47. Finckh A, Dudler J, Wermelinger F, Ciurea A, Kyburz D, Gabay C, Bas S, SCQM PO (2010) Influence of anti-infliximab antibodies and residual infliximab concentrations on the occurrence of acquired drug resistance to infliximab in rheumatoid arthritis patients. Joint Bone Spine 77: 313–318
48. Yamada A, Sono K, Hosoe N, Takada N, Suzuki Y (2010) Monitoring functional serum antitumor necrosis factor antibody level in Crohn's disease patients who maintained and those who lost response to anti-TNF. Inflamm Bowel Dis 16:1898–1904
49. Reich K, Nestle FO, Papp K, Ortonne JP, Evans R, Guzzo C, Li S, Dooley LT, Griffiths CE, investigators Es (2005) Infliximab induction and maintenance therapy for moderate-to-severe psoriasis: a phase III, multicentre, double-blind trial. Lancet 366: 1367–1374
50. Adişen E, Aral A, Aybay C, Gürer MA (2010) Anti-infliximab antibody status and its relation to clinical response in psoriatic patients: a pilot study. J Dermatol 37:708–713
51. Sugita S, Yamada Y, Mochizuki M (2010) Relationship between serum infliximab levels and acute uveitis attacks in patients with Behçet disease. Br J Ophthalmol 95:549–552

Letters to the Editor

Research Correspondence

Voriconazole for Fungal Corneal Ulcers

Dear Editor:

There are promising reports in literature suggesting that newer generation triazole agents like voriconazole may overcome the significant shortcomings of commonly used antifungals such as poor ocular penetration, fungistatic nature, drug stability, lack of broad-spectrum coverage, and suboptimal aqueous concentration.[1–4] To the best of our knowledge, no study has compared efficacy of voriconazole with conventional antifungal treatment in severe keratomycosis, in a prospective randomized clinical trial. In this prospective randomized study, we compared the efficacy of oral and topical voriconazole with that of conventional antifungal treatment.

We prospectively included 45 eyes of 45 patients having severe fungal corneal ulcer (Table 1; available at http://aaojournal.org) and randomly divided them into 3 groups (restricted to 15 patients each): Group I received oral and topical voriconazole 1%, group II received oral voriconazole and topical natamycin 5% suspension, and group III received oral itraconazole and topical natamycin 5% suspension (Table 2; available at http://aaojournal.org).

Primary outcome measures were time to resolution of epithelial defect, infiltrates, and hypopyon (if present). Secondary outcome measures were nature of corneal opacity and best corrected visual acuity at the end of 3 months after enrollment.

Predisposing risk factors at baseline were noted in 34 (75.5%) eyes (Table 3; available at http://aaojournal.org). The most common risk factor noted was trauma to cornea in 23 (51.1%) eyes. Organisms were isolated in 17 (37.8%) eyes through culture. We isolated *Fusarium solani* in 6 (13.3%) eyes, *Aspergillus flavus* in 5 (11.1%) eyes, *Aspergillus niger* in 3 (6.6%) eyes, *Curvularia lunata* in 2 (4.4%) eyes, and *Acremonium spp* in 1 (2.2%) eye (Table 4; available at http://aaojournal.org).

The mean time for disappearance of hypopyon was 9.8±1.7, 12.3±3.6, and 16±10.52 days in groups I, II, and III respectively ($P = 0.231$). The mean time of resolution of infiltrates was 36.8±10.66, 38.81±8.94, and 36.7±10.42 days in groups I, II, and III respectively ($P = 0.860$). The mean time of closure of epithelial defect was 31.1±11.4, 29.18±8.25, and 31.8±11.4 days in group I, II, and III respectively ($P = 0.837$) (Table 5; available at http://aaojournal.org). At the end of 3 months, treatment success was achieved in 10 eyes (66.7%) in group I, 11 eyes (73.3%) in group II, and 10 eyes (66.7%) in group III respectively ($P = 0.902$). We did not note any significant difference in the incidence of complications including corneal perforation between the 3 groups (Table 6; available at http://aaojournal.org). In our study, we found a comparable rate of healing of epithelial defects and infiltrates in all the groups. Although not statistically significant, we observed a faster rate of resolution of hypopyon in patients receiving oral and topical voriconazole.

At the end of 3 months, leukomatous corneal opacity was observed in 9 (60%), 11 (73.3%), and 9 (60%) eyes in group I, II, and III respectively ($P = 0.4$). Also, improvement in visual acuity in healed subjects was similar in the study groups and depended on the location of the corneal opacity and its relation with the visual axis (Table 7; available at http://aaojournal.org).

In our study, logistic regression analysis identified age ≥45 years (95% confidence interval [CI], 1.15–60; $P = 0.036$) and epithelial defect area ≥36 mm^2 (95% CI, 2.56–151.49; $P = 0.004$) as the most significant risk factors for primary treatment failure in severe keratomycosis (Table 8; available at http://aaojournal.org). Although not statistically significant, we also found presence of hypopyon at baseline in all patients with treatment failure. One study identified ulcer size greater than 14 mm^2, presence of hypopyon and growth of *Aspergillus* species as the risk factors for poor treatment outcome in fungal keratitis treated with 5% natamycin monotherapy.[5] In our study, 4 of the 5 fungal ulcers caused by *Aspergillus* species responded well to voriconazole treatment. Thus, voriconazole may offer better efficacy compared with conventional antifungal drugs against corneal ulcers caused by *Aspergillus* species.

This is the first prospective randomized study on the role of voriconazole in treatment of fungal corneal ulcers. We found voriconazole effective and well-tolerated drug in treatment of severe keratomycosis. Further studies with a larger sample size might be helpful to establish voriconazole as a first line of treatment in keratomycosis.

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References

1. Reis A, Sundmacher R, Tintelton K. Successful treatment of ocular invasive mould infection (fusariosis) with the newer antifungal voriconazole. Br J Ophthalmol 2000;84:932–3.
2. Bunya V, Hammersmith K, Rapuano C, et al. Topical and oral voriconazole in the treatment of fungal keratitis. Am J Ophthalmol 2007;143:151–3.
3. Granados JM, Puerto N, Carrilero MJ. Efficiency of voriconazole in fungal keratitis caused by candida albicans. Arch Soc Esp Oftalmol 2004;79:565–8.
4. Stewart R, Quah SA, Neal TJ, Kaye SB. The use of voriconazole in treatment of Aspergillus fumigatus keratitis. Br J Ophthalmol 2007;91:1095–6.
5. Prajna L, Namperumalsamy VP, Kabra A, et al. Risk factors for treatment outcome in fungal keratitis. Ophthalmology 2006;113:526–30.

Spontaneous Regression of Intraocular Lymphoma

Dear Editor:

Intraocular lymphoma (IOL) is a rare malignancy of the eye, often seen in the context of central nervous system lymphoma. Cytological examination of intraocular specimens

remains the gold standard for the diagnosis of primary IOL, but measurement of the interleukin (IL)-10 concentration and gene rearrangement can be helpful.[1] We herein report an IOL case showing spontaneous regression, verified by clinico-histopathological analyses.

An 80-year-old man complained of blurred vision in his left eye (OS). On presentation, his visual acuity was counting fingers OS in April 2010. Ophthalmological findings were hypopyon and vitreous opacity OS. The right eye was normal. Since infectious endophthalmitis or uveitis was initially suspected, systemic antibiotics and corticosteroids were administered; however, ocular symptoms did not improve. After vitrectomy including silicon oil tamponade in October 2010, since an elevated retinal lesion was noted, he was referred to our hospital in November 2010. His visual acuity was hand motion OS. Slit-lamp examination demonstrated corneal epithelial edema without hypopyon OS. Fundus examination revealed an irregular-shaped yellowish elevated lesion with retinal hemorrhage located in the superior region of the optic disc (Fig 1A; arrow; available at http://aaojournal.org). Concentrations of IL-10, in vitreous samples obtained in October 2010, were 2111 pg/ml. Monoclonality of IgH gene rearrangement was confirmed with polymerase chain reaction method. Vitrectomy was performed to collect vitreous fluid submitted for cytological examination on January 28, 2011. Cytology demonstrated atypical lymphoid cells with a high nuclear/cytoplasm ratio (Fig 1B; available at http://aaojournal.org). From clinico-pathologic findings, a diagnosis of IOL was made. Systemic evaluation by imaging modalities and lumbar puncture displayed no abnormalities except for in the left eye. After vitrectomy, the visual acuity became no light perception OS. After informed consent was obtained, enucleation was eventually performed OS 2 weeks after the final vitrectomy.

A central cut section of the enucleated eye demonstrated an elevated lesion near the optic disc (Fig 2A; arrow, available at http://aaojournal.org), and retinal detachment. In a histological section of the elevated lesion, there was marked cell infiltration predominantly in the subretinal space (Fig 2B; available at http://aaojournal.org). At a high magnification, small lymphoid cells infiltrated the subretinal space with no cellular atypia (Fig 2C; available at http://aaojournal.org), even in deeper cut sections. A variety of small lymphoid cells were positive for CD20, a marker for B cells. Moreover, CD8-positive T lymphocytes massively infiltrated the subretinal space (Fig 2D; available at http://aaojournal.org). Monoclonality of IgH gene rearrangement was not observed in DNA isolated from the paraffin-embedded eyeball section. This patient was healthy without systemic metastases or recurrence 8 months after enucleation.

Based on searches in Medline and in PubMed, to the best of our knowledge, no articles have reported spontaneous regression of IOL in the English literature. Goto et al[2] reported a case of presumed spontaneous regression of IOL in a Japanese patient. In their report, retinal exudates, clinically supposed to be IOL, were histologically necrotic tissue without lymphoma cell infiltration, verified by a subretinal biopsy. Cytological, and IgH gene rearrangement examinations, and IL concentrations showed nothing of note.[2] Therefore, the previous report could not show the spatial-temporal process regarding the definite diagnosis of IOL and its spontaneous disappearance.

In this case, based on the presence of atypical lymphoid cells by cytology, monoclonal IgH gene rearrangement, and a high IL-10 concentration in the vitreous fluid, we diagnosed the patient with IOL before enucleation. Surprisingly, the enucleated eye demonstrated no atypical lymphoid cells even in deeper cut sections. A monoclonal IgH gene rearrangement was not detected in DNA samples isolated from the enucleated eye, indicating reactive lymphocyte infiltration. Since these results suggest that the enucleated eye did not contain lymphoma cells, we believe ours is the first report showing the complete spontaneous regression of IOL after vitrectomy, verified by clinico-histopathological analyses.

Histologically, diffuse large B-cell lymphoma, a high-grade non-Hodgkin lymphoma (NHL), can be a common subtype of primary IOL.[3] Indeed, complete spontaneous regression of such high-grade NHL is an extremely rare phenomenon.[4,5] The leading hypothesis regarding the spontaneous regression of NHL involves modulation of the host's immune system.[4,5] In this case, CD8-positive T lymphocytes massively infiltrated the subretinal space in the enucleated eye, suggesting that CD8+T cells are one of the possible factors responsible for the spontaneous regression of IOL.

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References

1. Rajagopal R, Harbour JW. Diagnostic testing and treatment choices in primary vitreoretinal lymphoma. Retina 2011;31:435–40.
2. Goto H, Murase K, Usui M. Case of spontaneous regression of intraocular lymphoma demonstrated by subretinal biopsy. Nippon Ganka Gakkai Zasshi 2006;110:226–31.
3. Horie Y, Saito W, Kase S, Yoshida K, et al. A case of primary central nervous system lymphoma with intraocular recurrence following optic nerve disorder. Retinal Cases Brief Reports 2007;1:165–8.
4. Iihara K, Yamaguchi K, Nishimura Y, et al. Spontaneous regression of malignant lymphoma of the breast. Pathol Int 2004;54:537–42.
5. Abe R, Ogawa K, Maruyama Y, et al. Spontaneous regression of diffuse large B-cell lymphoma harbouring Epstein-Barr virus: a case report and review of the literature. J Clin Exp Hematop 2007;47:23–6.

Measurement of Peripheral Eye Length

Dear Editor:

Recent evidence support the importance of peripheral refraction and eye shape in the development of myopia.[1,2] The method for peripheral refraction appears to be well established; however, the measurement of peripheral eye length (PEL) is less successful. Magnetic resonance imaging (MRI) and optical low-coherence interferometry have been used, but they are expensive, less accessible, and therefore limit their use in clinical assessment. In this study, we propose a new method to measure the PEL, using IOLMaster (Carl Zeiss Meditec,

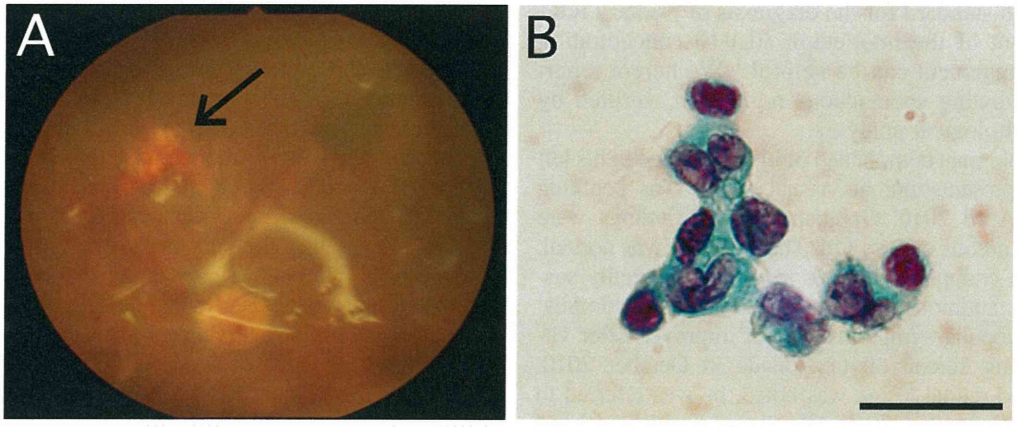

Figure 1. Funduscopic examination (**A**) and cytological findings in the vitreous fluid (**B**). A yellowish elevated lesion is present in the superior region of the optic disc (**A**, arrow). An irregular-shaped site of retinal hemorrhage can be observed near the elevated lesion and superior arcade (**A**). Cytology demonstrates atypical lymphoid cells with a high nuclear/cytoplasm ratio (**B**). Several atypical cells are hyperchromatic and show prominent nucleoli (**B**). Bar indicates 50 μm.

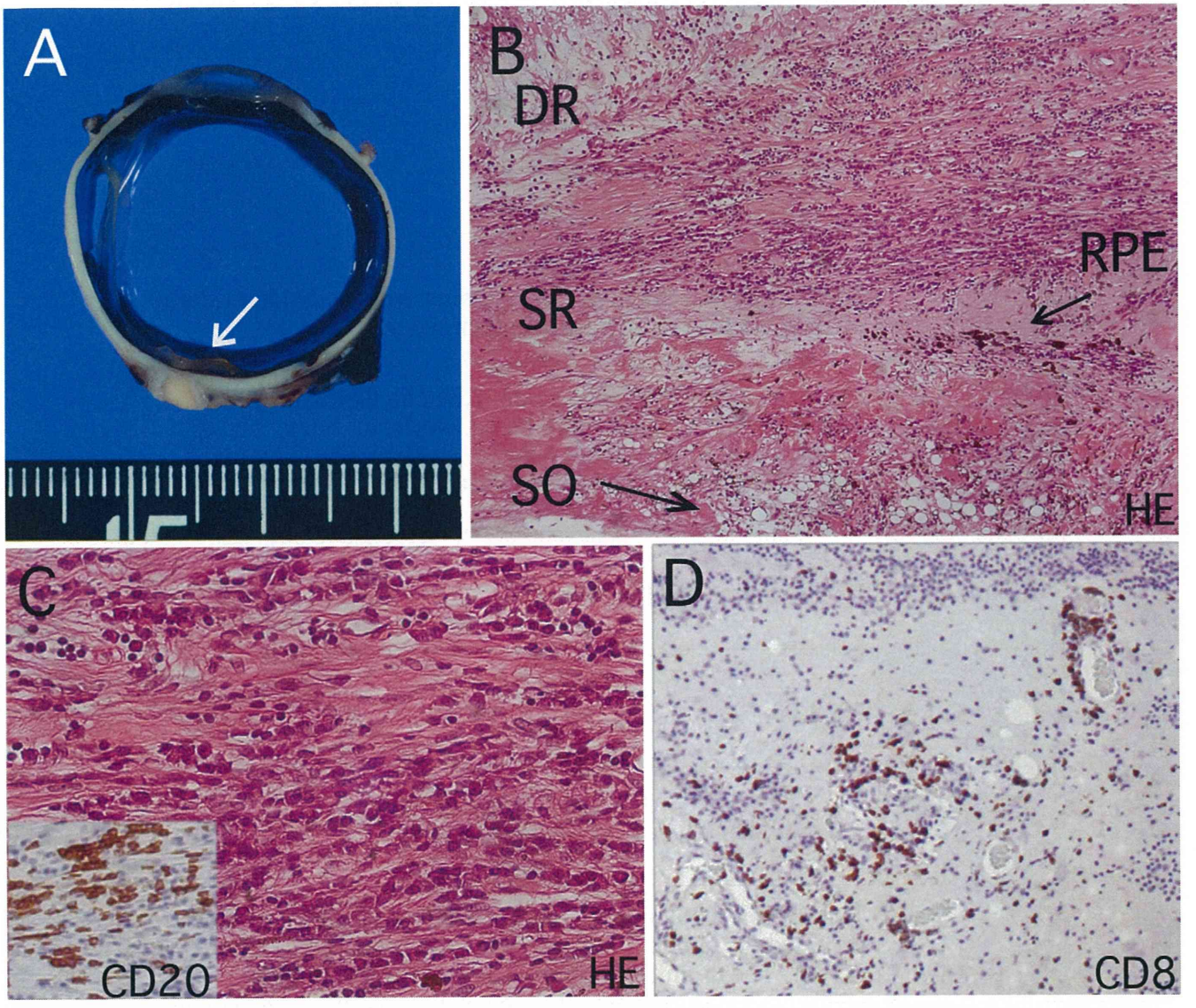

Figure 2. Gross and histological findings (**A–C**) and immunohistochemistry for CD20 (**C** insert), and CD8 (**D**) in the enucleated eye. A central cut section of the enucleated eye demonstrates a flat elevated lesion near the optic disc (**A**, arrow). In a histological section of the elevated lesion, there is marked cell infiltration predominantly in the subretinal space (SR) beneath the degenerative retina (DR), where vacuolated lesions indicating silicon oil (SO) deposition and retinal pigment epithelial (RPE) cell proliferation are intermingled (**B**). At a high magnification, lymphoid cells infiltrate the subretinal space with no cellular atypia (**C**). A variety of lymphoid cells are positive for CD20, a marker for B cells, (**C**, insert). The massive infiltration of CD8-positive T lymphocytes is noted in the subretinal space (**D**). HE = hematoxylin and eosin.

Title: Replication of a microsatellite genome-wide association study of Behcet disease in a Korean population

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Running head:

Microsatellite GWAS of Behect disease

Key messages

- Replication study of the microsatellite GWAS was performed.
- *HLA-B*51* gene was strongly associated with Behcet disease in Korean population.
- One of the six microsatellite marker was significantly associated with Korean population.

Abstract

Objectives: Behcet disease is one of the major etiologies of uveitis causing blindness in Asian countries. A genome-wide association study identified 6 microsatellite markers as disease susceptibility loci for Japanese patients with Behcet disease. To confirm our recent results, these microsatellite markers were examined in Korean population as a replication study.

Methods: Study participants included 119 Behcet patients and 141 controls. All were enrolled in Korea. Association between the 6 reported microsatellite markers (D3S0186i, D6S0014i, D6S0032i, 536G12A, D12S0645i, and D22S0104i) and Behcet disease was analyzed. HLA-B was genotyped by sequence-based typing methods.

Results: A microsatellite marker located near the *HLA-B* region demonstrated significant association with Behcet disease ($P = 0.028$). The genotype and phenotype frequencies of the *HLA-B*51* gene were significantly increased in patients (23.1% and 39.5%, respectively) compared with healthy controls (11.2% and 20.1%, respectively; $P < 0.001$).

Conclusions: Microsatellite analysis revealed that the *HLA-B*51* gene was strongly associated with Behcet disease in a Korean population.

Key words:

Microsatellite, GWAS, Behcet disease, replication study, HLA-B, Korean

Introduction

Behcet disease is a chronic, systemic, inflammatory disorder characterized by 4 major symptoms consisting of oral aphthous ulcers, genital ulcers, skin lesions, and recurrent ocular inflammation [1]. The disease is occasionally associated with inflammation in the vascular and/or central nervous systems and joints. Behcet disease is found predominantly between East Asia and the Mediterranean basin along the historic Silk Road; however, it is uncommon in the American continents, Oceania, and sub-Saharan Africa [2]. The distribution of intraocular inflammation and uveitis also differ in different regions of the world [3, 4].

In 1973, the first genetic factor was reported between Behcet disease and human leukocyte antigen (HL-A) 5 [5]. The nomenclature of HL-A5 was later changed to HLA-B5. Today, HLA-B5 is comprised of 3 subantigens: HLA-B51, -B52, and -B53. Susceptibility to Behcet disease is strongly associated with *HLA-B*51*, as reported in different ethnic groups [2, 5, 6]. Populations with a high prevalence of *HLA-B*51* lie predominantly north of the equator, spanning Japan and Western Europe between 30° and 45° N [7]. The area is completely consistent with the countries where Behcet disease is common. Meanwhile, many other susceptibility genes, such as *HLA-A, -E, -F, and –G; tumor necrosis factor (TNF) -a; toll like receptor (TLR) 4;interleukin (IL)-1, -8, -10, -12, and -18; IL23R;* and *CD28* have been reported in relation to Behcet disease [8-13]. Most recently, reports from ourselves and others of genome-wide association studies (GWAS) using 500,000 single nucleotide polymorphism (SNP) microarrays have identified 2 new disease susceptibility loci for Behcet disease on Chr.1p31.3 and 1q32.1, aside from the HLA class I region, in Japanese, Turkish, Korean, UK Caucasian, and Greek populations [11, 12]. These results are compatible with the fact that approximately one-half of Behcet patients are *HLA-B*51*-negative, and most *HLA-B*51* carriers never suffer from Behcet disease in their lifetime [2, 14].

However, there is little doubt but that the *HLA-B* region still demonstrates the strongest association with Behcet disease among all ethnic groups. A GWAS using microsatellite markers demonstrated that 6 (D3S0186i, D6S0014i, D6S0032i, 536G12A, D12S0645i, D22S0104i) of

23,465 markers differed significantly between patients and healthy subjects by using pooled DNA and individual typing methods in the Japanese population [8]. One of these significant 6 markers is located near the *HLA-B* region. Therefore, it is critically important to examine these indicated microsatellite markers among the people along the historic Silk Road, considering the prominent high prevalence of both the disease and the *HLA-B*51* gene there. In the present study, these microsatellite markers were investigated in a Korean population as a replication study, and the association was also examined between clinical features and the gene frequencies.

Methods

Participants

In the present study, 119 Behcet patients and 141 healthy controls were enrolled. All the patients were from Seoul National University Hospital, Seoul, Korea and fulfilled the diagnostic criteria of the International Study Group for Behcet disease (ISGBD). Informed consent was obtained from all participating individuals. The ethical committee of Seoul National University Hospital approved the study. The procedures used conformed to the tenets of the Declaration of Helsinki. All of the patients and control subjects were Korean.

Genotyping

Genomic DNA was prepared from peripheral blood specimens using the QIAamp DNA Blood Mini Kit (Qiagen, Tokyo, Japan). Each polymerase chain reaction (PCR) product for the 6 MS markers (D3S0186i, D6S0014i, D6S0032i, 536G12A, D12S0645i, D22S0104i) was amplified by PCR reactions. Each MS marker was amplified using two primers: forward 5'- AGC TCT TCC TAA CTG ATA AGG AAG -3' and reverse 5'- GTA AAG GTT GCT AGG TCC TGT T -3' for D3S0186i, forward 5'- CCA TAT GCT AGA AAT TAT GGT ACT -3' and reverse 5'- GTT TCA CTA TGT TGG CCA G -3' for D6S0014i, forward 5'- TAA GTC TAA GAA TGT GAG ACC AAC -3' and reverse 5'- GTA ATG CTG ATA ACG TTT ACT GTC -3' for D6S0032i, forward 5'- GTG TGC

TTG TGT CTG TTA ATT G -3' and reverse 5'- ACA CTA TAT TGT TAG CAA GTT ACT GAA C -3' for 536G12A, forward 5'- GGC AGA GAC AGT GTC TTT CTC -3' and reverse 5'- AGG TCA AGT GCA TGT TTG AC -3' for D12S0645i, and forward 5'- TAA GGC TGA GTA GCA GTC TAC ATA -3' and reverse 5'- TCA TTA AAG AAC TGG ATC TAC CAT -3' for D22S0104i. The reaction mixture was subjected to 5 min at 94°C; followed by 35 cycles of 1 min for denaturing at 94°C, 1 min for annealing at 57°C or 60°C, and 2 min for extension at 72°C; and 10 min for final elongation at 72°C using a PCR thermal cycler, GeneAmp System 9700 (Applied Biosystems, Foster City, CA, USA). Each forward primer was labeled at the 5' end with 6-FAM, NED, or VIC to determine the number of MS repeats. Fragment length analysis was performed using an ABI3130 DNA sequencer (Applied Biosystems) and the number of MS repeats was estimated with GeneMapper v3.5 software using GS500(-250)Liz (Applied Biosystems) as a size marker.

Genotyping of the *HLA-B* gene was performed using PCR sequence-based typing methods, and the data were analyzed using Assign software. *HLA-B* typing by sequence-based typing methods was performed by using HLA typing kit (Allele SEQR HLA-B®, Abbott, Japan).

Statistical analysis

Allele frequencies were calculated by direct counting. The significance of the association was assessed using the chi-square test. The strength of all *P*-values was derived from a two-sided test; $P < 0.05$ was considered statistically significant.

Results

There was a significant difference between patients and healthy controls in one of 6 microsatellite markers. The allele frequency of "308" of the microsatellite marker D6S0032i was statistically higher among Behcet patients (14.3%) compared with healthy subjects (8.2%) [$P = 0.028$, odds ratio (OR) = 1.86, 95% confidence interval (CI) = 1.06-8.66] (Table 1).

Genotype and phenotype frequencies at the *HLA-B* locus were examined, and 23 antigens were identified in 119 patients and 141 controls. The genotype frequency of *HLA-B*51* was significantly higher among patients (23.1%) than among healthy controls (11.2%) [P = 0.0003, OR = 2.39, 95% CI = 1.48-9.23]: in particular, *HLA-B*5101* [P = 0.0006, OR = 2.31, 95% CI = 1.42-9.12] (Table 1). The phenotype frequency of *HLA-B*51* was significantly higher among patients (39.5%) than among healthy controls (20.1%) [P = 0.00064, OR = 2.59, 95% CI = 1.49-9.74] (Table 1).

Discussion

To confirm recent GWAS studies in Japan [8], it is important to repeat population specific analysis and other population data analysis. In the present study, we determined the reproducibility of the recent Japanese GWAS results, and successfully found the new disease susceptibility gene by using microsatellite markers in a Korean population. Microsatellite polymorphisms have been used in the genetic association study because they are highly polymorphic, relatively simple, and inexpensive [15]. Recent technologies have reduced the necessary time and cost to genotype SNPs [16]. However, because of their lower variability, SNPs may be considered as carrying relatively less information at each locus. Furthermore, the microsatellite mutation rate was estimated to be 10^{-2} – 10^{-5} each generation; more frequent than that of SNPs (2.5×10^{-8}) [17]. That is to say, although microsatellite analysis has the great advantages of higher polymorphism and sensitivity, it also carries the disadvantage of relatively high genotyping error rates due to its highly polymorphic nature [18]. Therefore, it is necessary to replicate the obtained susceptible genes in other ethnic groups by other means. This is why we have organized the present study.

The marker D6S0032i, located at 1.1 Mb telomeric of *HLA-B* within the HLA class I region, showed statistical significance in Korean Behcet patients. However, there was no significant difference between patients and controls for the other 5 markers located near the *ROBO1* (roundabout, axon guidance receptor, homolog 1), *HLA-L and B, PPIL4* (peptidylprolyl isomerase–

like 4), *SOX5* (sex determining region Y–box 5), and *IGLV1-40 (IGL variable 1-40)* genes. The previous Turkish GWAS using fewer than 400 microsatellite markers reported chromosomes 12p12–13 and 6p22–24 as the susceptible loci for Behcet disease [19]. However, our recent GWAS in Japanese people using 23,465 microsatellite markers disagreed with that Turkish study.

In this type of study, however, there will be sources of bias that may distort the results. In this study, we have identified the following possibilities:

(1) Classification bias: We adopted International Study Group (ISG) Criteria in this study, however, Japanese GWAS study adopted Japan Behcet Disease Research Committee's Criteria. The use of different standardised criteria may have led to misclassification when comparing the frequencies of systemic features.

(2) Ascertainment bias: All samples in the current work were collected by rheumatologists, however in the Japanese GWAS, patients were mainly recruited at eye clinics. The patients with ocular lesions was significantly fewer in this study (37.8%) than recent Japanese GWAS study (89.3%) (P =0.0000067) [9]. It is possible that some patients with quite mild ocular inflammation might be misidentified as no ocular manifestations at rheumatology clinics when they did not consult ophthalmologists .

There are 3.61% of genetic differences between Japanese and Koreans [20]. Additional replication studies in other ethnic groups may be required for better understanding the mechanisms, etiology, and characteristics of Behcet disease.

In conclusion, we performed a microsatellite analysis in a population of Korean Behcet patients as a replication study, and demonstrated significant association with the marker located near the *HLA-B* region. The *HLA-B*51* gene is significantly associated with patients with ocular manifestations.

Acknowledgements

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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References

1. Ohno S, Nakamura S, Hori S, et al. Efficacy, safety, and pharmacokinetics of multiple administration of infliximab in Behcet's disease with refractory uveoretinitis. J Rheumatol. 2004; 31: 1362-8.
2. Ohno S, Ohguchi M, Hirose S, et al. Close association of HLA-Bw51 with Behcet's disease. Arch Ophthalmol. 1982; 100: 1455-8.
3. Kitaichi N, Miyazaki A, Iwata D, et al. Ocular features of Behcet's disease: an international collaborative study. Br J Ophthalmol. 2007; 91: 1579-82.
4. Kitaichi N, Miyazaki A, Stanford M R, et al. Low prevalence of juvenile-onset Behcet's disease with uveitis in East/South Asian people. Br J Ophthalmol. 2009; 93: 1428-30.
5. Ohno S, Aoki K, Sugiura S, Nakayama E and Itakura K. Letter: HL-A5 and Behcet's disease. Lancet. 1973; 2: 1383-4.
6. Ohno S, Asanuma T, Sugiura S, et al. HLA-Bw51 and Behcet's disease. JAMA. 1978; 240: 529.
7. Verity D H, Marr J E, Ohno S, Wallace G R and Stanford M R. Behcet's disease, the Silk Road and HLA-B51: historical and geographical perspectives. Tissue Antigens. 1999; 54: 213-20.
8. Meguro A, Inoko H, Ota M, et al. Genetics of Behcet disease inside and outside the MHC. Ann Rheum Dis. 2010; 69: 747-54.
9. Meguro A, Ota M, Katsuyama Y, et al. Association of the toll-like receptor 4 gene polymorphisms with Behcet's disease. Ann Rheum Dis. 2008; 67: 725-7.
10. Horie Y, Meguro A, Ota M, et al. Association of TLR4 polymorphisms with Behcet's disease in a Korean population. Rheumatology (Oxford). 2009; 48: 638-42.
11. Mizuki N, Meguro A, Ota M, et al. Genome-wide association studies identify IL23R-IL12RB2 and IL10 as Behcet's disease susceptibility loci. Nat Genet. 2010; 42: 703-6.
12. Remmers E F, Cosan F, Kirino Y, et al. Genome-wide association study identifies variants in the MHC class I, IL10, and IL23R-IL12RB2 regions associated with Behcet's disease. Nat Genet. 2010; 42: 698-702.
13. Jiang Z, Yang P, Hou S, et al. IL-23R gene confers susceptibility to Behcet's disease in a Chinese Han population. Ann Rheum Dis. 2010; 69: 1325-8.
14. Mizuki N, Inoko H, Mizuki N, et al. Human leukocyte antigen serologic and DNA typing of Behcet's disease and its primary association with B51. Invest Ophthalmol Vis Sci. 1992; 33: 3332-40.
15. Ball A D, Stapley J, Dawson D A, et al. A comparison of SNPs and microsatellites as linkage mapping markers: lessons from the zebra finch (Taeniopygia guttata). BMC Genomics. 2010; 11: 218.
16. Syvanen A C. Toward genome-wide SNP genotyping. Nat Genet. 2005; 37 Suppl: S5-10.
17. Tishkoff S A and Verrelli B C. Patterns of human genetic diversity: implications for human evolutionary history and disease. Annu Rev Genomics Hum Genet. 2003; 4: 293-340.

18. Pompanon F, Bonin A, Bellemain E and Taberlet P. Genotyping errors: causes, consequences and solutions. Nat Rev Genet. 2005; 6: 847-59.
19. Karasneh J, Gul A, Ollier W E, Silman A J and Worthington J. Whole-genome screening for susceptibility genes in multicase families with Behcet's disease. Arthritis Rheum. 2005; 52: 1836-42.
20. Miller R D, Phillips M S, Jo I, et al. High-density single-nucleotide polymorphism maps of the human genome. Genomics. 2005; 86: 117-26.

Table 1. Genotype frequencies of the D6S0032i, and genotyped and phenotyped HLA-B allele frequencies in the patients and healthy controls of the Korean population

	Patients		Controls				
D6S0032i	2n=238	%	2n=280	%	Odds (95%CI)	$\chi 2$	P values
304	7	2.9	10	3.6	0.82 (0.31-8.80)	0.16	0.69
308	**34**	**14.3**	**23**	**8.2**	**1.86 (1.06-8.66)**	**4.84**	**0.028**
313	109	45.8	163	58.2	0.61 (0.43-7.64)	7.95	0.0048
321	88	37	84	30	1.37 (0.95-7.56)	2.82	0.093
Genotype	2n=238	%	2n=278	%	Odds (95%CI)	$\chi 2$	P values
B*51	**55**	**23.1**	**31**	**11.2**	**2.39 (1.48-9.23)**	**13.2**	**0.00028**
B*5101	**52**	**21.8**	**30**	**10.8**	**2.31 (1.42-9.12)**	**11.73**	**0.00062**
B*5102	3	1.3	1	0.4	3.54 (0.22-10.0)	1.35	0.24.
Phenotype	n=119	%	n=139	%	Odds (95%CI)	$\chi 2$	P values
B*51	**47**	**39.5**	**28**	**20.1**	**2.59 (1.49-9.74)**	**11.64**	**0.00064**

* Significant p values are in bold

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Intravitreal Injection of Bevacizumab in a Case of Occlusive Retinal Vasculitis Accompanied by Syphilitic Intraocular Inflammation

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Key Words
Bevacizumab · Occlusive retinal vasculitis · Syphilis · Jarisch-Herxheimer reactions · Treponema

Abstract
Background: We report a rare case of syphilitic intraocular inflammation with occlusive retinal vasculitis treated with intravitreal injection of bevacizumab (IVB) in addition to conventional therapy for syphilis.
Case: A 24-year-old woman who complained floaters in both her eyes showed occlusive retinal vasculitis OU. According to the high titer of the Treponema antigen and characteristic cutaneous eruption, she was diagnosed as secondary syphilis.
Observation: She was treated with oral amoxicillin and retinal photocoagulation OU. Then, administration of prednisolone was required to the intraocular inflammations considered as Jarisch-Herxheimer reactions. IVB was also performed toward exacerbated retinal neovascularization and showed transient effects.
Conclusion: We experienced a rare case of occlusive retinal vasculitis accompanied by syphilis intraocular inflammation. IVB was considered to be effective as an adjunctive therapy for inflammatory neovascularizations.

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Introduction

Syphilis is one of the sexually transmitted diseases, and due to the educational instruction and development of antibiotics, the number of the syphilis patients has decreased markedly in the last half-century in Japan. According to the recent epidemiological survey, syphilis is in less than 1% the cause of intraocular inflammation in Japan (published in Japan). However, it is still an important disease and always should be kept in mind as one of the differential diagnoses. Syphilitic intraocular inflammation shows nonspecific ocular findings such as iridocyclitis, retinal vasculitis, and retinal pigment epithelium atrophy. However, occlusive retinal vasculitis as an ocular finding seen in syphilis is rare and so far only one case has been reported [1].

In this report, we show a rare case of occlusive retinal vasculitis accompanied by syphilis intraocular inflammation, in which intravitreal injection of bevacizumab (IVB; Roche, Basel, Switzerland) seemed to be effective for suppressing retinal neovascularization.

Case Report

A 24-year-old woman visited an ophthalmology clinic with the complaint of floaters in both eyes and was referred to Gifu University Hospital, Japan, in October 2007. Decimal visual acuity was 1.5 OU. No inflammatory cells were detected in the anterior chamber. Diffuse vitreous opacity, slight redness of optic disc and sheathing of retinal artery OU, and retinal hemorrhage adjacent to retinal veins in the inferior quadrant OD and in the superior quadrant OS were seen. Fluorescein angiography showed non-perfusion area and dye leakage indicating retinal neovascularization (**fig. 1**).

QuantiFERON®-TB assay for cellular immunity to *Mycobacterium tuberculosis* was performed, but the result was negative. Syphilis lipid antigen (rapid plasma reagin; RPR) and Treponema antigen (*Treponema pallidum* latex immunoassay; TPLA) showed high titer; 16 times and 2,280 TU, respectively. She showed characteristic cutaneous syphilis and was diagnosed as secondary syphilis.

After she moved to Hokkaido, Japan, she was immediately orally administered amoxicillin 1,500 mg, and retinal photocoagulation was performed to the non-perfusion area OU. Oral amoxicillin was administered for 8 weeks, and TPLA decreased to 104.3 TU and has never risen again. However, anterior chamber cells and vitreous hemorrhages appeared OS and fluorescein angiography revealed increasing dye leakage from retinal vessels. Then, oral prednisolone (40 mg; 0.7 mg/kg/day) was administered 4 weeks after antibiotics administration.

Despite these intensive therapies, the retinal neovascularization gradually exacerbated and fluorescence leakage from neovessels increased. And additional photocoagulation could not be performed due to repeated small vitreous hemorrhages. Therefore, IVB 1.25 mg was performed OU on April 23 with the approval of the Ethics Committee of the University Hospital and the informed consent of the patient. On May 23, 4 weeks after IVB, fluorescence leakage from new vessels markedly decreased (**fig. 2**). On June 17, 8 weeks after IVB, due to the increase of fluorescence leakage from new vessels, a second IVB was performed OU.

Fluorescence leakage decreased afterwards, and finally the leakage disappeared. However, in August, severe vitreous hemorrhage occurred OS, and pars plana vitrectomy with endophotocoagulation was performed. After the operation, fluorescence leakage and intraocular inflammation have never been seen OS until now. Vitreous hemorrhage never occurred OD. Final visual acuity in November was 1.2 OD, and 1.0 OS. Prednisolone was administered with gradual decrease of the dose until February 2009.

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Discussions

As for therapy for occlusive retinal vasculitis, treatment for a primary disease should be aimed (in this case, syphilis), together with photocoagulation to the non-perfusion area, and corticosteroids to control the inflammation. We performed antibiotic treatment for syphilis, photocoagulation to the non-perfusion area and steroid medicine in this case. However, the activity of neovascularization was so high that repeated vitreous hemorrhages occurred. Therefore, we performed IVB as an adjunctive therapy.

It has been reported that high concentration of vascular endothelial growth factor (VEGF) is detected from the vitreous cavity in some retinal diseases [2], and intravitreal injection of bevacizumab, which is a recombinant humanized monoclonal antibody against VEGF, is effective for the diseases such as diabetic retinopathy, branch retinal vein occlusion and age-related macular degeneration [3]. In addition, there is a report describing the effectiveness of IVB for inflammatory ocular neovascularization [4], and in this case we obtained a similar effective result.

In this case, administration of antibiotics worsened the intraocular inflammations including exacerbation of neovascularization. This phenomenon seemed to be Jarisch-Herxheimer reactions (JHR) [5]. JHR is a paradoxical worsening phenomenon after antibiotic therapy, and it is speculated to be caused by endotoxin released from dead organisms killed by antibiotics. Although prednisolone was administered toward JHR at the peak stage of the inflammation, this therapy was not effective enough to suppress retinal neovascularizations.

Although IVB has only transient effects and needs to be performed repeatedly, it is considered to be a useful tool as an adjunctive therapy for inflammatory neovascularizations with JHR, as far as JHR is also a transient phenomenon.

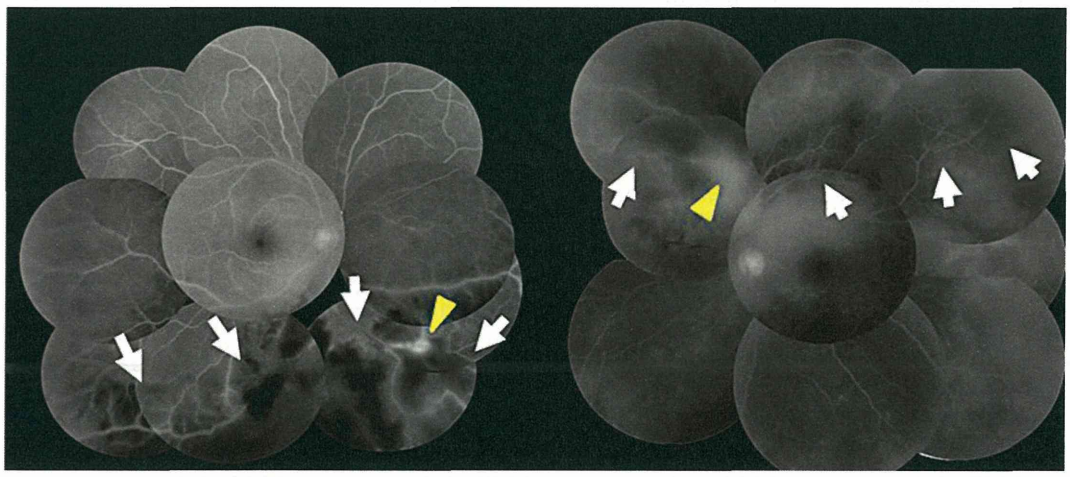

Fig. 1. Fluorescein angiography. Fluorescein angiography showed non-perfusion area (white arrows) and dye leakages (yellow arrows).