consent was obtained after the nature and possible consequences of the study had been explained. Where applicable, the research was approved by the institutional human experimentation committee.

The general characteristics of patients with glaucoma and controls are presented in Table 1. The mean age at the time of blood sampling was 64.8±12.0 years (mean±SD) in POAG patients, 58.8±13.2 years in NTG patients, and 69.7±11.2 in normal subjects. The mean age of control subjects was significantly older than POAG patients ($p<0.001$) or NTG patients ($p<0.001$). We purposely selected older control subjects (>40 years) to reduce the likelihood of including control subjects who might later develop glaucoma.

The characteristics of glaucoma patients that we examined included age at diagnosis, untreated maximum IOP (defined as IOP at diagnosis), and visual field defects at the initial examination (defined as visual field score at diagnosis). The severity of visual field defects was scored from 1 to 5 [35,36]. Data obtained with different types of perimeters were combined using a five-point scale as follows: 1, no alterations; 2, early defect; 3, moderate defect; 4, severe defect; and 5, light perception only or no vision [14,34].

All patients underwent serial ophthalmic examinations including IOP measurements by Goldmann applanation tonometry, visual fields by Humphrey perimetry (30-2) or Goldmann perimetry, gonioscopy, and optic disc examination including fundus photography. All patients were diagnosed with glaucoma based upon the presence of typical optic disc damage with glaucomatous cupping (cup:disc ratio >0.7) and loss of the neuroretinal rim tissue; reproducible visual field defects compatible with glaucomatous cupping; and open angles according to gonioscopy. POAG was diagnosed if the patient had an IOP >21 mmHg at any time during the follow-up period. Patients with exfoliative glaucoma, pigmentary glaucoma, or corticosteroid-induced glaucoma were excluded. OAG patients were diagnosed with NTG when three criteria were met: an untreated peak IOP less than or equal to 21 mmHg at all times including the three baseline measurements and those obtained during daylong testing (every 3 h from 6 am to midnight); a peak IOP that was consistently less than or equal to 21 mm Hg throughout the follow-up period with or without medication after diagnosis; and absence of known secondary causes for glaucomatous optic neuropathy, such as a previously elevated IOP resulting from trauma, steroid administration, or uveitis.

Control subjects were recruited from Japanese individuals who had no known eye abnormalities except for cataracts. There were 240 subjects, and all were >40-years-of-age. Their IOP was below 20 mmHg. They had no glaucomatous disc change, and no family history of glaucoma.

Genotyping: Genomic DNA was isolated from peripheral blood lymphocytes by standard methods. Four SNPs were assessed in all participants: Ser49Gly and Arg389Gly in the *ADRB1* gene; and Arg16Gly and Gln27Glu in the *ADRB2* gene. To investigate the Ser49Gly polymorphism, we genotyped 745 subjects using polymerase chain reaction (PCR) amplification followed by restriction fragment length polymorphism analysis [37]. The primer pair used to amplify the DNA was 5'-CGC TCA GAA ACA TGC TGA AGT CC-3' (sense) and 5'f-GGA CAT GAT GAA GAG GTT GGT GAG-3' (antisense). Amplified DNA fragments were digested with a restriction endonuclease, *Eco*O109I (Takara Bio Inc., Shiga, Japan). After digestion of the amplified DNA fragments by the restriction enzyme, genotypes were identified by electrophoreisis on 3% agarose gels and made visible with ethidium bromide staining and ultraviolet illumination.

Genotypes for the other three SNPs were identified with the Invader assay (Third Wave Technologies, Inc., Madison, WI), which was developed recently for high-throughput genotyping of SNPs [14,38]. Oligonucleotide sequences of the primary probes and Invader probes used are listed in Table 2.

Table 1.

Parameters	Control (n=240)	POAG (n=211)	NTG (n=294)
Gender (male/female)	114/126	123/88	144/150
Age at diagnosis (years)		58.1±12.1	55.8±13.1
Age at blood sampling (years)	69.7±11.2	64.8±12.0	58.8±13.2
Untreated maximum IOP (mmHg)		26.5± 6.0	16.6± 2.5
Visual field score at diagnosis		3.1± 0.9	2.8± 0.7
Family history of glaucoma	0/240 (0.0%)	63/211 (29.9%)	93/294 (31.6%)
Medical characteristics			
Hypertension	55/240 (22.9%)	48/211 (22.8%)	57/294 (19.4%)
Ischemic heart disease	15/240 (6.3%)	9/211 (4.3%)	11/294 (3.7%)
Lipid metabolism disorders	14/240 (5.8%)	15/211 (7.1%)	16/294 (5.4%)

Demographic, ocular, and general medical characteristics in glaucoma patients and control subjects. A total of 745 Japanese subjects were examined. No significant difference was observed in prevalence of systemic diseases between POAG, NTG, and control groups. Data for age at diagnosis, age at blood sampling, untreated maximum IOP, and visual field score at diagnosis are expressed as mean±standard deviation. Data for systemic diseases and history of glaucoma are counts with percentages in parentheses.

Table 2.

Polymorphism	Nucleotide change	Target	Probe	Probe	Sequence
ADRB1 Arg389Gly	C to G	Antisense	Wild	C probe	Flap1-CGACTGCTCTGCTG
			Mutant	G probe	Flap2-GGACTGCTCTGCTG
			Invader	Invader	CCCGACTTCCGCAAGGCCTTCCAGT
ADRB2 Arg16Gly	A to G	Sense	Wild	A probe	Flap1-TATTGGGTGCCAGCA
			Mutant	G probe	Flap2-CATTGGGTGCCAGC
			Invader	Invader	TCGTGGTCCGGCGCATGGCTTCA
ADRB2 Gln27Glu	C to G	Antisense	Wild	C probe	Flap1-CAAAGGGACGAGGTGT
			Mutant	G probe	Flap2-GAAAGGGACGAGGTGT
			Invader	Invader	GCCGGACCACGACGTCACGCAGT

Sequences of primary probes and Invader oligonucleotides used in assays. Three polymorphisms were detected among all participants. Genotyping of the polymorphisms was performed by the Invader assay using the probes listed.

Statistical analysis: χ^2 analysis was used to determine whether the population was in Hardy-Weinberg equilibrium. Frequencies of genotypes and alleles were compared between patients and controls by χ^2 analysis. The odds ratio and 95% confidence interval (CI) were also calculated. The Bonferroni correction was used to adjust for multiple comparisons for static analysis of each SNP. A value of $p<0.0125$ was considered to be significant. Associations between clinical characteristics (age, IOP, and visual field score at diagnosis) and genotypes were assessed by multivariate analyses carried out with a logistic regression model. Statistical analysis was performed with SPSS (version 12.0; SPSS Inc., Chicago, IL). A value of $p<0.05$ was considered to be significant.

RESULTS

The allele and genotype frequencies are shown for the four SNPs in glaucoma patients and controls in Table 3. Each genotype frequency was consistent with a population in Hardy-Weinberg equilibrium. The Arg389Gly polymorphism of the *ADRB1* gene was significantly associated with NTG, showing a significantly different allele frequency in NTG patients and control subjects, with the Gly allele being less frequent in the NTG patients (p=0.004, odds ratio=1.570, 95% CI=1.154-2.137).

The distribution of the Arg389Gly genotype also differed significantly between NTG patients and control subjects (p=0.006); the difference was more significant in a recessive genetic model (Arg/Arg+Arg/Gly versus Gly/Gly, p=0.006) than in a dominant model (Arg/Arg versus Arg/Gly+Gly/Gly, p=0.023).

The other three SNPs studied (Ser49Gly, Arg16Gly, and Gln27Glu polymorphisms) did not show a significant difference between any patient group and controls (Table 3).

Characteristics of patients with glaucoma were examined assuming dominant and recessive models for each polymorphism. In the recessive model, no significant difference was detected in the three characteristics in patients with OAG for any polymorphism (data not shown). Differences in the dominant model of polymorphisms of *ADRB2*/Arg16Gly and *ADRB2*/Gln27Glu in association with three clinical characteristics are shown in Table 4 and Table 5.

For the Arg16Gly polymorphism in the *ADRB2* gene in POAG patients, patients in the Arg/Gly+Gly/Gly group (i.e., Gly16 carriers) were significantly younger at diagnosis than patients in the Arg/Arg group (i.e., non-Gly16 carriers; 56.7±11.7 years versus 62.9±12.7, $p<0.001$; Table 4). This association was not observed in NTG patients or in OAG patients overall. For the Gln27Glu polymorphism in the *ADRB2* gene, the IOPs at diagnosis were significantly higher in OAG patients with Gln/Glu+Glu/Glu (27Glu carriers; 24.2±9.2 mm Hg) than in those with Gln/Gln (non-27Glu carriers; 20.2±5.9 mm Hg, $p<0.001$; Table 5).

DISCUSSION

Two functional polymorphisms of *ADRB1*, Ser49Gly [39] and Arg389Gly [26,27] have been identified. Arg389Gly is located within a region important for receptor G-protein coupling and subsequent agonist-stimulated adenylyl cyclase activation [40]. In vivo studies have shown that the Arg389 variant of *ADRB1* mediates a more pronounced response to agonist stimulation than the Gly389 variant, suggesting the Arg389Gly polymorphism is of functional importance. A potential association be-

TABLE 3.

		Allele frecuency (percentage)					Genotype frequency (percentage)			
Polymorphism	Allele	OAG	POAG	NTG	Control	Genotype	OAG	POAG	NTG	Control
		(n=1010)	(n=422)	(n=588)	(n=480)		(n=505)	(n=211)	(n=294)	(n=240)
ADRB1	Ser	872(86.3)	362(85.8)	510(86.7)	415(86.5)	Ser/Ser	374(74.1)	155(73.5)	219(74.5)	176(73.3)
Ser49Gly	Gly	138(13.7)	60(14.2)	78(13.3)	65(13.5)	Ser/Gly	124(24.6)	52(24.6)	72(24.5)	63(26.3)
						Gly/Gly	7(1.4)	4(1.9)	3(1.0)	1(0.4)
		p=0.949	p=0.769	p=0.895			p=0.405	p=0.313	p=0.666	
		(n=1002)	(n=420)	(n=582)	(n=474)		(n=501)	(n=210)	(n=291)	(n=237)
ADRB1	Arg	821(81.9)	332(79.0)	489(84.0)	365(77.0)	Arg/Arg	335(66.9)	132(62.9)	203(69.8)	143(60.3)
Arg389Gly	Gly	181(18.1)	88(21.0)	93(16.0)	109(23.0)	Arg/Gly	151(30.1)	68(32.4)	83(28.5)	79(33.3)
						Gly/Gly	15(3.0)	10(4.8)	5(1.7)	15(6.3)
		p=0.026	p=0.462	p=0.004*			p=0.050	p=0.728	p=0.006*	
		(n=950)	(n=406)	(n=544)	(n=472)		(n=475)	(n=203)	(n=272)	(n=236)
ADRB2	Arg	470(49.5)	189(46.6)	281(51.7)	224(47.5)	Arg/Arg	100(21.1)	39(19.2)	61(22.4)	53(22.5)
Arg16Gly	Gly	480(50.5)	217(53.4)	263(48.3)	248(52.5)	Arg/Gly	270(56.8)	111(54.7)	159(58.5)	118(50.0)
						Gly/Gly	105(22.1)	53(26.1)	52(19.1)	65(27.5)
		p=0.474	p=0.789	p=0.182			p=0.178	p=0.580	p=0.062	
		(n=938)	(n=388)	(n=550)	(n=466)		(n=469)	(n=194)	(n=275)	(n=233)
ADRB2	Gln	881(93.9)	358(92.3)	523(95.1)	432(92.7)	Gln/Gln	415(88.5)	164(84.5)	251(91.3)	199(85.4)
Gln27Glu	Glu	57(6.1)	30(7.7)	27(4.9)	34(7.3)	Gln/Glu	51(10.9)	30(15.5)	21(7.6)	34(14.6)
						Glu/Glu	3(0.6)	0(0.0)	3(1.1)	0(0.0)
		p=0.382	p=0.810	p=0.111			p=0.179	p=0.969	p=0.031	

Allele and genotype frequency of *ADRB1* and *ADRB2* polymorphisms in Japanese glaucoma patients and controls. Data shown are counts with percentage in parentheses. Frequencies of genotypes and alleles were compared between patients and controls by χ^2 analysis. The Bonferroni correction was used to adjust for multiple comparisons for static analysis of each SNP. The Arg389Gly polymorphism of the *ADRB1* gene was significantly associated with NTG, showing a significantly different allele frequency in NTG patients and control subjects (p=0.004). Distribution of the Arg389Gly genotype also differed significantly between NTG patients and control subjects (p=0.006). P value from χ^2 test. The asterisk indicates a $p<0.0125$, Bonferroni correction.

tween Arg389Gly of the *ADRB1* gene and disease susceptibility has been suggested so that the Arg389 homozygote genotype was significantly more frequent in patients with acute myocardial infarction [27] and hypertension [41]. In Caucasian woman, the Arg389 allele was associated with greater body weight, higher body mass index (BMI), and more fat mass [42]. In Japanese patients with dilated cardiomyopathy, the Gly389 allele was reported to be associated with a decreased risk of ventricular tachycardia [43]. Agonist stimulation of β-adrenergic receptors was reported to increase the sarcoplasmic reticulum Ca^{2+} content in cardiac myocytes [44]. ADRB signaling regulates activity of several Ca^{2+}-regulatory proteins, and alterations in ADRB signaling have shown associations with heart disease [45].

The Arg389Gly polymorphism of the *ADRB1* gene had significantly different allele and genotype frequencies in NTG patients than in subjects without eye disease (p=0.004 and 0.006, respectively). Arg389 was significantly more frequent among patients with NTG than in control subjects (84% versus 77%), while Gly389 was significantly more prevalent among controls than NTG patients (23% versus 16%, p=0.004). However, no significant differences were seen between the clinical characteristics of glaucoma patients with the two polymorphisms of the *ADRB1* gene. The increased response to agonist stimulation by the Arg389 variant of ADRB1 [40] may increase the intracellular Ca^{2+} content excessively [44,45]. This could then induce apoptosis in retinal ganglion cells. Thus, the Gly389 allele may be protective against NTG.

Table 4.

		Genotype		
Phenotype	Clinical characteristics	Arg/Arg	Arg/Gly+Gly/Gly	p value
OAG	Age at diagnosis (years)	57.9±12.7 (n=100)	56.3±12.7 (n=371)	0.085
	IOP at diagnosis (mm Hg)	20.3± 5.8 (n=90)	20.8± 6.5 (n=335)	0.469
	Visual field score at diagnosis	2.8± 0.7 (n=99)	2.9± 0.8 (n=375)	0.508
POAG	Age at diagnosis (years)	62.9±12.7 (n=39)	56.7±11.7 (n=162)	<0.001*
	IOP at diagnosis (mm Hg)	26.3± 4.9 (n=33)	26.3± 6.0 (n=147)	0.973
	Visual field score at diagnosis	3.0± 0.9 (n=38)	3.1± 0.9 (n=164)	0.898
NTG	Age at diagnosis (years)	54.7±11.7 (n=61)	56.0±13.5 (n=209)	0.531
	IOP at diagnosis (mm Hg)	16.8± 2.5 (n=57)	16.6± 2.4 (n=188)	0.581
	Visual field score at diagnosis	2.7± 0.5 (n=61)	2.8± 0.7 (n=211)	0.266

Clinical characteristics of glaucoma patients according to genotype for Arg16Gly in the *ADRB2* gene. Differences in the dominant model of polymorphisms of *ADRB2*/Arg16Gly in association with three clinical characteristics are shown. Associations between clinical characteristics (age, IOP, and visual field score at diagnosis) and genotypes were assessed by multivariate analyses carried out with a logistic regression model. For the Arg16Gly polymorphism in the *ADRB2* gene in POAG patients, patients in the Arg/Gly+Gly/Gly group were significantly younger at diagnosis than patients in the Arg/Arg group (56.7±11.7 years versus 62.9±12.7, p<0.001). P values were calculated with logistic regression analysis. The asterisk indicates a p<0.05.

Table 5.

		Genotype		
Phenotype	Clinical characteristics	Gln/Gln	Gln/Glu+Glu/Glu	p value*
OAG	Age at diagnosis (years)	56.7±12.7 (n=412)	57.1±12.3 (n=53)	0.448
	IOP at diagnosis (mm Hg)	20.2± 5.9 (n=374)	24.2± 9.2 (n=45)	<0.001*
	Visual field score at diagnosis	2.9± 0.8 (n=414)	2.9± 0.8 (n=54)	1.000
POAG	Age at diagnosis (years)	58.4±12.3 (n=162)	56.3± 2.2 (n=30)	0.272
	IOP at diagnosis (mm Hg)	26.0± 5.1 (n=144)	28.6± 9.1 (n=28)	0.038*
	Visual field score at diagnosis	3.1± 0.9 (n=163)	3.1± 0.9 (n=30)	0.837
NTG	Age at diagnosis (years)	55.6±12.8 (n=250)	58.2±12.6 (n=23)	0.986
	IOP at diagnosis (mm Hg)	16.6± 2.5 (n=230)	17.1± 2.0 (n=17)	0.447
	Visual field score at diagnosis	2.8± 0.7 (n=251)	2.8± 0.6 (n=24)	0.692

Clinical characteristics of glaucoma patients according to genotype for Gln27Glu in the *ADRB2* gene. Differences in the dominant model of polymorphisms of *ADRB2*/Gln27Glu in association with three clinical characteristics are shown. Associations between clinical characteristics and genotypes were assessed by multivariate analyses carried out with a logistic regression model. For the Gln27Glu polymorphism in the *ADRB2* gene, IOPs at diagnosis were significantly higher in OAG patients with Gln/Glu+Glu/Glu (24.2±9.2 mmHg) than in those with Gln/Gln (20.2±5.9 mmHg, p<0.001). P values were calculated with logistic regression analysis. The asterisk indicates a p<0.05.

With respect to the *ADRB2* gene, two functionally significant polymorphisms, Arg16Gly and Gln27Glu, have been identified [28-31]. Potential association of *ADRB2* genetic polymorphisms have been suggested to be associated with susceptibility to certain disease states: a strong association between asthma and the Gly16 polymorphism [28-30]; a higher frequency of the Glu27 polymorphism in patients with type II diabetes mellitus [46] and obesity [47]; and a higher frequency of the Arg27 polymorphism in subjects with hypertensive family-history [31].

As ADRB2 is expressed predominately in human nonpigmented ciliary epithelial cells [15], trabecular meshwork cells [22], and the optic nerve head [24,25], we investigated the associations between *ADRB2* polymorphism and susceptibility to glaucoma. As ADRB2 is significantly increased in the optic nerve head following neuronal injury in animal models [24] and in POAG patients [25], it may be associated with neural cell death. When Gungor et al. [32] studied the distribution of three *ADRB2* polymorphisms in patients with primary congenital glaucoma and POAG, they found no association between Arg16Gly, Gln27Glu, or Thr164Ile polymorphisms and susceptibility to glaucoma. Our case-control association study also showed no association between Arg16Gly or Gln27Glu polymorphisms of the *ADRB2* gene and susceptibility to glaucoma. In the Japanese population, the Thr164Ile substitution was not detected in 508 subjects in a study of obesity, hypertriglyceridemia, and diabetes mellitus [46]. In our study, we also did not detect the Thr164Ile substitution (data not shown).

However, we found that in POAG patients, Gly16 allele carriers for the *ADRB2* gene were significantly younger at diagnosis than patients without this allele ($p<0.001$). The *ADRB2* Gly16 allele affected the age of POAG diagnosis without directly affecting the maximum untreated IOP. The maximum untreated IOP is not always associated with development of glaucoma. Diurnal variations or pressure spikes of the IOP also could certainly be associated with development of glaucoma [48]. It is not unusual that Arg16Gly polymorphism is associated with age of POAG diagnosis, but not with maximum untreated IOP.

Furthermore, pericytes are contractile cells of the capillary wall and might influence the hemodynamics of capillaries. They are particularly abundant in the retina and the optic nerve head [49]. In vitro, pericytes were demonstrated to relax in response to isoproterenol and beta-adrenergic agonist in a dose-dependent manner [50]. After a 24 h exposure to isoproterenol, Arg16 underwent a 26% reduction in receptor density, while Gly16 underwent a 41% reduction [51]. The Arg16Gly polymorphism is significantly associated with agonist-promoted down-regulation events [30,51]. These might help to explain the younger age at onset of POAG in Gly16 carriers.

The untreated maximum IOP at diagnosis was significantly higher in OAG patients carrying the Glu27 allele for the *ADRB2* gene than in patients not carrying Glu27 ($p<0.001$). The Gln27Glu polymorphism may be associated with untreated maximum IOP in OAG patients. The same genetic variant appears to be contributing to the IOP in both POAG and NTG. Even though nonsignificant in NTG, the trend is in same direction, and clearly these individuals contributed to the overall significance of the OAG results. OAG is a multifactorial disease including IOP-dependent and non-IOP-dependent risk factors. For the IOP, the Gln27Glu polymorphism could be a common genetic risk factor in POAG and NTG.

Interestingly, an in vitro study demonstrated that amino acids 16 and 27 conferred an opposing effect on agonist-promoted down-regulation events [51]. The Arg16Gly polymorphism is significantly associated with agonist-promoted down-regulation events, but the Gln27Glu polymorphism was resistant to such downregulation after long-term exposure to the agonist. On the other hand, the conformational alterations of Glu27 led to depressed receptor degradation. We hypothesized that if patients with Glu27 allele had dysfunction of TM cells, IOP would increase.

Two functional *ADRB2* polymorphisms, Arg16Gly and Gln27Glu, were associated with different clinical phenotypes such as age at diagnosis and untreated maximum IOP, respectively. *ADRB2* polymorphisms may thus have a modifying effect in OAG as a genetically determined factor in its pathophysiology. Further studies are needed to understand the role of the *ADRB* polymorphisms in patients with OAG, especially in aqueous humor dynamics and responsiveness to optic nerve damage.

In conclusion, we have found that the Arg389Gly polymorphism of the *ADRB1* gene had significantly different allele and genotype frequencies in patients with NTG than in controls. In glaucoma patients, the two polymorphisms in the *ADRB2* gene did not show significant genotype distribution differences from controls. However, the age at diagnosis was significantly younger in POAG patients carrying Gly16 of the *ADRB2* gene than in those lacking Gly16. In addition, the IOPs at diagnosis were significantly higher in OAG patients carrying 27Glu for the *ADRB2* gene than in those lacking 27Glu. Our results suggest that *ADRB1* and *ADRB2* gene polymorphisms may influence pathophysiology of OAG in Japanese patients.

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