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Vitrectomy for Endophthalmitis After Intraocular Lens Implantation

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Purpose: To report the results of vitrectomy and intraocular lens (IOL) removal for the treatment of endophthalmitis after IOL implantation.

Methods: We reviewed 14 eyes of 14 patients who underwent pars plana vitrectomy because of postoperative endophthalmitis. Culture results, surgical methods, and visual outcome are presented.

Results: The cultures grew *Enterococcus faecalis* (n = 3), *Staphylococcus epidermidis* (n = 2), *Propionibacterium acnes* (n = 1), and gram-negative bacillus (n = 3). The eyes infected with *E. faecalis* had poor visual outcome. Eleven eyes treated by the combination of pars plana vitrectomy and IOL removal did not have a recurrence. The remaining 3 eyes on which only vitrectomy was performed had a recurrence, and the additional procedures consisting of vitrectomy and IOL removal could result in eradicating endophthalmitis.

Conclusions: A higher rate of *E. faecalis* was detected and these eyes had severe inflammation and poor visual outcome. Combined vitrectomy and IOL removal may be a more certain method to prevent recurrence. **Jpn J Ophthalmol 2000;44:439–442**

Key Words: Endophthalmitis, *Enterococcus faecalis*, intraocular lens implantation, pars plana vitrectomy.

Introduction

Postoperative endophthalmitis is one of the most feared complications following intraocular lens (IOL) implantation. Although different treatment protocols have been proposed, the best treatment for endophthalmitis and the prevention of its recurrence have not been established.

We present our experience with 14 eyes of 14 patients with endophthalmitis after IOL implantation who were referred to the Nagoya University Hospital. The microbiologic and antibiotic sensitivity patterns, visual outcome, and treatment opinion including vitrectomy and IOL removal will be presented.

Materials and Methods

We reviewed the medical records and cultures of 14 cases of endophthalmitis occurring in eyes following IOL implantation and treated at the Nagoya University Hospital (Tables 1 and 2). All cases were referred to us between December 1992 and April 1998. The 14 patients ranged in age from 55 to 83 years (mean = 70.9 ± 8.5 years), and included 6 men and 8 women. Four patients had diabetes. The original surgery that led to endophthalmitis was phacoemulsification through the limbus and IOL implantation in 13 cases, and secondary IOL implantation in 1 case. The materials of the IOLs were polymethyl methacrylate (n = 7), polyacrylic (n = 3) and silicone (n = 4). The type of incision performed in IOL implantation was corneal in 3 eyes and sclerocorneal in 11 eyes, including 3 eyes with sutureless technique (corneal incision [n = 2], sclerocorneal incision [n = 1]). The duration between the initial operation and the onset of inflammation was within 1 week in 9 cases, within 6 weeks in 3 cases, and over 6 weeks in 2 cases. The initial vitrectomy was performed with 1 week in 6 cases, within 1 month in 5 cases, and over 1 month in 3 cases from the onset of endophthalmitis. The best corrected visual acuity before vitrectomy ranged from light perception to 0.1. The cases that had a long period from the onset of endophthalmitis to the initial vitrectomy had been treated with subconjunctival steroids and intravenous antibiotics at other hospitals.

The clinical diagnosis of endophthalmitis was based on intraocular inflammation greater than expected during the postoperative period, and was made because of symptoms including blurred vision, pain, ocular redness, and lid swelling. In addition, there were clinical signs of hypopyon, vitreous opacification, chemosis, corneal edema and infiltrations. The degree of inflammation was considered to be sufficient to warrant surgical intervention to treat the endophthalmitis and to obtain cultures. Vitrectomy was performed in those patients who appeared to have an aggressive infection characterized by severe, rapidly progressive intraocular inflammation.

Intraocular specimens for culture were obtained during vitrectomy. Vitreous and aqueous humor were cultured in all cases, and the IOL and the posterior

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Table 1. Patient History

Case No.	Age (y)	Sex	Previous Surgery*	Time Between Surgery and Inflammation Onset	Interval Between Inflammation Onset and Vitrectomy	Diabetes Mellitus
1	70	F	IOL†	3 days	3 weeks	–
2	77	F	PEA, IOL	6 days	1 day	–
3	62	M	PEA, IOL	4 days	1 day	–
4	72	F	PEA, IOL	5 days	4 weeks	–
5	83	M	PEA, IOL	5 days	2 days	–
6	55	F	PEA, IOL	11 months	1 month	–
7	82	M	PEA, IOL	2 days	1 day	–
8	68	F	PEA, IOL	5 days	2 days	+
9	78	M	PEA, IOL	3 days	2.5 months	+
10	68	M	PEA, IOL	10 days	5 days	–
11	72	M	PEA, IOL	4.5 months	11 months	–
12	78	F	PEA, IOL	20 days	1.5 months	–
13	69	F	PEA, IOL	1.5 months	10 days	+
14	58	F	PEA, IOL	6 days	3 weeks	+

*PEA: Phacoemulsification; IOL: intraocular lens implantation.
†Secondary implantation.

capsule were cultured when they were removed. IOLs were removed from 11 of the 14 eyes at the initial vitrectomy. In the remaining 3 eyes, IOLs were removed following vitrectomy as additional procedures because of the recurrence of endophthalmitis. Undiluted aqueous humor (0.2–0.3 mL) was obtained by paracentesis with a 27-gauge needle through the corneal limbus at the beginning of vitrectomy. Vitreous biopsy consisted of 0.3–0.5 mL of vitreous humor at the beginning of vitrectomy with an aspiration/cutter inserted through a pars plana incision. Samples were plated onto blood agar, chocolate agar, MacConkey agar, and thioglycolated broth and maintained at 37°C for bacterial growth. Sabouraud agar and blood agar at 25°C were used to enhance fungal isolation.

The therapy after the initial intervention included broad spectrum systemic antibiotics (piperacillin, ceftazidime [CAZ], or minocycline [MINO]) and topical antibiotics (ofloxacin [OFLX], or norfloxacin [NFLX]). The specific antibiotic was determined with the identification of the microorganism in the culture and antibiotic sensitivity pattern. If the inflammation did not appear to be subsiding, additional vitrectomy was performed immediately.

The follow-up period was from 8 to 48 months (mean = 14.6 ± 10.4 months). The postoperative visual acuity reported here was measured at the latest examination.

Results

The cultures were positive in 9 of 14 patients (64.3%), and were negative in 5. The cultures from IOL or posterior capsule were positive despite the culture-negativity of the aqueous and vitreous in 3 cases. Only a single species grew in all cases.

Enterococcus faecalis was cultured from 3 eyes (21.4%), *Staphylococcus epidermidis* from 2 eyes, and *Propionibacterium acnes* from 1 eye. The remaining 3 isolates were gram-negative bacteria. The multi-resistant organisms were methicillin-resistant *S. epidermidis* (minimum inhibitory concentration [MIC], [μg/mL]; oxacillin > 8, cefmetazole = 8, gentamicin [GM] > 8, MINO ⩽ 1, OFLX ⩽ 0.5, vancomycin [VCM] ⩽ 4) and *Stenotrophomonas maltophilia* (MIC; sulfamethoxazole/trimethoprim [ST] > 2, CAZ > 16, GM > 8, imipenem > 8, MINO ⩽ 1, OFLX = 2).

A visual acuity equal to or better than 0.1 was obtained in 9 cases (64.3%). Three eyes infected with *E. faecalis* and 1 eye with *Pseudomonas aeruginosa* had worse visual outcomes; light perception, counting fingers, 0.06 and 0.1, respectively. Three of the five culture-negative eyes achieved 0.8 or better vision. The other 2 culture-negative eyes had recurrence of endophthalmitis and the final visual acuity was hand motion and 0.2. Visual acuity remained counting fingers in the eye infected with *S. maltophilia* because of tractional retinal detachment due to diabetic retinopathy, which was found during vitrectomy. Atrophy of the optic disc was found in 5 cases whose visual acuity was worse than 0.06 (cases 1, 2, 3, 7, and 10).

The three eyes without IOL removal had recurrence of endophthalmitis, and the inflammation abated after IOL removal as an additional proce-

Table 2. Patient Data

Case No.	Causative Organisms*	Specimens†	Surgical Procedure‡	Preoperative Visual Acuity§	Postoperative Visual Acuity§
1	*Enterococcus faecalis*	Aqueous	IOL removal, Vit	LP	LP
2	*E. faecalis*	Capsular bag	IOL removal, Vit	LP	CF
3	*E. faecalis*	Aqueous, IOL, capsular bag, vitreous	IOL removal, Vit	HM	0.06
4	*Staphylococcus epidermidis*	Vitreous	Vit*	0.01	0.3
5	MRSE	IOL	IOL removal, Vit	HM	0.7
6	*Propionibacterium acnes*	Capsular bag	IOL removal, Vit	0.1	0.6
7	*Pseudomonas aeruginosa*	Aqueous	IOL removal, Vit	HM	0.1
8	*Stenotorophomonas maltophilia*	Aqueous, IOL	IOL removal, Vit	HM	CF
9	GNF-GNR	IOL, vitreous	IOL removal, Vit	0.01	0.3
10	Negative	–	Vit**	HM	HM
11	Negative	–	Vit*	0.1	0.2
12	Negative	–	IOL removal, Vit	0.1	0.8
13	Negative	–	IOL removal, Vit	HM	1.0
14	Negative	–	IOL removal, Vit	0.1	1.5

*MRSE: Methicillin-resistant *Staphylococcus epidermidis*; GNF-GNR: glucose nonfermentative gram-negative rods.
†IOL: Intraocular lens.
‡Vit: Vitrectomy; Vit*: Vit→Vit→Vit, IOL removal: Vit**: Vit→Vit, IOL removal.
§LP: Light perception; HM: hand motion; CF: counting fingers.

dure. In the end, all 14 eyes underwent combined vitrectomy and IOL removal, and none had recurrence of inflammation. No postoperative complications including retinal detachment or bullous keratopathy were found. Four eyes that had no recurrence after more than 6 months follow-up had a secondary IOL implantation (cases 5, 12, 13, and 14), and to date, endophthalmitis had not recurred.

Discussion

The cultures were positive in 9 cases (64.3%) which is comparable to the result reported in similar cases.[1] *E. faecalis* was isolated in 3 cases (21.4%) in our series. Shimizu and Shimizu[2] recently reported that *E. faecalis* was obtained from 6 eyes of 13 patients (43%), even though previous studies have reported that the incidence of *E. faecalis* endophthalmitis is rare.[1] In our series, eyes infected with *E. faecalis* had poor visual outcomes, ranging from light perception to 0.06, as has been reported for *E. faecalis* endophthalmitis.[2] To prevent endophthalmitis by *E. faecalis*, Shimizu and Shimizu[2] recommend irrigation solution with imipenem/cilastatin during IOL implantation. This procedure remains controversial.

We performed vitrectomy immediately after endophthalmitis had been diagnosed to remove the abscess containing the infectious organisms and the toxins produced by the organisms, as described in a previous report.[1] In our series, however, 4 of the 5 eyes with an interval of more than 2 weeks from the onset of inflammation to vitrectomy were referred to our institution after treatment with intravenous or subconjunctival injections of steroids by the initial ophthalmologists. Fortunately, these eyes did not have a poor visual outcome because the causative organisms may not have had strong virulence.

The removal of IOL in the treatment of endophthalmitis remains controversial. Recent reports on chronic low-grade endophthalmitis after IOL implantation have demonstrated the role played by various low-virulence microorganisms growing on the IOL and in the capsular bag. Busin et al.[3] reported that primary IOL removal with partial or total capsulectomy provides a surgical approach to the treatment of chronic low-grade endophthalmitis not responsive to medical therapy. In acute endophthalmitis, it has been reported that an IOL is usually removed when it disturbs visualization of the fundus during vitrectomy.[4] In the present series, IOLs in 3 eyes were removed because of recurrence after initial vitrectomy. These eyes had no further recurrence for as long as 6 months. One of the causative organisms in these eyes was *S. epidermidis*. It has been reported that *S. epidermidis* produces biofilm,[5] which is one of the causes of recurrence. In addition, the fact that a culture from an IOL or posterior capsule was positive despite the culture-negativity of the aqueous and vitreous in 3 of our cases may indicate the adhesion of bacteria. Therefore, the advan-

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tages of IOL removal may be the elimination of the source of bacteria, facilitation of performance of vitrectomy, and collection of additional specimens from the IOL and capsule for culture. Although controlled, prospective studies may be necessary to answer the question of the advantages of removing the IOL, all these measures may prevent recurrence.

Multi-resistant bacteria grew in two eyes. One was methicillin-resistant *S. epidermidis*, and the other was multi-resistant *S. maltophilia*. Fortunately, both of these bacteria were not very virulent, although virulent bacteria such as methicillin-resistant *S. aureus* or vancomycin-resistant *E. faecalis* often cause severe endophthalmitis resulting in poor visual acuity. In these cases, antibiotic therapy may not be effective, and it may be better to perform vitrectomy to remove the infectious organisms. In the eyes that had worsening of inflammation or infection after initial treatment in the Endophthalmitis Vitrectomy Study,[6] re-cultures were positive in more eyes with intravitreal antibiotic injection than in eyes after the initial vitrectomy. Although the results of the Endophthalmitis Vitrectomy Study showed that immediate vitrectomy is beneficial only in the eyes with light perception, they added that vitrectomy could remove the causative organisms and sterilize the eyes. It may be important not only to select effective antibiotics but also to perform vitrectomy to remove the infectious organisms.

In summary, we reported 14 cases of endophthalmitis after IOL implantation. A higher rate of *E. faecalis* was detected and these eyes had severe inflammation and poor visual outcome. Vitrectomy may be helpful to sterilize the eyes infected with multi-resistant bacteria because antibiotics may not be effective in these eyes. The combined method of vitrectomy and IOL removal seemed to be a more certain method to prevent recurrence.

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Serum Prolactin Levels in Behçet's Disease

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Purpose: Evaluation of serum prolactin levels in Behçet's disease patients in Turkey.

Methods: Serum prolactin levels were measured by radioimmunoassay (RIA) in 17 patients with ocular findings, and 20 patients without ocular findings of Behçet's disease, and in 17 healthy volunteers.

Results: The average prolactin levels were measured as 9.53 ng/mL in patients with ocular findings, 8.84 ng/mL in patients without ocular findings, and 9.59 ng/mL in healthy controls. There was no statistical significance among these three groups. Also, the average levels were 9.84 ng/mL in remission periods and 7.54 ng/mL in attacks.

Conclusions: In some studies, it has been suggested there is a correlation between high serum prolactin levels and activation of some autoimmune diseases, such as systemic lupus erythematosus and rheumatoid arthritis. However, we found no such correlation in Behçet's disease. On the contrary, prolactin levels were lower in attacks than in remissions. **Jpn J Ophthalmol 2000;44:442–445**

Key Words: Behçet's disease, prolactin, radioimmunoassay.

Introduction

In the early 1980s, it was determined that cellular immunity played an important role in endogenous uveitis and also that Cyclosporine A (CsA) had a se-

lective effect on T cell subgroups. For this reason, CsA has gained widespread use in the last 15 years.[1]

Although its effectiveness on patients with severe endogenous uveitis was proven, CsA also had severe side effects, mainly on kidneys. Also, the therapy regimen with this drug was too expensive. These factors have limited its clinical use and combined therapies with steroids and bromocriptine have been used more often in many countries, including Turkey.[2]

Bromocriptine is a prolactin inhibitor and has a direct antiproliferative effect on T lymphocyte replication in vitro. Prolactin in an immunomodulator hormone and is supposed to prevent CsA binding to the receptors located in lymphocytes. Inhibition of prolactin should make CsA more effective at lower doses, and so keep adverse effects to a minimum.[3]

In this clinical study, we examined the serum prolactin levels in Behçet's disease patients, hoping to obtain clues to clarify the action mechanism of bromocriptine.

Materials and Methods

This study was carried out on three different groups, which included 54 patients. In the first group there were 17 patients with ocular findings (ocular Behçet's disease), who were followed and treated in the retina department of the eye clinic of the Akdeniz University Medical Faculty. In the second group, there were 20 patients without ocular findings (extraocular Behçet's disease) who were referred by the dermatology department of the same hospital. The third group contained 17 cases with refractive errors and without any systemic disease, forming our age- and sex-matched controls.

The demographic properties of all three groups are shown in Table 1. Patients with ocular Behçet's disease in the first group were 6 women and 11 men. Mean age was 39.64 years and mean duration of disease was 10.2 years. Patients with extraocular Behçet's disease in the second group were 8 women and 12 men. Mean age was 37.35 years and mean duration of disease was 7.41 years. Healthy volunteers of the third group were composed of 8 women and 9 men. Mean age was 39.94 years.

Patients who showed signs and symptoms of activation both clinically (anterior or posterior uveitis, aphthous stomatitis, genital ulcers, arthritis) and serologically (increase in erythrocyte sedimentation rate or in C-reactive protein) were considered as having an attack. There were 4 women and 8 men in this group and 11 women and 16 men in the remission group.

Special care had been taken in the medical treatment of patients so as not to affect the serum prolactin levels. Patients in the remission stage with no systemic or local therapy (corticosteroid or immunosuppressive) during the last 1 month were included. During attacks, blood samples were taken before the systemic treatment was given. Patients treated with neuroleptics, opium and ergot derivatives, reserpine, estrogen, seratonin, and dopamine antagonists were excluded from the study. Patients in the last 6 months of pregnancy or with history of abortion were also excluded.

The aim of the study was explained to all participants before the start of the study and informed consent was received from each patient. Serum prolactin levels were measured by radioimmunoassay (RIA) and samples were taken at early morning hours (between 9:00 AM and 11:00 AM). Normal values were 2.10–17.70 ng/mL for men, and 2.80–29.20 ng/mL for women.

All data have been analysed by the Statistical Product and Service Solutions packet program. One- and two-way analyses of variance (ANOVA) were used for the significance of the difference between groups. The Mann–Whitney *U*-test was used for nonparametric test groups. *P* values were accepted significant at .05 for all analyses.

Table 1. Demographic Data and Serum Prolactin Levels of Patient and Control Groups

	No. of Cases			Age (y)		Follow-up period (y)		Prolactin levles (ng/mL)†		
Groups*	Male	Female	Total	Mean	±SD	Mean	±SD	Mean	±SD	*P* Value
OBD	11	6	17	39.64	10.96	10.2	10.45	9.53	7.58	
EOBD	12	8	20	37.35	7.53	7.41	4.44	8.84	5.08	.91
Controls	9	8	17	39.94	10.49	–	–	9.59	6.03	
Total	32	22	54	38.88	9.55	8.80	8.3	–	–	

*OBD: Ocular Behçet's disease, EOBD: extraocular Behçet's disease.

†Without taking into account attacks and remissions.

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Table 2. Serum Prolactin Levels on Attacks and Remissions in Two Patient Groups and in Male and Female Cases in Three Groups

	Prolactin Levels on Attacks (ng/mL)			Prolactin Levels on Remissions (ng/mL)				Prolactin Levels in Men (ng/mL)		Prolactin Levels in Women (ng/mL)		
Groups*	No. of Cases	Mean	±SD	No. of Cases	Mean	±SD	*P* Values	Mean	±SD	Mean	±SD	*P* Values
OBD	6	7.11	2.91	13	10.48	8.48	.76	8.94	7.70	10.61	7.94	
EOBD	6	7.97	2.92	14	9.21	5.82	.71	7.63	2.77	10.65	7.19	.26
Controls	–	–	–	–	–	–		6.62	2.55	12.24	7.09	
Total	12	7.54	2.81	27	9.84	7.18	.39	7.84	5.0	11.26	7.05	.04

*OBD: Ocular Behçet's disease, EOBD: extraocular Behçet's disease.

Results

Serum prolactin levels in all three groups are shown in Table 1 without taking into account the attacks and remissions.

As seen in this table, average serum prolactin levels in ocular Behçet's patients are 9.53 ng/mL and in extraocular Behçet's patients are 8.84 ng/mL. The average levels in the control group are 9.59 ng/mL and there are no statistically significant differences between the three groups ($P > .05$).

Serum prolactin levels in the remission and attack groups are shown in Table 2. Average prolactin levels in 13 serum samples of patients with ocular findings are 10.48 ng/mL in remissions and in 6 samples are 7.11 ng/mL in attacks. In extraocular Behçet's patients these values are 9.21 ng/mL in remissions and 7.97 ng/mL in attacks. Although in general, the levels in attack (7.54 ng/mL) are less than in remission (9.84 ng/mL), the difference is not significant ($P > .05$).

The effect of sex difference on serum prolactin levels is also shown in Table 2. Both in women and men, the difference between the average prolactin levels in all three groups is not significant ($P > .05$). But the general average values are 11.26 ng/mL in women and 7.84 ng/mL in men and the differences between these two values is significant ($P < .05$).

Discussion

Prolactin is a peptide hormone released from the pituitary. It has many effects on living organisms, from osmoregulation to carbohydrate and fat metabolism and milk and mucin secretion. In recent years, the effects on the immune system has become more important. It has an immunostimulator role in the differentiation of lymphocytes in thymus and spleen. Prolactin receptors were shown on T and B cell lymphocytes in peripheral blood and the possible role in humoral and cellular immunity was postulated.[4]

In vitro studies also have showed that lymphocytes were important target cells for prolactin. It prevents the CsA binding to the receptors on lymphocytes. Cyclosporin A is an immunosuppressive agent and the decrease of prolactin levels by bromocriptine makes the CsA more effective in lower doses.[3] Additionally, bromocriptine has a direct antiproliferative effect on T lymphocyte replication in vitro and also reduces the lymphocyte response to an antigenic stimulus in vivo in rats.[4]

Since the 1980s, CsA has been widely used for Behçet's disease and other endogenous uveitis. Bromocriptine has been added to therapy to reduce the side effects of CsA. In spite of the antiprolactin effects of bromocriptine, we could not find any study about prolactin levels in Behçet's disease in the literature. Since the 1990s, numerous articles have been published about the relationship between hyperprolactinemia and some autoimmune collagen diseases, such as systemic lupus and erythematosus and rheumatoid arthritis.[4,5].

This clinical study was planned to determine the serum prolactin levels in Behçet's disease patients. In this way, we hoped to obtain more clues to clarify the mechanism of bromocriptine. No significant difference was found between the patient and the control groups. We believe that serum prolactin levels did not play an important role in clinical findings of Behçet's disease. The levels in attacks were lower than in remissions (although not statistically significant). However, owing to the limited number of patients with attacks, and also the relatively small number of women in this group, we could not make more definite conclusions on this subject. Our results should be confirmed by studies with many more patients and a balanced sex distribution.

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The Gly367Arg Mutation in the Myocilin Gene Causes Adult-Onset Primary Open-Angle Glaucoma

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Purpose: To present the phenotype of a family whose members showed the Gly367Arg mutation in the myocilin gene and developed primary open-angle glaucoma (POAG).

Methods: The proband developed POAG when she was 45 years old. Examination of the myocilin gene revealed that the patient had a mutation causing amino-acid change (Gly367Arg) in the myocilin gene. The available family members were given clinical and genetic examinations.

Results: Eight members of this family carried the same mutation. The age of disease onset of POAG in these patients with the mutation averaged 36.7 years. Four young members with the mutation, with an average age of 20.8 years, had not yet developed POAG.

Conclusion: The Gly367Arg mutation of the myocilin gene in the pedigree causes the development of POAG in adulthood. **Jpn J Ophthalmol 2000;44:445–448** © 2000 Japanese Ophthalmological Society

Key Words: Adult-onset primary open-angle glaucoma, glaucoma genetics, myocilin gene.

Introduction

The myocilin gene, also called trabecular meshwork inducible glucocorticoid response gene, was identified in 1997[1,2] as the gene responsible for primary open-angle glaucoma (POAG). Since then, studies of this gene developed rapidly, and more than 20 mutations of the gene have been reported around the world. Most of the family members with myocilin gene mutation were reported to develop juvenile-onset POAG, but some adult-onset POAG family members were also reported.[3]

In our previous report, we examined the myocilin gene in Japanese POAG patients and found two families with two novel mutations of Pro370Leu and Gly367Arg.[4] The POAG with Pro370Leu mutation was of the juvenile onset type.4 The patient with Gly367Arg mutation was diagnosed as having POAG at age 45, but we could not examine her relatives at that time and the characteristics of this type of POAG with the Gly367Arg mutation were unclear.

Recently, we had an opportunity to examine the myocilin gene and the clinical features of this pedigree. In this pedigree wherein examination of a total of 13 members was possible, 8 members carried the Gly367Arg mutation and developed POAG in adulthood.

Materials and Methods

A 50-year-old Japanese woman with the Gly367Arg mutation in the myocilin gene and her family members were examined at the Department of Ophthalmology of the University of Tokyo. Her mother, her aunt, and 4 of her cousins were reported to have POAG. Thirteen members of this family, including 3 POAG patients, agreed to participate in this study.

Genomic DNA was extracted from their peripheral blood samples using QIA Amp Blood Kit (QIAGEN, Chatsworth, CA, USA). A DNA fragment encoding a peptide of myocilin (amino acid residue 317–476) was amplified by the polymerase chain reaction (PCR) method with the following primers: ATACTGCCTAGGCCACTGGA (5′-sense direction) and CATGCTGCTGTACTTATAGCGG (3′-antisense direction). Polyermerase chain reaction was performed in a total volume of 100 μL that consisted of 150 ng template, 8 μL of the 2.5 mM deoxynucleotides mixture, 10 pmol of each primer, 10 μL of 10× buffer containing 15 mM $MgCl_2$, and 0.5 μL of *Taq* polymerase (Ampli*Taq* Gold; Perkin Elmer, Foster City, CA, USA). Amplification conditions were as follows: 10 minutes of preincubation at 94°C, followed by 35 cycles for 30 seconds at 94°C, for 30 seconds at 55°C, and for 1 minute at 72°C. The nucleotide sequences of both strands of the PCR products were directly determined by the terminator cycle sequencing method using fluorescent

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dideoxynucleotides and an automatic DNA sequencer (model 373S; Perkin Elmer).

Routine ophthalmic examinations were carried out on each subject. Examinations included slit-lamp examination, gonioscopy, intraocular pressure (IOP) measurements, indirect ophthalmoscopy, and optic nerve head examination with direct ophthalmoscopy. The visual field test using the Humphrey Field Analyzer Program 30-2 (Humphrey Instruments, San Leandro, CA, USA) was added when the subject had high IOP (≥22 mm Hg), or was suspected of having glaucomatous optic nerve head change, or had the myocilin gene mutation.

This study was approved by the Ethical Committee of the University of Tokyo School of Medicine. Each patient was given sufficient explanation about the study, and a written informed consent was obtained.

Results

Of the 13 members examined, 3 (1 man and 2 women) were diagnosed with POAG (patients A,B,C in Figure 1 and Table 1). Nine had normal eyes (patients E—M in Figure 1 and Table 1), and 1 was blind in both eyes. The cause of blindness was unknown, since his past history was unclear and fundus examination was not possible due to corneal opacity (patient D in Figure 1 and Table 1). His IOP was 14 mm Hg in both eyes.

All the POAG patients and the blind man carried a heterozygous mutation of adenine (A) substitution for guanine (G) at the position of the first nucleotide of the codon corresponding to the 367th amino acid residue of the myocilin gene, resulting in an amino acid change from glycine to arginine (Gly367Arg). Furthermore, 4 of the members with normal results in ocular examinations carried the same mutation (patients E–H in Figure 1 and Table 1). These 4 subjects were 18–23 years old. No glaucomatous optic disc change was found, and their IOPs were between 15 and 20 mm Hg.

The other 5 members (3 men and 2 women) of this family did not carry the mutation and showed normal results in ophthalmological examinations (patients I–M in Figure 1 and Table 1).

The Proband and Her Children

In 1994, a 45-year-old woman was aware of halo vision and visited an ophthalmologist. At that time, her IOP was 45 mm Hg in the right eye and 50 mm Hg in the left eye. She was diagnosed as having POAG, and treated with 0.5% timolol, 1.25% epinephrine, and 2% pilocarpine. The next month, she was referred to the

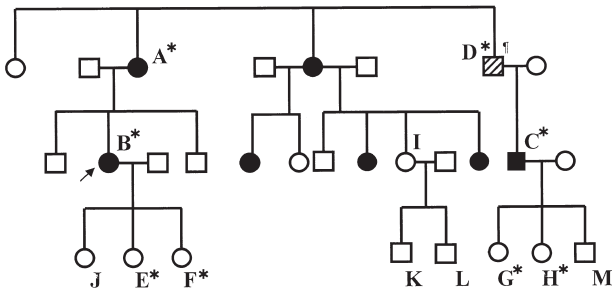

Figure 1. Pedigree. Arrow: the proband; ●, ■: glaucoma patients; ○: woman; □: man. A–M: subjects examined in this study. *Subjects with Gly367Arg mutation. ▨: bilateral blindness with corneal opacities, cause unknown.

University of Tokyo School of Medicine because of uncontrolled IOP. At her first visit to the glaucoma clinic, her IOP was 25 mm Hg in the right eye and 24 mm Hg in the left eye. She had normal open angle and glaucomatous optic disc change with cup/disc ratio of 0.9 × 1.0 in the right eye and 0.95 × 0.95 in the left eye.

Trabeculectomy was performed on both eyes together with subconjunctival administration of mitomycin C (0.04%, 3 minutes), after which her IOP was controlled between 15 and 18 mm Hg with no antiglaucoma medications.

The proband had 3 daughters. Two of them carried the Gly367Arg mutation (patients E and F in Figure 1 and Table 1). They were 23 and 22 years old. Their IOP was 15–17 mm Hg, and they did not show apparent glaucomatous disc change. Results of visual field tests (Humphrey 30-2) were normal.

The other daughter did not carry any mutation, and ocular examination revealed normal results.

The Cousin of the Proband and His Children

A cousin of the proband, a 51-year-old man, had POAG and carried the Gly367Arg mutation. In 1983, when he was 36 years old, he first recognized halo vision. He visited an ophthalmologist and was diagnosed as having POAG. His IOP before treatment is unknown. In 1986, his IOP was 32 and 33 mm Hg in the right and left eyes, respectively, although he had been treated with 1% pilocarpine, 0.5% timolol, and 1.25% epinephrine eyedrops, and 250 mg of oral acetazolamide per day. He underwent trabeculectomy in both eyes with the adjunctive use of 5-fluorouracil injection. Since then, his IOP has been around 5 mm Hg in the right eye and 11 mm Hg in the left eye. Fundus examination revealed damage of 0.95 × 1.0 in the

Table 1. Clinical Features of Family with Gly367Arg Mutation

Patient	MYOC Gene Mutation	Eye Disease	Age at Examination	Age at Diagnosis	Highest Intraocular Pressure (mm Hg)	Intraocular Pressure with Medication (mm Hg)	Halo Vision	Surgical Treatment
A	+	Glaucoma	83	29	Unknown	Unknown	+	+
B	+	Glaucoma	50	45	50	25	+	+
C	+	Glaucoma	51	36	Unknown	33	+	+
D	+	Unknown*	79	nd	Unknown	nd	–	–
E	+	None	22	nd	15	nd	–	–
F	+	None	23	nd	17	nd	–	–
G	+	None	18	nd	18	nd	–	–
H	+	None	20	nd	20	nd	–	–
I	–	None	44	nd	18	nd	–	–
J	–	None	19	nd	13	nd	–	–
K	–	None	14	nd	18	nd	–	–
L	–	None	16	nd	16	nd	–	–
M	–	None	23	nd	16	nd	–	–

MYOC: Myocilin; nd: not determined.
*Blind.

right eye and 0.95 × 0.95 in the left eye in the cup/disc ratio of the optic disc, with severe visual field defect.

He has 1 son and 2 daughters, and all of them had normal IOP (16–20 mm Hg) and normal optic discs. Of these 3 children, 2 daughters (patients G and H in Figure 1 and Table 1) carried the Gly367Arg mutation, but their visual field showed no apparent glaucomatous change. The son did not have the mutation.

The Mother of the Proband

The mother of the proband is an 84-year-old woman and carries the Gly367Arg mutation. She remembers that she had recognized halo vision when she was 29 years old, but she did not remember her IOP level at that time. She has undergone several operative procedures in the past, including trabeculectomy in both eyes, but her IOP has remained uncontrolled in both eyes. Ocular examination performed by us revealed that her IOP was 50 mm Hg in the right eye and only light perception remained. Her left eye was phthisical.

Discussion

In this family, 8 of the 13 members examined carried a heterozygous mutation of the myocilin gene. Three of them had POAG and 4 of them had normal eyes when examined recently. No POAG patient was found without the mutation.

The IOP of the proband was extremely high before treatment. The other 2 patients experienced halo vision, and they might have had very high IOPs previously, too. Conservative treatment was not effective for them, so surgery was required to normalize their IOPs. These characteristics are compatible with those of the other reported families with the myocilin gene mutation.

The average age of onset of the above 3 patients was 36.7 years and the average age of the carriers was 20.8 years. The patients, therefore, can be diagnosed as having adult-onset POAG, defined conventionally as chronic open-angle glaucoma with onset after 35 years of age.[5] It is known that the penetrance of the myocilin gene is rather high[3], and the 4 young carriers are considered to be at a high risk of developing glaucoma in the future.

The function of the myocilin protein in the regulation of the IOP has not yet been established. The myocilin protein was identified as a protein or glycoprotein expressed by trabecular meshwork cells under the administration of dexamethasone, but so far no steroid-induced glaucoma patients were reported to carry the myocilin gene mutation. The relationship between the response to steroids and the myocilin gene is unclear. Further investigation is required in both clinical and biological aspects to understand the exact role of the myocilin gene in the pathogenesis of glaucoma.

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