

Fig. 5. Cell proliferative response and cytokine production of lymphocytes from regional lymph nodes of hIRBP-immunized and OPN-siRNA-treated mice. A. ^3H -thymidine incorporation by primed lymphocytes. Lymphocytes were obtained from B6 mice immunized with hIRBP and treated with OPN-siRNA (\bullet) or control-siRNA (\circ). Lymphocytes were incubated with indicated dose of hIRBP peptide and with ^3H -thymidine for the last 16 h. B. IFN- γ and TNF- α produced in the culture supernatant. C. IL-17, IL-2 and GM-CSF produced in the culture supernatant. The results are presented as mean \pm standard deviation. Statistical significance is determined using two-tailed Student's *t*-test (**, $P < 0.01$, * $P < 0.05$). Data are representative of two separate experiments with the similar result.

We also examined whether OPN-siRNA treatment could reverse ongoing EAU. Mice were immunized by IRBP_{1–20} peptide at day 0 and treated with two injections of OPN-siRNA at day 7 and day 8 after EAU induction. At the 7-day time point, uveitogenic effector cells had already been primed and could induce EAU (Agarwal et al., 2000). As anticipated with this report, the reversal regimen was not effective for amelioration of the ongoing disease (Fig. 4A and B). These results suggested that the effects of OPN-siRNA were induced by the blockade of upsurge of OPN following immunization and thus preventing generation of primed T cells more than by inhibiting the effector function of induced T cells. However, the ineffectiveness of OPN blockade with siRNA after disease onset may not ruin its application for ongoing diseases. This is because the consecutive priming and generation of autoreactive T cells may take place even in chronic diseases. Moreover, co-administration of anti-OPN Ab may also compensate the effect of siRNA administration after onset, which should be pursued in further investigation.

We then examined the antigen-specific proliferative responses of lymphocytes upon *ex vivo* hIRBP peptide restimulation in the preventive regimen. Proliferation of hIRBP peptide-primed cells

was slightly reduced by administration of OPN-siRNA *in vivo*. This finding is partially compatible with the result with the anti-OPN antibody (Kitamura et al., 2007). Notably, the production of IFN- γ and TNF- α was significantly reduced in the culture supernatants of the OPN-siRNA-treated group compared to that of the control group. The suppressed production of IFN- γ and TNF- α appeared to be interpreted by the blockade of Th1 cells, which led to the amelioration of EAU, a Th1-mediated autoimmune model (Caspi et al., 1988; Caspi, 2002; Schiffelers et al., 2005). IFN- γ induces macrophage activation and nitric oxide production, which leads to destruction of retina in EAU (Hoey et al., 1997). TNF- α provokes inflammatory responses (Green and Flavell, 1999) and TNF p55 receptor deficient mice are resistant to EAU (Calder et al., 2005).

OPN has been recognized as a key player in the Th1-responses for several reasons. First, the expression of OPN is mediated by T-bet, which is indispensable for the polarization of Th1 immune response (Shinohara et al., 2005). The secreted OPN further affects the expression of IL-12 (enhancement) and IL-10 (inhibition) that favors Th1-deviation (Ashkar et al., 2000). Second, the specific form of OPN, intracellular OPN, could induce IFN- α secretion from

plasmacytoid dendritic cells (pDC) in the presence of CpG in TLR9-MyD88- and IRF7-dependent manner (Shinohara et al., 2006). IFN- α also favors Th1-deviation. Third, OPN, especially the NH₂-terminal fragment of OPN cleaved by thrombin, promotes adhesion and migration of leukocytes and neutrophils and directly binds to $\alpha_9\beta_1$, which interacts with vascular cell adhesion molecule-1 (VCAM-1) in extravasation of neutrophils at sites of acute inflammation (Taooka et al., 1999). Thus, the migration of inflammatory cells might be blocked by the reduction of OPN content in the tissue.

It has been reported that OPN enhances survival of activated T cells by inhibiting transcription factor Foxo3a, activating NF- κ B, and altering pro-apoptotic proteins (Hur et al., 2007). Thus, OPN function seems to be superfluous and not only supports Th1-deviation but also plays complex roles in immunological responses. This finding may explain the various influences on the manifestation of autoimmune diseases observed in different disease models.

On the other hand, there were conflicting results that EAU development was aggravated in IFN- γ KO and IFN- γ receptor KO mice (Fukushima et al., 2005; Hikita et al., 2006), which implied that IFN- γ might inhibit generation of pathogenic Th17 cells in EAU.

Recently, a new insight with Th17 cells has emerged for the pathogenesis of EAU (Amadi-Obi et al., 2007). In the present study, OPN-siRNA treatment suppressed not only Th1 cytokines but also IL-17 production, which could also account for the amelioration of EAU. These results suggest that OPN represents a good therapeutic target to ameliorate uveoretinitis as shown in our previous (Kitamura et al., 2007) and present studies. From a clinical viewpoint, OPN blockade seems to be not only potent but also beneficial for the treatment of human uveoretinitis without serious side effect, an obstacle of anti-TNF antibody therapy (Ohno et al., 2004). Thus far, no reports have demonstrated that OPN deficiency deteriorates host defense in mice (Rittling et al., 1998; Sato et al., 2005).

To date, OPN blockade has been shown to ameliorate various disease models in mice (Chabas et al., 2001; Jansson et al., 2002; Yumoto et al., 2002; Yamamoto et al., 2003; Hikita et al., 2006; Kitamura et al., 2007). Concordantly, OPN was elevated in human counterparts, including pulmonary sarcoidosis (Maeda et al., 2001), rheumatoid arthritis (Ohshima et al., 2002), and multiple sclerosis (Comabella et al., 2005). We thus presume that OPN blockade is also effective in these diseases. It is important to develop a safe and feasible technique for siRNA delivery to render the RNAi treatment applicable to human patients, as the hydrodynamic method is rather intense. Several novel techniques are being developed for the efficient introduction and interference for siRNA especially for *in vivo*-use (Liu et al., 1999; Howard and Kjems, 2007). The mechanistic elucidation and technical excellence will drive the RNAi for the treatment of immunological diseases with equal or better chance of use than monoclonal antibodies targeted to various molecules involved in disease development.

Disclosure

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Association of *TLR4* polymorphisms with Behçet's disease in a Korean population

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Objectives. HLA-B51 is strongly associated with Behçet's disease (BD) in any ethnic background. We recently reported that another gene, Toll-like receptor-4 (*TLR4*) is also implicated in BD in a Japanese population. To confirm these results, we investigated polymorphisms in the *TLR4* gene in Korean patients with BD.

Methods. In this study, 119 patients with BD and 141 healthy controls were enrolled; every participant was a Korean. Nine single nucleotide polymorphisms previously detected in *TLR4* by direct sequencing were analysed for an association with BD.

Results. The most frequent haplotype, TAGCGGTAA, was significantly increased in *HLA-B*51*-positive BD patients (49.5%), compared with healthy control participants [32.3%; $P=0.029$; odds ratio (OR) = 2.01; 95% CI 1.25–3.23]. This haplotype was also significantly increased in BD patients with arthritis (48.2%; $P=0.003$; OR = 1.96; 95% CI 1.26–3.26). There were no significant differences in the allele and genotype frequencies of patients and controls for each single nucleotide polymorphism.

Conclusions. The haplotype of *TLR4* may increase the risk for developing BD and the complication of arthritis in the Korean population.

Key words: Behçet's disease, Toll-like receptor 4, Korea, Polymorphism.

Introduction

Behçet's disease (BD) is a refractory, multisystemic inflammatory disorder characterized by oral aphthous ulcers, ocular lesions, skin lesions and genital ulcers [1]. This disease is occasionally associated with inflammation throughout the body including the vascular system and joints [2]. In patients with BD, serum TNF and IFN- γ are significantly elevated [3, 4]. The strong association of HLA-B51 with BD was first described in 1973 and has been confirmed in patients from many ethnic groups [5, 6]. Epidemiologically, BD is scattered throughout the world but a higher prevalence has been found among Asian populations along the Silk Route, stretching into the countries of the Mediterranean region [6]. BD appears to be influenced by many susceptible genes, including endogenous and exogenous elements, because most HLA-B51-positive individuals do not suffer from BD throughout their lives, and ~50% of the BD patients are negative for HLA-B51 [6, 7]. These observations suggest that there are other susceptible genes in addition to those that have been reported, which include *TNF- α* , *IFN- γ* , *IL-1 α* , *-1 β* , *-2*, *-8*, *-10* and *-12* and *CD28* [5, 8, 9].

The Toll-like receptor (*TLR*)4 gene on chromosome 9q32–33 spans ~13 kb and contains three exons encoding a protein consisting of 222-amino acid protein. The TLR proteins are a family of phylogenetically conserved receptors that recognize

both self and non-self molecular patterns and play an important role in both the innate and adaptive immune systems [10–12]. Among TLR family members, TLR4 has been the most exhaustively investigated; it has been shown to be a principal receptor for lipopolysaccharide (LPS)-recognition. Single nucleotide polymorphisms (SNPs) in *TLR4* have been reported to be associated with endotoxin hyporesponsiveness and Gram-negative infections, and they affect the risk for various inflammatory diseases, such as atherosclerosis, Crohn's disease, ulcerative colitis, RA and prostate cancer [13–20].

Recently, we reported that *TLR4* is significantly associated with BD in Japanese patients [21]. To confirm the reproducibility of this result, and to extend the investigation into the Korean population, we explored the association of *TLR4* with BD in Korean patients using the same nine SNPs examined in the Japanese study. The information on linkage disequilibrium (LD) and haplotype structure defined by SNPs in a population is essential for the design of disease association studies. Genetic structure constructed by LD patterns and haplotype structure may be different among populations. Although differences in genetic structure between Korean and Japanese populations remain unclear, recent reports have presented strong genetic affinities [22, 23]. In the context of genetic similarity between Koreans and Japanese, we attempted to analyse disease susceptibility of the *TLR4* gene for BD in Korean patients and differences in the haplotype structure in those SNPs examined.

Materials and methods

Participants

We recruited 119 Korean patients with BD from Seoul National University Hospital, Seoul, Korea. All the patients fulfilled the diagnostic criteria of the International Study Group for BD (ISGBD) [24], and the definition of the clinical manifestations followed the criteria by ISGBD [24]. Arthritis was defined as any episode of joint pain not related to direct trauma that was confirmed by a physician as the presence of either swelling or tenderness or pain on motion of the involved joints. The healthy blood donors were randomly recruited as healthy controls. Informed consents were obtained from all of the individuals. This study was approved by the ethical committee of Seoul

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University Hospital. The procedures used conformed to the tenets of the Declaration of Helsinki.

Genotyping

DNA was prepared from peripheral blood specimens using the QIAamp DNA Blood Mini Kit (Qiagen, Tokyo, Japan). Nine previously described SNPs (rs10759930, rs1927914, rs1927911, rs12377632, rs2149356, rs11536889, rs1554973, rs7037117 and rs7045953) from the *TLR4* region were examined [21]. Each PCR product for the nine SNPs was amplified by standard PCR reactions (Table 1).

After purification using ExoSAP-IT (USB Corporation, Cleveland, OH, USA), the PCR products were sequenced with Big Dye terminator v3.1 (Applied Biosystems, Foster City, CA, USA) using either sense or anti-sense primers (Table 1). The BigDye XTerminator Purification Kit was used to purify the DNA from the sequencing reactions. The sequencing reactions were analysed using an ABI3130 sequencer (Applied Biosystems).

Statistical analysis

Differences in genotype frequency differences between case and control genotypes were assessed by using the χ^2 -test and Fisher's exact test. The strength of LD between SNPs was quantified by the standardized disequilibrium (D') [25]. The maximum likelihood estimates of haplotype frequencies were estimated by pairs of unphased genotypes using the expectation-maximization (EM) algorithms and P -values were corrected by using the 1000 permutations test in Haploview software [26]. All P -values were derived from a two-sided test, and those that were <0.05 were considered to be statistically significant.

TABLE 1. PCR primers, product sizes and sequence primers for *TLR4* polymorphisms

SNP	ID	Forward primer Reverse primer	Product size (bp)	Sequence primer
rs10759930	1	ATGGCACTATGTAGGCTGAT ACCCTCTTTTATCCTTGGAC	295	Forward
rs1927914	2	GCTTTTAGGACAGTGTCTGG CTTTGACATGGAGGTTGTTT	251	Reverse
rs1927911	3	GATGACACACAAATCCATGA GGGAAAGTAGGCTAGAAAGC	400	Forward
rs12377632	4	AATCGATACCATCACTCAGG GCCCTAATTCAGAATCTCCT	473	Forward
rs2149356	5	AAGCATGTAATAGCCTTGGA TGTTCACTTCACCTCCTTTT	419	Forward
rs11536889	6	CTCACTGCCAGGAGAACTAC GAAGAAGGTTCCAATTTCT	341	Reverse
rs1554973	7	GGAGCTTCAAACAAAGGATA ACTCACCCATAAGGACAGTG	330	Reverse
rs7037117	8	TGCAAATGCAGTTTTGTTAC ATTCTTAAGCCAAGCATTG	470	Forward
rs7045953	9	CCCCATTGAGTTATAGGTGA TATTGGAGGCTCTGTCTCAT	196	Forward

Results

The patient group included 61 males (51.3%) and 58 females (48.7%). Oral aphthous ulcers were observed in all patients. Genital ulcers, skin lesions, ocular lesions, deep vein thrombosis and arthritis were observed in 77.3, 80.7, 37.8, 16.8 and 47.9% of the patients, respectively. We found 39.5% of the patients to be *HLA-B*51* positive (Table 2).

The magnitude of LD between any two of the nine SNPs showed an extremely high value among all SNPs, with pair-wise LD valued at $D' > 0.68$ in controls and > 0.74 in cases (Fig. 1). There was no significant difference between the allele or genotype frequencies of the cases and controls. In our clinically stratified analysis, we investigated the presence of some of the clinical features, such as oral aphthous ulcers, genital ulcers, skin lesions, ocular lesions, deep vein thrombosis and arthritis. None of these clinical findings was significantly associated with the nine SNPs (data not shown).

Table 3 shows haplotype frequencies of the patients with BD and healthy controls. Only possible haplotypes were estimated to have a frequency > 0.05 in both case and control groups using an EM algorithm. The patients with BD were divided into two groups, those with or those without *HLA-B*51* and arthritis. The healthy controls were also divided into with or without *HLA-B*51*.

Table 4 summarizes the analysis of the haplotypes, comparing BD patients with controls. The most frequent haplotype, TAGCGGTAA, was significantly increased in *HLA-B*51*-positive patients compared with healthy controls [odds ratio (OR) = 2.01; 95% CI 1.25, 3.23; $P = 0.029$]. This haplotype was also increased in patients with BD complicated by arthritis when compared with healthy controls (OR = 1.96; 95% CI 1.26, 3.05; $P = 0.0030$) or BD patients without arthritis (OR = 2.01; 95% CI 1.19, 3.40; $P = 0.037$) (Table 4).

Discussion

The TLR4 protein is expressed in a wide variety of human cells, and acts as a receptor in the activation of the innate/adaptive immune system in response to both endogenous and exogenous ligands. This transmembrane signalling receptor is the primary

TABLE 2. Clinical features of the Korean patients with BD

	Cases, n (%)
Sex	
Male	61
Female	58
Oral aphthous ulcers	119 (100)
Genital ulcers	92 (77.3)
Skin lesions	96 (80.7)
Ocular lesions	45 (37.8)
Deep vein thrombosis	20 (16.8)
Arthritis	57 (47.9)
<i>HLA-B*51</i>	47 (39.5)

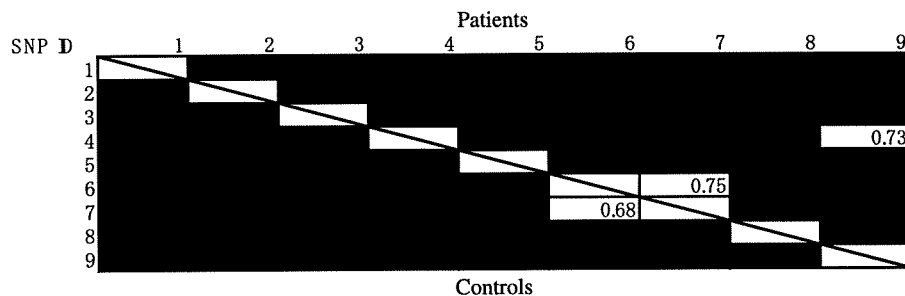


FIG. 1. D' score for the nine SNPs studied across the *TLR4* haplotype. Upper triangle, patient population; lower triangle, control population. A black cell means $D' > 0.8$.

TABLE 3. Haplotype frequencies for TLR4 in BD patients and healthy controls

Haplotype		BD				Controls			
		(%; n = 119)	<i>B*51(+)</i> (%; n = 47)	<i>B*51(-)</i> (%; n = 72)	Arthritis(+) (%; n = 57)	Arthritis(-) (%; n = 62)	(%; n = 141)	<i>B*51(+)</i> (%; n = 28)	<i>B*51(-)</i> (%; n = 113)
Hap 1	TAGCGGTAA	39.7	49.5	35.4	48.2	32.2	32.3	30.4	33.5
Hap 2	TAGCGCTAA	22.4	13.3	26.4	20.2	24.1	26.1	23.1	25.0
Hap 3	CGATTGTAA	11.7	5.8	13.2	9.6	13.7	13.8	12.5	15.0
Hap 4	CGATTGTGA	8.1	9.6	7.6	7.0	8.9	11.4	16.1	11.2
Hap 5	CGATTGCGG	8.0	9.5	6.9	7.9	8.0	7.8	8.9	7.2

*B*51*: HLA-B*51.

TABLE 4. Analysis of the haplotypes in TLR4

	Hap 1 OR (95% CI); <i>P</i> -value	Hap 2 OR (95% CI); <i>P</i> -value	Hap 3 OR (95% CI); <i>P</i> -value	Hap 4 OR (95% CI); <i>P</i> -value	Hap 5 OR (95% CI); <i>P</i> -value
BD vs Control	1.35 (0.95, 1.94); 0.173	0.84 (0.56, 1.26); 1	0.75 (0.45, 1.25); 1	0.63 (0.35, 1.15); 0.763	1.13 (0.59, 2.18); 1
<i>B*51(+)</i> BD vs Control	2.01 (1.25, 3.23); 0.029	0.46 (0.24, 0.88); 0.17	0.60 (0.28, 1.29); 0.744	0.77 (0.36, 1.68); 0.845	0.81 (0.31, 2.16); 1
<i>B*51(-)</i> BD vs Control	1.09 (0.71, 1.67); 1	1.12 (0.71, 1.76); 1	0.86 (0.48, 1.55); 1	0.61 (0.30, 1.23); 0.817	0.98 (0.45, 2.16); 1
<i>B*51(+)</i> BD vs <i>B*51(-)</i> BD	1.84 (1.08, 3.13); 0.228	0.41 (0.20, 0.83); 0.202	0.70 (0.30, 1.61); 1	1.28 (0.51, 3.21); 0.76	0.83 (0.28, 2.43); 1
<i>B*51(+)</i> Control vs <i>B*51(-)</i> Control	0.87 (0.46, 1.64); 1	0.89 (0.45, 1.77); 1	1.04 (0.47, 2.30); 1	1.16 (0.47, 2.84); 0.76	1.39 (0.48, 4.01); 1
<i>B*51(+)</i> BD vs <i>B*51(+)</i> Control	2.25 (1.12, 4.52); 0.071	0.51 (0.21, 1.20); 0.887	0.55 (0.21, 1.49); 0.945	0.74 (0.26, 2.11); 0.869	0.63 (0.18, 2.22); 1
<i>B*51(-)</i> BD vs <i>B*51(-)</i> Control	1.06 (0.68, 1.65); 1	1.09 (0.68, 1.75); 1	0.82 (0.45, 1.50); 1	0.67 (0.32, 1.41); 1	1.06 (0.46, 2.43); 1
Arthritis(+) BD vs Control	1.96 (1.26, 3.05); 0.003	0.73 (0.43, 1.25); 0.843	0.61 (0.30, 1.23); 0.764	0.55 (0.25, 1.23); 0.787	1.13 (0.50, 2.57); 1
Arthritis(+) BD vs Arthritis(-) BD	2.01 (1.19, 3.40); 0.037	0.77 (0.41, 1.42); 1	0.67 (0.30, 1.51); 1	0.77 (0.30, 1.99); 1	0.98 (0.38, 2.52); 1

P-values were corrected by 1000 permutation test. Significant *P*-values are in bold.

recognition molecule for LPS and HSP, both of which are reported to trigger BD. HSPs, widely distributed in nature, are highly immunogenic molecules. Human HSPs are expressed on cell membranes in response to physiological stress and microbial challenge. Recent reports have indicated that HSPs of microorganisms were frequently found in the oral flora and serum of BD patients and they were considered to be implicated in the development of BD through cross-reactive immunopathological responses [27–30]. Notably, the expression of HSPs is up-regulated at lesions of BD and the serum level of HSPs is significantly higher in BD patients than in controls [31–33]. Furthermore, HSPs stimulated $\gamma\delta$ T-cell responses from BD patients but not in controls *in vitro* [31, 34]. TLR4 reportedly recognizes and interacts with HSP and LPS, regarded as antigens in BD. Recent studies have reported TLR4 polymorphisms and the risk of various diseases including inflammatory diseases [16, 18, 35]. It has been reported that two non-synonymous TLR4 SNP sites, Asp299Gly (rs4986790) and Thr399Ile (rs4986791), are associated with an elevation of serum cytokines in the Caucasian population [18]. However, no other studies have detected these non-synonymous mutations in Asian populations, including Koreans [36]. Thus, we investigated nine intronic SNPs, the same SNPs described in our earlier work focused on a Japanese population [21].

In the present study, we examined TLR4 because it was implicated in that previous study as a candidate gene in Japanese patients with BD [21]. It is widely accepted that HLA-B*51 affects the development of BD, and the frequency of HLA-B*51 is much higher in male patients, patients with ocular lesions and complete-type BD patients [6]. The association of the nine SNPs with BD

could not be explained by any single SNP, but the data in this study show that the frequency of the TAGCGGTAA haplotype is significantly increased in HLA-B*51-positive BD. TLR4 is located on the long arm of chromosome 9, whereas HLA is on the short arm of chromosome 6; thus, to elucidate the genetic influence of HLA-B*51 on TLR4, we divided HLA-B*51-positive participants from controls. Among healthy controls, there were no significant differences between healthy participants with and without HLA-B*51. In addition, there were no significant differences between HLA-B*51-positive patients and controls. These results suggest that the most frequently occurring haplotype, TAGCGGTAA, may be influenced by HLA-B*51 and dependently associated with HLA-B*51-positive BD patients.

The TLR4 gene has been well investigated in the context of RA [18, 35, 37], and arthritis classified as one of the minor symptoms of BD. In fact, 40% of the BD patients have the complication of arthritis, and it is the most common minor symptom [38]. In the present study, we successfully examined 57 BD patients (47.9%) with arthritis. When we examined the haplotype frequencies for BD patients with and without arthritis, the most frequently occurring haplotype, TAGCGGTAA, which is associated with a higher risk of BD, was significantly increased in patients with arthritis. Although the pathogenesis of BD and RA is still unknown, the arthritis in RA is chronic inflammation accompanied by constitutional symptoms such as fever, fatigue, malaise, myalgia, decreased appetite and weight loss. In most cases, RA is a chronic progressive disease that can cause extensive joint damage and deformity [39]. On the other hand, the arthritis in BD is usually self-limiting and non-erosive [40]. Nevertheless, these two diseases may share some genetic or immunological mechanisms.

It has been reported that intronic SNPs play important roles in disease development [41]. Other studies analysing complex traits have indicated that haplotypes within the gene interact and have a large effect on the observed phenotype, which could be explained by SNPs [42, 43]. Our present results support the hypothesis that the *TLR4* haplotype, TAGCGGTAA, increases the risk for BD, as well as the complication of arthritis. Although there have been many reports of a negative association of non-synonymous SNPs [Asp299Gly (rs4986790) and Thr399Ile (rs4986791)] with BD, a positive association has also been demonstrated in an analysis of intronic SNPs similar to that undertaken in the present study [35, 44, 45]. Our results are consistent with the previous results showing that genetic contributions to inflammatory diseases can be successfully detected by an analysis of intronic SNPs among Asian populations. A recent report has suggested that the *TLR4* polymorphisms may only be maintained by evolutionary pressure from infectious disease [46]. It is intriguing to consider whether or not the function and the polymorphisms of *TLR4* can be changed alternatively against exogenous elements. Analysis of *TLR4* may be helpful in elucidating the aetiology of BD along the historic Silk Road.

Previously, we reported a significant association at a single SNP (rs70377117), especially in incomplete BD patients, but did not show a significant haplotype link among Japanese patients [21]. However, we successfully found a significant difference of haplotype frequencies among Korean BD patients in the current work. Thus, there is a small variation between the results of these two studies, although our findings are, in general, in close agreement with those for the Japanese population [21]. There are three possible sources of bias that could distort these comparisons between results. First, the Japanese investigation involved 200 patients and 102 controls, and there were 119 patients and 141 controls in the current work. Having an insufficient number of samples may have resulted in the small variation between the two studies. A larger sample size for each population would be needed in follow-up studies. Secondly, in the present work, we used the ISGBD criteria; however, the other study selected patients based on the Japanese Committee's Criteria. This use of different criteria may have led to some minor differences in comparisons of the haplotype frequencies. Finally, the nature of the populations was different between the two reports: ocular features were seen in only 37.8% of the participants in this work, but 89.5% in the Japanese paper. Also, the arthritis rate was 47.9% in the present study, but only 35.5% in the other investigation [21]. All samples in the current work were collected at the rheumatology clinic, but patients were recruited in the ophthalmology clinic in the Japanese population study. Different patient characteristics may also have been a source of bias.

In conclusion, we have identified *TLR4* as a susceptible gene for *HLA-B*51*-positive BD and the complication of arthritis in Koreans using a case-control study of SNPs. The present findings are consistent with the interpretation that the immune response against various exogenous and/or endogenous *TLR4* ligands plays an important role in the development of BD.

Rheumatology key message

- *TLR4* is a susceptible gene for *HLA-B*51*-positive BD and for the complication of arthritis in Koreans.

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Evaluation of *PTPN22* polymorphisms and Vogt-Koyanagi-Harada disease in Japanese patients

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Purpose: Vogt-Koyanagi-Harada (VKH) disease is an autoimmune disorder against melanocytes. Polymorphisms of the protein tyrosine phosphatase non-receptor 22 gene (*PTPN22*) have recently been reported to be associated with susceptibility to several autoimmune diseases. In this study, genetic susceptibility to VKH disease was investigated by screening for single nucleotide polymorphisms (SNPs) of *PTPN22*.

Methods: A total of 167 Japanese patients with VKH disease and 188 healthy Japanese controls were genotyped by direct sequencing methods for six SNPs (rs3811021, rs1217413, rs1237682, rs3761935, rs3789608, and rs2243471) of *PTPN22* including the uncoding exons.

Results: The six SNPs in *PTPN22* showed no significant association with susceptibility to VKH disease or its ocular, neurologic, or dermatological manifestation.

Conclusions: Further studies are needed to clarify the genetic mechanisms underlying VKH disease.

Vogt-Koyanagi-Harada (VKH) disease is one of the most frequent forms of uveitis in Japan [1]. It is characterized as bilateral panuveitis accompanied by neurologic and skin lesions [2,3]. This disease is considered to be an autoimmune disease against melanocytes [4,5]. Though the etiology of VKH disease still remains unknown, genetic factors may play an important role in susceptibility as indicated by an established association between VKH disease and specific human leukocyte antigen (*HLA*)-*DRB1* alleles [6,7].

The protein tyrosine phosphatase non-receptor 22 gene (*PTPN22*) is located on chromosome 1p13.3-p13.1, and it encodes the lymphoid-specific phosphatase (Lyp) that is important in the negative control of T-cell activation and development [8-10]. Recently, it was reported the single nucleotide polymorphism (SNP), R620W (rs2476601), in *PTPN22* increased susceptibility to several autoimmune diseases including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and insulin dependent diabetes mellitus (IDDM) [11-15]. In the *PTPN22* risk variant (rs2476601), this substitution disrupts an interaction between Lyp and the protein tyrosine kinase, Csk, and may translate biologically to

a potential for 'hyperreactive' pathogenic T-cell responses [8].

This R620W mutation was not observed in the Japanese population [16,17]. Therefore, in this study, we analyzed six SNPs, which belong to the same haplotype block as R620W (rs2476601) in *PTPN22*. For the efficacy of the linkage analysis, we chose six SNPs of which minor allele frequencies were more than 15% from the database of Japanese Single Nucleotide Polymorphisms [18,19].

METHODS

We recruited 167 VKH (72 males and 95 females) patients and 188 healthy controls for this study. All patients and control subjects were Japanese. Patients were diagnosed according to the "Revised Diagnostic Criteria for VKH Disease" [3] at the Uveitis Survey Clinic of the Hokkaido University Hospital (Sapporo, Japan) and Yokohama City University Hospital (Yokohama, Japan). All patients and control subjects were informed of the study's purpose, and their consent obtained. The study was approved by the ethics committee from each institute participating in this study.

DNA was prepared from peripheral blood specimens using the QIAamp DNA Blood Mini Kit (Qiagen, Tokyo, Japan). Six SNPs (rs3811021, rs1217413, rs1237682, rs3761935, rs3789608, and rs2243471) from the *PTPN22* region were examined (Figure 1). Each of the six SNPs was amplified by standard polymerase chain reactions (PCRs; Table 1). After purification using ExoSAP-IT (USB

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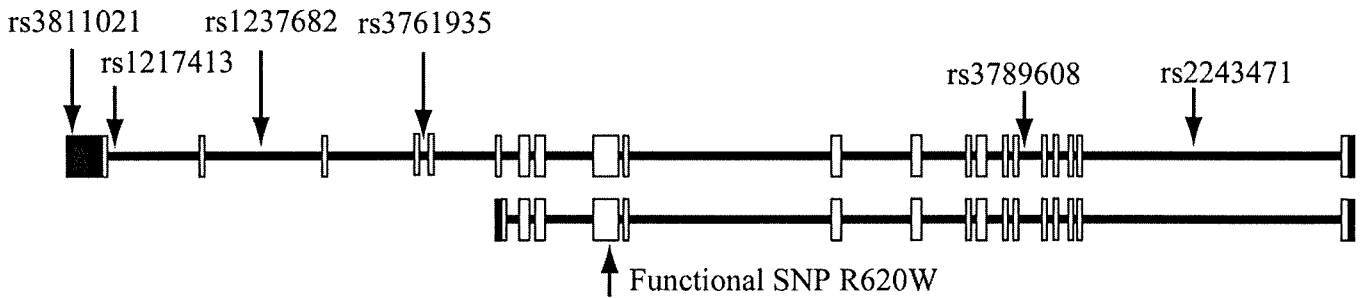


Figure 1. *PTPN22* structure with two transcript isoforms and six SNP. Six SNP variants with minor allele frequencies 15% from the database of Japanese Single Nucleotide Polymorphisms. The black and white areas in the exons indicate the UTR and coding region, respectively.

TABLE 1. PCR PRIMERS FOR *PTPN22* SNPs.

SNP	(SNP#)	position	Primers	Product size (bp)	sequence primer
rs3811021	(SNP1)	114158186	F: TGGGTTGCAATACAAACTGCTC R: TCAATTTGCCCTATTGGACTTC	600	Forward
rs1217413	(SNP2)	114159273	F: TTGCAGGTGTACTTGCAGCC R: TTGAAGGATTTCTGGACCGAC	552	Forward
rs1237682	(SNP3)	114165627	F: AAGGAGGCACAGATTCCACAC R: TGACCATGCCAATATACCAACTG	589	Forward
rs3761935	(SNP4)	114174051	F: AAAGTTCCGGCATGTTTCC R: TGGTGATTGTCGGCTAAGATTG	595	Reverse
rs3789608	(SNP5)	114199311	F: CATCATGGTCTGGCCAATTC R: TGAGGTGGAGTTCTAACCACAAG	589	Forward
rs2243471	(SNP6)	114207525	F: GACAAGACTGAATTGTACGAGCG R: CACCATCTCCAGCCTCTCAC	577	Forward

The position of the SNPs is cited from the NCBI database.

Corporation, Cleveland, OH), the PCR products were sequenced with Big Dye Terminator v3.1 (Applied Biosystems, Foster City, CA) using either sense or antisense primers (Table 1). The BigDye XTerminator Purification Kit (Applied Biosystems) was used to purify the DNA from sequencing reactions. The sequencing reactions were analyzed using an ABI3130 sequencer (Applied Biosystems).

Statistical analysis: For statistical analyses, the Hardy–Weinberg equilibrium was tested for each SNP among the control subjects. Genotype frequency differences between the case and control genotypes were assessed by the χ^2 test. The calculation of linkage disequilibrium (LD) and pair-wise LD (D' value) between SNPs of the *PTPN22* region and the haplotypes was performed with Haploview software, version 3.32. The maximum likelihood estimates of haplotype frequencies were estimated by pairs of unphased genotypes using the expectation-maximization (EM) algorithms in the R package ‘haplo.stats’ [20].

RESULTS

Allele frequencies for the six SNPs covering the gene were in Hardy–Weinberg equilibrium in both the patients and controls. The allelic frequency of each SNP in both groups was nearly equal, and no association was detected when compared independently (odds ratio, OR 1.14–1.35; Table 2). Stratifying the patients by the presence of diffuse choroiditis, sunset glow fundus, nummular chorioretinal depigmented spots, neurologic auditory involvement, meningismus,

tinnitus, cerebrospinal fluid pleocytosis, or integumentary findings also revealed no evidence of association in VKH disease (data not shown). We calculated pairwise D' values for all SNP pairs in *PTPN22* (Figure 2). The pairwise D' values in the gene were nearly 1 among almost all SNP pairs, indicating the SNPs were highly associated with each other and the entire *PTPN22* was contained within a single LD block. Haplotype analysis predicted and revealed that *PTPN22* was not associated with VKH disease in this Japanese cohort (data not shown).

DISCUSSION

In the present study, we analyzed polymorphisms of the new candidate gene, *PTPN22*, in Japanese patients with VKH

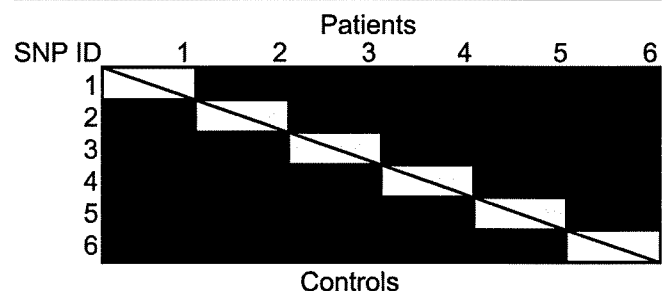


Figure 2. D' score for the six SNPs studied across the *PTPN22* haplotype. Black cells indicate that D' is greater than 0.9. Upper: patient population, lower: control population. The figure indicates that the six SNPs were in all the same haplotype block.

TABLE 2. GENOTYPE FREQUENCIES IN VKH PATIENTS AND CONTROLS.

SNP	Allele	VKH (n=168)	Percentage	Control (n=187)	Percentage	Odds ratio (95% CI)	p
rs3811021	C/C	2	1.2	9	4.8	0.24 (0.05–17.47)	0.05
	T/C	56	33.5	67	35.8	0.90 (0.58–7.38)	0.65
	T/T	109	65.3	111	59.4	1.29 (0.84–7.53)	0.25
rs1217413	C	60		85		0.75 (0.51–7.41)	0.12
	A/A	26	16	36	19.4	0.79 (0.45–7.63)	0.41
	A/G	77	47.2	91	48.9	0.93 (0.61–7.43)	0.75
	G/G	60	36.8	59	31.7	1.25 (0.80–7.56)	0.32
rs1237682	A	129		163		0.84 (0.62–7.25)	0.26
	C/C	26	15.5	33	18.4	0.83 (0.47–7.63)	0.53
	T/C	79	47	89	49.7	0.94 (0.62–7.34)	0.77
	T/T	59	35.1	57	31.8	1.20 (0.77–7.53)	0.42
rs3761935	C	131		155		0.87 (0.64–7.24)	0.37
	G/G	3	1.8	10	5.3	0.33 (0.09–12.76)	0.08
	T/G	55	33.3	67	35.8	0.90 (0.58–7.39)	0.62
	T/T	107	64.8	110	58.8	1.29 (0.84–7.54)	0.25
rs3789608	G	61		87		0.75 (0.52–7.41)	0.12
	T/T	2	1.2	9	4.8	0.24 (0.05–17.57)	0.05
	C/T	57	34.1	68	36.4	0.91 (0.59–7.38)	0.66
	C/C	108	64.7	110	58.8	1.26 (0.82–7.50)	0.29
rs2243471	T	61		86		0.74 (0.51–7.41)	0.11
	A/A	27	16.7	37	20.2	0.79 (0.46–7.62)	0.4
	A/G	79	48.8	88	48.1	1.03 (0.67–7.36)	0.9
	G/G	56	34.6	58	31.7	1.14 (0.73–7.48)	0.57
	A	133		162		0.88 (0.65–7.24)	0.39

The above table is the genotype and allele frequencies of the VKH patients and healthy controls. There are no differences between patients and controls.

disease. The gene encodes an important negative regulator of T cell activation [9]. An SNP of *PTPN22*, R620W (rs2476601) was reported to be associated with several autoimmune diseases such as RA, SLE, and IDDM [11,12, 14,15]. However, this SNP, which disrupts an interaction between Lyp and the protein tyrosine kinase, Csk, does not exist as a polymorphism in the Japanese population [9,10, 12]. Therefore, in this study, we examined six other SNPs to evaluate the susceptibility locus of *PTPN22*. *HLA-DRB1* is a common genetic factor in autoimmune diseases (RA and IDDM). Therefore, there may be other common genetic factors in VKH disease [21].

VKH disease is considered to be an autoimmune disease against melanocytes [2-5]. In early studies, activated T lymphocytes were elevated and attacked melanocytes of ocular choroidal tissue in patients in the active phase of VKH disease [22]. Antigen-specific T-cell assay revealed that peptide fragments of the tyrosinase family proteins (tyrosinase, tyrosinase related protein 1 and 2) proliferated in T lymphocytes collected from VKH patients [4,5]. These proteins are found in human melanocytes. These antigen-specific T cell responses were detected in cells collected from *HLA-DRB1*04* positive VKH patients only but not from *HLA-DRB1*04* negative patients or *HLA-DRB1*04* positive healthy people [7,23]. In the Japanese population, 40% of healthy people have *HLA-DRB1*04* [7]. However, people having VKH disease represent only 0.01% of the Japanese population [1,7,24,25]. In addition, some patients with VKH disease are *HLA-DRB1*04* negative [7]. Thus, it is believed

*HLA-DRB1*04* is a major susceptible gene in VKH disease. However, other minor genetic factors still remain unclear. To find other susceptible genes, we studied the tyrosinase gene (*TYR*), tyrosinase related protein 1 gene (*TYRP1*), tyrosinase related protein 2 gene (*TYRP2*), and interferon (*IFN-γ*), but we could not find any association with these genes and VKH disease [7,26]. Genetic influences of VKH were also investigated in other countries, but the etiology of the disease seems to be unresolved [27-29].

In this study, we found no association between *PTPN22* and VKH disease in the individuals studied. Our results suggest that further molecular genetic studies are needed to detect novel genetic loci and predisposing genes and to elucidate the true genetic mechanisms underlying VKH disease.

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Clinical features of intraocular inflammation in Hokkaido, Japan

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ABSTRACT.

Purpose: We aimed to investigate the clinical features of intraocular inflammation/uveitis in Hokkaido, Japan.

Methods: We retrospectively reviewed the medical records of 1240 uveitis patients (511 men, 729 women) who visited Hokkaido University Hospital, Sapporo, Japan between 1994 and 2003.

Results: Mean age at disease onset was 41.7 ± 17.8 years in men and 45.7 ± 18.3 years in women. Anterior, posterior and combined anterior and posterior segment intraocular inflammation accounted for 45.1%, 4.7% and 50.2% of cases, respectively. Sarcoidosis was the most frequent aetiology (14.9%), followed by Vogt-Koyanagi-Harada (VKH) disease (9.7%) and Behçet's disease (6.7%). Aetiologies in 49.8% patients were unknown. In sarcoidosis, women represented 72.4% of patients, and disease onset occurred at 35.1 ± 19.0 years of age in men and 50.3 ± 16.5 years in women. In VKH disease, 54.2% of patients were women, and disease onset took place at 45.9 ± 15.8 years in men and 46.4 ± 14.1 years in women. In Behçet's disease, men accounted for 56.6% of patients, and disease onset occurred at 35.5 ± 8.5 years in men and 44.5 ± 11.5 years in women.

Conclusions: Women were more prone to developing sarcoidosis compared with men. By contrast, men were more prone to developing Behçet's disease. The mean age at disease onset in both sarcoidosis and Behçet's disease was significantly lower in men than in women.

Key words: Behçet's disease – intraocular inflammation – sarcoidosis – uveitis – Vogt-Koyanagi-Harada disease

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Introduction

Uveitis, or intraocular inflammation, includes several clinical entities in which innate and acquired immune

responses play key roles. Intraocular inflammation often leads to visual failure or visual loss. The main cause of visual loss in intraocular inflammation is generally cystoid macular oedema,

although blindness resulting from intraocular inflammation can be caused by several processes, including macular oedema, macular degeneration, retinal detachment, choroidal atrophy and optic atrophy (Rothova et al. 1996). In developed countries, intraocular inflammation surveys of patient populations have shown that patterns of prevalence change over time. The prevalence of intraocular inflammation from all causes is around 40/100 000, with an incidence of around 15/100 000 population (Baarsma 1992). Intraocular inflammation affects all age groups, but occurs with greater frequency in the 20–40-year-old age group, which means the disease can have considerable socioeconomic impact (Suttorp-Schulten & Rothova 1996). The aetiological characteristics of intraocular inflammation vary with age and sex and, furthermore, frequencies differ around the world. Genetic and racial factors may play a significant part in the distribution of specific entities, such as Vogt-Koyanagi-Harada disease (VKH disease), Behçet's disease, HLA (human leucocyte antigen)-B27-associated uveitis, HTLV-1 (human T-cell leukaemia/lymphoma virus)-associated uveitis (HAU) and bird-shot retinochoroidopathy.

In the current study, we conducted a retrospective analysis of patients with intraocular inflammation who initially visited our clinic. We analysed the characteristics of the intraocular

inflammation observed over a 10-year period.

Materials and Methods

Patients

Between January 1994 and December 2003, 1240 new patients (511 men, 729 women) with uveitis visited the Uveitis Survey Clinic at Hokkaido University Hospital, Sapporo, Japan. We reviewed all medical records of the patients and obtained information on each patient's age at disease onset, gender, primary site of inflammation and clinical diagnosis. According to the International Ocular Inflammation Society (IOIS) proposal, we categorized uveitis/intraocular inflammation as anterior segment intraocular inflammation (ASII), posterior segment intraocular inflammation (PSII) or combined anterior and posterior segment intraocular inflammation (CAPSII). Patients with postoperative or traumatic endophthalmitis were not included. The study was conducted in accordance with the tenets of the Declaration of Helsinki.

In the present study, the aetiological diagnosis was only accepted if it met well defined criteria and fulfilled IOIS classification requirements (BenEzra 2001). To diagnose sarcoidosis, we adopted the criteria established by the Diffuse Pulmonary Disease Research Committee of Japan (Yamaguchi et al. 2004). We diagnosed VKH disease using criteria defined by Sugiura (1978), one of the most frequently used sets of criteria in the world. For diagnosis of Behçet's disease, we used criteria established by the Behçet's Disease Research Committee of Japan (1986) and the International Study Group for Behçet's Disease (1990). Diagnoses of other aetiologies were made by careful observations of ocular involvement, clinical course and accompanying systemic disease, combined with laboratory analyses. Unknown or suspicious aetiologies were all categorized as 'unclassified aetiologies'.

Statistical analysis

Average ages of patient groups were compared using the Mann-Whitney *U*-test and statistical significance was set at $p < 0.01$.

Results

Characteristics of uveitis patients

The distribution of intraocular inflammation was examined according to gender and age brackets (Fig. 1A). During the study period, we examined data for 1240 new patients. All patients were ethnically Japanese. Men and women accounted for 41.2% ($n = 511$) and 58.8% ($n = 729$) of subjects, respectively. The average age at disease onset was 41.7 ± 17.8 years in men and 45.7 ± 18.3 years in women, with an overall average age of 44.1 ± 18.2 years. The peak age range at which subjects visited our clinic was 50–60 years in women and 20–30 years in men. The distribution of the anatomical location of the primary site of inflammation was examined (Fig. 1A). Numbers of patients with ASII, PSII and CAPSII were 559 (45.1%), 58 (4.7%) and 623 (50.2%), respectively. Distribution of CAPSII differed in men and women: frequencies of CAPSII peaked at 20–30 years of age in men and at 50–60 years of age in women.

We then examined the aetiologies of intraocular inflammation (Table 1). Idiopathic, suspicious or unknown aetiologies were all categorized as 'unclassified' and accounted for nearly

half of all uveitis patients (49.8%). Among confirmed aetiologies, sarcoidosis ($n = 185$, 14.9%) was the most frequent aetiology, followed by VKH disease ($n = 120$, 9.7%), Behçet's disease ($n = 83$, 6.7%) and HLA-B27-associated uveitis ($n = 50$, 4.0%).

Frequent causes of uveitis

We detailed the characteristics of three major causes of uveitis in our clinic: sarcoidosis, VKH disease and Behçet's disease. Sarcoidosis was the most frequent disease among the aetiologies ($n = 185$, 14.9%). Women accounted for 134 patients (72.4%) and men for 51 (27.6%). Average age at disease onset was lower in men (35.1 ± 19.0 years) than in women (50.3 ± 16.5 years) ($p < 0.0001$). Furthermore, women had two peaks of disease onset, with the first occurring at 20–30 years of age and the second at 50–70 years. Although the peak at 20–30 years was observed in men, the combined sarcoidosis patient group showed a bimodal distribution (Fig. 2A).

The second most frequent aetiology was VKH disease ($n = 120$, 9.7%). Women were more prone to develop this disease ($n = 65$, 54.2%) compared with men ($n = 55$, 45.8%). Mean age at disease onset was

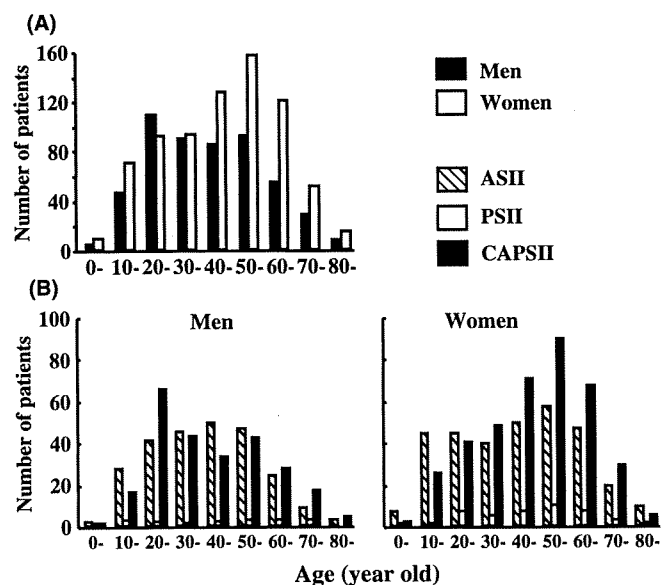


Fig. 1. (A) Distribution of uveitis patients presenting during 1994–2003 according to sex and age. (B) Distribution of uveitis patients according to sex and anatomical location of the primary site of inflammation. ASII = anterior segment intraocular inflammation; PSII = posterior segment intraocular inflammation; CAPSII = combined anterior and posterior segment intraocular inflammation.

Table 1. Aetiologies of uveitis patients at Hokkaido University Hospital between 1994 and 2003.

Aetiology	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	Total	%
Sarcoidosis	13	17	17	16	18	12	21	28	16	27	185	14.9
VKH disease	13	11	12	12	7	10	12	19	11	13	120	9.7
Behçet's disease	13	14	9	6	6	3	7	13	9	3	83	6.7
HLA-B27-associated uveitis	5	3	9	2	2	6	4	9	4	6	50	4.0
Fuchs' heterochromic iridocyclitis	4	4	3	5	3	2	3	2	4	1	31	2.5
Posner-Schlossman's syndrome	4	1	1	4	1	2	2	2	2	1	20	1.6
Acute retinal necrosis	2	1	1	3	4	2	3		3		19	1.5
Endophthalmitis	3	2	2	1		6	4				18	1.5
VZV iridocyclitis	2		2	2	2	2		1		2	13	1.0
HTLV-1-associated uveitis	1	1		1	2		3	2	2		12	1.0
HSV keratouveitis	2				3	2		2	2		11	0.9
Toxoplasmosis		1	2		1		1	1	1	1	8	0.7
Chronic iridocyclitis in young girls	1	1					1	4	1		8	0.7
TINU				1	1	1	1	2	1		7	0.6
MEWDS			1			1		2		1	5	0.4
Intermediate uveitis		2	1			1	1				5	0.4
Posterior scleritis			1					2		2	5	0.4
Others (<i>n</i> < 5)	3	2	2	1	1	5	2		4	3	23	1.9
Unclassified	64	51	49	44	40	52	58	88	89	82	617	49.8
Total number of patients	130	111	112	98	91	107	123	177	149	142	1240	100

VKH = Vogt-Koyanagi-Harada; HLA-B27 = human leucocyte antigen-B27; VZV = varicella zoster virus; HTLV = human T-cell lymphotropic virus; HSV = herpes simplex virus; TINU = tubular interstitial nephritis and uveitis; MEWDS = multiple evanescent white dot syndrome.

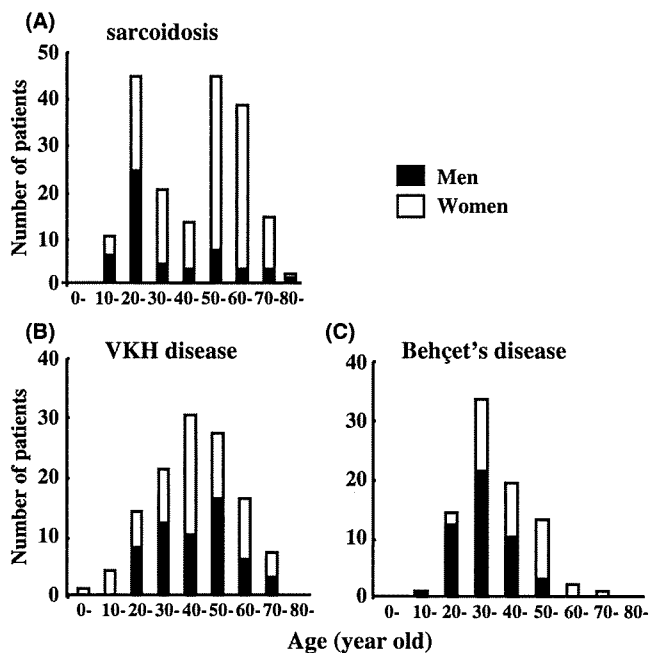


Fig. 2. Distribution according to sex and age of patients with (A) sarcoidosis, (B) Vogt-Koyanagi-Harada disease and (C) Behçet's disease.

45.9 ± 15.8 years in men and 46.4 ± 14.1 years in women. This difference was not statistically significant. Those most susceptible to this disease were people aged 40–50 years (Fig. 2B).

The third most common aetiology was Behçet's disease (*n* = 83, 6.7%), for which we found a statistically significant greater number of male

(*n* = 47, 56.6%) than female (*n* = 36, 43.4%) patients. The average age at disease onset was found to be significantly lower in men (35.5 ± 8.5 years) than in women (44.5 ± 11.5 years) (*p* < 0.0001). The peak age for disease onset was 30–40 years (Fig. 2C). Young and middle-aged Japanese males may represent a Behçet's disease-prone population.

Next, we calculated the age and sex distribution of the unclassified aetiologies of uveitis. Of all patients, 236 (38.2%) were men and 381 (61.8%) were women (Fig. 3A). The most common site of inflammation was ASII (352 subjects, 57.0%), followed by CAPSII (*n* = 235, 38.1%) and PSII (*n* = 30, 4.9%) (Fig. 3B).

Discussion

In the present study, we reviewed the medical records for 1240 patients newly diagnosed with intraocular inflammation. Hokkaido, in which our clinic is located, is Japan's northernmost island and has a population of 5.6 million. Our clinic is one of the biggest uveitis centres in the world, and is also the only one of its kind on Hokkaido Island. As our clinic is the second/third referral eye centre for the region, most uveitis patients in the region except those with very mild symptoms should be referred to it. We expect that our present results reflect, with a reasonable degree of precision, the epidemiology of intraocular inflammation in the Japanese population.

Sarcoidosis, VKH disease and Behçet's disease were the most frequent aetiologies seen in our clinic, although the frequencies differed for each year. These results were consistent with

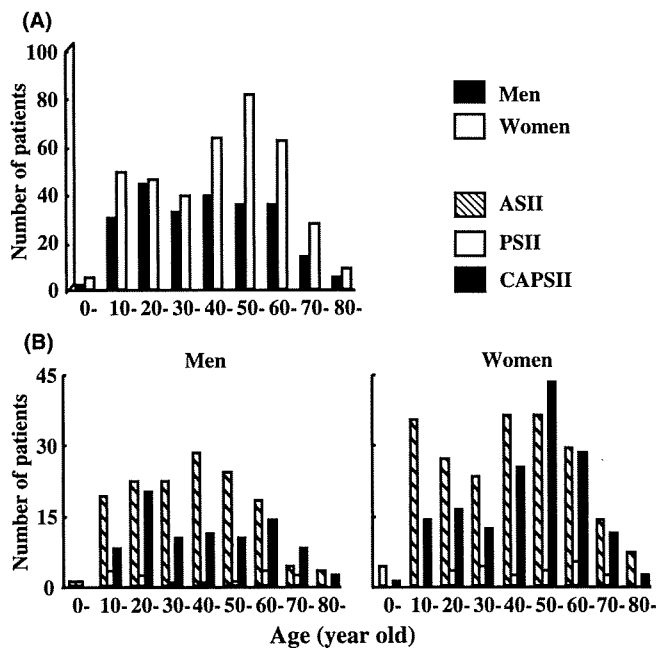


Fig. 3. (A) Distribution of 'unclassified' uveitis patients according to sex and age. (B) Distribution of unclassified uveitis patients according to anatomical location of the primary site of inflammation. ASII = anterior segment intraocular inflammation; PSII = posterior segment intraocular inflammation; CAPSII = combined anterior and posterior segment intraocular inflammation.

those of a recent report (Goto et al. 2007). It is also known that frequencies vary to some extent even within Japan. People in Kyushu, the southernmost part of Japan, have a much higher prevalence of HAU than in other areas because the frequency of virus carriers is quite high in Kyushu compared with that in other regions of Japan (Nakao et al. 1989; Goto et al. 1995). In the present study, frequencies of two of the diseases, sarcoidosis and Behçet's disease, changed during the decade under study. The number of new patients with sarcoidosis increased and the number of Behçet's disease patients decreased. Our statistical results exhibited the same tendencies reported at other centres in Japan (Wakabayashi et al. 2003; Akiyama et al. 2006). However, these results were appreciably different from those of American and European studies. A relatively high percentage of Japanese patients had CAPSII (50.2%) and ASII (45.1%), but a low percentage had PSII (4.7%). By contrast, the most common types of uveitis were PSII in the USA and ASII in Europe (Wakefield & Chang 2005). In addition, the frequency of HLA-B27-associated uveitis was lower in Japanese subjects, but

frequencies of Behçet's disease and VKH disease were much higher in Japanese than in European patients (Päivönsalo-Hietanen et al. 1994). The low prevalence rate of HLA-B27-associated uveitis was not unexpected, given that only 0.5% of the Japanese population is HLA-B27-positive, reflecting an incidence that is only 10% of that of other ethnic groups (Yamaguchi et al. 1995). Further, we had no patients with birdshot retinochoroiditis because few people in the Japanese population carry the HLA-A29 gene. Moreover, only a few patients with intermediate uveitis were observed. These results showed geographical and racial differences in the aetiologies of endogenous uveitis.

We can further describe the characteristics of the three major aetiologies: sarcoidosis, VKH disease, and Behçet's disease. Sarcoidosis is a multisystem granulomatous disease of unknown aetiology (Liu et al. 2006; Khanna et al. 2007). Diagnosis is almost entirely based on histologically proven granuloma in any tissue, including lung or skin, or by clinical criteria. Our results showed that 72.4% of sarcoidosis patients were women and many patients were aged in either their 20s or 50s, which is in

agreement with a previous report (Bonfioli & Orefice 2005). As new diagnostic techniques, transbronchial lung biopsy (TBLB) and analysis of bronchoalveolar lavage fluid (BALF) have facilitated confirmation of this diagnosis in patients with sarcoidosis. We should note that our records included many young patients (< 20 years old). However, ocular symptoms sometimes appear earlier than a definitive histological diagnosis and TBLB and/or BALF were rarely performed in very young patients. Thus, it is possible that some undiagnosed sarcoidosis patients were included in the group of 'unclassified' subjects. The true incidence of sarcoidosis may be higher than that reported here.

Vogt-Koyanagi-Harada disease is a multisystem autoimmune disorder in which the T cell-mediated autoimmune response plays a key role. The diagnostic system established by Sugiura (1978) has long been used to make accurate diagnoses of VKH disease. As new diagnostic criteria were proposed in 2001 (Read et al. 2001), we diagnosed VKH disease using both sets of criteria in this study. Fortunately, we encountered few issues with these diagnoses because the concordance rate between the two sets is very high, as has been previously reported (Kitamura et al. 2005). Patients are typically 20–50 years of age and have no history of either surgical or accidental ocular trauma.

Behçet's disease is a chronic, relapsing vasculitis that can affect most organ systems. A heritable risk factor for Behçet's disease was first identified as an association with HL-A5, now called HLA-B51 (Ohno et al. 1973). Prevalences vary geographically, and the disease is more common in countries in the Mediterranean basin, the Middle East and East Asia. The geographical distribution of HLA-B51 among healthy subjects also roughly corresponds with global disease distribution (Verity et al. 1999). In the present study, 56.6% of Behçet's disease patients were men and many of them were in their 30s and 40s. These results were comparable with those of our recent large-scale, international, collaborative study (Kitaichi et al. 2007). According to a nationwide survey of Behçet's disease patients in Japan, the incidence of this disease has recently decreased (Yoshida et al.

2004). The prevalence of this disease was 7.5/1 000 000 in 1990, compared with 8.9/1 000 000 in 1984. Reflecting this trend, the number of patients we observed in Hokkaido with Behçet's disease had also decreased. In fact, we found 152 new Behçet's disease patients (88 men, 64 women) during 1984–93 (data not shown), but only 83 new patients during 1994–2003. However, Behçet's disease is still a principal cause of visual failure as a result of recurrent ocular attacks.

Because some systemic diseases, such as sarcoidosis and Behçet's disease, are sometimes confined to the eye for some years, longer follow-up periods might increase the numbers of sarcoidosis and/or Behçet's disease patients identified in the future. In conclusion, we report on a large-scale, single-centre study of uveitis in Japan. We hope that this study can serve as a basis for future comparisons of the differences in incidences between countries and races.

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