Jpn J Ophthalmol 2007;51:353–359

DOI 10.1007/s10384-007-0464-z

CLINICAL INVESTIGATION

Long-term Therapeutic Outcome of Acute Primary Angle Closure in Japanese

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Abstract

Purpose: To investigate the long-term clinical course of acute primary angle closure (APAC) and acute primary angle-closure glaucoma (APACG) in Japanese patients.

Methods: We retrospectively reviewed our records of 66 consecutive APAC or APACG eyes observed between February 1992 and December 2003 (mean follow-up, 42.1 months). Immediately after the diagnosis, all patients had received similar medications to halt the acute attack. Subsequently, laser iridotomy or surgical peripheral iridectomy and/or laser iridoplasty were conducted. If intraocular pressure (IOP) control was poor under maximum tolerable ocular hypotensive agents, trabeculectomy with adjunctive mitomycin C (MMC) was undertaken.

Results: After laser therapy, the probability of success, defined as an IOP of <21 mmHg with or without medications, was 81.2% ± 6.2%. In the ten eyes that were trabeculectomized, the probability of success based on the same criterion was 40.0% ± 29.7%. Multivariate analysis revealed that the degree of synechial angle closure ($P = 0.029$) and the preexistence of chronic glaucomatous optic neuropathy ($P = 0.015$) significantly influenced the need for subsequent filtering surgery.

Conclusions: Without the intervention of filtering surgery, 84.6% of eyes with APAC or APACG maintained IOP control with or without antiglaucoma medications. However, APAC and APACG eyes that eventually received trabeculectomy were predisposed to an uncontrollable IOP, even with the intraoperative application of MMC. The severity of APAC or APACG in Japanese may be affected by an underlying creeping angle closure. **Jpn J Ophthalmol** 2007;51:353–359 © Japanese Ophthalmological Society 2007

Key Words: acute primary angle-closure, acute primary angle-closure glaucoma, filtering surgery, Japanese, laser therapy

Introduction

In East Asian countries, primary angle-closure glaucoma or primary angle closure is more common than in Western countries,[1] and often leads to severe bilateral vision impairment.[2,3] The acute forms, acute primary angle closure (APAC) and acute primary angle-closure glaucoma (APACG), can constitute an ophthalmic emergency, requiring early diagnosis followed by appropriate treatment to pull the peripheral iris away from the chamber angle. The classical initial treatment for APAC or APACG includes medication therapy using miotics and carbonic anhydrase inhibitors, laser peripheral iridotomy or surgical peripheral iridectomy, and, in certain cases, filtering surgery.

Recently, it has been reported that primary phacoemulsification and intraocular lens implantation (PPI) for uncontrolled APAC or APACG might safely and effectively reduce intraocular pressure (IOP) and improve visual acuity.[4,5] However, Jacobi and associates[5] reported that five patients (5/43, 11.4%) who underwent PPI required subsequent IOP-lowering surgical intervention for IOP control.[5] Even when the IOP was reduced following the elimination

Received: February 27, 2007 / Accepted: June 7, 2007
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of a relative pupillary block, 24%–72% of cases have been reported to show a recurrent IOP rise occurring months to years later, mainly due to extensive synechial angle closure.[6–12] These observations led us to reconsider the value of the classical treatment. In the present study, we investigated the therapeutic outcomes of the classical treatment in a Japanese population in order to reassess the long-term efficacy of this treatment. We also sought to identify the risk factors that indicate a need for surgical procedures to reduce IOP with the goal of identifying new therapeutic approaches for APAC or APACG.

Materials and Methods

We retrospectively reviewed the clinical records of all patients with APAC or APACG who were treated in our department at the Gifu University Graduate School of Medicine between February 1992 and December 2003, and summarized their clinical course after laser and/or surgical intervention. The study was approved by the Institutional Review Board of the Gifu University Graduate School of Medicine. This study included 66 eyes of 57 patients observed for at least 3 months, selected from the 83 consecutive eyes with APAC or APACG treated at our hospital during the above period. Eyes with any other secondary causative factors for developing angle closure, such as aqueous misdirection syndrome, uveitis, and lens-induced glaucoma, were excluded.

The initial ocular diagnostic examinations included visual acuity measurement, slit-lamp examination, measurement of corneal endothelial cell density, IOP measurement with Goldmann applanation tonometry, funduscopy, and the evaluation of angle structure and width by a Goldmann two-mirror gonioscopic lens. In cases of suspected plateau iris or other ocular abnormalities, ultrasound biomicroscopy was also performed.

The diagnosis and definition of APAC or APACG was made based on the criteria of Foster et al.[13] Briefly, APAC was defined as the condition of an eye, in the absence of glaucomatous optic neuropathy (GON), in a patient who had at least two of the following symptoms: ocular or periocular pain, nausea or vomiting, past episodes of intermittent blurring of vision with halos, presenting IOP of more than 21 mmHg, and at least three of the conjunctival hyperemia, corneal epithelial edema, mid-dilated nonreactive pupil, shallow anterior chamber, and the presence of occludable angle with or without peripheral anterior synechiae (PAS). Similarly, APACG was classified as APAC with GON.

Immediately after the diagnosis, all patients underwent the following definitive initial medical management: topical applications of 2% pilocarpine 3–4 times every 5 min and a beta-blocker, and unless contraindicated systemically, intravenous administration of 500 ml of 20% mannitol and 500 mg acetazolamide. In addition, dexamethasone was given topically to inhibit any anterior chamber reaction. After the initial medical therapy, the ocular condition was reexamined. Subsequently, laser peripheral iridotomy (LPI) and/or laser iridoplasty was performed. If severe corneal epithelial edema or a decrease of corneal endothelial cell density was noted, surgical peripheral iridectomy to halt the acute attack was performed. When IOP control was poor under maximum tolerable ocular hypotensive agents (IOP > 30 mmHg), trabeculectomy (TLE) with adjunctive mitomycin C (MMC) was undertaken. Conventional trabeculectomy was performed with a limbal-based flap of the conjunctiva and Tenon's capsule. A half-thickness 4 mm × 4 mm scleral flap was dissected to clear the cornea. Thereafter, 0.04% MMC was instilled intraoperatively, with an exposure time of 5 min, followed by washing with 250 ml of saline solution. After excision of the trabeculum, a peripheral iridectomy was performed. The scleral flap and conjunctiva were sutured firmly with 10-0 nylon.

The IOP data were analyzed by using the Kaplan-Meier life table method to calculate the cumulative probability of successfully controlled IOP, defined as an IOP of <21 mmHg with or without ocular hypotensive agents. Poorly controlled IOP was considered to have reached its end point when the IOP was found to be ≥21 mmHg during three consecutive clinical examinations. When comparing the demographic data between trabeculectomized and nontrabeculectomized eyes, we used both eyes except in cases when the acute attacks affected both eyes simultaneously. Data were analyzed using an unpaired *t* test, Mann-Whitney *U* test, and Fisher's exact probability test. For the multivariate analysis, which was used to evaluate the independence of prognostic factors, we randomly selected one of the eyes in bilateral cases and employed a Cox proportional hazards regression analysis. A *P* value of less than 0.05 was considered to be statistically significant. All statistical analyses were performed with STATA software version 8.2 (Stata, College Station, TX, USA).

Results

Table 1 shows the demographic data for all of the patients. At presentation, mean patient age was 68.4 ± 8.4 years (range, 50–85 years). There were 48 women and nine men. In five patients, the acute attacks affected both eyes simultaneously. APAC had developed in the fellow eye of five patients before the prophylactic LPI. Although two eyes had received previous prophylactic LPI, an acute IOP rise occurred in these eyes as a result of occluded iridotomies. The mean IOP during the attack in the affected eye was 54.2 ± 11.2 mmHg (range, 34 to >80 mmHg). The mean duration of symptoms before the appropriate treatment was 2.6 days (range, 0–23 days). The extent of PAS, which was identified with the aid of an indentation gonioscopic lens after the cessation of the acute attack, varied between 0.0 and 12.0 h. Ten eyes were judged to have signs of preexisting chronic angle-closure glaucoma (APACG) if the cup-to-disc ratio was greater than 0.8. The mean follow-up period was 42.6 ± 31.9 months (range, 4–147 months). Immediately after the initial medical therapy, the IOP

Table 1. Patient background

Sex (male/female)	11 eyes, 9 patients/55 eyes, 48 patients
Age (years)	68.4 ± 8.4 (50–85)
IOP during the attack (mmHg)	54.2 ± 11.2 (34 to >80)
Duration of symptoms (days)	2.6 ± 4.5 (0–23)
APAC/APACG	56 eyes/10 eyes
Extent of PAS (clock hours)	5.3 ± 4.6 (0.0–12.0)
Duration of follow-up (months)	42.0 ± 32.1 (4–147)

Values are means ± SD (range)
IOP, intraocular pressure; PAS, peripheral anterior synechiae; APAC, acute primary angle closure; APACG, acute primary angle-closure glaucoma.

Table 2. Postoperative complications after laser peripheral iridotomy

Complication	No. of cases (%) $n = 62$
Bullous keratopathy[a]	3 (4.8)
Corneal burn	1 (1.6)
Hyphema	1 (1.6)

[a]Two eyes received both laser peripheral iridotomy and trabeculectomy.

Table 3. Postoperative complications after trabeculectomy

Complication	No. of cases (%) $n = 10$
Shallow anterior chamber	3 (30.0)
Choroidal detachment	1 (10.0)
Hyphema	2 (20.0)
Leaking bleb	2 (20.0)
Retinal hemorrhages	3 (30.0)
Bullous keratopathy[a]	2 (20.0)

[a]Two eyes received both laser peripheral iridotomy and trabeculectomy.

decreased to 22.0 ± 13.5 mmHg (range, 3–56 mmHg). During the acute attack, one eye developed anterior ischemic optic neuropathy, while nonischemic central retinal vein occlusion was seen in two eyes.

For further analysis regarding the therapeutic outcome, one eye was excluded because it had no light perception in the initial ocular examinations, and we recommended no further aggressive laser or surgical management in this case. Thereafter, 58 eyes received only LPI, four eyes received LPI and laser gonioplasty, and three eyes received surgical peripheral iridectomy (PI) due to hazy media or decreased corneal endothelial cell density. Laser gonioplasty was employed in three eyes to halt the acute attack, and in one eye with unrecognized plateau iris during an attack. Of the 66 eyes studied, ten eventually required filtering surgery with intraoperative use of MMC for IOP control.

As shown in Table 2, laser peripheral iridotomy caused some complications, such as corneal burn and transient hyphema. In addition, incomplete iridotomy was found in six eyes (9.7%). On the other hand, further postoperative complications after trabeculectomy were noted in some cases. Transient shallow anterior chamber was found in three patients; choroidal detachment in one patient; retinal hemorrhages, presumably caused by drastic IOP reduction, in three patients; transient hyphema in two patients; and leaking bleb several years later in one patient (Table 3). The bullous keratopathy that developed in two eyes was treated by both LPI and TLE. Because of the very short periods between LPI and TLE in these cases, we could not identify whether this complication was attributable to the LPI or TLE, or to acute IOP elevation during the attack. Of the ten trabeculectomized patients, one required additional combined trabeculectomy and cataract surgery, and one required bleb revision for IOP control.

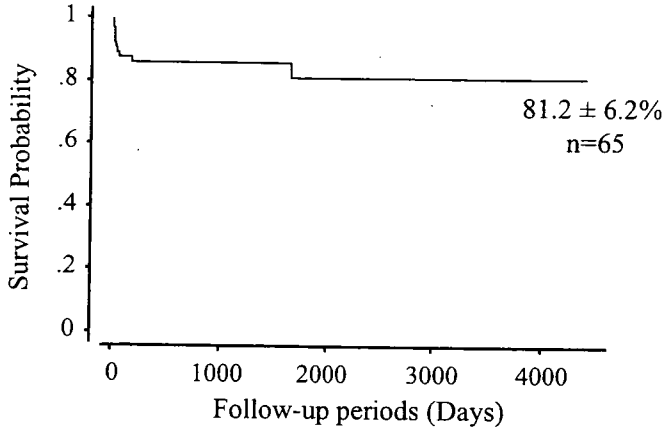

Figure 1. Kaplan-Meier curve showing the probability of success after laser therapy or surgical peripheral iridectomy. After laser therapy or surgical peripheral iridectomy, the probability of success, defined as an intraocular pressure of <21 mmHg with or without medication, was 81.2 ± 6.2%.

After laser therapy or surgical peripheral iridectomy, the probability of success, defined as an IOP of <21 mmHg with or without medications, was 81.2 ± 6.2% (Fig. 1). No further medical or surgical treatment was needed in 31 eyes (31/65, 47.7%), and only medication treatment was required in 24 eyes (24/65, 36.9%). Of the 24 eyes that received only further medical treatment, 17 received pilocarpine, 14 beta-blockers, 9 prostaglandin analogs, and 4 oral or topical carbonic anhydrase inhibitors. In the ten eyes (10/65, 15.4%) trabeculectomized owing to inadequate IOP control, the

Table 4. Comparison of patient backgrounds between trabeculectomized and nontrabeculectomized eyes

	Nontrabeculectomized	Trabeculectomized	*P* value
Sex (male/female)	8 eyes / 43 eyes	2 eyes / 8 eyes	0.6626
Age (years)	68.1 ± 8.4 (50–85)	69.8 ± 9.1 (52–82)	0.5726
IOP during the attack (mmHg)	54.4 ± 11.8 (34 to >80)	55.5 ± 7.1 (46–70)	0.7337
Duration of symptoms (days)	2.1 ± 3.7 (0–21)	4.4 ± 6.9 (0–23)	0.1435
APAC/APACG	47 eyes / 4 eyes	5 eyes / 5 eyes	0.0039
Extent of PAS (clock hours)	5.3 ± 4.6 (0.0–12.0)	10.1 ± 2.1 (8.0–12.0)	0.0007
Duration of follow-up (months)	41.8 ± 31.0 (4–147)	53.8 ± 41.2 (5–108)	0.4188

Values are means ± SD (range).
IOP, intraocular pressure; PAS, peripheral anterior synechiae; APAC, acute primary angle closure; APACG, acute primary angle-closure glaucoma.

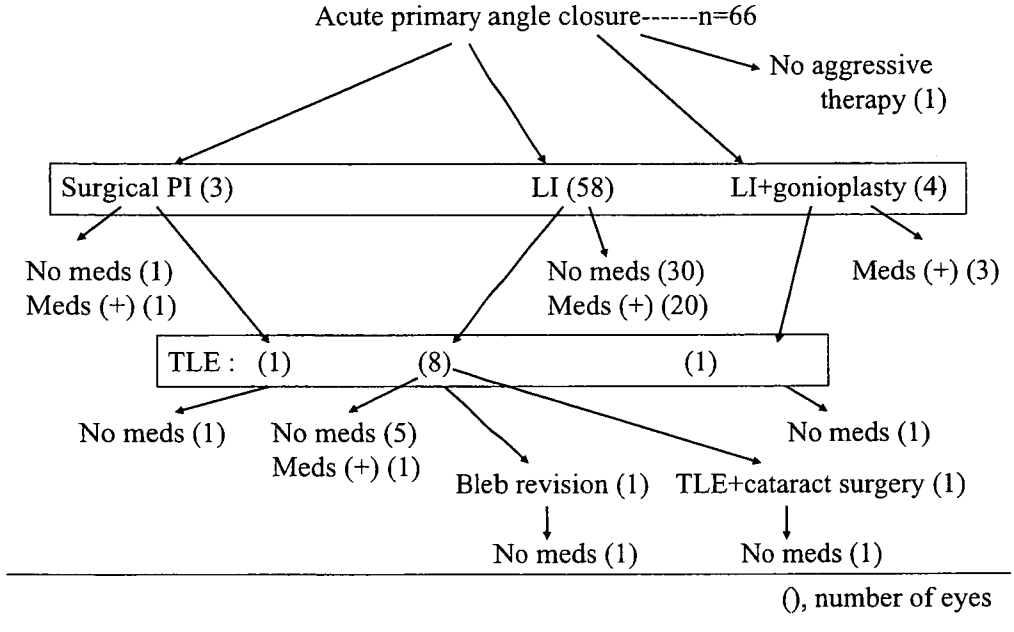

Figure 2. Flow chart of therapeutic outcome in eyes with acute primary angle closure. After laser intervention, no further medical or surgical treatment was needed in 31 eyes (31/65; 47.7%), and only topical treatment was required in 24 eyes (24/65; 36.9%). Ten eyes (10/65; 15.4%) were inevitably trabeculectomized due to inadequate control of intraocular pressure (IOP). Of these, one required additional combined trabeculectomy and cataract surgery, and one required bleb revision for IOP control. Number of eyes is shown in parentheses. *PI*, peripheral iridectomy; *LI*, laser iridotomy; *TLE*, trabeculectomy.

probability of success based on the same criterion was only 40.0 ± 29.7% (Figs. 2 and 3).

Differences in the extent of PAS and glaucoma type between the trabeculectomized and nontrabeculectomized eyes were statistically significant (Table 4). The Cox proportional hazards model revealed that the extent of PAS showed a significant hazard ratio with regard to the required trabeculectomy after LPI or surgical PI (hazard ratio: 1.366, $P = 0.029$; Table 5) and preexisting chronic glaucomatous optic neuropathy (hazard ratio: 6.474, $P = 0.015$; Table 5).

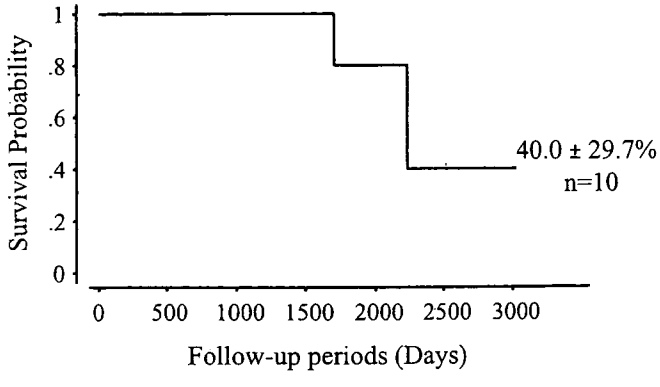

Figure 3. Kaplan-Meier curve showing the probability of success after trabeculectomy with mitomycin C. In the ten eyes trabeculectomized owing to uncontrolled IOP, the probability of success, defined as an IOP of <21 mmHg with or without medication, was only 40.0 ± 29.7%.

Discussion

The present findings demonstrated that, with the classical therapeutic modality, 84.8% of all APAC or APACG eyes could maintain good IOP control without a need for trabeculectomy, a finding apparently compatible with the reported results of the newly developed PPI procedure[4,5]

Table 5. Hazard ratios of potential risk factors for required filtering surgery after laser therapy

Risk factor	Hazard ratio	95% confidence interval	*P* value
Sex (male)	2.173	0.053–3.980	0.481
Age	1.018	0.932–1.112	0.695
IOP during the attack	1.007	0.925–1.096	0.878
Duration of symptoms	1.008	0.891–1.140	0.902
APAC/APACG (APACG)	6.474	0.035–0.691	0.015
Extent of PAS	1.366	1.033–1.806	0.029

Values are means ± SD (range).
IOP, intraocular pressure; PAS, peripheral anterior synechiae; APAC, acute primary angle closure; APACG, acute primary angle-closure glaucoma.

with respect to its IOP-reducing effect. We also found that the extent of PAS and preexisting chronic glaucomatous optic neuropathy were indicators of a risk of developing subsequent uncontrolled IOP after the elimination of the relative pupillary block.

Until recently, the nomenclature of primary angle closure was not uniform, and the literature contained little information about glaucomatous optic nerve damage in the many articles concerning primary angle closure. Recently, a new definition of angle closure has been proposed,[13,14] and is gradually being recognized worldwide. According to this definition, the term "glaucoma" is used only for cases with glaucomatous optic neuropathy and its corresponding visual field loss. In the present study, we retrospectively classified the eyes into two groups: those with APAC and those with APACG, on the basis of clinical records. However, in some cases we had difficulty determining whether a patient had preexisting glaucomatous optic nerve head or visual field abnormalities. The visual field following the acute attack usually shows a nonspecific change,[15,16] and often returns to normal after the attack ceases.[16] Additionally, hazy media or a swollen optic disc during the attack often leads to a misdiagnosis, especially when there are early glaucomatous optic disc changes such as localized optic nerve head excavation (notching) or slight generalized optic nerve head expansion.

In this series of Japanese subjects, we demonstrated that laser therapy alone could prevent a recurrent IOP rise in approximately 50% of APAC eyes. The remainder received further ocular hypotensive therapy to maintain an IOP of 21 mmHg or less, although a drainage procedure was required in 15.4% of eyes. Playfair and Watson[6] and Krupin et al.[7] reported that controlled IOP was achieved with surgical PI or LPI alone in 72% and 76% of eyes, respectively, and with antiglaucoma agents in 84% and 96% of eyes, respectively. In 137 APAC patients who underwent LPI or surgical PI as a primary treatment, Buckley et al.[17] showed that good IOP was maintained in 80 eyes (58.4%) that required no further medications, while 21 eyes (15.3%) ultimately required trabeculectomy. A recent study from the United Kingdom[18] found that no additional treatment after LPI was required in 44% of cases, and that further medical or surgical intervention was required in 21% and 35% of cases, respectively. On the other hand, Aung and associates[19] reported from Singapore that 58.1% of eyes with APAC developed an IOP rise after resolution of the acute attack, and that 32.7% eventually underwent filtering surgery because of poor IOP control. They suggested that the relatively high proportion of required trabeculectomy reflected racial differences in the outcome of LPI after APAC in Asian populations. The proportion of eyes requiring no further medical therapy after laser therapy was similar in our study, while the number requiring filtering surgery was much smaller in our study.

We speculate that the comparatively lower proportion of APAC eyes requiring trabeculectomy in the present study might reflect the use of a therapeutic approach including latanoprost and dorzolamide during the last decade in Japan. Indeed, two clinical trials have been reported regarding the efficacy of latanoprost in patients with chronic PACG.[20,21] Although the use of latanoprost may aggravate the inflammatory response in the anterior chamber,[22] it might be a therapeutic option for APAC, especially after complete cessation of an acute attack. In our consecutive series, of 24 eyes that had only further medication treatment following laser therapy or surgical PI, 13 eyes received latanoprost and/or dorzolamide.

Generally, APAC is recognized to be more severe in Asians than in Western populations,[23] possibly owing to the longer duration of symptoms,[24] the thickness and rigidity of the irides, or genetic and anatomic predisposition to angle closure.[24,25] In eyes with dark irides, so-called creeping angle closure is thought to be the most common type of angle closure.[26–28] Accordingly, the disease usually progresses asymptomatically, eventually resulting in circumferential permanent angle closure and persistent intraocular pressure elevation. However, in a previous biomicroscopy study of the fellow eyes of acute primary angle-closure patients in a Japanese series,[29] we showed that appositional angle closure beginning in the vicinity of Schwalbe's line is the exclusive predominant type rather than creeping angle closure. Multivariate analysis of the association of each factor with subsequent medically uncontrolled IOP after laser therapy identified the extent of organic angle closure and putative preexisting chronic glaucomatous optic neuropathy as potential risk factors. Therefore, one plausible

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reason for the lower incidence of cases that ultimately required filtering surgery in the present study might be the comparatively low appositional angle closure starting at the bottom of the chamber angle in Japanese subjects.

It is well recognized that performing surgery on a congestive eye is far more hazardous than surgery on a quiet eye. In addition, there is a high risk of failure associated with any ocular surgery on inflamed eyes with increased IOP. Success in IOP control with or without ocular hypotensive agents after trabeculectomy was reported in 21 patients (65.6%) with medically failed APAC.[30] This result is consistent with the present findings, although fewer of our patients required filtering surgery. However, of the ten trabeculectomized eyes, two (20%) developed corneal decompensation several years postoperatively. Furthermore, unilateral cataract progression presumably due to the LPI or drainage procedure occurred in five cases, with one of these requiring cataract extraction. On the other hand, the recently introduced PPI procedure for APAC have been reported to result in some intraoperative or early postoperative complications, including iris prolapse, vitreous loss, and IOP elevation to >25 mmHg.[5] Additionally, in one study, of 43 APAC eyes that underwent PPI, five (11.5%) required subsequent surgical intervention to lower IOP.[5] In the present study, we showed that the degree of PAS and the putative preexistence of chronic glaucomatous optic neuropathy were significant indicators of poor IOP control. Therefore, it may be crucial for clinicians to differentiate, prior to surgical intervention, between eyes predisposed to recurrent IOP increase and those that are not.

Our present study has many limitations, including small sample size, a retrospective study design, and the variability of follow-up periods. The initial treatment was performed in a definitive manner; however, in several cases a beta-blocker and hyperosmotic agents had not been used because of the patient's systemic condition. The meaning of the term "maximum tolerable ocular hypotensive agents" has been changing yearly as new drugs have become available in Japan. Additionally, during follow-up after the cessation of acute attack, patients had received various topical ocular hypotensive medications. Moreover, although we conducted ultrasound biomicroscopy in cases of suspected plateau iris based on conventional gonioscopy, we did not use ultrasound biomicroscopy in all PAC cases. Regrettably, the follow-up period varied (range, 4–147 months) because of the tendency for APAC patients to be older and the retrospective nature of this study.

Nevertheless, with the classical treatment for APAC or APACG eyes in a Japanese population, 84.6% of eyes could maintain good IOP control with antiglaucoma drugs or no medications, and without the intervention of filtering surgery. Additionally, we showed that the outcome in these cases could be predicted on the basis of putative preexisting chronic glaucomatous optic neuropathy and the extent of permanent angle closure. A prospective large-scale intensive investigation is required to identify a treatment that will improve the prognosis and reduce post- and intraoperative complications in cases of acute APAC.

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A Pilot Study to Detect Glaucoma With Confocal Scanning Laser Ophthalmoscopy Compared With Nonmydriatic Stereoscopic Photography in a Community Health Screening

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Purpose: To assess the efficacy and practical usefulness of the Heidelberg Retina Tomograph II (HRT II) compared with nonmydriatic stereoscopic photography in a public glaucoma screening.

Methods: We examined 1173 local residents, aged 40 years or older, who visited a community health screening in Komatsu City. Initial glaucoma screening consisted of noncontact pneumotonometry, nonmydriatic stereoscopic fundus photography, and HRT II. When glaucoma was suspected, the subjects were referred for a definitive examination, in which slit-lamp biomicroscopic examination, Goldmann applanation tonometry, Humphrey 30-2 test, gonioscopy, and optic nerve head evaluation were performed.

Results: A total of 97.2% (2279/2345) of the nonmydriatic stereoscopic optic disc photographs could be interpreted and 93.4% (2189/2345) were good images. HRT II measurements were successful in 99.0% (2322/2345) of eyes, and acceptable images were obtained in 91.9% (2154/2345) of eyes. On the basis of clinical diagnoses, 94 eyes of 60 participants were diagnosed with glaucoma. The sensitivity of nonmydriatic stereoscopic photographs for personal-level analysis and eye-level analysis was 95.8% and 95.5%, respectively. Using Moorfield's regression analysis, HRT sensitivity and specificity were 72.3% to 91.5% and 84.0% to 93.1%, respectively, for personal-level analysis, and 60.3% to 72.6% and 89.7% to 95.6%, respectively, for eye-level analysis.

Conclusion: Although HRT II did not detect glaucoma as well as optic nerve stereophotographs in this Japanese population, it may play a role in community health screening.

Key Words: Heidelberg Retina Tomograph II, nonmydriatic stereoscopic photography, public glaucoma screening

(*J Glaucoma* 2007;16:531–538)

Received for publication July 19, 2006; accepted February 11, 2007.

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No author of this manuscript had a proprietary or financial interest in it.

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More than 50% of glaucoma patients remain undiagnosed in the United States[1] and Europe.[2] Recently, a Japanese population screening study[3] revealed that the prevalence of primary open-angle glaucoma (POAG) in the Japanese population older than 40 years was estimated to be 3.9%, of which 93.3% of POAG patients were previously undiagnosed. Therefore, a mass screening method for glaucoma is urgently needed. Three-dimensional evaluation of the optic disc by glaucoma specialists is thought to be the ideal method but subjective techniques are routinely used to evaluate the optic disc and retinal nerve fiber layer, and test results clearly depend on the examiner's experience. Consequently, it would be useful to introduce objective quantitative measurements. Recently, a number of new devices have been developed for this purpose,[4–7] including the Heidelberg Retina Tomograph II (HRT II; Heidelberg Instruments, Heidelberg, Germany), a confocal scanning laser ophthalmoscope that allows a rapid 3-dimensional topographic analysis of the optic disc and retina without pupillary dilatation.

The purpose of this study was to investigate the efficacy and practical usefulness of the HRT II compared with nonmydriatic stereoscopic photography in a public glaucoma screening.

SUBJECTS AND METHODS

Study Population

This study was approved by the Ethical Committee of Kanazawa University Graduate School of Medical Science. Subjects were recruited at an annual community health screening project in Komatsu City, Japan, conducted from June 2003 to July 2003. To be eligible to participate in the health checkup, a citizen must be older than 40 years and without an opportunity to receive a health checkup at an office. At the checkup, we posted a bill about glaucoma screening at the building entrance and arranged to use a venue nearby. Three thousand subjects participated in the community health checkup, of which 1173 subjects participated in the glaucoma screening. Written informed consent was obtained from all participants in the study.

Initial Screening Examination

The screening methods used in this study were implemented in 2 stages. After obtaining informed consent, an initial screening was performed, which included recording of personal data (sex, age, personal and family histories of glaucoma, systemic and local disease, and subjective ocular symptoms), autorefractometry, tonometry, digital nonmydriatic stereoscopic photography, and HRT II.

Intraocular pressure (IOP) was measured using noncontact pneumotonometry (NCT) (Canon, TX-F, Tokyo, Japan). Refractive status was measured using an autorefractometer (Nidek, ARK-730A, Gamagori, Japan). Nonsimultaneous 30-degree-field stereoscopic digital color images of optic nerve heads were obtained without pupillary dilatation using the IMAGEnet digital fundus camera system (Topcon, NSW6S, Tokyo, Japan). Two sequential photographs of each eye were taken with a lateral shift in camera position to obtain a stereo effect. All participants underwent imaging (scanning field, 15×15 degrees) with an HRT II. All images were obtained by 1 of 2 trained technicians. Magnification errors were corrected using patients' corneal curvature measurements. The contour line of the optic disc edge was drawn by consensus between 2 of the authors (S.O., H.T.) while viewing stereoscopic photographs of the optic disc.

Evaluation of Optic Nerve Head

The optic nerve was evaluated from nonsimultaneous stereoscopic digital photographs of the optic disc using a 3-dimensioanl Viewer System (Topcon, 3D Viewer and Stereo Viewer System, Tokyo, Japan), which consisted of electronic shutter glasses (StereoGraphics Corp, Crystal Eyes 3, San Rafael, CA), an emitter (StereoGraphics Corp, E-2), and a 19-inch flat screen monitor (Nanao, T766, Matto, Japan). The photographic quality was classified as "good quality," "poor quality," or "not available." Poor quality was when obtaining a stereo view failed or was poor, but still assessable. The subjects with poor quality stereoscopic photographs were referred for a definitive examination except those were judged to be obviously normal by monoscopic fundus photography.

HRT II Confocal Scanning Laser Ophthalmoscopy

For every participant, 3 topographic images were obtained, combined, and automatically aligned to generate one mean topographic image for analysis. Moorfield's regression analysis (MRA),[5] incorporated in the HRT II software (version 1.6), was applied for this screening. The imaging quality of HRT II was classified as "acceptable (topographic standard deviation $\leq 50\,\mu m$)," "unacceptable (topographic standard deviation $> 50\,\mu m$)," or "not available."

Definitive Examination

A definitive examination was performed, masked to the initial screening results, when the subject was suspected to have glaucoma. The criteria for definitive examination eligibility are summarized in Table 1. When at least one finding suggested the presence of glaucoma, the subjects were recruited for definitive examination.

In the definitive examination, slit-lamp biomicroscopic examination, Goldmann applanation tonometry, visual field test with a Humphrey Field Analyzer II 30-2 SITA Standard program (Carl Zeiss Meditec Inc, Dublin, CA) and gonioscopy using a 4-mirror gonioscope (Menicon, PG-410, Nagoya, Japan) were performed. The pupil was dilated with 0.5% tropicamide and 0.5% phenylephrine hydrochloride and fundus examination was carried out with a slit-lamp and super field NC lens (Volk Optical Inc, Mentor, OH) or a 4-mirror gonioscope. Patients with occludable angles (20 subjects), or with driving responsibilities after the examination (3 subjects), were not dilated for this examination.

Evaluation of Visual Field

Two examiners evaluated the test results from the Humphrey Field Analyzer. We initially set the reliability criteria at fixation loss, $\leq 33\%$ and false-positive and false-negative, $\leq 20\%$. However, only 73.7% (370 eyes) of the visual field data collected in the definitive examinations (502 eyes of 251 subjects) passed these criteria, so we applied the criteria in accordance with a recent Japanese population study (fixation loss, $> 50\%$; false-positive and false-negative, $> 50\%$).[3] Fifty-seven eyes (11.4%) failed these criteria. The criteria for abnormal visual fields were based on a recent Japanese population study.[3] Briefly, abnormal visual field data were defined by the presence of at least one abnormal hemifield, which was determined by the criteria proposed by Anderson and Patella.[8] A hemifield was judged to be abnormal when the pattern deviation probability plot showed a cluster of 3 or more nonedge contiguous points, having sensitivity with a probability of less than 5% in the upper or lower hemifield, and in one of these, a probability of less than 1%.

Diagnosis of Glaucoma

A glaucoma specialist (K.S.) determined the final diagnosis on the basis of optic disc appearance, including

TABLE 1. The Criteria for Definitive Examination Eligibility

(1) IOP of 21 mm Hg or higher in either eye
(2) Any findings regarding the presence of abnormality including glaucomatous change [one or more of the following existed: the vertical cup/disc ratio of the optic nerve head was more than or equal to 0.6, the rim width at the superior portion (11-1 h), or inferior portion (5-7 h) was less than or equal to 0.2 of disc diameter, the difference in the vertical cup/disc ratio was more than or equal to 0.2 between both eyes, or a nerve fiber layer defect or splinter disc hemorrhage was found] as seen in stereoscopic fundus photographs
(3) A "borderline" or "outside normal limits" classification as detected by HRT II
(4) Failure to take stereoscopic fundus photographs or obtain HRT II images

nerve fiber layer defects, results of Humphrey Field Analyzer examinations, and clinical findings that were obtained through initial screening and definitive examinations. The criteria for glaucoma diagnosis were based on previous population studies.[3,9] Category 1 glaucoma was diagnosed when the vertical cup/disc ratio of the optic nerve head was more than or equal to 0.7, or the rim width at the superior portion (11 to 1 h) or inferior portion (5 to 7 h) was less than or equal to 0.1 of disc diameter, or the difference in vertical cup/disc ratio was more than or equal to 0.2 between both eyes, or a nerve fiber layer defect was found, and the hemifield-based visual field abnormality was compatible with optic disc appearance or nerve fiber layer defect. When the visual field test result was not reliable or available, category 2 glaucoma was diagnosed. This was where the vertical cup/disc ratio of the optic nerve head was more than or equal to 0.9, or the rim width at the superior portion (11 to 1 h) or inferior portion (5 to 7 h) was less than or equal to 0.05, or the difference in vertical cup/disc ratio was more than or equal to 0.3 between both eyes. Suspect glaucoma was diagnosed when the cup/disc ratio of the eye was 0.7 or more but less than 0.9, or the rim width at the superior portion (11 to 1 h) or inferior portion (5 to 7 h) was 0.1 or less but more than 0.05 of the disc diameter, or the difference in vertical cup/disc ratio was 0.2 or more but less than 0.3 between both eyes, or a nerve fiber layer defect was found, and the visual field test was not reliable or available or did not show a compatible hemifield-based defect. In definitive diagnosis, anomalous discs, including tilted discs and superior segmental optic hypoplasia, were carefully excluded. The glaucoma status of each person was classified on the basis of the more affected eye.

Main Outcome Measures

To evaluate the practical usefulness of HRT II and nonmydriatic stereoscopic photography for mass screening, the imaging status of each instrument for each diagnostic category and by age were calculated. The grouping was performed for the HRT II examination results based on the MRA. "Borderline" outcomes were treated as test positive (only normal was test negative, MRA 1) and borderline outcomes were treated as test negative (normal, borderline results were considered test negative, MRA 2). After these groupings, sensitivities, specificities, positive predictive value and negative predictive value for participants (person-level analysis) and for individual eyes (eye-level analysis), were calculated between the MRA diagnosis of HRT II and clinical diagnosis gold standards. Sensitivities of stereoscopic photographs for participants and for individual eyes were also calculated. Data from unacceptable topographic images, unacceptable photographs, and subjects who did not undergo definitive examinations were excluded from this data analysis.

Data Analysis

The privacy of personal medical information was protected at the data analysis center of Kanazawa University Graduate School of Medical Science. Statistical analysis was performed using StatMate III software (ATMS, Tokyo, Japan). Differences between groups were evaluated using Student t test. The χ^2 test was used to compare proportions.

RESULTS

The average age of all participants (435 male, 738 female) was 61.6 ± 10.5 years (mean ± standard deviation). Of the 1173 subjects (2345 eyes) examined in the initial screening, 296 (25.2%) were referred for definitive examination, but 45 declined or were unable to participate. As a result, 251 subjects (84.8%) underwent definitive examination. On the basis of the clinical diagnosis, 94 eyes of 60 participants were diagnosed with definite glaucoma and 41 eyes of 29 participants were diagnosed with suspect glaucoma. After the definitive examination, diagnoses of 6 subjects were changed. One subject diagnosed with glaucoma was changed to superior segmental optic hypoplasia and 1 subject diagnosed with glaucoma was converted to suspect glaucoma. In addition, 4 subjects with suspect glaucoma were subsequently diagnosed as normal.

The rate of previously undiagnosed glaucoma was 90.0% (54/60). The number of POAG subjects was 58. Of these, 55 subjects were diagnosed with category 1 criteria, and 3 were diagnosed with category 2 criteria. Of the 58 POAG subjects, 50 (86.2%) had an IOP of 21 mm Hg or less without medication (both in initial screening and definitive examination in both eyes) and 8 had an IOP of more than 21 mm Hg. No subjects were found to have primary angle-closure glaucoma. There were 2 secondary glaucoma subjects who were diagnosed with category 1 and exfoliation glaucoma. The average age of all

TABLE 2. Prevalence Rate of Definitive Glaucoma in the Screened Population

	Glaucoma (%; 95% Confidence Interval)		
Age Groups (y)	Male	Female	All
40-49	0/46 (0%, 0-0)	3/113 (2.7%, −0.3-5.7)	3/159 (1.9%, −0.2-4.0)
50-59	2/91 (2.2%, −0.8-5.2)	5/201 (2.5%, 0.3-4.7)	7/292 (2.4%, 0.6-4.2)
60-69	8/174 (4.6%, 1.5-7.7)	10/236 (4.2%, 1.6-6.8)	18/410 (4.4%, 2.4-6.4)
70 and older	13/103 (12.6%, 6.2-19.0)	19/164 (11.6%, 6.7-16.5)	32/267 (12.0%, 8.1-15.9)
All subjects	23/414 (5.6%, 3.4-7.8)	37/714 (5.2%, 3.6-6.8)	60/1128 (5.3%, 4.0-6.6)

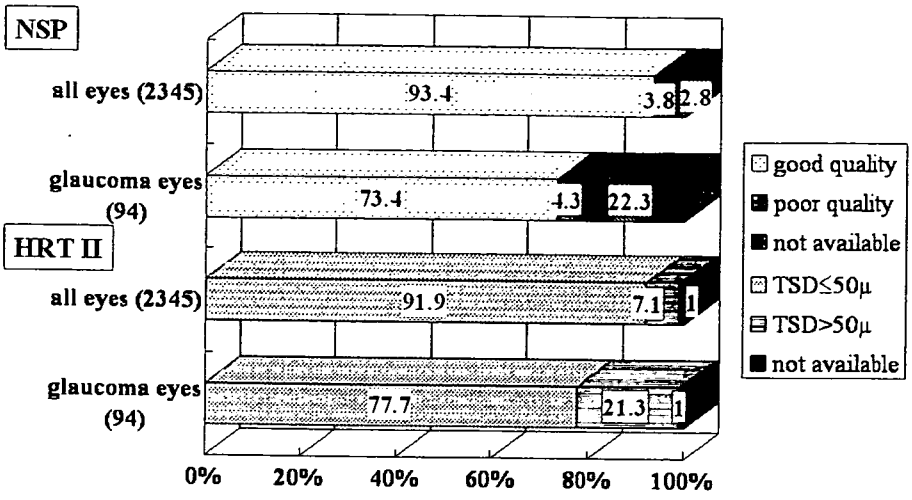

FIGURE 1. Comparison of images acquired by nonmydriatic stereoscopic photography and HRT II for diagnosis. NSP indicates nonmydriatic stereoscopic photography; TSD, topographic standard deviation.

glaucoma patients was 69.1 ± 10.2 years (mean ± standard deviation). For the 45 subjects who did not undergo definitive examination, diagnosis was based on the initial screening and 10 subjects met the criteria of category 2. However, these 45 subjects were excluded from the data analysis. Table 2 shows the age-specific prevalence of glaucoma in this screened population. The estimated prevalence of POAG and exfoliation glaucoma was 5.1% (58/1128) (95% confidence interval, 3.8%-6.4%) and 0.2% (2/1128) (95% confidence interval, −0.1%-0.5%), respectively.

The imaging status of each instrument for each diagnostic category and by age is shown in Figure 1 and Table 3, respectively. The distribution of the quality of HRT II images obtained is shown in Figure 2. Figure 3 is a Venn diagram that summarizes the number of eyes that could not be interpreted by stereoscopic photography, those eyes that could not be scanned by HRT II, and both. Of the 66 eyes that could not be interpreted by stereoscopic optic disc photography, glaucoma was diagnosed in 21 eyes (31.8%). In the 23 eyes that could not be scanned by HRT II, glaucoma was diagnosed in 1 eye (4.3%). Figure 4 is a Venn diagram to show which eyes had poor quality stereoscopic photography, or unacceptable quality (above 50 μm) HRT II, and both.

We reviewed the methods for detecting definitive glaucoma. When subjects with not available HRT II scans and not available stereoscopic photographs were considered along with subjects defined as test positive, glaucoma was detected in 58 subjects (58/60, 96.7%) by nonmydriatic stereoscopic photography, in 52 subjects (52/60, 86.7%) by HRT II imaging and in 2 subjects (2/60, 3.3%) by elevated IOPs. When the subjects with not available HRT II scans and not available nonsimultaneous stereoscopic photographs were excluded, glaucoma was detected in 46 subjects (46/48, 95.8%) by nonmydriatic stereoscopic photography and in 52 subjects (52/60, 86.7%) by HRT II imaging. When the subjects with poor quality nonsimultaneous stereoscopic photographs and unacceptable HRT II scans were excluded, glaucoma was detected in 46 subjects (46/48, 95.8%) by nonmydriatic stereoscopic photography and in 43 subjects (43/47, 91.5%) by HRT II imaging.

The sensitivity, specificity, positive predictive value, and negative predictive value of HRT II are shown in Table 4. The sensitivity of nonmydriatic stereoscopic photography for personal-level analysis was 95.8% and that for eye-level analysis was 95.5%. Although the sensitivity for eye-level analysis of nonmydriatic stereoscopic fundus photography was significantly higher than that of HRT II (both MRA 1 and MRA 2, $P < 0.001$), the sensitivity for personal-level analysis was not significantly higher than that of HRT II when borderline outcomes were treated as test positive ($P = 0.654$).

TABLE 3. The Image Status of Each Instrument by Age

	NSP			HRT II		
Age Groups (y)	Good Image Eyes	Poor Image Eyes	Not Available Eyes	TSD ≤ 50 μm	TSD ≥ 50 μm	Not Available Eyes
40-49	321 (99.7%)	0 (0%)	1 (0.3%)	310 (96.3%)	11 (3.4%)	1 (0.3%)
50-59	577 (95.2%)	20 (3.3%)	9 (1.5%)	585 (96.5%)	16 (2.7%)	5 (0.8%)
60-69	785 (92.4%)	46 (5.4%)	19 (2.2%)	785 (92.4%)	62 (7.2%)	3 (0.4%)
70 and older	506 (89.3%)	24 (4.2%)	37 (6.5%)	474 (83.6%)	79 (13.9%)	14 (2.5%)
All subjects	2189 (93.4%)	903 (3.8%)	66 (2.8%)	2154 (91.9%)	168 (7.2%)	23 (0.9%)

NSP indicates nonmydriatic stereoscopic photography; TSD, topographic standard deviation.

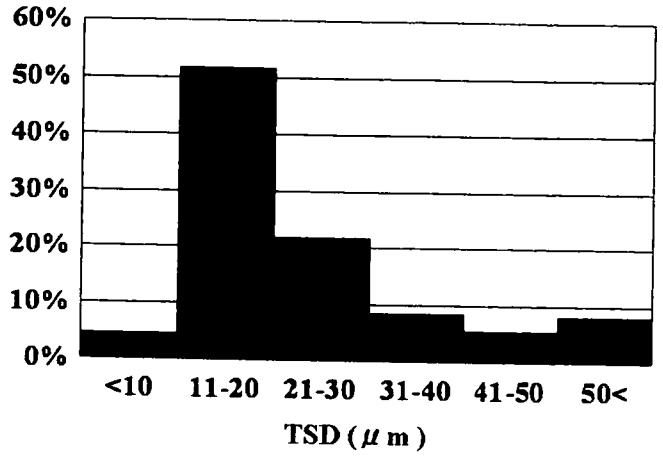

FIGURE 2. Distribution of topographic standard deviation (TSD) of HRT II in both eyes of all the subjects who participated.

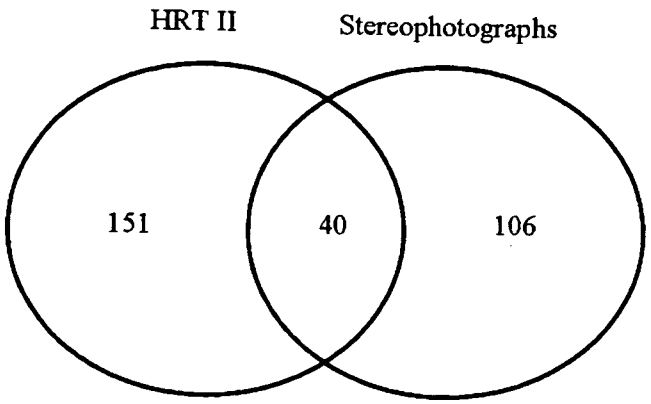

FIGURE 4. Venn diagram showing the number of eyes which had poor quality stereoscopic photography, or unacceptable quality (above 50 μm) HRT II, or both.

DISCUSSION

The fact that most glaucoma is undiagnosed[1–3] may partly be attributed to the lack of an ideal screening method. A mass glaucoma screening method that is safe, specific, sensitive, and acceptable to participants is needed. Recently, frequency-doubling technology (FDT) perimetry was shown to be an effective method for glaucoma screening,[10,11] but evaluating the optic disc or nerve fiber layer directly may be a preferable method to eliminate variability due to subjective judgment. Several novel digital imaging technologies[4–7] have been developed for the structural investigation of the optic nerve head. Scanning laser tomography, optical coherence tomography, and scanning laser polarimetry discriminated between glaucomatous and normal eyes with clinically useful sensitivities and specificities in hospital-based studies.[4–7] In addition, Harasymowycz et al[12] reported that HRT II using MRA or cup shape measurements may be a valid screening tool to detect clinically diagnosed glaucomatous optic nerve damage in high-risk populations. It was reported that the measurement of optic disc parameters on stereoscopic fundus photographs was limited by difficulties in obtaining high-quality photographs on all subjects, even if the pupils are dilated.[13] Recently, the digital nonmydriatic fundus camera was improved, and Detry-Morel et al[14] reported that a total of 98.1% of optic disc photographs taken by the nonmydriatic fundus camera could be interpreted and therefore could be a useful and quick method to screen for glaucomatous damage in a community. Furthermore, recent improvement in the digital nonmydriatic fundus camera enables stereophotographs of the optic disc to be taken without pupillary dilatation. In the present study, we evaluated the efficacy of HRT II scanning using MRA compared with nonmydriatic stereoscopic fundus photography in a population-based glaucoma screening.

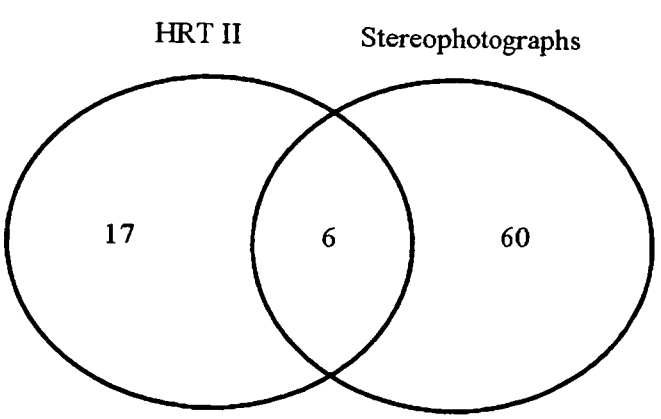

FIGURE 3. Venn diagram showing the number of eyes which could not be interpreted by stereoscopic photography, or which could not be scanned by HRT II, or both.

Nonmydriatic stereoscopic photographs and HRT II scans were successfully obtained in the large majority of eyes (97.2% and 99.0%, respectively). Good quality photographs (enabling good stereopsis) were obtained in 93.4% of eyes by a nonmydriatic stereoscopic camera. Acceptable images (topographic standard deviation no greater than 50 μm) were obtained in 91.9% of eyes by HRT II. These results were equal to HRT II in a population-based glaucoma screening for high-risk population (acceptable quality, 88%).[12] Therefore, both instruments were deemed acceptable for mass glaucoma screening.

HRT sensitivity and specificity were 72.6% and 89.7%, respectively, when borderline outcomes were treated as test positives (MRA 1) and 60.3% and 95.6%, respectively, when borderline outcomes were treated as test negatives (MRA 2) for eye-level analysis. These results were similar to those reported by Ford et al,[15] although the MRA of HRT II was based on the data from white subjects while our study population was Japanese. As Vitale et al[16] showed previously, at the

TABLE 4. Agreement Between HRT II-MRA and Clinical Diagnosis

	Sensitivity (95% Confidence Interval)	Specificity (95% Confidence Interval)	Positive Predictive Value (95% Confidence Interval)	Negative Predictive Value (95% Confidence Interval)
Person-level analysis (n = 977)				
MRA 1	43/47 (91.5%, 84.6-88.8)	781/930 (84.0%, 77.2-82.2)	43/192 (22.4%, 19.8-25.0)	781/785 (99.5%, 99.1-99.9)
MRA 2	34/47 (72.3%, 63.8-69.6)	866/930 (93.1%, 88.1-91.9)	34/98 (34.7%, 31.7-37.7)	866/879 (98.5%, 97.8-99.2)
Eye-level analysis (Right eye and left eye, n = 2053)				
MRA 1	53/73 (72.6%, 70.7-74.5)	1777/1980 (89.7%, 88.4-91.0)	53/256 (20.7%, 18.9-22.5)	1777/1980 (98.9%, 98.4-99.4)
MRA 2	44/73 (60.3%, 58.2-62.4)	1896/1980 (95.6%, 94.7-96.5)	44/128 (34.4%, 32.3-36.5)	1896/1980 (98.5%, 98.0-99.0)
Right eye (n = 1029)				
MRA 1	25/36 (69.4%, 66.6-72.2)	900/993 (90.6%, 88.4-92.4)	25/118 (21.2%, 18.7-23.7)	900/911 (98.8%, 98.1-99.5)
MRA 2	20/36 (55.6%, 52.6-58.6)	955/993 (96.2%, 95.0-97.4)	20/58 (34.5%, 31.6-37.4)	955/971 (98.4%, 97.6-99.2)
Left eye (n = 1024)				
MRA 1	28/37 (75.7%, 73.1-78.3)	877/987 (88.9%, 88.4-91.0)	28/138 (20.3%, 17.7-22.9)	877/886 (99.0%, 98.4-99.6)
MRA 2	24/37 (64.9%, 62.0-67.8)	941/987 (95.3%, 94.7-96.5)	24/70 (34.3%, 31.4-37.2)	941/954 (98.6%, 97.9-99.3)

MRA 1: "borderline" outcomes were treated as test positive; MRA 2: "borderline" outcomes were treated as test negative.

personal-level sensitivity increased and specificity decreased, relative to eye-level analysis. Indeed, in this study, HRT sensitivity increased without much impact on specificity when borderline outcomes were treated as test positives (MRA 1) at personal-level analysis. Although HRT was not sensitive enough for eye-level analysis, HRT might be useful for glaucoma screening for personal-level analysis.

Although the ratio of a good image obtained with nonmydriatic stereoscopy was 93.4% that was obtained for glaucoma was only 73.4%. By the same token, definite glaucoma was detected in 31.8% (21/66) of eyes in which stereoscopic photographs could not be interpreted. In other words, those eyes in which stereoscopic photographs could not be interpreted seemed to be a high-risk group for glaucoma. Conversely, of the 23 eyes for which HRT II imaging data could not be obtained, definite glaucoma was diagnosed in only one eye (4.3%). In addition, of the 199 eyes for which acceptable images could not be obtained, definite glaucoma was diagnosed in 21 eyes (21/199, 10.6%). Therefore, detection number by HRT II (52/60) exceeded the detection number by stereoscopic photography (46/48) as long as interpretable photographs or images were obtained.

Most subjects with POAG (92.3%) had an IOP below 21 mm Hg in the Tajimi study.[3] In our study, 86.2% (50/58) of all patients with POAG had an IOP below 21 mm Hg and a single measurement of IOP detected only 3.3% of all glaucoma. A recent Japanese population study[3] reported the mean IOP of a Japanese population to be 14.6 ± 2.7 mm Hg (standard deviation) in the right eye and 14.5 ± 2.7 mm Hg in the left eye. Therefore, our cut-off IOP value in this study might be not appropriate. If the cut-off IOP value was 17 or 19 mm Hg, the glaucoma detection rate using IOP was 18.3% (11/60) or 11.6% (7/60), respectively. Thus, our results are consistent with the conclusion of past reports that measurement of IOP alone is a poor tool to detect glaucoma.[14,17,18]

The estimated prevalence of POAG (5.1%) in this study appeared to be higher than that of a recent Japanese population-based glaucoma survey (3.9%).[3] This difference could be partly because our study population may have been biased by the use of volunteers, probable overrepresentation of subjects interested in glaucoma, and the older age of our subjects [61.6 ± 10.5 y (mean ± standard deviation)] compared with the participants in the Japanese survey [58.4 ± 11.8 y (mean ± standard deviation)].[3] Since the efficiency of screening methods was the main interest of our study, rather than estimation of the prevalence of glaucoma, a random sample of eligible participants was not requested.

In this study, there were several limitations owing to the population-based and large-scale study. First, we did not perform definitive examinations of participants whose eyes appeared normal in the initial screening examinations. Subjects with glaucoma may therefore have been overlooked. In a recent Japanese population study,[3] 4 out of 119 glaucoma patients were detected by FDT alone, suggesting that 3.4% (4/119) of glaucoma patients were overlooked by monoscopic fundus photography. In this study, we evaluated stereoscopic fundus photographs of optic discs to estimate vertical cup-to-disc ratio, which is reported to be more reliable than monoscopic evaluation.[19] However, we still cannot deny the possibility that we too overlooked a few glaucoma patients. Moreover, it is likely that obtaining a pair of images that enable a stereo view would fail, particularly in more elderly subjects.[13] In fact, in this study a total of 6.6% of the photographs did not enable a good stereo view, 10.7% of which were from subjects aged 70 years or older. Of 126 subjects where good quality photographs in at least one eye could not obtained, 71 subjects were judged to be normal by monoscopic fundus photography. The remaining 55 subjects were referred for a definitive examination and 14 were diagnosed as definite glaucoma. Therefore, it is unlikely that we overlooked considerable number of glaucoma.

Second, we could not perform definitive examinations on all subjects recruited for definitive examinations. However, there was no significant difference between the average age of subjects who had definitive examinations [61.4 ± 10.4 y (mean ± standard deviation)] and those who did not [66.1 ± 11.1 y (mean ± standard deviation)] ($P = 0.083$, Student t test). Also, the proportion of glaucoma patients in the group undergoing a definitive examination (23.9%, 60/251) was similar to that of the group that failed to attend the definitive examination (22.2%, 10/45) ($P = 0.807$, χ^2 test), in whom glaucoma was identified in the initial screening. Therefore, we think that this second limitation did not greatly influence the efficiency of HRT II in this screening.

Third, we performed visual field tests once and almost all the subjects were inexperienced in these tests. It was reported in an Ocular Hypertension Treatment Study[20] that most visual field abnormalities were not verifiedon retest so the reliability of the visual field test is limited. However, in definitive examinations we determined the final glaucoma diagnosis not only on visual field tests but also on disc appearance judged by a glaucoma specialist. Of the subjects classified as category 1, all fulfilled the rather conservative criteria of at least one abnormal hemifield and a compatible optic disc appearance, a nerve fiber layer defect, or both. Glaucoma subjects in category 2 may also include some preperimetric or suspected glaucoma patients even though more strict diagnostic criteria were applied to optic disc appearance.

This study showed that the use of nonmydriatic stereoscopic fundus photography and HRT II is a suitable approach for an initial glaucoma mass screening because images of both eyes can be rapidly acquired without the need for pupillary dilatation. This screening could conceivably be performed in the absence of a trained ophthalmologist and without pupillary dilatation. The evaluation of stereophotographs is subjective and test results clearly depend on the examiner's experience. In addition, good photographs were harder to obtain on glaucoma eyes than normal eyes, results that are similar to a Baltimore Eye Survey.[13] Compared with optic disc stereophotographs, HRT II provided objective and quantitative data and measurements were successful in almost all glaucomatous eyes. In this study, we defined regions of interest (ROIs) by drawing contour lines on stereoscopic photographs. However, if HRT II were to be used as a screening instrument, then disc photos would not be available. Watkins and Broadway[21] reported no difference in the defined ROI when 2 experienced observers defined ROIs from stereoscopic or nonstereoscopic optic disc photographs, or without any photographic guide. Therefore, contour lines may be drawn with or without a photographic aid, and the results are unaffected. However, in this study, we used stereoscopic optic disc photographs to draw contour lines to reduce dependency on operator's skill.

In this study, costs associated with recruiting eligible subjects or management of contact information were negligible because this study was performed as part of a community health screening project. Personnel costs were $8765 whereas direct costs were almost $2600. Since we detected 54 new definite glaucoma patients, the estimated cost of the initial screening per new case was approximately $210. However, this does not include medical equipment.

Although HRT II did not detect glaucoma as well as optic nerve stereophotographs in this Japanese population, it may play a role in community health screening. Furthermore, HRT II retains the potential to increase the detection rate of glaucoma in conjunction with other diagnostic techniques such as FDT.

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Association between Genetic Polymorphisms of the Prostaglandin $F_{2\alpha}$ Receptor Gene and Response to Latanoprost

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Purpose: To evaluate the relationship between polymorphisms of the prostaglandin $F_{2\alpha}$ receptor (FP receptor) gene and the effectiveness of topical latanoprost treatment in normal volunteers.

Design: Prospective nonrandomized trial.

Participants: One hundred normal volunteers were recruited into the study.

Methods: Baseline intraocular pressures (IOPs) of both eyes of 100 normal subjects were measured at 3 time points. Latanoprost (0.005%) was applied to one eye once daily for 7 days. Diurnal IOP was measured again on day 7. Response to latanoprost was evaluated by percent IOP reduction in the treated eye minus IOP fluctuations of the nontreated eye. We classified subjects by the mean diurnal percent IOP reduction (%ΔIOP) into 3 groups: low responders (%ΔIOP<10), medium responders (10≤%ΔIOP<25), and high responders (%ΔIOP≥25). Single-nucleotide polymorphisms (SNPs) in the FP receptor gene were searched, and the genotype was determined mainly by direct DNA sequencing. A promoter assay with a reporter luciferase gene was also performed.

Main Outcome Measures: Mean diurnal percent IOP reduction and genotyping of SNPs in the FP receptor gene.

Results: Ten SNPs were identified in this study. One, rs3753380, was located in the promoter region of the FP receptor gene and was significantly correlated with %ΔIOP (CC, 20.3%±1.5% [mean ± standard error]; CT + TT, 15.6%±1.2%; $P = 0.0316$). Mean diurnal percent IOP reduction was not associated with the other SNPs. When the category classified by %ΔIOP was analyzed, not only rs3753380 but also rs3766355, an SNP in intron 1, were associated with the degree of response to latanoprost. The promoter assay revealed that the C allele of rs3766355 and T allele of rs3753380 were found in constructs with lower transcriptional activity of the FP receptor gene.

Conclusions: rs3753380 and rs3766355, SNPs in the promoter and intron 1 regions of the FP receptor gene, correlate with a response to short-term latanoprost treatment in normal volunteers. The genotype of these SNPs may be an important determinant of variability in response to latanoprost. *Ophthalmology 2007;114:1039–1045*

Latanoprost, a prostaglandin $F_{2\alpha}$ analog, is widely used to lower intraocular pressure (IOP) in patients with glaucoma, although in some cases the response is unexpectedly weak. Although Aung et al[1] and Scherer[2] showed that some patients fail to respond to latanoprost treatment, it is currently unknown what is responsible for weak responses to latanoprost in different patients.

Originally received: August 4, 2006.
Accepted: March 12, 2007. Manuscript no. 2006-874.

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Presented at: Joint Meeting of the American Academy of Ophthalmology and Asia Pacific Academy of Ophthalmology, November 2006, Las Vegas, Nevada.

Financial support: Japan Society for the Promotion of Science, Tokyo, Japan (Grant-in-Aid for Scientific Research no. 17591826).

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Recently, genetic polymorphisms of β-adrenergic receptors,[3–6] angiotensin-converting enzyme,[7,8] and angiotensin II type 1 receptor[8,9] were reported to affect the degree of response to certain drugs. Schwartz et al[10] reported that a single-nucleotide polymorphism (SNP) at codon 389 in the β_1-adrenergic receptor gene correlated with the degree of response to betaxolol in normal volunteers.

Because latanoprost is a highly selective agonist for the prostaglandin $F_{2\alpha}$ receptor (FP receptor),[11] the reduction of IOP by latanoprost is considered to be mainly caused by the FP receptor. The FP receptor is a 359–amino acid protein containing 7 transmembrane domains characteristic of G protein–coupled receptors.[12,13] Later cDNA analysis showed an alternative splice variant, hFPs, which has a 71–base pair (bp) insert that causes a frame shift resulting in a truncated receptor lacking the seventh transmembrane domain and intracellular carboxyl tail.[14] The FP receptor is expressed in ocular tissues, such as corneal epithelium, ciliary epithelium, the circular portion of the ciliary muscle, and the stroma and smooth muscle cells of the iris.[15–17]

ISSN 0161-6420/07/$–see front matter
doi:10.1016/j.ophtha.2007.03.025

Activation of the FP receptor leads to G_q-mediated IP_3 generation and increases in intracellular calcium[12,18,19] and triggers an increase of matrix metalloproteinases.[20–22] Up-regulation of matrix metalloproteinases degrades the extracellular matrix in the ciliary muscle[23,24] and increases uveoscleral outflow of aqueous humor, resulting in a reduction of IOP.[11,25]

It was also shown that the FP receptor is essential for early IOP response to latanoprost in the mouse eye because latanoprost had no effect on IOP in homozygous FP receptor knockout mice.[26] Therefore, it is plausible that genetic polymorphisms of the FP receptor gene may influence the degree of response to latanoprost.

In this study, we examined whether IOP reduction by latanoprost was affected by SNPs of the FP receptor gene in healthy volunteers.

Materials and Methods

Subjects

The study protocol was approved by the institutional review board of Kanazawa University School of Medical Science and followed the guidelines of the Declaration of Helsinki. We recruited 100 Japanese volunteers without a medical history of eye diseases and obtained written informed consent from each (82 male, 18 female; mean age, 24.3±0.2 years (mean ± standard error [SE]).

Intraocular Pressure Measurement and Latanoprost Treatment

The schedule of examinations was as follows. At 9 AM, 1:30 PM, and 6 PM on day 0, baseline IOPs of both eyes were measured with a Goldmann applanation tonometer (Carl Zeiss, Inc., Jena, Germany). Central corneal thickness and refraction were measured by a pachymeter (DGH-500, DGH Technology, Inc., Exton, PA) and autorefractor/keratometer (ARK-2000, Nidek Co. Ltd., Aichi, Japan), respectively. At 9 AM, 0.005% latanoprost (Xalatan, Pfizer Japan Inc., Tokyo, Japan) was applied to one eye once daily for 7 days. The other eye served as a nontreated control. Diurnal IOP was measured again on day 7. The IOP was measured by the same investigator (MT) throughout this study.

Intraocular pressure reduction at each time point was expressed according to the formula percent IOP reduction = $([T\text{pre} - T\text{post}] - [C\text{pre} - C\text{post}])/T\text{pre} \times 100$, where T and C are the IOPs of treated and control eyes, respectively, and *pre* and *post* correspond to pretreatments and posttreatments on day 0 and day 7, respectively. Intraocular pressure reduction was shown as the mean diurnal percent IOP reduction (%ΔIOP). Based on %ΔIOP, subjects were classified as low responders (%ΔIOP<10), medium responders (10≤%ΔIOP<25), or high responders (%ΔIOP≥25). Conjunctival hyperemia in the treated eye was evaluated on day 7.

Screening and Genotyping of Single-Nucleotide Polymorphisms in the Prostaglandin $F_{2\alpha}$ Receptor Gene

Single-nucleotide polymorphisms, which were located in part of the promoter and coding regions including a newly identified exon of the FP receptor gene, were screened from genomic DNA from 24 randomly selected subjects. Known SNPs in the promoter, intron 1, and 3′-untranslated regions of the gene, reported in the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/SNP), were also selected.

Genomic DNA was extracted from peripheral leukocytes. The primers for polymerase chain reaction (PCR) amplification of the FP receptor gene were synthesized based on sequence information[12,14,27] (NCBI database, NT 026943 in Bild122). Polymerase chain reaction was performed using KOD -plus- (Toyobo Co., Ltd., Osaka, Japan) according to the manufacturer's protocol with some modifications; 500 ng of genomic DNA was used as a template, and concentrations of magnesium sulfate and primers were adjusted to 0.8 to 1 mmol/l and 0.4 μmol/l, respectively. The amplified fragments were electrophoresed on an agarose gel, recovered from the gel using SUPREC-01 (Takara Bio Inc., Shiga, Japan), and sequenced using a dye terminator cycle sequencing kit (DYEnamic ET Terminator, Amersham Biosciences Corp., Piscataway, NJ) and an automated DNA sequencer (ABI PRISM 377 DNA Sequencer, Applied Biosystems, Foster City, CA).

Genotyping of an SNP (rs3753380) in the promoter region of the FP receptor gene was performed by a method based on single-strand conformation polymorphism. After the PCR reaction, 1 μl of each PCR mixture was combined with 3 μl of loading buffer (98% formamide, 10 mmol/l ethylenediaminetetra-acetic acid, 0.3% bromophenol blue, and 0.3% xylene cyanol) and heated at 90° C for 5 minutes. The samples were electrophoresed on a nondenaturing 10% or 12% polyacrylamide gel containing 0.5 × TBE (45 mmol/l Tris, 45 mmol/l boric acid, 1 mmol/l ethylenediaminetetra-acetic acid, pH 8.5) buffer and 5% glycerol at 4° C. After electrophoresis, DNA was stained with SYBR Gold Nucleic Acid Gel Stain (Molecular Probes Inc., Eugene, OR) and visualized with an ultraviolet transilluminator.

The SNP at A938G (K313R) in exon 4 of the FP receptor gene was detected by restriction fragment length polymorphism. The amplified fragments (515 bp) were digested with Eco81I overnight at 37° C and electrophoresed on a 1.5% agarose gel. The fragments containing an A allele were not digested, whereas those containing a G allele were digested and generated 2 fragments, 228 bp and 287 bp.

Genotyping of the other SNPs was carried out by direct sequencing as described above.

Construction of the Prostaglandin $F_{2\alpha}$ Receptor–Luciferase Fusion Gene and Reporter Assay

The region from the promoter to exon 2 of the FP receptor gene was amplified from genomic DNA by PrimeSTARHS DNA polymerase (Takara Bio) using a set of primers, FPEcoRV2F (5′-GCTGATATCCTCAGAAATAACATCACACATC-3′) (−2416 to −2394) and FPHindIIIR (5′-GCGAAGCTTCTCAAAACTGT-GCAGGATTGCAG-3′) (+1632 to +1655).[27] The nucleotide position was counted from the transcription start site determined by Zaragoza et al.[27] However, the nucleotide number in this study differed from that in their data, because their DNA sequence had extra nucleotides compared with the NCBI database (NT 026943). The amplified fragment was digested with EcoRV and HindIII and inserted into the EcoRV and HindIII–digested Bluescript SK⁻. The nucleotide sequences of the cloned fragments were confirmed by DNA sequencing as above. The AccI (filled by T4 DNA polymerase)–HindIII fragment was inserted into the SmaI and HindIII–digested sites of the pGL3-Basic vector (Promega Corp., Madison, WI). Plasmids were purified by the Plasmid Midi Kit (Qiagen K. K., Tokyo, Japan) and quantified using a spectrophotometer.

HeLa cells (RIKEN Bio Resource Center, Ibaragi, Japan) were plated in each well of a 12-well culture plate (1.6×10^5 cells/well) and grown overnight in minimum essential medium containing 10% calf serum. Cells were co-transfected with 800 ng of the reporter plasmid; 16 ng of the pRL-TK vector (Promega), which served as an internal control; and 800 ng of Bluescript SK$^-$ using Lipofectamine 2000 (Invitrogen Corp., Carlsbad, CA). After 24 hours of incubation, firefly and renilla luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega) and a Lumat LB 9507 luminometer (Berthold Japan Co., Ltd., Tokyo, Japan).

Statistical Analyses

The relationship between %ΔIOP and genotype was analyzed using a nonparametric statistic, the Mann–Whitney *U* test. Statistical analyses between IOP or corneal thickness and genotype were performed using Student's *t* test (Stat View, version 5, Hulinks Inc., Tokyo, Japan). For analysis of categorical data (with the low responders, medium responders, and high responders classification), the chi-square test (Stat Mate III, ATMS Co., Ltd., Tokyo, Japan) was used. The Hardy–Weinberg equilibrium test and estimation of haplotype frequency based on the expectation–maximization algorithm[28] were performed using SNPAlyze (version 3.2, Dynacom Co., Ltd., Kanagawa, Japan). Student's *t* tests were performed to examine the difference between the transcriptional activities of different haplotype combinations of 4 SNPs in the FP receptor gene. A value of $P<0.05$ was considered statistically significant.

Results

Intraocular Pressure Decrease by Latanoprost Treatment

A total of 100 normal subjects (82 male, 18 female) were treated with latanoprost in one eye. The mean diurnal IOP in the treated eyes significantly decreased, from 14.0±0.2 mmHg (mean ± SE) on day 0 to 11.4±0.2 mmHg on day 7 ($P<0.0001$), and a significant IOP reduction was observed at each diurnal time point. In contrast, IOP in the control eyes was not changed (13.5±0.2 mmHg on day 0 and 13.5±0.2 mmHg on day 7). Mean diurnal percent IOP reduction in the treated eye was 18.1±1.0. Mean central corneal thickness and refraction were 557.8±3.1 μm and −4.0±0.3 diopters, respectively. Fifty-two percent of the treated eyes had conjunctival hyperemia on day 7. There was no significant correlation between %ΔIOP and central corneal thickness, refraction, or conjunctival hyperemia.

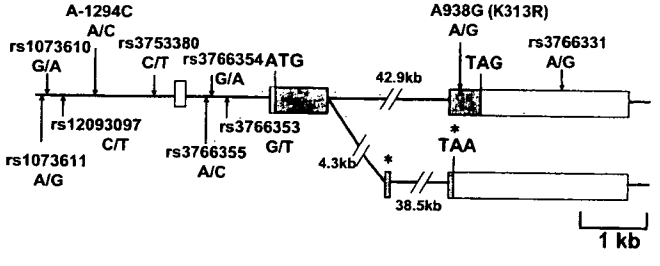

Figure 1. Structure of the prostaglandin $F_{2\alpha}$ receptor gene and the location of single-nucleotide polymorphisms (SNPs) identified in this study. The description of each SNP is available and searchable by the identification number (rsx) shown (http://www.ncbi.nih.gov/SNP). A−1294C and A938G are newly identified SNPs. Alleles (major/minor) and position of the SNPs are shown. Exons are boxed, and the coding regions are displayed with dark shading. Start and stop codons are also shown. *The coding region and stop codon produced by alternative mRNA splicing. kb = kilobase.

Table 1. Genotype of Each Single-Nucleotide Polymorphism (SNP) in the Prostaglandin $F_{2\alpha}$ Receptor Gene and Mean Diurnal Percent Intraocular Pressure Reduction

SNP Identification No.	Major Homogenotype	Minor Carrier	P*
rs1073611	17.5±1.0 (86)	21.4±2.9 (14)	0.295
rs1073610	17.5±1.0 (86)	21.4±2.9 (14)	0.295
rs12093097	17.9±1.1 (88)	19.7±2.5 (12)	0.494
A−1294C	17.6±1.0 (90)	22.3±2.9 (10)	0.173
rs3753380	20.3±1.5 (52)	15.6±1.2 (48)	0.0316
rs3766355	19.6±2.4 (25)	17.6±1.0 (75)	0.362
rs3766354	17.5±1.3 (57)	18.8±1.6 (43)	0.791
rs3766353	16.8±1.3 (46)	19.2±1.4 (54)	0.263
A938G	18.0±1.0 (96)	19.8±2.5 (4)	0.712
rs3766331	18.6±1.2 (74)	16.7±1.8 (26)	0.512

Numbers of subjects are shown in parentheses.
*Mann–Whitney *U* test.

Correlation between Mean Diurnal Percent Intraocular Pressure Reduction and Single-Nucleotide Polymorphisms

Ten SNPs in the promoter, coding, and 3′-untranslated regions of the FP receptor gene were identified and subjected to further analyses (Fig 1). Two SNPs, A−1294C in the promoter region and A938G (K313R) in exon 4, were newly identified, whereas the 8 other SNPs shown were previously reported (NCBI database). There were no polymorphisms in 19 additional SNPs that were reported previously[12,29] (NCBI database).

The relationships between %ΔIOP and SNPs of the FP receptor gene are shown in Table 1. The genotype distributions of all SNPs were in Hardy–Weinberg equilibrium ($P>0.05$), except rs1073611. Among all 10 SNPs studied, we identified a correlation between %ΔIOP by latanoprost and the genotype of one SNP, rs3753380. The %ΔIOP of subjects carrying the

Table 2. Characteristics of Subjects According to Genotypes at rs3753380

Genotypes	CC	CT + TT	P*
n (female)	52 (10)	48 (8)	0.74
Age (yrs)	24.3±0.3	24.3±0.4	1
IOP of treated eye			
Baseline (mmHg)	13.7±0.3	14.3±0.3	0.16
Day 7	10.9±0.3	12.0±0.3	0.0097
IOP of control eye			
Baseline	13.2±0.3	13.9±0.3	0.11
Day 7	13.2±0.3	13.8±0.3	0.17
Central corneal thickness of treated eye (μm)	557.2±4.8	558.5±4.0	0.84

IOP = intraocular pressure.
*t test.

Table 3. Allele and Genotype Distribution of rs3753380 and rs3766355

	Allele		Genotype			Allele		Genotype		
	C	T	CC	CT	TT	A	C	AA	AC	CC
rs3753380										
Low responders	23	15	7	9	3					
Medium responders	87	33	29	29	2					
High responders	35	7	16	3	2					
rs3766355										
Low responders						16	22	5	6	8
Medium responders						60	60	11	38	11
High responders						27	15	9	9	3

major homogenotype (CC) at rs3753380 was 20.3±1.5 (mean ± SE), whereas that of CT + TT was 15.6±1.2 (P = 0.0316). The mean diurnal IOP of the treated eyes on day 7 was significantly lower in subjects with the CC genotype (10.9±0.3 mmHg) than in those with CT + TT (12.0±0.3 mmHg) (P = 0.0097), whereas the baseline IOP level was not associated with the SNP genotype. Gender, age, and corneal thickness did not significantly differ between the CC and CT + TT genotypes (Table 2). Mean diurnal percent IOP reduction by latanoprost was not correlated with genotypes of the other SNPs.

Association of the Prostaglandin $F_{2\alpha}$ Receptor Gene Polymorphisms with Response to Latanoprost

To determine if we could predict the response to latanoprost in each patient by genetic polymorphisms of the FP receptor gene, we classified subjects by %ΔIOP into 3 groups, as described in "Materials and Methods." There were 19, 80, and 21 low, medium, and high responders, respectively. No significant differences in baseline IOPs of treated eyes, pretreatment and posttreatment IOPs of the control eyes, and corneal thickness were observed among the 3 groups.

We analyzed the relationship between each SNP of the FP receptor gene and the category of %ΔIOP. Given the allele and genotype distributions of rs3753380 and rs3766355 (Table 3), the polymorphisms in rs3753380 and rs3766355 were significantly associated with the degree of response to latanoprost (Table 4). Significant associations with response to latanoprost were found in high responders versus others (rs3753380, CC vs. CT + TT [P = 0.013; odds ratio (OR), 3.82]; rs3766355, AA vs. AC + CC [P = 0.033; OR, 2.95]) and in low responders versus others (rs3766355, CC vs. AA + AC [P = 0.019; OR, 3.48]). There was also a significant association in low responders versus medium responders versus high responders in rs3753380 (CC vs. CT + TT, P = 0.030). No significant differences were observed between the categories and frequencies of alleles or genotypes of the other 8 SNPs (data not shown).

Reporter Assay of the Prostaglandin $F_{2\alpha}$ Receptor Gene

Because there were associations between the genotypes of 2 SNPs (rs3753380 and rs3766355) and response to latanoprost, we estimated the haplotype frequency of these 2 SNPs and 2 adjacent SNPs, rs3766354 and rs3766353. Of the 2^4 (16) possible combinations of these SNPs, only 6 haplotypes were found by expectation–maximization algorithm analysis. Furthermore,

Table 4. Association Analyses of rs3753380 and rs3766355 with Response to Latanoprost

	Allele	Genotype			
	C:T	CC:CT + TT		TT:CC + CT	
rs3753380 Groups Compared	χ^2 (P)	χ^2 (P)	*Odds Ratio (95% CI)*	χ^2 (P)	*Odds Ratio (95% CI)*
Low responders vs. medium responders vs. high responders	5.20 (0.074)	7.00 (0.030)			
Low responders vs. others	3.37 (0.066)	2.16 (0.14)	0.47 (0.17–1.31)	1.37 (0.24)*	3.61 (0.74–17.72)
High responders vs. others	3.13 (0.077)	6.23 (0.013)	3.82 (1.28–11.45)	<0.01 (0.98)*	1.56 (0.28–8.66)
	Allele	**Genotype**			
	A:C	AA:AC + CC		CC:AA + AC	
rs3766355 Groups Compared	χ^2 (P)	χ^2 (P)	*Odds Ratio (95% CI)*	χ^2 (P)	*Odds Ratio (95% CI)*
Low responders vs. medium responders vs. high responders	4.20 (0.12)	5.01 (0.082)			
Low responders vs. others	1.66 (0.20)	0.022 (0.88)	1.09 (0.35–3.40)	5.53 (0.019)	3.48 (1.18–10.22)
High responders vs. others	3.48 (0.062)	4.52 (0.033)	2.95 (1.06–8.22)	0.44 (0.51)*	0.53 (0.14–1.98)

CI = confidence interval.
*Yates correction.

4 of the 6 haplotypes accounted for 95% of the subjects (haplotypes 1–4 in Fig 2).

To explore whether these SNPs had an influence on transcriptional activity of the FP receptor gene, a DNA fragment from the promoter region (−640) to exon 2 (+1655) that contained each of the 4 haplotype combinations was prepared and fused to the firefly luciferase gene. Reporter constructs were introduced into HeLa cells, and the luciferase activity of each was measured. As shown in Figure 2, the relative activity of constructs with haplotypes 3 and 4 was significantly reduced compared with that of constructs with haplotypes 1 and 2.

Discussion

Effect of Intraocular Pressure Reduction by Latanoprost in Normal Subjects

It was reported that baseline IOP had an influence on IOP reduction by latanoprost.[30,31] To reduce this effect, we evaluated the efficacy of latanoprost as a percent IOP reduction. Furthermore, we subtracted the IOP fluctuation of the control eyes from the IOP reduction of the treated eyes at each time point to assess the true pharmacological component of IOP reduction. We also measured IOP at 3 time points and evaluated the %ΔIOP to reduce the possible effect of diurnal IOP variations. The %ΔIOP of all subjects was 18.1±1.0 (mean ± SE), and 19% of subjects were judged as low responders (%ΔIOP<10). Aung et al[1] reported that nonresponders (percent IOP reduction < 15) were 5.4% of all subjects, whereas Scherer[2] reported that nonresponders (percent IOP reduction < 20 or IOP reduction < 5 mmHg) were 25% of all subjects. The difference of the ratio of nonresponders or low responders in these reports may be attributed to differences of study design, including subjects, definition of IOP response, and duration of latanoprost treatment.

Association between Single-Nucleotide Polymorphisms in the Prostaglandin $F_{2\alpha}$ Receptor Gene and Effect of Latanoprost

We found a significant correlation between the rs3753380 SNP and %ΔIOP by latanoprost, whereas the other FP receptor gene SNPs were not associated with %ΔIOP. When subjects were classified by %ΔIOP into low, medium, and high responders, rs3766355 was also associated with a response to latanoprost. The results of the promoter assay showed the association between the C allele at rs3766355 in intron 1 and reduced expression of the FP receptor–luciferase reporter in HeLa cells. Although we did not find a significant correlation between the rs3766355 SNP and %ΔIOP, the result of the reporter assay is consistent with the findings that the presence of the C allele or absence of the A allele is associated with the lower responders to latanoprost. As for rs3753380, the T allele, which is associated with reduced %ΔIOP and lower responders, was found only in haplotype 3, one of the haplotype combinations with low transcriptional activity. Therefore, the C allele of rs3766355 and T allele of rs3753380 may cause reduced response to latanoprost through downregulation of FP receptor gene expression.

Zaragoza et al[27] deduced the binding sites of the transcription factors SP-1, GATA-1, STAT-1, AP-1, NFκB, and NF-IL-6 from the 5′-untranslated region of the FP receptor gene. However, none of these putative binding sites was associated with the rs3753380 and rs3766355 SNPs. In addition to the primary transcription start site, the bovine and rat FP receptor genes have one or two additional transcription start sites in intron 1, which is located upstream of the ATG initiator codon.[32,33] Therefore, it is possible that intron 1 affects transcriptional regulation of the human FP receptor gene, although this is not yet well characterized. The results of the reporter assay support this possibility, although further studies are required to elucidate how the rs3753380 and rs3766355 SNPs affect transcriptional activity of the FP receptor gene. At present, although the molecular mechanism by which the 2 SNPs rs3753380 and rs3766355 reduce IOP response to latanoprost is unclear, analysis of these SNPs may help to predict the degree of response to latanoprost.

Single-Nucleotide Polymorphisms in the Coding Region of the Prostaglandin $F_{2\alpha}$ Receptor Gene

In this study, we have identified 2 new SNPs (A−1294C and A938G) in addition to the 8 known SNPs. Stjernschantz[29] reported 4 SNPs—C63T (T21), C213T (S71), A573G (E191), and A1012G (I338V)—but no polymorphisms of these SNPs were found in our preliminary analysis of 24 subjects. This may be due to different characteristics of the subjects, such as race. One of the new SNPs, A938G, is located adjacent to the seventh transmembrane domain and results in an amino acid substitution (K313R). Mutations in this seventh transmembrane domain region have been shown to significantly affect FP receptor protein function—for example, the R291L mutation abolished ligand binding and localization to the plasma membrane, and the D300Q mutation caused loss of discrimination between prostaglandin $F_{2\alpha}$ and prostaglandin D_2.[34] Although A938G did not correlate with a response to latanoprost, it is possible that this SNP affects other functional aspects of the FP receptor such as ligand binding.

Other Genes

The FP receptor is involved in IOP regulation via a pathway consisting of various proteins, including prostaglandin transporter; fatty acid amide hydrolase, which is responsible for the activation of all prostaglandin prodrugs[35]; FP receptor regulatory protein[36]; and matrix metalloproteinases.[20–22] As a preliminary experiment, we analyzed the correlation between %ΔIOP and polymorphisms that were reported previously: T396A in prostaglandin transporter[37]; P129T in fatty acid amide hydrolase[38]; T277S, N576K, and I837V in FP receptor regulatory protein (NCBI database); −1607 insG in matrix metalloproteinase 1[39]; C-1306T in matrix metalloproteinase 2[40]; −1171 delA in matrix metalloproteinase 3[41]; and C-1562T[42] and CA repeats (−131 to −90) in matrix metalloproteinase 9.[43] However, we found no significant correlation. It remains possible that new SNPs that are associated with IOP reduction by latanoprost will be identified in these genes.