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ORIGINAL ARTICLE

Proinflammatory Effect of TWEAK/Fn14 Interaction in Human Retinal Pigment Epithelial Cells

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ABSTRACT

Purpose: To investigate the expression and function of fibroblast growth factor-inducible 14 (Fn14) in human retinal pigment epithelial cells.

Methods: A human retinal pigment epithelial cell line (RPE cells: ARPE-19) was used. Expression of Fn14 protein was assessed by flow cytometry. An antibody array and ELISA were used to detect chemokines and cytokines in the supernatant of RPE cells cultured with or without stimulation by TWEAK and/or TGF-β_1. To explore the mechanism by which TWEAK stimulates RPE cells, we investigated phosphorylation of MAP kinase in TWEAK-stimulated cells. We also investigated whether TWEAK induced the migration of RPE cells by performing an *in vitro* wound assay.

Results: RPE cells showed constitutive surface expression of Fn14 protein. FGF, VEGF, and TGF-β_1 did not induce Fn14 expression by RPE cells. TWEAK increased the production of IL-8 and MCP-1 by RPE cells via Fn14, and TGF-β_1 augmented TWEAK-induced production of these chemokines. TWEAK induced the phosphorylation of MAP kinase in RPE cells and promoted the migration of these cells via MAP kinase.

Conclusion: TWEAK/Fn14 interaction may have proinflammatory effects in RPE cells.

KEYWORDS: Chemokine; Fn14; MAP kinase; Retinal pigment epithelial cells; TWEAK

INTRODUCTION

Tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK) is a type II transmembrane protein belonging to the TNF family that was originally identified as a relatively poor inducer of apoptosis.[1] Recent studies have shown that TWEAK is a multifunctional cytokine. TWEAK can be cleaved to generate a soluble factor with multiple biological activities, including stimulation of cell proliferation,[2–4] migration,[5,6] angiogenesis,[2,7–10] apoptosis,[11] and inflammatory cytokine production.[12,13] These actions are principally mediated via a TWEAK receptor named fibroblast growth factor-inducible 14 (Fn14). Fn14 is a type I transmembrane protein with a single cysteine-rich domain in the extracellular region and a short cytoplasmic region containing a TNF receptor-associated factor (TRAF)-binding motif.[6,14] This receptor mediates signaling via the NF-kB and mitogen-activated protein kinase (MAPK) pathways.[15,16] TWEAK is pro-inflammatory cytokine, so persistent TWEAK-Fn14 axis activation may play a role in human diseases that involve an excessive or abnormal inflammatory response. Recent studies using rodent models of human disease have indicated that TWEAK-dependent Fn14 signaling may contribute to the development of three autoimmune inflammatory diseases, which are rheumatoid arthritis,[17,18] systemic lupus erythematosus,[19–21] and multiple sclerosis.[22–24] Furthermore, there have

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been reports that soluble TWEAK in the serum and urina serves as a biomarker for several diseases, including atherosclerosis,[25] hemophagocytic lymphohistocytosis,[26] and lupus nephritis.[27] Therefore, the interaction between TWEAK and Fn14 may regulate various physiological processes, but it seems to play a particularly important role in inflammation. However, the pathological role of TWEAK in the retina is largely unknown, and the expression and function of Fn14 in RPE cells has not been investigated to our knowledge. Therefore, the present study was performed to assess the expression and role of Fn14 in RPE cells.

MATERIALS AND METHODS

Antibodies and Ligands

Anti-human Fn14 monoclonal antibody (mAb) with or without phycoerythin (PE)-conjugation and isotype control mouse IgG with or without PE-conjugation were purchased from e Bioscience (San Diego, CA, USA). This mAb without PE-conjugation has a blocking activity. Phospho-p38 MAP kinase (Thr 180/Tyr 182) rabbit polyclonal antibody was obtained from Cell Signaling Technology (Danvers, MA, USA). This antibody detects endogenous levels of p38 MAP kinase only when activated by phosphorylation at threonine 180 and throsine 182. These Abs were used for flow cytometry. Recombinant human soluble TWEAK, TGF-β_1, FGF-2, and VEGF were purchased from R&D Systems (Minneapolis, MN, USA).

Cell Culture

ARPE-19 (a human RPE cell line) was purchased from the American Type Culture Collection (No. CRL-2302; Rockville, MD, USA). The cells were maintained in Dulbecco's modified essential medium and Ham's F12 medium (DMEM/F12; 1:1, Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS), penicillin at 100 U/mL, and streptomycin at 100 mg/mL. Cells were cultured at 37°C in an atmosphere of 10% CO_2 and 90% moist air until confluence was reached. Media were changed twice a week. Cell viability was assessed by trypan blue dye exclusion.

Flow Cytometry

To detect the expression of Fn14 protein in RPE cells, we used flow cytometry with a FACScan (BD Biosciences, San Jose, CA, USA). After being washed with buffer (FACS PBS [pH 7.4], 0.5% BSA, and 0.02% sodium azide), 10^6 cells were treated with Fc-block (BD Biosciences) for 15 min and then were incubated with mAb targeting human Fn14 or isotype control mouse IgG for 1 hr at room temperature. The cells were subsequently washed twice more with the same buffer and analyzed. To gate out dead cells, staining with a propidium iodide (PI) kit was performed according to the manufacturer's instructions (BD Biosciences). To investigate MAPK phosphorylation in TWEAK-stimulated cells, we also performed flow cytometry with FITC-conjugated anti-rabbit IgG (BD PharMingen, San Diego, CA, USA) as the secondary antibody. For analysis of intracellular expression, a cell fixation-permeabilization kit (Cytofix/Cytoperm; BD Biosciences) was used. Data were analyzed with the accompanying software (Cell Quest; BD Biosciences).

Antibody Array Analysis

The conditioned medium obtained from cultured RPE cells was analyzed with an antibody array kit (Ray Bio; Human Angiogenesis Antibody I Kit; RayBiotech Inc., Norcross, GA, USA) according to the manufacturer's instructions. This kit could simultaneously detect 20 different molecules, including chemokines, cytokines, soluble cytokine receptors, and growth factors.

The antibody array was placed into an 8-well tray, washed twice with Tris-buffered saline, and incubated for 30 min at room temperature with 2 mL/well of 1 × blocking buffer. Then 1 mL of supernatant from RPE cells cultured in a 6-well plate (35 mm in diameter) was added to each array and incubation was performed overnight at 4°C. After the samples were decanted, the antibody arrays were washed three times (for 5 min each) with 2 mL of 1 × washing buffer I at room temperature, followed by washing twice (for 5 min each) with 1 × washing buffer II at room temperature. Next, 1 mL of a 1:250 dilution of biotinylated antibody was added to each array, and incubation was done overnight at 4°C with shaking. After further washing, the array was incubated for 1 hr at room temperature with 2 mL/well of a 1:1000 dilution of HRP-conjugated streptavidin. After thorough washing, each array was incubated at room temperature for 5 min with a mixture of 1 × detection buffers C and D. Finally, the arrays were exposed to autoradiography film (BioMax Light Film; Eastman Kodak, Tokyo, Japan) for 1 min before processing. The signal intensity of individual spots was determined by densitometry with analytical software (Gel-Pro; Media Cybernetics, Inc., Silver Spring, MD, USA).

Cytokine and chemokine production was investigated after cells were washed twice with PBS and cultured in serum-free DMEM with or without TWEAK stimulation (100 ng/mL). The supernatant

was harvested after 24hr and analyzed by using the antibody array.

Enzyme-Linked Immunosorbent Assay

To detect IL-8 and monocyte chemoattractant protein-1 (MCP-1) in the conditioned medium of RPE cells, we used ELISA kits according to the manufacturer's instructions (Quantikine; R&D Systems). RPE cells were grown to subconfluence in DMEM/F12 with 10% FCS, washed twice with PBS, and then incubated in serum-free basal DMEM/F12 for 24hr with or without exposure to TWEAK (100ng/mL) and/or TGF-β (10ng/mL). Subsequently, the supernatants were harvested for ELISA.

Wound Assay

To determine the effect of TWEAK on cell migration, confluent monolayers of RPE cells in 6-well plates were wounded with a pipette tip, washed twice with PBS, and incubated in serum-free DMEM/F12 medium with or without TWEAK (100ng/mL). Some cells were also incubated with TWEAK and PD98059 (30mM in 0.1% DMSO) for 24hr and 48hr at 37°C. Photographs were taken 0, 24, and 48hr after creation of the wounds.

Statistical Analysis

Results are expressed as the mean ± SE. The significance of differences was evaluated by Student's *t*-test (Excel Software; Microsoft, Redmond, WA, USA).

RESULTS

Flow Cytometric Analysis of Fn14 Expression by RPE Cells

We examined Fn14 expression by RPE cells. FACS analysis using anti-Fn14 mAb showed that RPE cells constitutively expressed Fn14 on the cell surface. Next, we examined the influence of adding FGF or VEGF (both at 10ng/mL for 24hr) to RPE cell cultures and found that Fn14 expression was not changed. In FACS analysis, we recognized an almost same mean intensity of positive area in control and experimental groups. Since TGF-β_1 synergistically enhanced the induction of chemokine production by TWEAK, we investigated whether Fn14 expression by RPE cells was influenced after exposure to TGF-β_1. When RPE cells were incubated with TGF-β_1 (10ng/mL) for 24hr, these cells did not show an increase of Fn14 expression by FACS analysis (Figure 1).

Phosphorylation of MAPK in RPE Cells by TWEAK

To explore the mechanism by which TWEAK stimulates human RPE cells, we investigated the phosphorylation of MAPK in TWEAK-stimulated RPE cells by FACS analysis. As shown in Figure 2, TWEAK (100ng/mL) induced the phosphorylation of MAPK in RPE cells after 10min of stimulation.

Assessment of Chemokine Production in RPE Cells by Antibody Array Analysis

To investigate production of chemokines and other factors by RPE cells, we assessed 20 molecules by antibody array analysis. A representative array that was used to analyze the culture supernatant of RPE cells is shown in Figure 3. IL-8 and MCP-1 proteins were detected in RPE cell culture medium. The RPE cells also produced cytokines and various growth factors, such as IL-6, tissue inhibitor of metalloproteinase (TIMP)-1, TIMP-2, angiogenin, EGF, and bFGF.

To examine whether signaling via TWEAK enhanced chemokine/cytokine production, we compared RPE cells treated with TWEAK (100ng/mL for 24hr) and untreated cells. The mean optical density of each positive spot from the culture supernatants of cells incubated with TWEAK was compared with that of the corresponding spot from the culture supernatant of unstimulated cells. This analysis showed that stimulation of RPE cells with TWEAK enhanced the production of IL-8. In contrast, MCP-1 production was slightly enhanced.

TGF-β_1 Enhances TWEAK-Induced Production of IL-8 and MCP-1by RPE Cells

We then investigated whether stimulation with TWEAK (100ng/mL) and/or TGF-β_1 (10ng/mL) enhanced the production of these chemokines by ELISA. As shown in Figure 4, TWEAK induced the production of both IL-8 and MCP-1, and this effect was almost completely inhibited by blocking the TWEAK/Fn14 interaction with an anti-Fn14 mAb (1mg/mL). These findings indicated that TWEAK stimulated RPE cells to produce IL-8 and MCP-1 via Fn14. Next, we examined the influence of TGF-β_1 on TWEAK-induced production of IL-8 and MCP-1 by RPE cells. Stimulation with TGF-β_1 (10ng/mL for 24hr) alone did not induce the

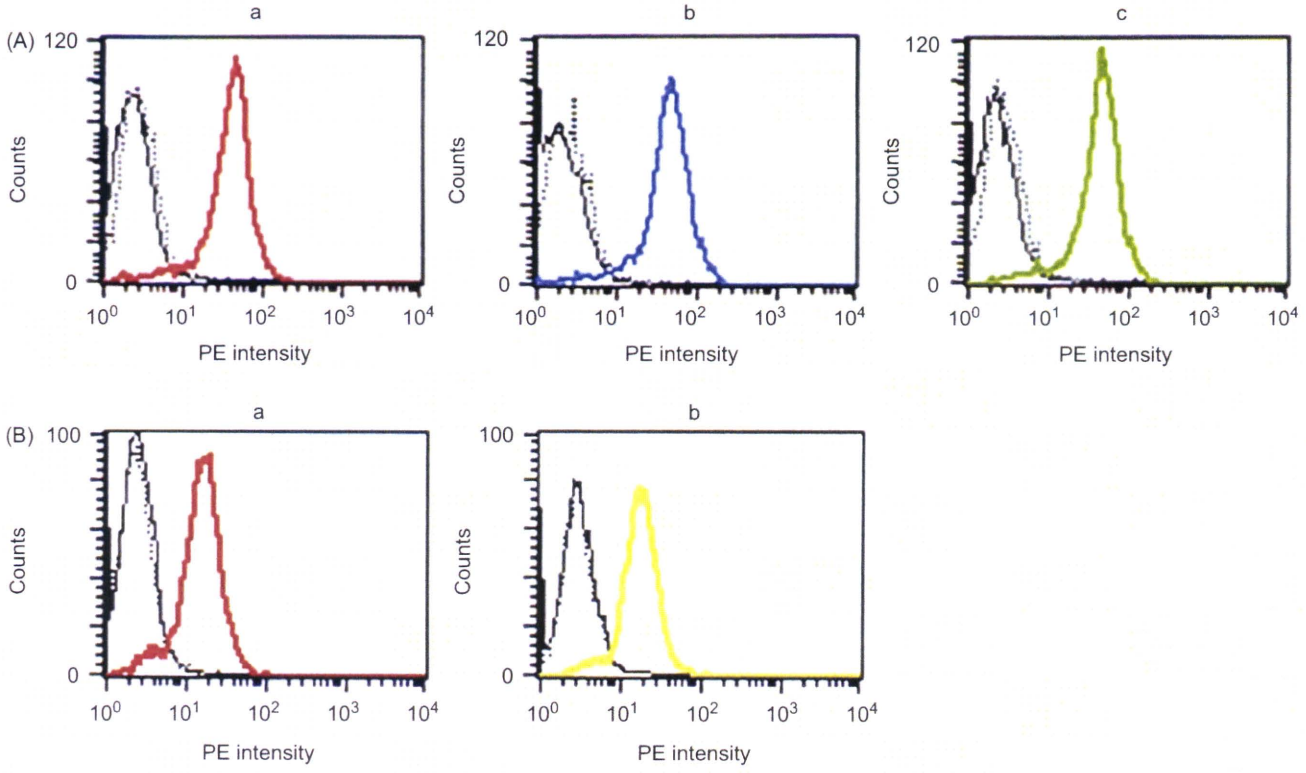

FIGURE 1 Flow cytometric analysis of Fn14 expression by RPE cells treated with or without FGF, VEGF, and TGF-β_1. The RPE cells constitutively expressed Fn14 on the cell surface. The expression of Fn14 of RPE cells treated with FGF, VFGF, and TGF-β_1 was not changed. Black trace: NoAb; Dotted trace: Control mouse IgG. Red, blue, green, or yellow trace: Data with anti-Fn14, mAb. (A) RPE cells treated with or without FGF and VFGF. (a) No treatment, (b) FGF (10 ng/mL), (c) VEGF (10 ng/mL). (B) RPE cells treated with or without TGF-β_1. (a) No treatment, (b) TGF-β_1 (10 ng/mL).

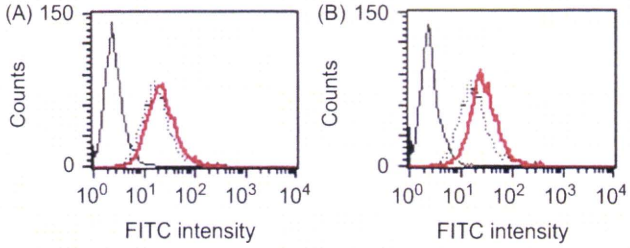

FIGURE 2 Flow cytometric analysis of phosphorylation of MAPK in RPE cells. TWEAK-induced phosphorylation of MAPK in RPE cells. Black trace: No Ab; Dotted trace: Control rabbit IgG; Red trace: Anti-MAPK Ab. (A) No treatment, (B) Cells treated with TWEAK (100 ng/mL).

production of these chemokines by RPE cells. However, TGF-β_1 augmented TWEAK-induced chemokine production by RPE cells, and this effect was almost completely inhibited by blocking the TWEAK/Fn14 interaction with an anti-Fn14 mAb.

Cell Migration

Using an *in vitro* wound assay, we examined the effect of TWEAK on cell migration. A uniform wound was made with a pipette head in culture dishes of confluent cells. After washing the culture extensively with PBS, TWEAK was added at a concentration of 100 ng/mL. Cells were incubated for 24 and 48 hr and then were photographed. As shown in Figure 5, cells at the edge of the wound migrated into the denuded area more extensively when TWEAK was added. This showed that TWEAK promoted the migration of RPE cells. Next, to determine whether MAPK played a crucial role in cell migration by TWEAK, we examined whether migration was inhibited by PD98059 (10 mM, a specific MAPK inhibitor). As can be seen in Figure 5, cells at the edge of the wound almost never migrated when PD98059 was added. Therefore, TWEAK promoted cell migration via MAPK.

DISCUSSION

The basal surface of each RPE cell is adjacent to the choroid, which contains circulating leukocyte. RPE cells exhibit polarized secretion of chemokines toward their basal side, suggesting that such chemokines may recruit leukocytes.[28,29] Our antibody array analysis revealed that RPE cells produced IL-8, MCP-1, IL-6, TIMP-1, TIMP-2, angiogenin, EGF, and b-FGF. Among

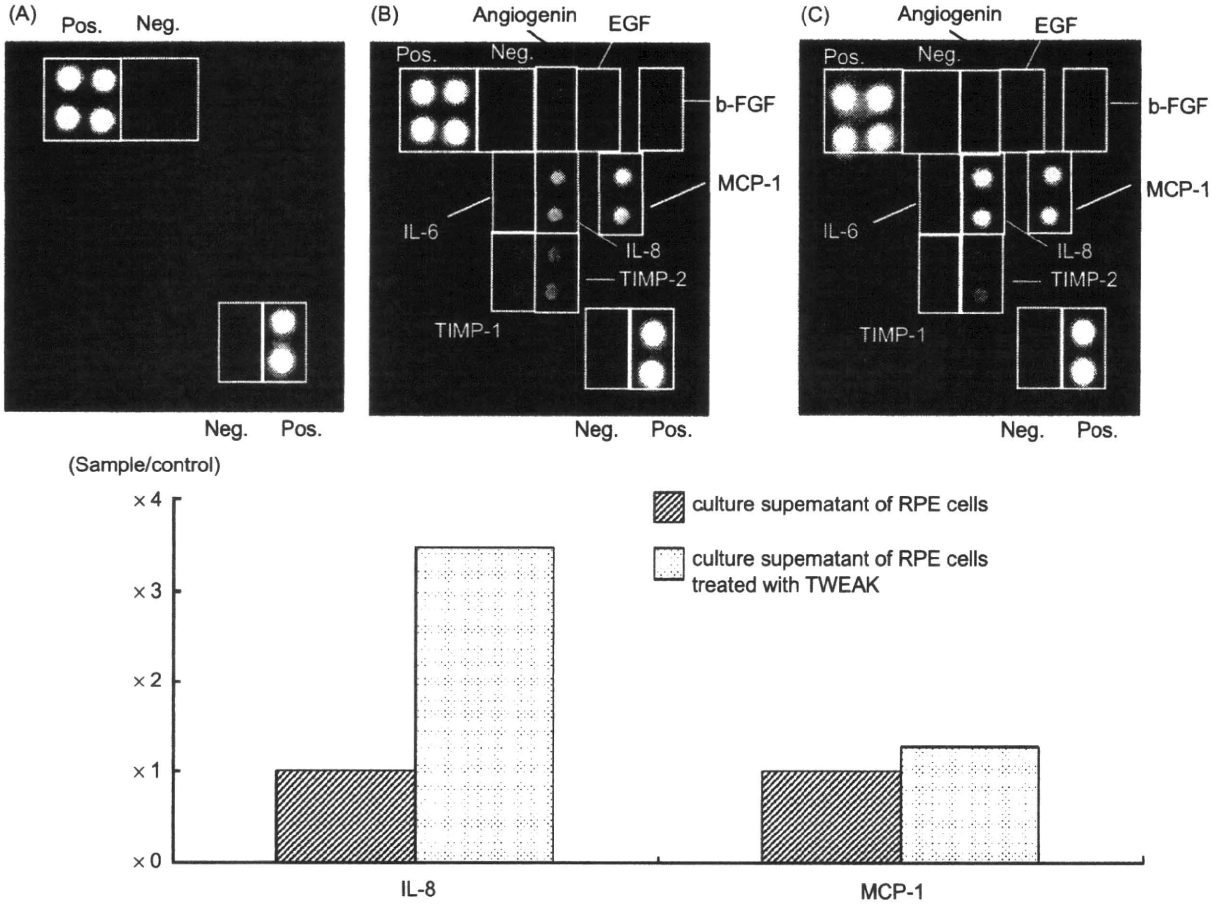

FIGURE 3 Chemokine production in RPE cells detected by antibody array analysis. The appearance of representative array for factors in the culture supernatant of RPE cells is shown. No chemokines are detected in basic medium (A). IL-8 and MCP-1 proteins were detected in RPE cells culture medium (B). RPE cells also produced cytokines and growth factors such as IL-6, TIMP-1, TIMP-2, angiogenin, EGF, and b-FGF (B). To examine whether TWEAK (100 ng/mL 24 hr) enhanced chemokine production, we compared RPE cells treated with TWEAK (C) and cells without any treatment (B). The mean optical intensity of positive spots from the culture supernatants of cells incubated with TWEAK was compared with that from the culture supernatants of unstimulated cells. Stimulation of RPE cells with TWEAK enhanced the production of IL-8. In contrast, MCP-1 production was slightly enhanced. (A) Medium only, (B) Culture supernatant of RPE cells, (C) Culture supernatant of RPE cells treated with TWEAK.

these chemokines, IL-8 and MCP-1 were expressed at much higher levels than the other chemokines. Furthermore, we showed that TWEAK increased the production of IL-8 and MCP-1 by RPE cells via Fn14. IL-8 is a chemoattractant for neutrophils and eosinophils,[30] while MCP-1 attracts monocytes[31] and lymphocytes.[32] These two chemokines are responsible for the majority of the chemotactic activity derived from RPE cells. Several studies have revealed that the levels of chemokines, including IL-8 and MCP-1, are higher in the vitreous of patients with proliferative vitreoretinal disease (PVR), proliferative diabetic retinopathy (PDR), and uveitis than in normal subjects.[33–48] These chemokines may stimulate the infiltration of neutrophils and monocytes into the eyes of patients with such disorders. Therefore, TWEAK may have an important role in the recruitment of circulating leukocytes in patients with vitreoretinal diseases.

Next, we examined the effect of TGF-β_1 on TWEAK-induced production of IL-8 and MCP-1 by RPE cells. TGF-β isoforms 1, 2, and 3 have been identified in the posterior segment of the eye by both molecular and immunohistochemical techniques,[49–51] with TGF-β_2 considered to be the predominant isoform at this site.[51] TGF-β_2 has been hypothesized to play a major role in PVR because its level is increased in the vitreous of patients with PVR.[52] However, TGF-β_1 may also have an important role in the pathogenesis of PVR and PDR. Bochaton-Piallat et al. investigated the expression of TGF-β_1 by scar-like epithelial membranes (ERMs) in the vitreous cavity of 23 patients during microsurgery for PVR or PDR.[53] Immunohistochemical investigation revealed strong TGF-β_1 expression in ERMs, indicating that TGF-β_1 also has an important role in the pathogenesis of PVR and PDR. In the present study, we showed that TGF-β_1

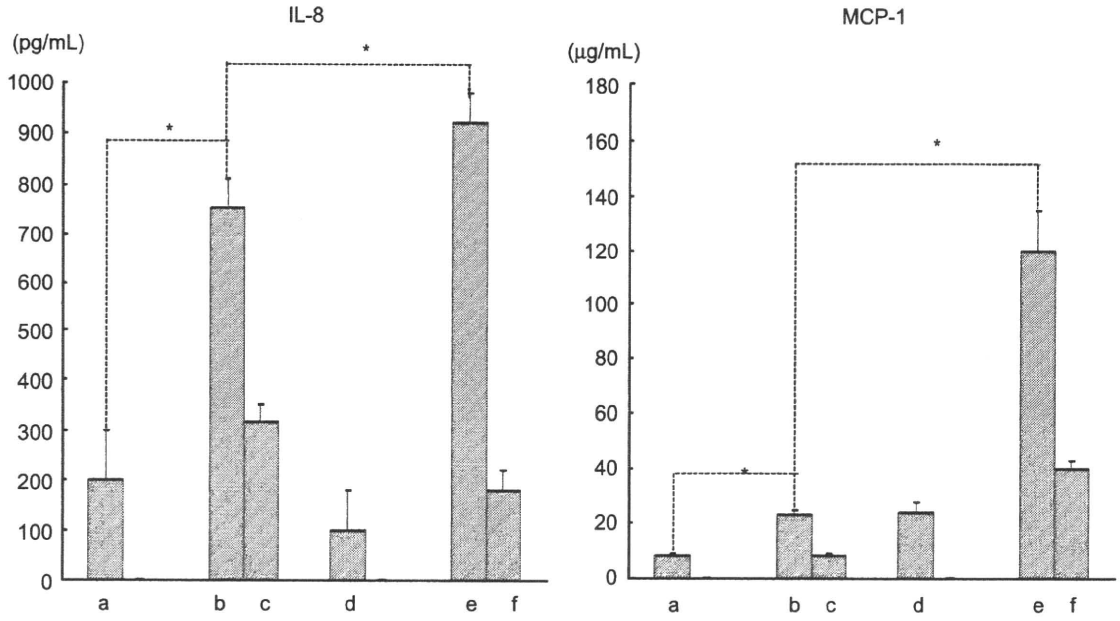

FIGURE 4 Chemokine production in RPE cells detected by ELISA. TWEAK (100 ng/mL) induced IL-8 and MCP-1 production by RPE cells, and TGF-β_1 (10 ng/mL) augmented TWEAK induced these chemokines. Anti-Fn14 blocking mAb (1 mg/mL) almost completely inhibited chemokines production by TWEAK. (A) No treatment, (B) TWEAK, (C) TWEAK + Anti-Fn14 mAb, (D) TGF-β_1, (E) TWEAK + TGF-β_1, (F) TWEAK + TGF-β_1 + Anti-Fn14 mAb.

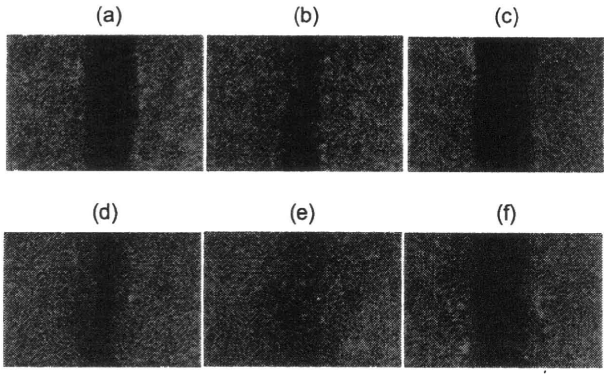

FIGURE 5 TWEAK induced migration of RPE cells in the *in vitro* wound assay. Confluent cell monolayers on a 6-well plate were scratched by a pipette tip to create a cell-free area, and TWEAK (100 ng/mL) alone or TWEAK (100 ng/mL) with PD98059 (30 mM) were added as described in METHODS. Wound closure was documented by photography 24 hr and 48 hr after the treatment. (A) No treatment (24 hr), (B) TWEAK (24 hr), (C) TWEAK and PD98059 (24 hr), (D) No treatment (48 hr), (E) TWEAK (48 hr), (F) TWEAK and PD98059 (48 hr).

augmented the TWEAK-induced production of IL-8 and MCP-1. However, the surface expression of Fn14 on RPE cells was not increased by TGF-β_1. At the present time, the precise mechanism through which TGF-β_1 promotes the increase of IL-8 and MCP-1 production by TWEAK is unclear. Because TGF-β_1 influences MAPK-mediated signaling, production of IL-8 and MCP-1 via MAPK phosphorylation may be augmented by TWEAK synergically with TGF-β_1.[54] Therefore, an increase of IL-8 and MCP-1 production in RPE cells may occur as a result of interference with signaling by TGF-β and TWEAK.

TWEAK expression is restricted to epithelial and mesenchymal cells, but they are expressed in a variety of cell and tissue types. When we examined the expression of TWEAK in RPE cells, its expression was recognized at the mRNA level but not at the protein level (data not shown). On the other hand, RPE cells constitutively expressed Fn14 on the surface. Because some growth factors or mitogens, including fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF), up-regulate Fn14 expression in vascular endothelial cells,[55] we examined the influence of FGF and VEGF on RPE cells and found that Fn14 expression was not changed. In our previous study, we found that TGF-β_1 also increased surface Fn14 expression by keratocytes (data not shown). However, Fn14 expression on RPE cells was not increased by TGF-β_1. Thus, it appears that Fn14 expression on RPE cells is relatively stable compared with that on vascular endothelial cells and keratocytes, although other growth factors/cytokines should also be tested for a possible role in the regulation of Fn14 in RPE cells. ARPE-19 cells are hybridomas by which the characteristics are not entirely the same as the original RPE cells. In our previous experiments, we detected the expression of Fn14 in primary cultured mouse RPE cells (data not shown). In the future, we think that fur-

ther investigation in the expression of Fn14 in primary cultured human RPE cells is necessary.

TWEAK also promoted the migration of RPE cells in the present *in vitro* wound assay. Several reports have indicated that MAPK, a serine/threonine protein kinase, has a critical role in the proliferation and migration of RPE cells stimulated by growth factors such as platelet-derived growth factor (PDGF), tumor necrosis factor-α (TNF-α), and hepatocyte growth factor (HGF).[56–60] Therefore, we examined the role of MAPK signaling in the effect of TWEAK on cell migration. Treatment of RPE cells with TWEAK resulted in MAPK phosphorylation within 10 min on FACS analysis. To examine the relation between MAPK phosphorylation and cell migration, we used inhibitors of the MAPK pathway. Blockade of this pathway by a specific MAPK inhibitor (PD98059) inhibited the migration of RPE cells stimulated by TWEAK. These data suggest that MAPK could be involved in the TWEAK-induced migration of RPE cells. With respect to the role of MAPK in retinal disease, it undergoes phosphorylation within 15 min after retinal detachment and remains phosphorylated for several days in RPE cells.[61] In the early stage of PVR of rabbit model, increased phosphorylation of MAPK was observed in the retina and RPE cells.[61] These results suggest that the interaction between TWEAK and MAPK may have a crucial role in various retinal diseases.

In summary, we demonstrated a novel effect of TWEAK on RPE cells. Our results suggested that the TWEAK/Fn14 interaction was involved in chemokine production, while TGF-β_1 augmented the TWEAK-induced production of IL-8 and MCP-1. Furthermore, TWEAK promoted the migration of RPE cells via phosphorylation of MAPK. However, the role of TWEAK in retinal diseases still remains unclear. We are currently examining TWEAK expression in vitreous fluid obtained from patients with various retinal diseases. In the future, TWEAK may be used as a biomarker of vitreoretinal diseases, and the TWEAK/Fn14 axis may be a potential therapeutic target for these diseases.

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Absence of Association between *COL1A1* Polymorphisms and High Myopia in the Japanese Population

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Purpose. The collagen type I alpha 1 (*COL1A1*) gene was recently reported to be associated with high myopia in the Japanese population. To validate this positive association, the tag single-nucleotide polymorphism (tSNP) approach was used.

Methods. Eight tSNPs, including rs2075555 and rs2269336 (previously reported to be high myopia–susceptible SNPs in the Japanese), were selected to tag the linkage disequilibrium blocks harboring the *COL1A1*. These tSNPs were genotyped by using an SNP assay. A total of 427 unrelated Japanese cases with high myopia (axial length, ≥26.50 mm in both eyes; the refraction of the 644 phakic eyes ranged from −5.0 to −36.0 D, with a mean ± SD of −13.61 ± 4.20 D) and 420 Japanese control subjects were recruited. Genotype and allele distributions were compared between the cases and controls by using the χ^2 test, with multiple testing corrections performed by the permutation test.

Results. There was no association noted between high myopia and rs2075555 ($P = 0.47$, $P_c > 0.99$) and rs2269336 ($P = 0.40$, $P_c > 0.99$). Meta-analysis of a previous Japanese study and new data obtained in a fixed-effect model indicated a mild significant association of high myopia with rs2075555 (odds ratio [OR], 1.19; 95% confidence interval [CI], 1.03–1.38, $P = 0.022$) and rs2269336 (OR, 1.18; 95% CI, 1.02–1.36, $P = 0.026$). No significant associations were seen with further tSNPs tests.

Conclusions. This study did not replicate the previously reported positive association between *COL1A1* and high myopia in the Japanese population, and thus the genetic risk associated with this gene, if any, is weaker than originally reported. (*Invest Ophthalmol Vis Sci.* 2009;50:544–550) DOI:10.1167/iovs.08-2425

Myopia is a common ocular disorder that is found worldwide. The most important contributor to myopic refraction is the axial length of the eyeball (i.e., longer eyes are more myopic),[1–3] and when the elongation of the eyeball is excessive, the condition is called high myopia. It is well known that high myopia is associated with many ocular complications[4] and is one of the major causes of blindness in many developed countries.[5–10] Thus, the economic and social burden of high myopia is an important public health problem.

Recent population-based studies have estimated the prevalence of high myopia in the elderly population to be approximately 1% to 5%,[2,11–16] and this prevalence has been increasing worldwide, especially in the younger East Asian population.[17–19] One possible explanation for the increase in high myopia in developed countries is a change in lifestyle. It has been reported that environmental factors such as near work and higher education can contribute to the development of high myopia. However, genetic factors also have been reported to be responsible for the development of high myopia[20] (for detailed review, see Refs. 21, 22). For example, several twin studies have shown that there is a high heritability of refraction and axial length.[23–28] There have been many studies in which investigators have attempted to use a genetic approach to identify the susceptible locus or genes for high myopia (for detailed review, see Refs. 22, 29, 30), with several genes now reported to have an association.[31–39] However, there are other studies in which the original findings for these genes were not replicated.[25,38,40–48]

Many animal studies on myopia have indicated that there is a local control mechanism of eye growth; hyperopic defocus produces signals from the retina through the retinal pigment epithelium and choroid to cause remodeling of the scleral tissue, and the secondary scleral remodeling results in axial elongation (for detailed review, see Refs. 21, 22, 49, 50). In mammals, the scleral tissue contains approximately 90% collagen by weight, predominantly type I[51] (Zorn M, et al. *IOVS* 1992;33:ARVO Abstract 1811; Norton TT, et al. *IOVS* 1995;36: ARVO Abstract 3517). Several animal studies have reported that mRNA expression of type I collagen in the sclera is reduced during the development of myopia.[52,53] The *COL1A1* (collagen type I, alpha 1) gene encodes the pro-α1 chains of type I collagen. This *COL1A1* is located on 17q21.33, where a myopia susceptibility locus (MYP5, 17q21-22) has been reported.[54] These pathologic, expression, and genetic studies indicated that *COL1A1* is a good candidate gene for myopia. In 2007, Inamori et al.[36] reported that the single-nucleotide polymorphisms (SNPs) rs2075555 and rs2269336 in *COL1A1* are significantly associated with high myopia in the Japanese population. However, Liang et al.[44] reported that the polymor-

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phisms of *COL1A1* are not significantly associated with high myopia in the Taiwanese population.

In the present study, we conducted a systematic case-control study to validate the association between the polymorphisms of the *COL1A1* gene (including previously reported susceptible SNPs) and high myopia in the Japanese population.

Methods

All investigations in this study adhered to the tenets of the Declaration of Helsinki. The Institutional Review Board and the Ethics Committee of the each institute approved the protocols of this study. All the patients were fully informed of the purpose and procedures of this study, and written consent was received from each patient.

Study Population

A total of 427 unrelated Japanese patients with high myopia (mean age ± SD, 57.6 ± 14.1 years; men/women, 31.4% vs. 68.6%) were recruited from the Center for Macular Diseases of Kyoto University Hospital, Fukushima Medical University Hospital, and the high myopia clinic of Tokyo Medical and Dental University Hospital. All underwent comprehensive ophthalmic examinations, including dilated indirect and contact lens slit lamp biomicroscopy, automatic objective refraction evaluation, and measurement of the axial length by applanation A-scan ultrasound (UD-6000; Tomey, Nagoya, Japan) or partial coherence interferometry (IOLMaster; Carl Zeiss Meditec, Dublin, CA). To be enrolled in the study, the patients with high myopia were required to have an axial length of ≥26.50 mm in both eyes. The axial lengths of the 854 eyes ranged from 26.50 to 36.32 mm (mean ± SD, 29.18 ± 1.68). Among the 854 eyes enrolled, 644 (75.4%) were phakic, 185 (21.7%) were pseudophakic, and 25 (2.9%) were aphakic. The mean refraction of the 644 phakic eyes ranged from −5.00 to −36.00 D (mean ± SD, −13.61 ± 4.20). To check the results in another axial length-based definition of high myopia, a subset with longer axial lengths was also defined. The inclusion criterion for this subset was axial length ≥ 28.00 mm in both eyes. A total of 278 patients were enrolled in this subset. The axial length of the 556 eyes in this subset was 29.95 ± 1.43 mm. There were 394 phakic eyes in this subset, with the refraction ranging from −7.25 to −36.00 D (−15.03 ± 4.14). If subjects had preexisting ocular diseases or a history of ocular surgery, with the exception of cataract surgery, they were excluded from the study.

As a population-based control, DNA samples from 420 subjects (mean age ± SD, 44.3 ± 12.1 years; men/women, 46.2% vs. 53.8%) were randomly selected from the Pharma SNP Consortium. The cohort had been recruited for previous genomic studies and was regarded as being representative of the general Japanese population.[55] All participants were Japanese and none of the subjects had any history of ocular diseases.

SNP Selection and Genotyping

To replicate the positive association of the SNPs with high myopia that has been reported in a previous Japanese study, we genotyped rs2075555 from intron 11 of the *COL1A1*, and rs2269336 from the 5′ upstream region of the *COL1A1*. The associated functions for these two SNPs have yet to be elucidated. To systematically examine the possible association between the polymorphisms of the *COL1A1* gene and the high myopic cases, we used the tag SNP (tSNP) approach. The public dbSNP database build 126 and the HapMap database phase 2 release 22 were used to extract the relevant sequencing information for the *COL1A1* gene and the genotyping information for the SNPs. Haplotypes and linkage disequilibrium (LD) blocks were inferred by a solid spine of LD with a minimum D′ of 0.8, according to Haploview version 4.0.[56] We selected eight tSNPs to tag the LD blocks harbored within and surrounding the *COL1A1* gene (Fig. 1A). Tagging of the LD blocks was based on the software Tagger (http://www.broad.mit.edu/mpg/tagger/ provided in the public domain by the Broad Institute, Massachusetts of Technology, Cambridge, MA), which used a minimum r^2 of 0.8 and a minimum minor allele frequency (MAF) of 20% in the Japanese population of the HapMap dataset. It has been reported in a Japanese study that two SNPs (rs2075555 and rs2269336) are high myopia-susceptible polymorphisms.[36] These SNPs were both included within the eight tSNPs. Genomic DNA was extracted from the leukocytes of the peripheral blood and purified (QuickGene-810; Fujifilm, Tokyo, Japan). All the tSNPs were genotyped with an SNP assay (*Taq*man; Applied Biosystems, Foster City, CA), according to the manufacturer's instruction.

Statistics

The statistical power calculation was performed using the module case-control for discrete traits of the Genetic Power Calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/ provided in the public domain by the Psychiatric and Neurodevelopmental Genetics Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA).[57] For the calculation, the type 1 error rate was set at 0.05 and the prevalence of high myopia in the general population was set at 1%. The HWE for the genotype distributions was examined by using the χ^2 test in each group. Differences in the observed genotype and allelic frequencies between the cases with high myopia and the control subjects were also examined by the χ^2 test. For the current experiment, we combined our results for the single SNP analysis of rs2075555 and rs2269336 with the results of a previous Japanese study,[36] in which the Mantel-Haenszel method based on the fixed-effect model was used to elucidate their predisposing effects on high myopia in a larger Japanese population. We performed the meta-analysis using the R software package Meta (http://cran.r-project.org/web/packages/rmeta/index.html/ provided in the public domain by The Comprehensive R Archive Network, hosted by the Department of Statistics and Mathematics, University of Vienna, Austria).

Differences in the estimated haplotype frequencies between the cases and the controls were also examined by the χ^2 test. These SNP and haplotype analyses were performed with Haploview ver. 4.0. The multiple testing correction for P (P_c) was performed by the permutation test (number of iterations, 10,000), also in Haploview, ver. 4.0. The level of statistical significance was set at $P < 0.05$ and $P_c < 0.05$.

Results

The distribution of the genotypes for the eight tSNPs among the cases with high myopia and the control subjects were all in HWE ($P > 0.05$). The results of the genotyping for rs2075555 and rs2269336 in the cases with high myopia and the control subjects are shown in Table 1. In this study, there were no significant differences noted for the genotype and allelic frequencies for these two SNPs in the *COL1A1* gene between the patient and the control cases. The results of the meta-analysis for rs2075555 and rs2269336 are shown in Table 2. The Mantel-Haenszel method showed the summary odds ratio (OR) to be 1.19 (95% confidence interval [CI], 1.03–1.38; $P = 0.022$) for rs2075555 and 1.18 (95% CI, 1.02–1.36; $P = 0.026$) for rs2269336, respectively. When we performed the subset analysis on the 278 cases with the longer axial lengths (≥28.00 mm in the both eyes), no new significant differences were found for rs2075555 and rs2269336 in our own study (data not shown). The summary OR for the meta-analysis using the subset was 1.27 (95% CI, 1.08–1.48; $P = 0.0035$) for rs2075555 and 1.25 (95% CI, 1.07–1.46; $P = 0.0051$) for rs2269336, respectively.

We also performed a systematic tSNP approach to assess the possible association between the *COL1A1* and high myopia in Japanese. The distributions of the allelic frequencies for all the eight tSNPs are given in Table 3. None of the eight tSNPs showed significant differences between the cases with high myopia and the control subjects with regard to the distribution of the genotype and allelic frequency. We also performed a

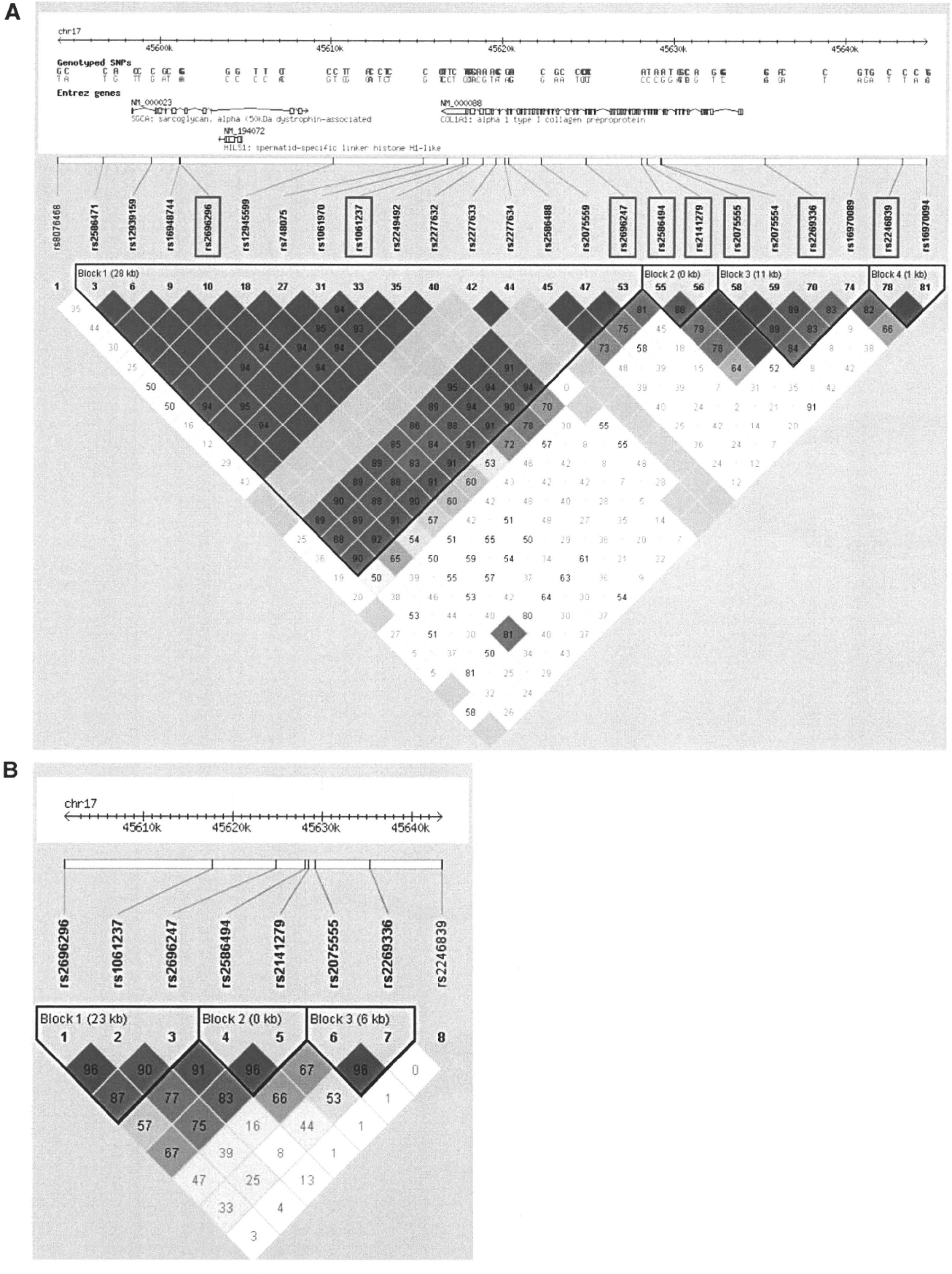

FIGURE 1. LD structure across the *COL1A1* region and selected tag SNPs. LD blocks were inferred by a solid spine of LD with a minimum D′ of 0.8. (**A**) LD structure in Japanese samples from the HapMap database. SNPs with MAF > 5% are displayed, with the selected 8 tSNPs shown in boxes. (**B**) LD structure for the samples obtained in the present study (427 unrelated Japanese cases with high myopia [axial length ≥ 26.50 mm in both eyes] and 420 healthy Japanese controls). Three haplotype blocks were identified. The distribution of the haplotypes from each of the three blocks is shown in Table 4.

TABLE 1. Frequencies of Genotypes and Alleles of rs2075555 and rs2269336 in the Current Study

	Genotype					Allele				
SNP ID*		Case (%)	Control (%)	P†	OR (95% CI)		Case (%)	Control (%)	P†	OR (95% CI)
rs2075555	CC	167 (39.2)	158 (37.7)	0.736	1.17 (0.79–1.75)	C	528 (62.0)	505 (60.3)	0.471	1.07 (0.88–1.31)
	CA	194 (45.5)	189 (45.1)		1.14 (0.77–1.68)	A	324 (38.0)	333 (39.7)		
	AA	65 (15.3)	72 (17.2)		1.00 (ref.)					
rs2269336	CC	141 (33.8)	125 (29.9)	0.444	1.14 (0.78–1.67)	C	469 (56.2)	453 (54.2)	0.400	1.09 (0.90–1.32)
	CG	187 (44.8)	203 (48.6)		0.93 (0.65–1.33)	G	365 (43.8)	383 (45.8)		
	GG	89 (21.3)	90 (21.5)		1.00 (ref.)					

The nucleotides were defined on the forward strand of the reference sequence by dbSNP Build 126.

* SNP ID in National Center for Biotechnology Information (NCBI; Bethesda, MD) dbSNP Build 126.

† The nominal probabilities were calculated by the χ^2 test.

subset analysis on cases with axial lengths ≥ 28.00 mm. However, no new significant differences were found for the subjects in our study (data not shown).

We identified three haplotype blocks in the *COL1A1* gene (Fig. 1B). The estimated haplotype frequencies in the cases with high myopia and the control subjects are shown in Table 4. The haplotype frequencies were not significantly different between the patients with high myopia and the control subjects after the multiple testing corrections. Before correction, only one haplotype in block 1 showed a trend for a mildly significant difference in distribution ($P = 0.014$, $P_c = 0.14$). However, a haplotype analysis using the subset with axial lengths ≥ 28.00 mm did not show significant results for any of the blocks, even before correction (data not shown).

In addition, to check the results of our analyses using the same inclusion criteria that were used in a previous Japanese study,[36] we performed another subset analysis on 261 binocular phakic cases with refractions < −9.25 D (mean refraction ± SD, −14.46 ± 3.94 D; mean axial length ± SD, 29.17 ± 1.60 mm). The allelic frequency distributions for all the eight tSNPs in the subset analysis are given in Table 5. No new significant differences were noted for the genotype and allelic frequencies for rs2075555 and rs2269336. A haplotype in block 1 (the same haplotype described above) showed a trend for a mildly significant different distribution ($P = 0.034$, $P_c = 0.33$). However, there were no tSNPs or haplotypes that showed any significant differences after the multiple testing corrections.

DISCUSSION

The results in this study did not show significant associations with high myopia of the two SNPs of the *COL1A1* gene (rs2075555 and rs2269336, which have been reported to be high-myopia-susceptible SNPs in the Japanese population[36]). A systematic examination using the tSNP approach to access the possible association between the *COL1A1* gene and Japanese high myopia also did not find any significant results. The power calculation results that were based on the multiplicative model showed that our own observations rejected the reported ORs of rs2075555 (OR, 1.36) and rs2269336 (OR, 1.31) from the previous Japanese study with 85.9% and 78.7% power, respectively.

In our study, we defined high myopia by axial length instead of refraction. On the other hand, in the previous Japanese study that was included in our current analyses, they defined high myopia as refraction < −9.25 D.[36] Thus, one possible explanation for the discrepancy that was observed between the previous Japanese study and our own observations might be related to the difference in the way that high myopia was defined. To further examine this possibility, we performed a subset analysis on binocular phakic cases that had refraction < −9.25 D in both eyes, and we found no further significant differences in the present study. High myopia is most commonly defined by refraction. However, corneal curvature and the intraocular lens may also affect the refraction. Among these multiple factors, the axial length is the most important contributor to myopic refraction.[1–3] Hence, we suggest that the axial length is a more appropriate parameter than refraction when assessing the association between the *COL1A1* gene and high myopia. However, our study could not show any significant result whether the axial length or the refraction was chosen as the parameter. We cannot conclude which of the two, axial length or refraction, is the more appropriate parameter to assess the association between the *COL1A1* gene and high myopia.

TABLE 2. Meta-analysis* of the *COL1A1* rs2075555 and rs2269336 in Japanese Subjects with High Myopia

		Case		Control			
SNP ID†	Source	Subject Number	Risk Allele‡ *n* (%)	Subjects (*n*)	Risk Allele‡ *n* (%)	OR (95% CI)	P§
rs2075555	Current study	426	528 (62.0)	419	505 (60.3)	1.07 (0.88–1.31)	0.47
	Inamori et al.[36]	328	422 (64.3)	326	372 (57.1)	1.36 (1.09–1.70)	0.0071
	Total	754		745		1.19 (1.03–1.38)	0.022
rs2269336	Current study	417	469 (56.2)	418	453 (54.2)	1.09 (0.90–1.32)	0.40
	Inamori et al.[36]	329	397 (60.3)	330	354 (53.6)	1.31 (1.06–1.64)	0.014
	Total	746		748		1.18 (1.02–1.36)	0.026

* This meta-analysis was performed using the Mantel-Haenszel method based on the fixed-effect model.

† SNP ID in NCBI dbSNP Build 126.

‡ Risk alleles of rs2075555 (A/C) and rs2269336 (C/G) are allele C and allele C, respectively. The nucleotides were defined on the forward strand of the reference sequence by the dbSNP Build 126. We confirmed through personal communication that the nucleotides for rs2269336 (C/G) in a previous study by Inamori et al.[36] were defined on the reverse strand of the reference sequence by dbSNP.

§ The nominal probabilities were calculated by the χ^2 test.

TABLE 3. Association of Eight Tagged SNPs of the *COL1A1* with High Myopia in the Current Study

SNP ID*	Position†	Ref‡	Var‡	Case–Control		OR (95% CI)	P§	
				Allele Counts	Allele Frequencies		Nominal	Corrected
rs2696296	45601230	G	A	427:421, 395:445	0.504, 0.470	1.14 (0.94–1.38)	0.171	0.886
rs1061237	45617774	T	C	469:377, 477:339	0.554, 0.585	0.88 (0.73–1.07)	0.214	0.933
rs2696247	45624902	A	G	589:257, 567:271	0.696, 0.677	1.10 (0.89–1.35)	0.386	0.997
rs2586494	45628154	A	C	407:441, 425:409	0.480, 0.510	0.89 (0.73–1.08)	0.224	0.940
rs2141279	45628463	T	C	270:574, 268:568	0.320, 0.321	1.00 (0.81–1.22)	0.977	1.000
rs2075555	45629290	A	C	324:528, 333:505	0.380, 0.397	0.93 (0.77–1.13)	0.471	1.000
rs2269336	45635355	C	G	469:365, 453:383	0.562, 0.542	1.09 (0.90–1.32)	0.400	0.998
rs2246839	45643395	C	T	321:527, 321:511	0.379, 0.386	0.97 (0.80–1.18)	0.759	1.000

Axial length ≥ 26.5 mm in both eyes.

* SNP ID in NCBI dbSNP Build 126.

† Position of the polymorphism in the reference sequence NT_010783.14.

‡ Ref and Var were the reference and variant nucleotides, respectively, that were defined on the forward strand of the reference sequence by dbSNP.

§ The nominal probabilities were calculated by the χ^2 test, and the multiple testing corrections were performed by the permutation test (number of iterations = 10,000).

Another difference between the previous study and our own observations is that we used a population-based control. The prevalence of high myopia in the general population has been estimated to be approximately 1% to 5% in elderly adults.[2,11–16] Even if the control subjects in our study had no history of ocular diseases, the possibility exists that some of the eyes might have had an axial length ≥ 26.50 mm without the presence of vision threatening complications. If this were the case, this would be a possible explanation for the negative results that we found for our case-control association study. To check the results for a different axial-length-based definition, we also performed a subset analysis on cases with longer axial lengths (≥28.00 mm in both of the eyes). However, no new significant differences were found in the present study. Further subset analyses by redefining the cutoff value of axial length (27.00, 27.50, 28.50, and 29.00 mm) did not show any significant results (data not shown). Thus, we can conclude that the results of the present study did not replicate the previously reported Japanese study, which found significant associations for rs2075555 and rs2269336 with high myopia.

The results of our meta-analysis suggested that there were mildly significant associations between these two SNPs and high myopia in the Japanese population. However, it should be noted that we combined the data of our own study with the data of a previous Japanese study, a study that was the first to report positive results.[36] There was a potential for publication bias in the first positive study, and indeed, the reported ORs in the first positive studies were higher than most of the results that have been reported for subsequent replication studies.[58] Therefore, actual ORs of these SNPs are estimated at up to the ORs that are suggested by the results of the meta-analysis in this study. We concluded that the genetic risk in the *COL1A1* gene, if any, is weaker than has been originally reported.

In conclusion, the present study failed to replicate the positive association between the polymorphisms of the *COL1A1* gene and high myopia that has been reported in a prior study involving Japanese subjects. To elucidate whether the *COL1A1* gene in the MYP5 locus is associated with high myopia in the Japanese population, additional genetic and molecular biological studies are needed.

TABLE 4. Association of Haplotypes across the *COL1A1* Region with High Myopia in the Current Study

Haplotype*	Frequency	Case–Control		OR (95% CI)	P†	
		Ratio Counts	Frequencies		Nominal	Corrected
Block 1						
ATA	0.489	403.5:450.5, 424.3:415.7	0.472, 0.505	0.88 (0.73–1.06)	0.179	0.889
GCG	0.293	243.0:611.0, 253.0:587.0	0.285, 0.301	0.92 (0.75–1.14)	0.452	0.998
GCA	0.132	130.0:724.0, 93.7:746.3	0.152, 0.112	1.43 (1.08–1.90)	0.014	0.142
GTA	0.062	57.3:796.7, 48.3:791.7	0.067, 0.057	1.18 (0.79–1.75)	0.411	0.997
ATG	0.017	11.8:842.2, 17.3:822.7	0.014, 0.021	0.67 (0.32–1.41)	0.288	0.980
Block 2						
AC	0.488	405.6:448.4, 420.6:419.4	0.475, 0.501	0.90 (0.75–1.09)	0.289	0.980
CT	0.315	269.6:584.4, 263.3:576.7	0.316, 0.313	1.01 (0.82–1.24)	0.922	1.000
CC	0.192	175.9:678.1, 150.2:689.8	0.206, 0.179	1.19 (0.94–1.52)	0.156	0.842
Block 3						
CC	0.545	474.3:379.7, 448.3:391.7	0.555, 0.534	1.09 (0.90–1.32)	0.369	0.993
AG	0.381	319.3:534.7, 326.4:513.6	0.374, 0.389	0.94 (0.77–1.14)	0.533	0.999
CG	0.067	54.8:799.2, 57.9:782.1	0.064, 0.069	0.93 (0.63–1.36)	0.699	1.000

Axial length ≥ 26.5 mm in both eyes. The nucleotides were defined on the forward strand of the reference sequence by dbSNP Build 126.

* Haplotypes and linkage disequilibrium (LD) blocks were inferred by a solid spine of LD with a minimum D′ of 0.8. The LD structure for the Japanese samples in the current study is shown in Figure 1(B).

† The nominal probabilities were calculated by the χ^2 test, with the multiple testing corrections performed by the permutation test (no. of iterations = 10,000).

TABLE 5. Association of Eight Tagged SNPs of *COL1A1* with a Subset of High Myopia in the Current Study

				Case–Control			*P*§	
SNP ID*	**Position†**	**Ref‡**	**Var‡**	**Allele Frequencies**	**Allele Counts**	**OR (95% CI)**	**Nominal**	**Corrected**
rs2696296	45601230	G	A	266:250, 395:445	0.516, 0.470	1.20 (0.96–1.49)	0.105	0.743
rs1061237	45617774	T	C	277:239, 477:339	0.537, 0.585	0.82 (0.66–1.03)	0.087	0.640
rs2696247	45624902	A	G	348:168, 567:271	0.674, 0.677	0.99 (0.78–1.25)	0.933	1.000
rs2586494	45628154	A	C	259:259, 425:409	0.500, 0.510	0.96 (0.77–1.20)	0.732	1.000
rs2141279	45628463	T	C	158:358, 268:568	0.306, 0.321	0.94 (0.74–1.19)	0.581	1.000
rs2075555	45629290	A	C	197:323, 333:505	0.379, 0.397	0.92 (0.74–1.16)	0.496	1.000
rs2269336	45635355	C	G	296:218, 453:383	0.576, 0.542	1.15 (0.92–1.43)	0.222	0.956
rs2246839	45643395	C	T	197:323, 321:511	0.379, 0.386	0.97 (0.77–1.22)	0.798	1.000

Eyes were binocular phakic and had refraction < -9.25 D.

* SNP ID in NCBI dbSNP Build 126.

† Position of the polymorphism in the reference sequence NT_010783.14.

‡ Ref and Var are, respectively, reference and variant nucleotides defined on the forward strand of the reference sequence by dbSNP.

§ The nominal probabilities were calculated by the χ^2 test, and the multiple testing corrections were performed by the permutation test (number of iterations = 10,000).

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Vision-Related Quality of Life and Visual Function after Vitrectomy for Various Vitreoretinal Disorders

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Purpose. To investigate vision-related quality of life (VR-QOL) in patients undergoing vitrectomy for various vitreoretinal disorders and to evaluate the relationship between VR-QOL and visual function.

Methods. The study included 100 normal control subjects and 299 patients with various vitreoretinal disorders including proliferative diabetic retinopathy (PDR), diabetic macular edema (DME), branch retinal vein occlusion (BRVO), central retinal vein occlusion (CRVO), macular hole (MH), epiretinal membrane (ERM), and rhegmatogenous retinal detachment (RD). The 25-item National Eye Institute Visual Function Questionnaire (VFQ-25) was answered by the patients with vitreoretinal disorders before and 3 months after pars plana vitrectomy, as well as by the normal control subjects. Clinical data were collected, including visual acuity, contrast sensitivity, and severity of metamorphopsia.

Results. Vitrectomy significantly improved the VFQ-25 composite score in all vitreoretinal disorders. Preoperative VFQ-25 composite scores in MH and ERM were significantly higher than those in PDR, DME, and BRVO. Postoperative VFQ-25 composite scores were significantly higher in MH, ERM, and RD than in PDR, DME, BRVO, and CRVO. A greater improvement in the VFQ-25 composite score was observed in ERM than in DME. Multiple regression analysis revealed that changes in contrast sensitivity had a significant correlation with changes in the VFQ-25 composite score in PDR and DME. Changes in metamorphopsia were significantly associated with changes in the VFQ-25 composite score in MH and ERM.

Conclusions. Vitrectomy significantly improved VR-QOL in various vitreoretinal disorders. The largest improvement in VR-QOL was observed in ERM and smallest improvement in DME. The visual function parameters associated with VR-QOL are different depending on vitreoretinal disorders. (*Invest Ophthalmol Vis Sci.* 2010;51:744–751) DOI:10.1167/iovs.09-3992

In ophthalmology, traditional objective clinical outcome measures such as visual acuity are increasingly being complemented with assessment of patients' perception of their visual function and quality of life. The National Eye Institute 25-Item Visual Function Questionnaire (VFQ-25) is a vision-related quality of life (VR-QOL) instrument designed to assess patients' perception of their visual function and QOL.[1] The VFQ-25 has been used to track the outcome of many ocular diseases.[2–16] Prior studies have reported the influence of vitrectomy on VR-QOL in patients with proliferative diabetic retinopathy (PDR), retinal detachment (RD), macular hole (MH), epiretinal membrane (ERM), and age-related macular degeneration (AMD).[10–16] Such studies have demonstrated that vitrectomy can improve patients' visual function as well as VR-QOL. A comparison of changes in VR-QOL after surgery among the various vitreoretinal diseases, however, has not been conducted. In the present study, VR-QOL data from 100 normal subjects and 299 patients with various vitreoretinal disorders were assessed by the VFQ-25. The purpose of this study was to compare VR-QOL among vitreoretinal disorders and to evaluate the relationship between VR-QOL and visual function in each disorder.

Methods

We included 99 patients with proliferative diabetic retinopathy (PDR), 38 patients with diabetic macular edema (DME), 20 patients with branch retinal vein occlusion (BRVO), 12 patients with central retinal vein occlusion (CRVO), 42 patients with macular hole (MH), 33 patients with epiretinal membrane (ERM), and 55 patients with rhegmatogenous retinal detachment (RD), all of whom were underwent pars plana vitrectomy at Tsukuba University Hospital between June 14, 2005, and April 20, 2007. One hundred volunteers served as normal control subjects. This research was conducted according to the tenets of the Declaration of Helsinki, and written informed consent was obtained from each suitable participant. This study was approved by the Institutional Review Board at the Tsukuba University Hospital. Exclusion criteria included patients with a history of vitreoretinal surgery and ocular disorders except for mild refractive errors and mild cataract. Patients who had undergone bilateral vitrectomy within 3 months were excluded.

The logarithm of minimum angle of resolution best-corrected visual acuity (logMAR BCVA), letter contrast sensitivity (CS), and severity of metamorphopsia were obtained before surgery and at 3 months after surgery.

CS was measured by the CSV-1000LV chart (Vector Vision, Columbus, OH). This instrument uses letter optotypes, all of which are the same size and of low spatial frequency (2.4 cyc/deg).[17] There were eight contrast levels (standard, 35.5%, 17.8%, 8.9%, 6.3%, 4.5%, 2.2%, and 1.1%), and each contrast level had three letters. The measurements were performed at a 2.5-m distance under full spectacle correction. The mean luminance was 81 cd/m^2. The test results were recorded, not as the contrast threshold or CS, but as the number of 24 letters correctly identified.[18,19]

The severity of metamorphopsia was evaluated in the patients with MH and ERM (M-Charts; Inami Co., Tokyo, Japan). The M (metamorphopsia)-Charts consist of 19 dotted lines with dot intervals ranging from 0.2° to 2.0° of visual angle. If the straight line is replaced with a dotted line and the dot interval is changed from fine to coarse, the distortion of the line decreases with the increasing dot interval, until the dotted line appears straight.[20,21] At first, vertical straight lines (0°) were shown to the patient. If the patient recognized the line as straight, the metamorphopsia score was 0. If the patient recognized the line as irregular or curved, then subsequent pages of the M-Charts, in which the dot intervals of the dotted line change from fine to coarse, were shown one after another. When the

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TABLE 1. Background Data of Normal Controls and Patients with Vitreoretinal Disorders

	NC	PDR	DME	BRVO	CRVO	MH	ERM	RD
Eyes, *n*	100	99	38	20	12	42	33	55
Men/women	56/44	53/46	23/15	9/11	9/3	20/22	16/17	40/15
Age, y	61.2 ± 9.9	57.7 ± 12.9	62.7 ± 9.0	64.1 ± 9.1	62.4 ± 11.3	64.3 ± 9.6	67.0 ± 8.4	52.3 ± 13.2*

Data are expressed as the mean ± SD. NC, normal control subjects.
* Significantly different from the other groups ($P < 0.05$, Fisher PLSD).

patient recognized a dotted line as being straight, the visual angle that separated the dots was considered to represent his or her metamorphopsia score for a vertical line. The M-Charts were rotated 90° and the same test was performed with the horizontal lines. The examinations were repeated three times for each subject, to evaluate reproducibility of the results, and the mean was used for data analyses. The examination was performed at 30 cm and the refraction of the eye was corrected exactly for this distance.

The indications for vitrectomy in the patients with PDR included recurrent or persistent nonclearing vitreous hemorrhage, traction or combined traction–rhegmatogenous retinal detachment, and adherent posterior hyaloid causing excessive macular traction. DME was defined by clinically significant macular edema according to the ETDRS guidelines, diagnosed by slit lamp biomicroscopy, and a central foveal thickness of ≥250 μm, as measured by optical coherence tomography (OCT, Stratus OCT 3000; Carl Zeiss Ophthalmic Systems-Humphrey Division, Dublin, CA), and vitrectomy was indicated when ≥3 months had passed after at least one session of laser treatment and when logMAR BCVA in the affected eye was 0.2 or worse. The indication for vitrectomy in BRVO and CRVO included persistent nonclearing vitreous hemorrhage and/or cystoid macular edema. The indication for vitrectomy in MH included stage II to IV full-thickness macular hole by means of slit lamp biomicroscopy with a 90-D fundus lens and OCT. ERM was defined as a translucent or semitranslucent membrane with macular thickening involving the center of the macula, with or without distortion and wrinkling of the inner retinal surface on biomicroscopy and OCT; vitrectomy was indicated if patients reported significant metamorphopsia. The indications for vitrectomy in RD included presence of causative horseshoe tears due to posterior vitreous detachment.

Surgical Procedures

All surgeries were performed by a single surgeon (FO) under sub-Tenon local anesthesia. The crystalline lens was removed by phacoemulsification and intraocular lens implantation was performed when required, followed by 20-gauge, three-port pars plana vitrectomy or 25-gauge transconjunctival vitrectomy. With conventional contact lenses, posterior hyaloid separation and removal of the posterior vitreous membrane were performed. Peripheral retinal examination with scleral depression was performed to search for a retinal tear or dialysis in all cases. In the patients with PDR, bimanual delamination, en bloc dissection, and segmentation techniques were used to remove proliferative tissues, and when required, 20% sulfur hexafluoride (SF_6) gas and/or silicone oil was injected. In the patients with ERM, the membrane was engaged and removed from the macula with a pick and intraocular forceps. In the patients with MH, the limiting membrane was peeled off with the aid of indocyanine green or triamcinolone acetonide, followed by injection 20% SF_6 gas. In the patients with RD, surgical procedures comprised release of vitreous traction around the breaks, internal drainage of the subretinal fluid, total gas–fluid exchange (20% SF_6), and endolaser photocoagulation. In the patients with macular edema due to DME, BRVO, and CRVO, triamcinolone acetonide (4 mg in 0.1 mL) was injected into the vitreous cavity, and 20 mg in 0.5 mL triamcinolone acetonide was administered into the sub-Tenon space of the superior temporal quadrant approximately 10 mm posterior to the limbus, at the end of surgery.

Visual Function Questionnaire (VFQ-25)

The patients answered the VFQ-25 before surgery and 3 months after surgery. The preoperative VFQ-25 was completed 1 to 2 days before surgery. In the patients with RD, preoperative evaluation by VFQ-25 was not performed, because of the rapid nature of its onset. The research staff explained the questionnaire to the patients, gave instructions verbally, and provided assistance when required. The completed questionnaires were reviewed for missing data by the research staff. Before surgery, all the missing items were filled out by the subjects themselves.

The VFQ-25 comprises 25 items that require the patient to assess the levels of difficulty of particular visual symptoms or day-to-day activities.[1] Each item is assigned to one of the following 12 subscales: general health, general vision, ocular pain, near activities, distance activities, social functioning, mental health, role difficulties, dependency, driving, color vision, and peripheral vision. The subscales are 0 to 100 points, where 100 indicates the highest possible function or minimal subjective impairment. The VFQ-25 composite score is calculated as the unweighted average response to all items, excluding the questions on general health.[1] The VFQ-25 used in this study was a Japanese version, with modifications to suit the Japanese culture and lifestyle. The modified NEI VFQ-25 questionnaire has been assessed for reliability and validity, and it has been shown to accurately measure VR-QOL in Japanese individuals.[22]

Statistical Analysis

The mean scores and standard deviations were calculated for each VFQ-25 subscale and the composite score, in the patients with vitreoretinal disorders and the normal control subjects. The Wilcoxon signed-ranks test was used to compare preoperative and postoperative results. The Mann-Whitney U test was performed to compare each subscale score and composite score between the patients with vitreoretinal disorders and the normal control subjects. Fisher's protected least-significant difference (PLSD) was performed to compare subscale

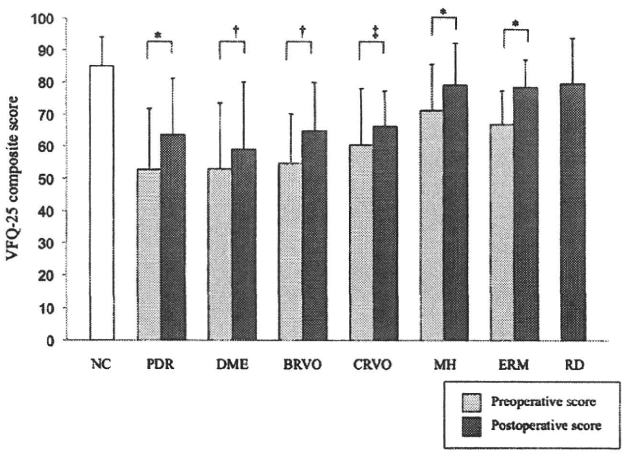

FIGURE 1. Preoperative and postoperative VFQ-25 composite scores in the patients with vitreoretinal disorders and in the normal control subjects. *$P < 0.0001$; †$P < 0.01$; ‡$P < 0.05$.