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GENETICS OF BEHÇET'S DISEASE INSIDE AND OUTSIDE THE MHC

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Abstract

Objectives: Behçet's disease (BD) is a rare, chronic, systemic, inflammatory disorder characterized by recurrent ocular, genital and skin lesions. Although its etiology is still uncertain, an intricate interplay between the environment (e.g. viruses) and the host seem to initiate and/or perpetuate the disease, although its mechanistics remains presently speculative. Since the identification of *HLA-B*5101* (and more recently of *MICA*) as a susceptibility locus for BD, the identification of additional genetic locus/loci, whether inside, or perhaps more importantly outside the MHC has clearly stalled. We hereby present the results of a first genomewide association study (GWAS) of BD.

Methods: 300 Japanese patients with BD and an equal number of controls were recruited. The samples were screened using a dense panel of 23,465 microsatellites (MS) covering the entire genome.

Results: We identified the six best (of a total of 147) positively associated MS with BD. Of these six, two were located within the HLA class I region itself. Although one of these was clearly reminiscent of the association with *HLA-B*, the second, not in linkage disequilibrium with the former was in the telomeric side of the class I region and remained to be formally identified. *In fine*, HLA genotyping and haplotype analysis conclusively led to the deciphering of a dual, independent, contribution of two HLA alleles to BD's pathogenesis: *HLA-B*5101* and *HLA-A*26*.

Conclusions: This GWAS highlights the premier genetic susceptibility locus for BD as the MHC itself, wherein reside two independent loci: *HLA-B* and *HLA-A*.

Behçet's disease (BD) is a rare, chronic, systemic inflammatory disorder characterized by four major symptoms consisting of recurrent ocular symptoms, oral and genital ulcers, and skin lesions (1). BD exists worldwide but is clearly more prevalent in countries along the ancient Silk route, spanning from Japan to the Middle East and the Mediterranean (2).

The etiology of BD remains uncertain, but as in many other inflammatory and/or immune-centered diseases, environmental factors are thought to trigger the pathology in individuals harboring a “favorable” genetic background. The sole solid genetic component of BD, persistent across the globe, is the human leukocyte antigen (HLA) class I antigen, *HLA-B51* (2). It remains to be firmly and formally established - as is the case of the quasi-totality of HLA-linked diseases - if this HLA allele itself or a closely linked gene(s) is the contributing genetic factor. Recently, we and others were able to show that *HLA-B*5101* itself, alone (3-5) or in conjunction with the closely linked *MICA*009* allele appear to be the major MHC-based contributing factor in the development of BD (5-7).

As much as the MHC is a must for any insight into the genetics of diseases having a more or less expansive immune component, it has been difficult, until recently, to venture beyond, as the tools to do so were not available. There are three main, more or less exclusive approaches, in order to track the genetic basis of diseases carrying an inherited component. Linkage studies are suited for Mendelian disorders, genomewide association study (GWAS) for complex diseases and finally the candidate gene approach which could be essentially employed as stand-alone and/or within the settings of the first two. As applied to BD, the candidate gene approach has allowed the identification - besides *HLA-B51* - of a number of disparate loci (8-10). Besides inherent caveats - i.e. candidate gene selection is by nature hypothesis driven - in such approach, most of the above mentioned efforts have in addition suffered from small sample size and a lack of replication studies. Although, non-Mendelian diseases are not well suited for linkage studies, Karasneh *et al.* did complete such analysis in BD using a set of 428 microsatellites (MS) in Turkish multicase families (11). The study reported the identification of several non-HLA susceptibility loci for the disease. However, the nonparametric linkage analysis performed in the study had several inherent weaknesses, including poor mapping resolution and low detection power. Finally and as alluded to previously, GWAS on the other hand are rightly suited for disease such as BD (12). Hence we embarked on a first GWAS in BD.

Materials and Methods

Patients and healthy controls

Detailed information about patients, controls and methods is presented in an online supplemental text file. In brief, 300 unrelated BD patients and 300 unrelated healthy controls, all of Japanese descent, were enrolled in this study. The diagnosis of BD was established according to standard criteria (13) proposed by the Japan Behçet’s Disease Research Committee. The patients and controls were equally distributed in groups of 100 versus 100 in

each and all of the three pooled DNA typing stages (Supplemental Table 1). After pooled DNA typing, individual DNA typing was performed on the same sample set. The study methodology complied with the guidelines of the Declaration of Helsinki. The study details were explained to all patients and controls before obtaining their consent to genetic screening.

GWAS

Pooled DNA construction

The protocol of Collins et al. (14) - upon slight modifications (15) - was used to perform MS typing on pooled DNA (14).

Genomewide MS genotyping

All MS markers and the genotyping methods for MS analysis used in this study have been previously described (16).

HLA genotyping and haplotype analysis

Twenty MS markers that cover the entire HLA class I region were used in this study. Genotyping of *HLA-A* and *HLA-B* genes was performed using the PCR-sequence-specific oligonucleotide probes (SSOP)-Luminex method (17). The genotyping of *HLA-E*, *HLA-F*, and *HLA-G* genes was performed by direct DNA sequencing (Supplemental Table 2) (18-20).

Results

Genomewide association study

The genetic basis of BD remains in large part untapped; once the HLA locus is discarded. Here we present the first GWAS of BD achieved through three rounds of pooled DNA screening using a comprehensive set 23,465 MS markers. In the first screen, using 23,465 markers, we found a signification association for 1,919 markers, as assessed by the Fisher's exact test. In the second screen, 390 of these 1,919 markers continued to show a significant association, but after the third screen, only 147 markers remained positive (Fig. 1, Supplemental Fig. 1, Supplemental Table 3). A total of 9 of these 147 markers had similar peak patterns among the first, second, and third case and control pools. To confirm, beyond doubt, the associations observed, we individually genotyped each of these nine markers in the same set of 600 screened individuals. Six markers remained positive after this individual screening step (Table 1, Fig. 1, Supplemental Fig. 1). Among them two - *D6S0014i* and *D6S0032i* - were located in the HLA class I region (6p21.3) - ~1.1 Mb and ~36 kb telomeric of the *HLA-B* gene, respectively - and were indeed most strongly associated with BD.

HLA genotyping and haplotype analysis

The location of the two most strongly BD-associated markers in the HLA class I region refocused our interest to the MHC. The HLA class I region spans 1.8 Mb from the

centromeric *MICB* to the telomeric *HLA-F* and contains in addition to these two "gatekeepers", the following MHC-I and related molecules (in the same centro-telomeric direction): *MICA, HLA-B, HLA-C, HLA-E, HLA-A* and *HLA-G*, in addition to a number of non-HLA related genes. Given the physical location of the two MS markers, the association with *D6S0032i* might be indeed reminiscent of that with *HLA-B* and that with *D6S0014i*, reflecting a yet to be identified HLA or non-HLA gene(s). Previous studies had indeed reported the association of BD with certain *HLA-A* alleles in some ethnic groups (21-23). Additionally, Park et al. showed that *HLA-E* and *HLA-G* polymorphisms contribute to the development of BD in Korea (24). However, to date, it remains to be shown whether the association between each gene (*HLA-A, HLA-E,* and *HLA-G*) and BD is a primary one or subsequent to LD with other genes, including *HLA-B*. In order to identify the genuine MHC-linked pathogenic BD locus/loci we had indeed to circumvent the LD across the region dictated by the only polymorphic loci tested to date i.e. HLA genes. In order to do so we performed a comprehensive MS mapping and HLA genotyping throughout the HLA class I region. Association, LD, and haplotype analyses were conducted using these data. To our knowledge, this is the first report of a genetic association study for BD that focuses on the entire ~2.0 Mb HLA class I region.

A total of 20 MS and 5 *HLA* genes in and around the HLA class I region were used to genotype the same above described 300 patients and 300 controls (Fig. 2*A, B*). For 22 of the 25 genes and MS, statistically significant differences in allele frequencies were observed between patients and controls. Among these, *HLA-B* ($Pc = 1.63 \times 10^{-24}$), *D6S2938* ($Pc = 5.75 \times 10^{-12}$), and *D6S2930* ($Pc = 1.15 \times 10^{-13}$) were most strongly associated with BD. In addition, genes and MS in/around the region from *HLA-F* to *HLA-G* were markedly associated with BD (*HLA-F*, $Pc = 1.83 \times 10^{-8}$; *HLA-G*, $Pc = 1.44 \times 10^{-6}$; *174B01*, $Pc = 9.91 \times 10^{-8}$; *180G06*, $Pc = 6.55 \times 10^{-7}$; *D6S2707*, $Pc = 8.37 \times 10^{-7}$). The frequencies of the identified *HLA-A, -B, -E, -F,* and *-G* alleles are listed in Supplemental Table 4. *HLA-B*5101* was the strongest susceptibility allele for the development of BD (Odds ratio (OR) = 5.50). In other HLA genes, *HLA-A*2601* (OR = 1.92, Pc = 0.016), *HLA-F*010101* (OR = 1.43, *Pc* = 0.011), and *HLA-G*010102* (OR = 2.08, $Pc = 1.44 \times 10^{-6}$) were positively associated with susceptibility to BD. Conversely, *HLA-A*3303* (OR = 0.34, *Pc* = 0.0035), *HLA-F*010102* (OR = 0.41, $Pc = 1.83 \times 10^{-8}$), and *HLA-G*010401* (OR = 0.41, $Pc = 3.67 \times 10^{-4}$) were negatively associated with susceptibility to BD. No association with *HLA-E* was observed.

A sizeable fraction of BD patients do not carry the *HLA-B*5101* allele. What is their genetic makeup? In order to dissect this fact, we stratified the patient and control populations according to their carriage or not of *HLA-B*5101*. There were 136 BD and 255 controls not carrying *HLA-B*5101* (Fig. 2*C*, Supplemental Table 4). No other *HLA-B* alleles were associated with BD. Three MS located within 260 kb telomeric of *HLA-B* (*D6S2938, D6S2930,* and *D6S0032i*) were only slightly associated with BD. In contrast, a strong association between BD and a 770 kb region including *HLA-A, -F,* and *-G* (from *D6S2770* to

D6S0014i) was observed, hence independently of *HLA-B*5101*. *D6S2838*, located between *HLA-A* and *HLA-G*, was most strongly associated with BD (OR = 2.75, *Pc* = 5.98 x 10^{-7}). *HLA-A*2601* (OR = 2.61, *Pc* = 5.11 x 10^{-4}), *HLA-F*010101 (*OR = 1.87, *Pc* = 1.55 x 10^{-4}*)*, and *HLA-G*010102* (OR = 2.24, *Pc* = 1.16 x 10^{-4}) were positively associated with susceptibility to BD, whereas *HLA-F*010102* (OR = 0.40, *Pc* = 2.09 x 10^{-5}) and *HLA-G*010401* (OR = 0.56, *Pc* = 2.41 x 10^{-3}) were negatively associated with susceptibility to BD.

Fig. 3 shows the overall LD patterns for the 20 MS and 5 HLA genes in 300 BD patients and 300 controls. Strong LD were observed throughout the ~380-kb region including *HLA-A*, *-F*, and *-G* (from 186A07 to 022G02), and strong associations were observed for *HLA-A-HLA-G* (*D'* of cases and controls ($D'_{cases\&controls}$) = 0.940 and 0.902, respectively), *HLA-A-HLA-F* ($D'_{cases\&controls}$ = 0.789 and 0.718), and *HLA-F-HLA-G* ($D'_{cases\&controls}$ = 0.662 and 0.746). *D6S0032i*, one of two positive MS markers located in the HLA class I region identified by our genome-wide case-control study, was most strongly associated with *HLA-B* ($D'_{cases\&controls}$ = 0.879 and 0.833). *HLA-B* exhibited a strong LD with *MICA-TM* (transmembrane) polymorphism ($D'_{cases\&controls}$ = 0.944 and 0.932), *D6S2938* ($D'_{cases\&controls}$ = 0.765 and 0.820) and *D6S2930* ($D'_{cases\&controls}$ = 0.747 and 0.779). On the other hand, *D6S0014i*, another positive marker identified by our genome-wide study, was more strongly associated with *HLA-A* ($D'_{cases\&controls}$ = 0.604 and 0.532) than with other HLA genes and exhibited the strongest LD with *D6S2770* ($D'_{cases\&controls}$ = 0.970 and 0.939). Interestingly, *D6S2770* exhibited a strong LD with most MS and with all HLA genes throughout the ~2.0 Mb region.

Table 2 shows the frequencies of *HLA-A-HLA-F-HLA-G* haplotypes in 300 BD patients and 300 controls. The frequency of the *HLA-A*2601-F*010101-G*010102* haplotype consisting of three alleles positively associated with BD susceptibility was significantly increased in BD patients (OR = 1.99, *Pc* = 0.028). Furthermore, the frequency of haplotypes containing *HLA-F*010101*, *HLA-G*010102*, and other *HLA-A*26* subtypes was increased in BD patients, but not significantly. The frequency of the haplotype consisting of *HLA-A*26*, *HLA-F*010101*, and *HLA-G*010102* was more significantly increased in BD patients (OR = 2.13, *Pc* = 2.77 x 10^{-4}). In contrast, the haplotype frequencies of *HLA-A*2402-F*010102-G*0101401* and *HLA-A*3303-F*010102-G*0101401* were significantly decreased in BD patients (OR = 0.47, *Pc* = 8.60 x 10^{-3}; OR = 0.33, *Pc* = 0.012, respectively). Table 2 also shows the frequencies of *HLA-A–HLA-F–HLA-G* haplotypes in *HLA-B*5101* non-carriers, 136 BD cases and 255 controls. The frequency of the *HLA-A*2601-F*010101-G*010102* haplotype was significantly increased in BD patients (OR = 2.68, *Pc* = 1.30 x 10^{-3}). Moreover, the *HLA-A*26-F*010101-G*010102* haplotype was most strongly associated with susceptibility to BD (OR = 3.08, *Pc* = 9.88 x 10^{-7}).

Discussion

Here we present the first GWAS of BD. Our GWAS effort employed 23,465 highly polymorphic MS markers to cover the euchromatic area (~90%) of the human genome at average intervals of 115.1 kb. Among the ultimate six positive markers which passed several filters, two - *D6S0014i* and *D6S0032i* - were located within the HLA class I region, respectively ~36 kb and ~1.1 Mb telomeric of *HLA-B.* Two other positive markers, *536G12A* and *D12S0645i*, were located on 6q25.1 and 12p12.1, respectively. These two chromosome regions were consistent with susceptibility regions, 6q25-26 and 12p12-13, that Karasneh et al. (11) by whole-genome linkage analysis using multicase families. The closest gene to *536G12A* is *PPIL4* (peptidylprolyl isomerase (cyclophilin)-like 4). *PPIL4* is a member of the cyclophilin family, a highly conserved family of proteins that exhibit peptidyl-prolyl cis-trans isomerase activity, and play a role in protein folding, immunosuppression by cyclosporin A, and infection of HIV-1 virions ans well as lymphocyte apoptosis (25-29). *D12S0645i* is located in intron 3 of the *SOX5* (SRY (sex determining region Y)-box 5) gene. *SOX5* is a member of the human *SOX* gene family; genes with critical roles in a number of embryonic developmental processes (30-35). *D3S0186i* and *D22S0104i* are located on 3p12 and 22q11.22, respectively. *D3S0186i* is located in intron 3 of the *ROBO1* (roundabout, axon guidance receptor, homolog 1) gene. ROBO1, an immunoglobulin superfamily member, encodes an axon guidance receptor and a cell adhesion receptor (36). *D22S0104i* is located in the immunoglobulin lambda (*IGL*) locus, containing a total of 88 *IGL* genes of which 39–43 are functional. *IGL* directs the synthesis of lambda-type light chains and plays an important role in the antibody response (37). There are 18 *IGL* genes within 100 kb from *D22S0104i*, and the nearest gene is *IGLV1-40* (IGL variable 1-40) located 1.4 kb centromeric of *D22S0104i*. Previous studies showed that the *IGL* locus or genes polymorphisms are associated with autoimmune disorders (38, 39). In addition, an association between BD and elevations of some immunoglobulins has been noted (40-43).

The intra-MHC positive markers did merit further exploration. In order to do so we investigated polymorphisms of 20 MS and 5 HLA genes in the HLA class I region and performed LD and haplotype analyses. We found that *HLA-B*5101* and MS near the *HLA-B* gene were most strongly associated with BD. In addition, the *HLA-A, HLA-F,* and *HLA-G* alleles and MS in the vicinities of these genes were also associated with BD. After stratification of individuals by their *HLA-B*5101* status and examining the *HLA-B*5101* non-carriers, *HLA-A*, *-F*, and *-G* alleles and MS near these genes remained strongly associated with susceptibility to BD. Hence, two independent, LD-strong segments at both ends of the MHC class I region i.e. respectively centered around *HLA-B* and a 380 kb region including *HLA-A*, *-F*, and *-G* were positively associated to BD. It is assumed that *HLA-B*5101* is one of such locus; the other being embedded within the 380 kb region harboring to date four expressed genes: *HLA-A*, *HLA-F*, *HLA-G*, and *HCG9* (44). Although polymorphism of *HCG9*, a gene of unknown functional relevance, was not analyzed in the present study, we showed that *HLA-A*, *HLA-F*, and *HLA-G* alleles were significantly associated with BD. In *HLA-F*,

*HLA-F*010101* was positively associated with susceptibility to BD, whereas *HLA-F*010102* was negatively associated with susceptibility to BD. The SNP that distinguishes between *HLA-F*010101* and *HLA-F*010102* results in a synonymous substitution, hence of no functional consequence. Therefore, if *HLA-F*010101* is the primary pathogenic gene that increases the risk for development of BD, *HLA-F*010102*, which has the same amino acid sequence, should also be positively associated with the risk for BD. However, these alleles had opposite effects. Furthermore, although *HLA-G*010102* conferred an increased risk of BD, the incidences of other *HLA-G*0101* alleles (*HLA-G*010101*, *-G*010103*, *-G*010105*, *-G*010107*, and *-G*010108*) were not similarly increased in BD patients. These results therefore suggest that the association with *HLA-F* and *HLA-G* alleles was due to LD - with a genuine, to be identified, pathogenic gene - and not a primary association.

HLA-F and *HLA-G* are located close to *HLA-A* where *HLA-A*2601* was positively associated with susceptibility to BD. Moreover, there is a strong LD between *HLA-F*/*-G* and *HLA-A*. In addition, the haplotype consisting of *HLA-A*2601*, *HLA-F*010101*, and *HLA-G*010102* was significantly increased in BD patients. Haplotypes consisting of other *HLA-A*26* subtypes, *HLA-F*010101*, *and HLA-G*010102* were also increased in BD patients, although not significantly. Therefore, the significantly increased incidences of *HLA-F*010101* and *HLA-G*010102* in BD patients likely result from LD with *HLA-A*26* subtypes, and it is therefore suggested that *HLA-A*26* represents an actual independent susceptibility allele for BD in the HLA class I region. In fact, the allele and phenotype frequencies of *HLA-A*26*, including all *HLA-A*26* subtypes, were strongly associated with BD. Especially, among the *HLA-B*5101* non-carriers of this study, the allele and phenotype frequencies of *HLA-A*26* were the most strongly associated with susceptibility to BD of all analyzed alleles (allele: 25.9% vs. 10.6%, OR = 2.96, $Pc = 3.63 \times 10^{-7}$; phenotype: 48.5% vs. 19.6%, OR = 3.86, $Pc = 3.20 \times 10^{-8}$) (Table 3).

In previous studies, we and other groups did show that BD is significantly associated with *HLA-A*26* in Japanese, Taiwanese, and Greek populations (21-23). In addition, it has been reported that the phenotype frequency of *HLA-A*26* was increased ~7 times in Saudi Arabian patients compared with healthy controls, but this difference was not significant (45). However, the role (primary or secondary) of *HLA-A*26* in the development of BD remained unclear because these studies did not include comprehensive analysis of the HLA class I region, and the genotyping was not performed on large samples. In other words these studies were unable to show if the association with the *HLA-A* locus was a primary one or secondary to association with *HLA-B*.

*HLA-A*26* is one of the most common HLA alleles in Asia with at least 38 distinct subtypes (http://www.ebi.ac.uk/imgt/hla/align.html). Moreover this allele has been documented to be involved in a variety of pathophysiological conditions (46-51). Our findings here confirm the results of previous studies and clarify that the *HLA-A*26* allele itself is a primary susceptibility gene involved in the development of BD. Park *et al.* (24) have reported

that the *HLA-G*010102* allele was significantly increased in Korean BD patients. However, judging from the LD analysis in this study, it is likely that the significantly increased incidence of *HLA-G*010102* in BD patients resulted secondarily from a strong LD with *HLA-A*26*, and *HLA-A*26* itself is significantly increased in Korean BD patients. On the other hand, the phenotype frequency of *HLA-A*26* was slightly increased in Palestinian, Jordanian, and Iranian BD patients compared with healthy controls (52, 53). Furthermore, in Ireland, Italy, and Turkey, the phenotype frequency of *HLA-A*26* was increased in healthy controls compared with BD patients, but not significantly (54-56). It is not straightforward to clarify why this association has not been found in all populations. Presently we can give several reasons for this 1) *HLA-A*26* is more common in Asia, especially Japan and Taiwan than in other areas. Therefore we could easily find the association between *HLA-A*26* and BD in Japan and Taiwan. 2) Previous studies didn't recruit enough samples to provide significant association results and didn't perform stratification according to *HLA-B*51* status; hence they could not detect the association between *HLA-A*26* and BD in these populations. 3) The environmental factor(s) required for development of BD as associated with the *HLA-A*26* antigen are unevenly distributed throughout the planet 4) In these populations other genetic and environmental factors are of major importance in the development of BD; outpacing largely the association between *HLA-A*26* and BD.

In conclusion, we have performed the first GWAS in BD and helped depict a first picture of the genetic constitution of BD susceptibility. It clearly, and perhaps somewhat unexpectedly, appears that the bulk of BD genetics remains in the MHC, where through extensive genotyping and haplotype analyses we were able to identify the *HLA-A* locus, as a second, independent, HLA-based susceptibility locus for BD. Functional studies could now be planned in order to unravel the joint action of two HLA alleles in BD pathogenesis.

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Figure Legends

Fig. 1. Flow chart of this genomewide association study of Behçet's disease with 23,465 microsatellite markers in 300 cases and 300 controls.

Fig. 2. Allele differences in HLA genes and MS between Japanese Behçet's disease (BD) patients and healthy controls. (*A*) Diagram of chromosome 6 showing the locations of HLA genes and MS examined in this study. MS are indicated by the following numbers: 1, *D6S0016i*; 2, *D6S2770*; 3, *D6S2910*; 4, *D6S0506i*; 5, *D6S0129i;* 6, *D6S0350i*; 7, *D6S2707*; 8, *D6S2837*; 9, *D6S2838*; 10, *D6S265*; 11, *D6S1103i*; 12, *D6S0014i*; 13, *D6S2840*; 14, *D6S2799*; 15, *D6S2956*; 16, *D6S2825*; 17, *D6S2938*; 18, *D6S2930*; 19, *D6S0032i*; 20, *MICA-TM*. (*B*) Allele differences between the Japanese BD and control groups in the HLA genes and MS used for association analysis. (*C*) Allele differences between the Japanese *HLA-B*5101* non-carrier BD and control groups in the HLA genes and MS used for association analysis. The vertical and horizontal axes are the corrected *P* (*Pc*) value and the location of genes and MS in the studied 2.0-MB region, respectively. Black and white circles denote genes and MS, respectively. The red line shows the significance threshold (i.e. *P*c = 0.05).

Fig. 3. Linkage disequilibrium (LD) values between each MS/gene in Behçet's disease patients and controls. LD measures are presented in *D'*, a standardized measure that ranges from 0 to 1. *D'* in patients and controls are shown at the top right and the bottom left, respectively. MS and genes are indicated by the following symbols: 1, *D6S0016i*; 2, *D6S2770*; 3, *D6S2910*; 4, *D6S0506i*; 5, *D6S0129i*; 6, *D6S0350i*; 7, *D6S2707*; 8, *D6S2837*; 9, *D6S2838*; 10, *D6S265*; 11, *D6S1103i*; 12, *D6S0014i*; 13, *D6S2840*; 14, *D6S2799*; 15, *D6S2956*; 16, *D6S2825*; 17, *D6S2938*; 18, *D6S2930*; 19, *D6S0032i*; 20, *MICA-TM;*.A, *HLA-A*; B, *HLA-B*; E, *HLA-E*; F, *HLA-F*; G, *HLA-G*.

Supplemental Fig. 1. Genomewide association study of Behçet's disease with 23,465 microsatellite markers. The figure shows *P* values by Fisher's exact test based on 2 x 2 contingency tables or on 2 x m contingency tables in the third screening. Circles indicate 147 markers showing a significant association in all three rounds of pooled DNA screenings. Red circles indicate six markers that showed a significant association in individual DNA screenings.

Table 1. Six positive microsatellite markers in the pooled DNA screening and in the individual DNA screening.

Marker	Chromosome	*P* value					Nearest gene
		Pooled DNA (2 x 2)			Individual DNA		
		1st	2nd	3rd	2 x 2	2 x m	
D3S0186i	3p12	＜0.0001	＜0.0001	＜0.0001	0.029	0.15	*ROBO1*
D6S0014i	6p21.3	0.002	0.0003	0.003	＜0.0001	＜0.0001	*FLJ45422*
D6S0032i	6p21.3	＜0.0001	0.0002	0.0005	＜0.0001	＜0.0001	*HLA-B*
536G12A	6q25.1	＜0.0001	＜0.0001	＜0.0001	0.031	0.111	*PPIL4*
D12S0645i	12p12.1	＜0.0001	＜0.0001	＜0.0001	0.024	0.364	*SOX5*
D22S0104i	22q11.22	＜0.0001	＜0.0001	0.006	0.019	0.125	*IGL@*

Table 2. Haplotype frequencies of *HLA-A*, *-F*, and *-G* in patients with Behçet's disease (BD) and controls.

Haplotype	Frequency		OR (95%CI)	*P*	*P*c
	BD	Controls			
All Subjects (BD : Controls = 300 : 300)					
*A*0201-F*010101-G*010101*	0.096	0.086	1.12 (0.75-1.65)		
*A*0206-F*010101-G*010101*	0.071	0.078	0.91 (0.59-1.39)		
*A*1101-F*010103-G*010401*	0.005	0.020	0.23 (0.06-0.86)	0.034	NS
*A*2402-F*010102-G*010401*	0.067	0.133	0.47 (0.31-0.69)	0.00016	0.0086
*A*2402-F*010103-G*010401*	0.254	0.243	1.06 (0.82-1.38)		
*A*2601-F*010101-G*010102*	0.130	0.070	1.99 (1.34-2.96)	0.00051	0.028
*A*2602-F*010101-G*010102*	0.030	0.015	2.04 (0.91-4.58)		
*A*2603-F*010101-G*010102*	0.033	0.018	1.82 (0.86-3.85)		
*A*2605-F*010101-G*010102*	0.003	0.000	-		
*A*3101-F*010103-G*010102*	0.107	0.064	1.75 (1.16-2.66)	0.0071	NS
*A*3101-F*010103-G*0103*	0.010	0.000	-	0.015	NS
*A*3303-F*010102-G*010401*	0.025	0.072	0.33 (0.18-0.61)	0.00022	0.012
*A*26-F*010101-G*010102*	0.197	0.103	2.13 (1.53-2.97)	5.34×10^{-6}	0.00028
*HLA-B*5101* non-carriers (BD : Controls = 136 : 255)					
*A*0201-F*010101-G*010101*	0.104	0.085	1.25 (0.76-2.07)		
*A*0201-F*010103-G*010101*	0.026	0.006	4.48 (1.15-17.47)	0.038	NS
*A*0206-F*010101-G*010101*	0.063	0.075	0.83 (0.46-1.50)		
*A*2402-F*010102-G*010401*	0.070	0.146	0.44 (0.26-0.75)	0.0017	NS
*A*2402-F*010103-G*010401*	0.256	0.244	1.07 (0.76-1.50)		
*A*2601-F*010101-G*010102*	0.174	0.073	2.68 (1.69-4.25)	2.89×10^{-5}	0.0013
*A*2602-F*010101-G*010102*	0.048	0.014	3.62 (1.43-9.19)	0.0071	NS
*A*2603-F*010101-G*010102*	0.033	0.014	2.47 (0.91-6.70)		
*A*3303-F*010102-G*010401*	0.030	0.083	0.34 (0.16-0.73)	0.0033	NS
*A*26-F*010101-G*010102*	0.256	0.100	3.08 (2.07-4.58)	2.30×10^{-8}	9.88×10^{-7}

Only haplotypes that reached frequencies >5% in BD patients or controls or that may have an effect on the development of BD are listed.

OR: odds ratio; CI: confidence interval; *P*c: corrected *P* value; NS: not significant.

Table 3. Allele and phenotype frequencies of *HLA-A*26* subtypes in all subjects and in *HLA-B*5101* non-carriers.

Allele/ phenotype	All subjects					*HLA-B*5101* non-carriers				
	No. (frequency)		OR (95%CI)	*P*	*P*c	No. (frequency)		OR (95%CI)	*P*	*P*c
	BD	Controls				BD	Controls			
Allele	n=600	n=600				n=272	n=510			
*A*2601*	79 (0.132)	44 (0.073)	1.92 (1.31-2.83)	0.00084	0.016	48 (0.178)	39 (0.076)	2.61 (1.66-4.10)	2.84×10^{-5}	0.00051
*A*2602*	18 (0.030)	9 (0.015)				13 (0.048)	7 (0.014)	3.63 (1.43-9.22)	0.0051	NS
*A*2603*	21 (0.035)	12 (0.020)				9 (0.033)	8 (0.016)			
*A*2605*	2 (0.003)	0 (0.000)				0 (0.000)	0 (0.0)			
*A*26*	120 (0.201)	65 (0.108)	2.07 (1.49-2.86)	9.99×10^{-6}	7.99×10^{-5}	70 (0.259)	54 (0.106)	2.96 (2.00-4.37)	4.54×10^{-8}	3.63×10^{-7}
Phenotype	n=300	n=300				n=136	n=255			
*A*2601*	77 (0.257)	42 (0.140)	2.12 (1.40-3.22)	0.00047	0.0089	46 (0.338)	37 (0.145)	3.01 (1.83-4.95)	1.21×10^{-5}	0.00022
*A*2602*	18 (0.060)	9 (0.030)				13 (0.096)	7 (0.027)	3.74 (1.46-9.62)	0.0048	NS
*A*2603*	21 (0.070)	12 (0.040)				9 (0.066)	8 (0.031)			
*A*2605*	2 (0.007)	0 (0.000)				0 (0.000)	0 (0.000)			
*A*26*	114 (0.380)	59 (0.197)	2.50 (1.73-3.62)	9.76×10^{-7}	7.81×10^{-6}	66 (0.485)	50 (0.196)	3.86 (2.45-6.10)	4.00×10^{-9}	3.20×10^{-8}

BD: Behçet's disease; OR: odds ratio; CI: confidence interval; *P*c: corrected *P* value; NS: not significant.

Materials and Methods

Patients and healthy controls

A total of 300 unrelated BD patients and 300 unrelated healthy controls, all of Japanese descent, were enrolled in this study. The diagnosis of BD was established according to standard criteria (1) proposed by the Japan Behçet's Disease Research Committee at the Yokohama City University, Hokkaido University, Kurume University, Yuasa Eye Clinic, and Fujioka Eye Hospital. BD was classified as complete-type or incomplete-type, according to these criteria. All control participants recruited from Tokai University were healthy volunteers unrelated to each other or to patients. The patients and controls were equally distributed in groups of 100 versus 100 in each and all of the three pooled DNA typing stages (Supplemental Table 1). After pooled DNA typing, individual DNA typing was performed on the same sample set. The study methodology complied with the guidelines of the Declaration of Helsinki. The study details were explained to all patients and controls before obtaining their consent to genetic screening. Genomic DNA was extracted from peripheral blood lymphocytes using the QIAamp DNA Blood Maxi Kit (Qiagen) under standardized conditions. Following extraction, DNA degradation and RNA contamination were assessed on a 0.8% agarose gel electrophoresis.

GWAS

Pooled DNA construction

The protocol of Collins et al. (2) - upon slight modifications (3) - was used to perform MS typing on pooled DNA (2). To confirm the suitability of pool DNA, as an time and cost saving alternative to individual DNA typing, we compared the allelic distribution of 10 MS markers typed using pooled vs. individual DNA (n=100). Allelic distribution was assessed using Fisher's exact test. No significant difference in allele frequencies were found between pooled and individual DNAs and the technique was therefore validated for large-scale use.

Genomewide MS genotyping

All MS markers and the genotyping methods for MS analysis used in this study have been previously described (4). PCR primers were designed for having a homogenous annealing temperature of 57°C. Forward primers were labeled at the 5' end with fluorescent reagent 6-FAM or HEX (Applied Biosystems). PCR on pooled DNA was carried out in 20 µl reactions containing 24 ng of pooled DNA, 0.5 units of AmpliTaq Gold DNA polymerase (Applied Biosystems), 2 µl of 10X reaction buffer

(100 mM Tris-HCl, pH 8.3, 50 0mM KCl, 15 mM $MgCl_2$), 2 µl of dNTP (2.5 mM each), and 20 pmol of forward and reverse primers. The amplification conditions consisted of an initial denaturation at 96°C for 9 min, annealing at 57°C for 1 min, and extension at 72°C for 1 min, followed by 40 cycles of denaturation at 96°C for 45 sec, annealing at 57°C for 45 sec, and extension at 72°C for 1 min, using a thermal cycler (GeneAmp PCR system 9700, Applied Biosystems). PCR on individual DNA was carried out in 20 µl reactions containing 1 ng of genomic DNA, 0.5 units of AmpliTaq Gold DNA polymerase, 2 µl of 10X reaction buffer, 2 µl of dNTP (2.5 mM each), and 20 pmol of each primer. The amplification conditions were essentially the same as described above except for a final extension of 5 min at 72°C after 40 cycles. The amplified products were denatured in formamide (Hi-Di, Applied Biosystems) at 95°C for 3 min and separated on a 3700 DNA analyzer (Applied Biosystems). Various data about the markers, such as the amplified peak positions and heights, were manually extracted by the PickPeak and MultiPeaks programs, developed by Applied Biosystems Japan, from the multipeak pattern in the chromatogram ABI .fas files.

Each stage compared a pool of 100 patient DNAs to that of 100 control DNAs. In the first screen, we used the totality of 23,465 markers. Among them, markers showing a significant association ($P < 0.05$) were subjected to the second screen. The markers showing a significant association in the second screen were subjected to the third screen. The markers remained positive in all three stages were assessed whether they had similar peak patterns among the first, second, and third case and control pools. The markers had similar peak patterns among each pool were subjected to the individual DNA screen and were genotyped in the totality of 600 individuals (the same 300 cases and 300 controls mentioned above).

Marker information

Our criteria for selection of MS markers were di-nucleotide repeats with >10 repeats; tri-, tetra-, and penta-nucleotide repeats with >5 repeats; and polymorphic MS markers with heterozygosity of >30%, but not those with heterozygosity of >85% to eliminate unstable and highly mutated MS markers. These markers cover the euchromatic area (~90%) of the human genome (3 Gb) at average intervals of 115.1 kb (3×10^9 kb × 0.90 ÷ 23,465 = 115.1 kb).

Statistical analysis

Statistical significance of differences was assessed using Fisher's exact test for 2×2 and 2×m contingency tables. The 2×2 and 2×m contingency tables for each

individual allele and 2×m contingency tables for each individual locus were used, where m refers to the number of marker alleles detected in the population. For the exact probability test, the Markov chain/Monte Carlo simulation method was employed to execute the Fisher's exact test for the 2×m contingency table. The significance level was set at $P < 0.05$ for all analyses. The strength of the association was estimated by odds ratio of risk and 95% confidence intervals. No correction for multiple comparisons was performed to minimize false-negative inferences.

HLA class I analysis

HLA genotyping and haplotype analysis

Twenty MS markers (*D6S0016i*, *D6S2770*, *D6S2910*, *D6S0506i*, *D6S0129i*, *D6S0350i*, *D6S2707*, *D6S2837*, *D6S2838*, *D6S265*, *D6S1103i*, *D6S0014i*, *D6S2840*, *D6S2799*, *D6S2956*, *D6S2825*, *D6S2938*, *D6S2930*, *D6S0032i*, and *MICA-TM*) that cover the entire HLA class I region were used in this study. The PCR reaction mixture contained 1 ng of genomic DNA, 2 μl of 10× PCR buffer, 2μl of dNTPs (2 mM each), 2μl of each primer (10 μM), and 0.5 U of AmpliTaq Gold (Applied Biosystems) in a total volume of 20 μl. The forward primer was labeled at the 5' end with 6-FAM, HEX, or TET (Applied Biosystems). After initial denaturation for 9 min at 95 °C, annealing for 1 min at the appropriate temperature (53 °C: *D6S2956*; 54 °C: *D6S0016i*, *D6S2838*, *D6S265*; 56 °C: *D6S2837*; 57 °C: *D6S0506i*, *D6S0129i*, *D6S0350i*, *D6S1103i*, *D6S2840*, *D6S2825*, *D6S2938*, *D6S2930*, *D6S0032i*; 59 °C: *D6S2910*, *D6S2799*; 60 °C: *D6S0014i*; 61 °C: *D6S2770*; 63 °C: *D6S2707*), and extension for 1 min at 72 °C, amplification was carried out in a GeneAmp PCR system 9600 (Applied Biosystems) for 40 cycles consisting of 45 s at 96 °C, 45 s at each annealing temperature, and 1 min at 72 °C, with a final extension of 5 min at 72 °C. The amplification products were analyzed on an ABI3730 DNA analyzer (Applied Biosystems) following standard protocols. Genotyping of *HLA-A* and *HLA-B* genes was performed using the PCR-sequence-specific oligonucleotide probes (SSOP)-Luminex method (5). The genotyping of *HLA-E*, *HLA-F*, and *HLA-G* genes was performed by direct DNA sequencing of exons 2 and 3 (*HLA-E*), 1, 2, 4, and 7 (*HLA-F*) and finally 2, 3, and 4 as well as by the status of a 14-bp deletion polymorphism in the untranslated region of exon 8 (*HLA-G*). PCR/sequencing primers for *HLA-E* and *HLA-G* were as described previously (6-8); primers for *HLA-F* typing were designed in this study (Supplemental Table 2). PCR amplification of *HLA-E*, to obtain a 812-bp fragment for DNA sequencing was carried out using HLA-E-ex2F and HLA-E-ex3R. PCR amplification of *HLA-F* to obtain a 456-bp fragment (including SNPs in exons 1 and 2) and a 1275-bp

fragment (including SNPs in exons 4 and 7) for DNA sequencing was carried out using HLA-F-ex1F/HLA-F-ex2R and HLA-F-ex4F/HLA-F-ex7R, respectively. PCR amplification of *HLA-G* to obtain a 1681-bp fragment (including SNPs in exons 2, 3, and 4) and a 210/224-bp fragment (including the 14-bp deletion polymorphism in exon 8) was carried out using HLA-G-ex2F/HLA-G-ex4R and HLA-G-ex8F/HLA-G-ex8R, respectively. Except for exon 8 of *HLA-G*, each 25 μl PCR reaction mixture contained 36 ng of genomic DNA, 2.5 μl 10 x buffer, 2μl dNTPs (2.5 mM each), 0.3 μl of each primer (10 pmol/μl), and 0.5 U AmpliTaq Gold. The PCR conditions were as follows: initial denaturation for 9 min at 95 °C, annealing for 1 min at the appropriate temperature (54 °C: 456-bp fragment of *HLA-F* and 1681-bp fragment of *HLA-G*, 63 °C: 812-bp fragment of *HLA-E*, 64 °C: 1275-bp fragment of *HLA-F*), and extension for 3 min at 72 °C, followed immediately by 35 cycles of denaturation for 30 s at 96 °C, 30 s at the appropriate annealing temperature, and 3 min at 72 °C, with a final extension for 5 min at 72 °C. The PCR products were subjected to nucleotide sequencing using the ABI PRISM BigDye terminator cycle sequencing kit (Applied Biosystems) and analyzed on an automated ABI3730 DNA analyzer. All primers except for HLA-G-ex8F/R were used for DNA sequencing. The PCR reaction mixture for the 14-bp deletion polymorphism in exon 8 of *HLA-G* contained 1 ng genomic DNA, 1 μl 10x buffer, 1μl dNTPs (2 mM each), 1μl of each primer (10 μM), and 0.25 U AmpliTaq Gold in a total volume of 10 μl. The PCR conditions were as follows: initial denaturation for 9 min at 95 °C, annealing for 1 min at 64 °C, and extension for 1 min at 72 °C, followed immediately by 35 cycles of denaturation for 30 s at 96 °C, 30 s at 64 °C, and 30 s at 72 °C, with a final extension for 5 min at 72 °C. The PCR products of exon 8 were analyzed by agarose gel electrophoresis (4% agarose 21; Nippon Gene).

Statistical analysis

Gene (allele) frequencies were estimated by direct counting. The significance of allele frequency differences between the patient and control groups was tested by the χ^2 method with the continuity correction and Fisher's exact probability test. All *P* values were derived from a two-sided test. Furthermore, the *P* value was corrected by multiplying by the number of observed alleles at the locus (corrected *P* [*Pc*] value). *Pc* values <0.05 were considered statistically significant. Haplotype frequencies and LD in the multi-locus analyses were calculated using PyPop (9). Haplotype frequencies were estimated using the iterative Expectation-Maximization algorithm. LD was measured using Hedrick's multiallelic *D'* statistic (10). *D'* weights the contribution to the LD of specific allele pairs by the product of their allele frequencies. The measure was

normalized to fall between zero and one, with higher values indicating stronger LD.

References for Supplemental Methods

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Supplemental Table 1. Characteristics of the participants in the study.

	The three stages of pooled DNA screening						Total	
	1st		2nd		3rd			
	BD	controls	BD	controls	BD	controls	BD	controls
Number of participants	100	100	100	100	100	100	300	300
Mean age at onset	33.9±10.1		35.2±9.9		33.6±11.3		34.2±10.5	
Male/female	57/43	57/43	58/42	58/42	58/42	58/42	173/127	173/127
Classification								
Complete BD	46		45		45		136	
Incomplete BD	54		55		55		164	
Major symptoms								
Oral ulcer	100		97		98		295	
Skin lesion	87		87		87		261	
Ocular lesion	89		88		91		268	
Genital ulcer	61		61		62		184	
Minor symptoms								
Arthritis	42		37		33		112	
Epididymitis	5		8		7		20	
GI lesion	16		10		10		36	
Vascular lesion	1		4		3		8	
CNS lesion	8		9		9		26	
*HLA-B*5101* carriers	56	15	55	15	53	15	164	45

BD: Behçet's disease; GI: Gastrointestinal; CNS: Central nervous system.

Supplemental Table 2. Primers used in the amplification and sequencing of *HLA-E*, *HLA-F*, and *HLA-G*.

gene	primer name	primer sequence	reference
HLA-E	ex2F	5' - TGAAGTATTTCCACACTTCCGTGTCCCGGCC - 3'	(18)
	ex2R	5' - AATCTGGGACCCGAAGATTCGAGGGGACCC - 3'	(18)
	ex3F	5' - CAAAATGCCCACAGGGTGGTGGCGACGGG - 3'	(18)
	ex3R	5' - GGAGATGGGAGAGTAGCCCTGTGGACCCTC - 3'	(18)
HLA-F	ex1F	5' - GCAGTTCCCAGGTTCTAAAGTCC - 3'	this study
	ex1R	5' - CAGAGGCCGTTTCTCTCTGG - 3'	this study
	ex2F	5' - CACTCCTTGAGGTATTTCAGCACC - 3'	this study
	ex2R	5' - GTGGTCCACTCCCAATACTGC - 3'	this study
	ex4F	5' - GACACAGAGCTTGTGGAGACC - 3'	this study
	ex4R	5' - CTGACCCTGCTGAAGAGCTC - 3'	this study
	ex7F	5' - CCACAGTTCCTCTAGCCACATC - 3'	this study
	ex7R	5' - GCTTCTCAGTCCCACACAAG - 3'	this study
HLA-G	ex2F	5' - TCCATGAGGTATTTCAGCGC - 3'	(19)
	ex2R	5' - TCCGTGGGGCATGGAGGT - 3'	(20)
	ex3F	5' - CCCAGACCCTCTACCTGGGAGA - 3'	(20)
	ex3R	5' - CTCTCCTTGTGCTAGGCCAGGCTGAGAGG - 3'	(20)
	ex4F	5' - CCATGAGAGATGCAAAGTGCT - 3'	(20)
	ex4R	5' - TGCTTTCCCTAACAGACATGAT - 3'	(20)
	ex8F	5' - GTGATGGGCTGTTTAAAGTGTCACC - 3'	(20)
	ex8R	5' - GGAAGGAATGCAGTTCAGCATGA - 3'	(20)

Supplemental Table 3. Summary of the three-phased genome screens using pooled DNA.

Chromosome	1st screening		2nd screening		3rd screening	
	No. analyzed MS	No. positive MS	No. analyzed MS	No. positive MS	No. analyzed MS	No. positive MS
1	1895	192	192	50	50	20
2	2053	153	153	41	41	10
3	1650	128	128	30	30	11
4	1554	127	127	26	26	11
5	1500	101	101	17	17	5
6	1369	127	127	24	24	13
7	1370	110	110	22	22	5
8	1103	95	95	19	19	7
9	956	68	68	15	15	3
10	1097	95	95	12	12	7
11	1076	68	68	17	17	11
12	1128	100	100	19	19	9
13	806	62	62	13	13	3
14	709	56	56	12	12	8
15	610	52	52	9	9	3
16	631	55	55	8	8	3
17	626	72	72	4	4	3
18	649	47	47	12	12	2
19	454	64	64	13	13	2
20	501	46	46	10	10	2
21	296	25	25	4	4	2
22	251	29	29	7	7	4
X	1105	46	46	5	5	3
Y	76	1	1	1	1	0
total	23465	1919	1919	390	390	147

MS: microsatellite

Supplemental Table 4. Allele frequencies of *HLA-A, -B, -E, -F*, and *-G* in all subjects and in *HLA-B*5101* non-carriers.

Allele	All subjects					HLA-B*5101 non-carriers				
	No. (frequency)		OR (95%CI)	*P*	*Pc*	No. (frequency)		OR (95%CI)	*P*	*Pc*
	BD (n=600)	Cont. (n=600)				BD (n=272)	Cont. (n=510)			
HLA-A										
*A*0101*	4 (0.007)	1 (0.002)				2 (0.007)	1 (0.002)			
*A*0201*	68 (0.113)	61 (0.102)				35 (0.130)	50 (0.098)			
*A*0203*	0 (0.000)	2 (0.003)				0 (0.000)	2 (0.004)			
*A*0206*	48 (0.080)	52 (0.087)				19 (0.070)	43 (0.084)			
*A*0207*	20 (0.033)	12 (0.020)				10 (0.037)	11 (0.022)			
*A*0210*	1 (0.002)	1 (0.002)				0 (0.000)	1 (0.002)			
*A*0301*	1 (0.002)	4 (0.007)				1 (0.004)	3 (0.006)			
*A*0302*	0 (0.000)	1 (0.002)				0 (0.000)	1 (0.002)			
*A*1101*	39 (0.065)	66 (0.110)	0.56 (0.37-0.85)	0.0077	NS	22 (0.081)	61 (0.120)			
*A*1102*	0 (0.000)	2 (0.003)				0 (0.000)	2 (0.004)			
*A*2401*	3 (0.005)	0 (0.000)				1 (0.004)	0 (0.000)			
*A*2402*	207 (0.346)	239 (0.398)				91 (0.337)	203 (0.398)			
*A*2420*	1 (0.002)	4 (0.007)				0 (0.000)	4 (0.008)			
*A*2601*	79 (0.132)	44 (0.073)	1.92 (1.31-2.83)	0.00084	0.016	48 (0.178)	39 (0.076)	2.61 (1.66-4.10)	$2.84x10^{-5}$	0.00051
*A*2602*	18 (0.030)	9 (0.015)				13 (0.048)	7 (0.014)	3.63 (1.43-9.22)	0.0051	NS
*A*2603*	21 (0.035)	12 (0.020)				9 (0.033)	8 (0.016)			
*A*2605*	2 (0.003)	0 (0.000)				0 (0.000)	0 (0.000)			
*A*3101*	70 (0.117)	45 (0.075)	1.64 (1.10-2.42)	0.014	NS	11 (0.041)	30 (0.059)			
*A*3303*	16 (0.027)	45 (0.075)	0.34 (0.19-0.61)	0.00018	0.0035	8 (0.030)	44 (0.086)	0.32 (0.15-0.70)	0.0038	NS
HLA-B										
*B*3501*	46 (0.077)	38 (0.063)				37 (0.136)	33 (0.065)	2.28 (1.39-3.73)	0.0014	NS
*B*4403*	20 (0.033)	43 (0.072)	0.45 (0.26-0.77)	0.0041	NS	9 (0.033)	42 (0.082)	0.38 (0.18-0.80)	0.0091	NS
*B*5101*	188 (0.313)	46 (0.077)	5.50 (3.89-7.77)	$4.29x10^{-26}$	$1.63x10^{-24}$	0 (0.000)	0 (0.000)			
*B*5201*	40 (0.067)	84 (0.140)	0.44 (0.30-0.65)	$3.89x10^{-5}$	0.0015	23 (0.085)	78 (0.153)	0.51 (0.31-0.84)	0.0070	NS
*B*5401*	17 (0.028)	46 (0.077)	0.35 (0.20-0.62)	0.00023	0.0088	12 (0.044)	43 (0.084)	0.50 (0.26-0.97)	0.040	NS
*B*5601*	2 (0.003)	11 (0.018)	0.18 (0.04-0.81)	0.022	NS	1 (0.004)	10 (0.020)			
HLA-E										
*E*010101*	172 (0.287)	176 (0.293)				94 (0.346)	157 (0.308)			
*E*010301*	227 (0.378)	253 (0.422)				116 (0.426)	217 (0.425)			
*E*010302*	201 (0.335)	171 (0.285)				62 (0.228)	136 (0.267)			
HLA-F										
*F*010101*	278 (0.463)	226 (0.377)	1.43 (1.13-1.80)	0.0028	0.011	141 (0.518)	186 (0.365)	1.87 (1.39-2.53)	$3.88x10^{-5}$	0.00016
*F*010102*	74 (0.123)	154 (0.257)	0.41 (0.30-0.55)	$4.58x10^{-9}$	$1.83x10^{-8}$	37 (0.136)	144 (0.282)	0.40 (0.27-0.60)	$5.22x10^{-6}$	$2.09x10^{-5}$
*F*010103*	247 (0.412)	214 (0.357)				93 (0.342)	175 (0.343)			
*F*0103*	1 (0.002)	6 (0.010)				1 (0.004)	5 (0.010)			
HLA-G										
*G*010101*	153 (0.255)	151 (0.252)				73 (0.268)	130 (0.255)			
*G*010102*	185 (0.308)	106 (0.177)	2.08 (1.58-2.73)	$1.31x10^{-7}$	$1.44x10^{-6}$	80 (0.294)	80 (0.157)	2.24 (1.57-3.19)	$1.06x10^{-5}$	0.00012
*G*010103*	16 (0.027)	29 (0.048)				9 (0.033)	26 (0.051)			
*G*010105*	1 (0.002)	1 (0.002)				1 (0.004)	1 (0.002)			
*G*010107*	0 (0.000)	1 (0.002)				0 (0.000)	1 (0.002)			
*G*010108*	13 (0.022)	9 (0.015)				3 (0.011)	1 (0.002)			
*G*0103*	6 (0.010)	0 (0.000)	-	0.031	NS	1 (0.004)	0 (0.000)			
*G*010401*	220 (0.367)	292 (0.487)	0.61 (0.48-0.77)	$3.34x10^{-5}$	0.00037	101 (0.371)	261 (0.512)	0.56 (0.42-0.76)	0.00022	0.0024
*G*010403*	2 (0.003)	9 (0.015)				2 (0.007)	8 (0.016)			
*G*0106*	4 (0.007)	1 (0.002)				2 (0.007)	1 (0.002)			
novel allele	0 (0.000)	1 (0.002)				0 (0.000)	1 (0.002)			

BD: Behçet's disease; Cont.: controls; OR: odds ratio; CI: confidence interval; *Pc:* corrected *P* value; NS: not significant. In HLA-B, only alleles associated with BD are listed.

Figure 1

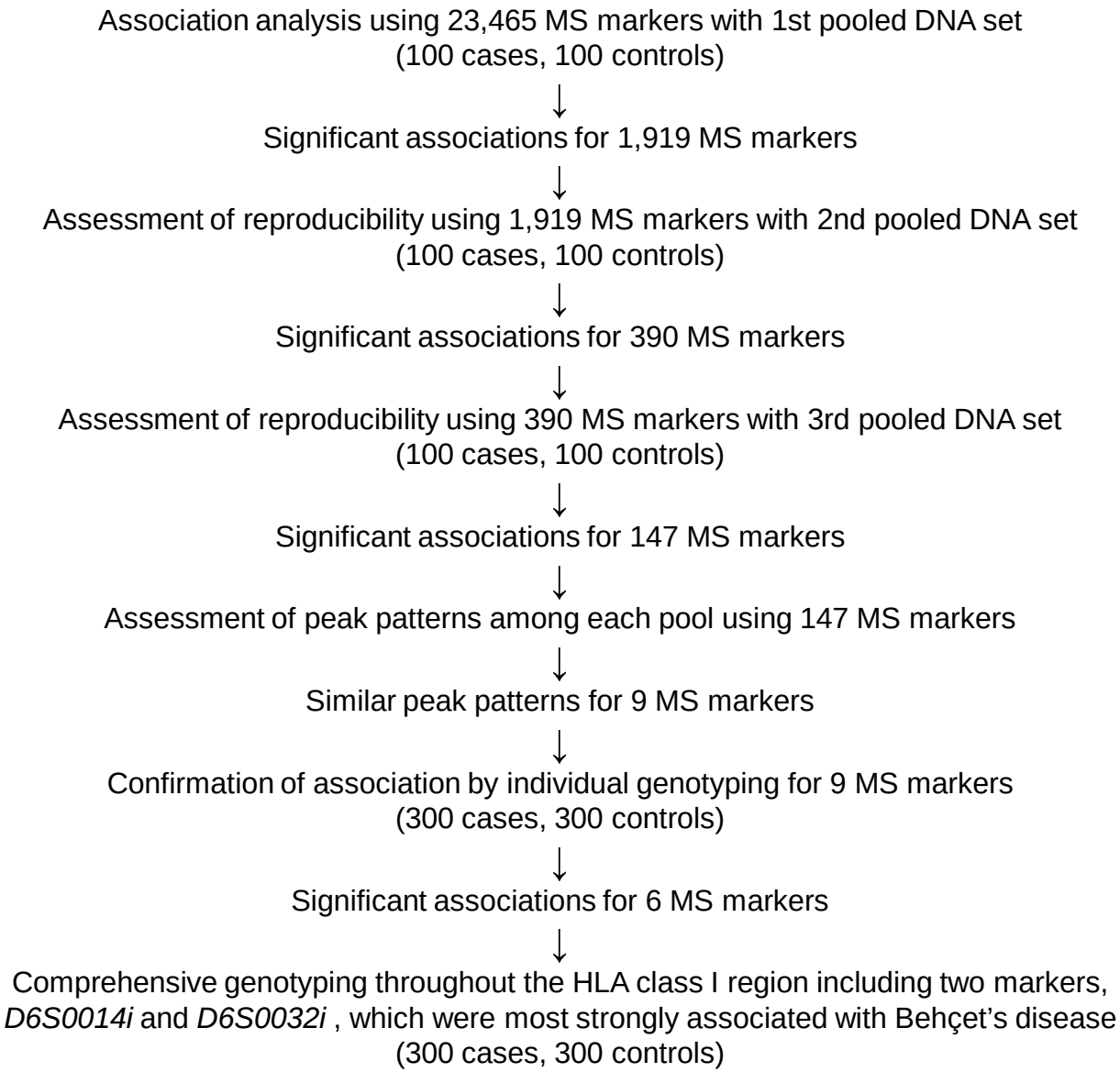


Figure 2

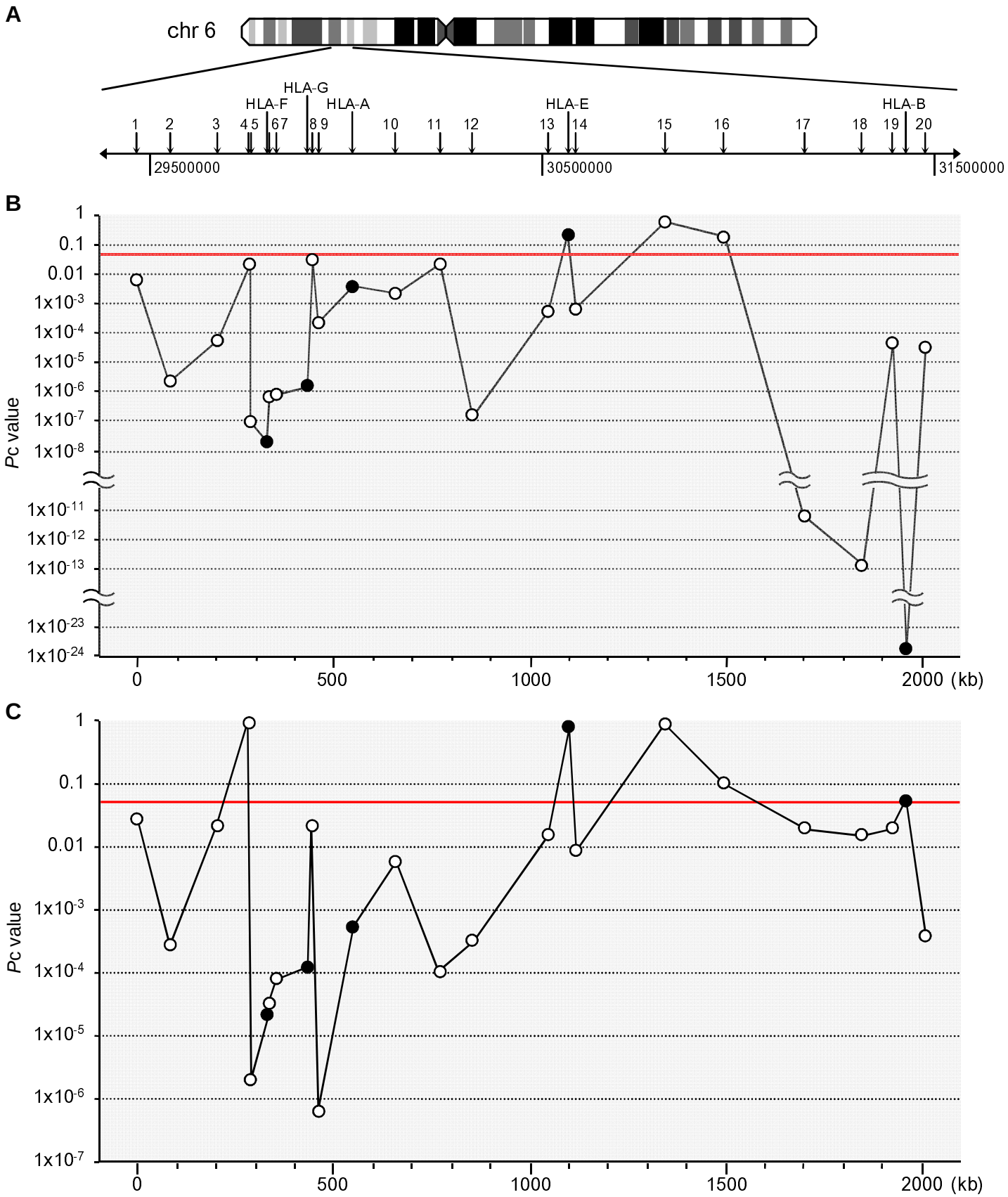
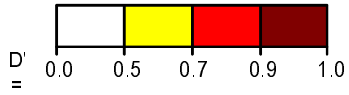


Figure 3

D'	1	2	3	4	5	F	6	7	G	8	9	A	10	11	12	13	E	14	15	16	17	18	19	B	20
1	-	0.704	0.533	0.458	0.420	0.392	0.377	0.520	0.330	0.204	0.148	0.429	0.288	0.274	0.298	0.378	0.200	0.158	0.163	0.280	0.309	0.379	0.307	0.491	0.170
2	0.719	-	0.648	0.950	0.911	0.890	0.915	0.920	0.900	0.831	0.982	0.682	0.769	0.530	0.970	0.664	0.876	0.476	0.602	0.709	0.767	0.716	0.538	0.813	0.816
3	0.527	0.605	-	0.545	0.642	0.568	0.439	0.744	0.357	0.237	0.584	0.557	0.620	0.460	0.421	0.434	0.531	0.704	0.416	0.413	0.350	0.387	0.359	0.504	0.249
4	0.549	0.958	0.507	-	0.897	0.789	0.770	0.735	0.514	0.439	0.975	0.672	0.484	0.351	0.284	0.469	0.412	0.376	0.226	0.296	0.320	0.364	0.251	0.437	0.205
5	0.537	0.976	0.581	0.915	-	0.877	0.809	0.750	0.719	0.524	0.971	0.774	0.640	0.422	0.507	0.537	0.388	0.401	0.312	0.356	0.394	0.364	0.308	0.459	0.229
F	0.462	0.966	0.600	0.743	0.876	-	0.941	0.830	0.662	0.607	0.972	0.789	0.696	0.393	0.215	0.484	0.345	0.147	0.236	0.246	0.277	0.355	0.285	0.411	0.233
6	0.458	0.970	0.526	0.736	0.850	0.905	-	0.806	0.648	0.590	0.828	0.776	0.680	0.369	0.328	0.501	0.365	0.216	0.291	0.308	0.301	0.382	0.273	0.418	0.262
7	0.554	0.969	0.771	0.774	0.828	0.882	0.865	-	0.817	0.648	0.783	0.774	0.696	0.391	0.538	0.536	0.440	0.373	0.353	0.362	0.411	0.415	0.324	0.501	0.375
G	0.315	0.958	0.355	0.439	0.751	0.746	0.751	0.845	-	0.751	0.987	0.940	0.730	0.349	0.557	0.464	0.167	0.317	0.196	0.321	0.246	0.331	0.173	0.396	0.207
8	0.308	0.955	0.416	0.377	0.608	0.693	0.674	0.730	0.822	-	0.575	0.784	0.701	0.373	0.339	0.419	0.175	0.146	0.207	0.244	0.288	0.334	0.205	0.384	0.220
9	0.256	1.000	0.707	0.920	0.840	0.957	0.815	0.708	0.856	0.680	-	0.990	0.725	0.584	0.654	0.575	0.501	0.547	0.273	0.242	0.485	0.308	0.172	0.370	0.301
A	0.456	0.780	0.600	0.594	0.729	0.718	0.769	0.769	0.902	0.844	0.988	-	0.867	0.458	0.604	0.565	0.342	0.350	0.322	0.372	0.388	0.388	0.312	0.466	0.312
10	0.389	0.763	0.618	0.421	0.637	0.665	0.680	0.680	0.754	0.738	0.741	0.860	-	0.457	0.436	0.543	0.181	0.229	0.210	0.371	0.288	0.356	0.337	0.412	0.285
11	0.299	0.528	0.480	0.366	0.427	0.443	0.437	0.396	0.392	0.403	0.581	0.469	0.464	-	0.333	0.503	0.375	0.382	0.254	0.271	0.335	0.329	0.280	0.371	0.191
12	0.421	0.939	0.610	0.227	0.538	0.433	0.424	0.511	0.491	0.427	0.606	0.532	0.481	0.364	-	0.518	0.321	0.295	0.176	0.219	0.267	0.289	0.203	0.404	0.086
13	0.440	0.783	0.594	0.478	0.534	0.418	0.475	0.554	0.391	0.377	0.579	0.526	0.527	0.442	0.449	-	0.545	0.548	0.390	0.459	0.398	0.457	0.360	0.552	0.385
E	0.302	0.896	0.340	0.276	0.444	0.332	0.353	0.470	0.337	0.321	0.216	0.371	0.249	0.348	0.307	0.587	-	0.584	0.279	0.270	0.346	0.351	0.265	0.482	0.196
14	0.284	0.474	0.648	0.272	0.342	0.197	0.216	0.322	0.251	0.198	0.423	0.338	0.243	0.257	0.269	0.560	0.608	-	0.328	0.423	0.179	0.279	0.176	0.439	0.129
15	0.296	0.839	0.299	0.301	0.346	0.332	0.382	0.437	0.232	0.256	0.124	0.319	0.248	0.352	0.134	0.457	0.323	0.313	-	0.263	0.279	0.296	0.189	0.438	0.178
16	0.363	0.753	0.523	0.339	0.375	0.266	0.344	0.414	0.307	0.323	0.324	0.455	0.389	0.348	0.193	0.499	0.386	0.494	0.354	-	0.481	0.388	0.412	0.559	0.459
17	0.399	0.824	0.407	0.398	0.495	0.478	0.510	0.552	0.364	0.415	0.559	0.451	0.341	0.341	0.309	0.508	0.441	0.344	0.407	0.488	-	0.662	0.555	0.765	0.656
18	0.432	0.765	0.371	0.357	0.386	0.327	0.387	0.446	0.366	0.354	0.220	0.423	0.363	0.352	0.277	0.463	0.356	0.337	0.443	0.424	0.624	-	0.534	0.747	0.591
19	0.324	0.583	0.407	0.226	0.336	0.292	0.351	0.368	0.257	0.272	0.336	0.384	0.292	0.382	0.177	0.379	0.249	0.301	0.296	0.472	0.487	0.570	-	0.879	0.543
B	0.530	0.844	0.542	0.480	0.552	0.474	0.514	0.584	0.472	0.479	0.466	0.549	0.512	0.497	0.485	0.612	0.510	0.499	0.506	0.607	0.820	0.779	0.833	-	0.944
20	0.289	0.794	0.288	0.252	0.351	0.342	0.369	0.391	0.344	0.362	0.340	0.331	0.283	0.172	0.223	0.411	0.366	0.232	0.364	0.490	0.681	0.578	0.470	0.932	-



A Simple PPT slide

Full of interesting information

And humorous anecdotes

Genetics of Behçet's disease inside and outside the MHC

Akira Meguro, Hidetoshi Inoko, Masao Ota, Yoshihiko Katsuyama, Akira Oka, Eiichi Okada, Ryoji Yamakawa, Takenosuke Yuasa, Tatsuhiko Fujioka, Shigeaki Ohno, Seiamak Bahram and Nobuhisa Mizuki

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Notes