Association Analysis of the Polymorphism of Human Leukocyte Antigen-A, -B and -E Gene with Behcet’s Disease in Japanese Cohort Using Sequencing-Based Typing Method

Abstract
Behcet’s disease (BD) is a chronic inflammatory autoimmune disease and strongly associated with human leukocyte antigen (HLA) gene such as HLA-B and -A. To determine the association of the HLA-A, HLA-B and HLA-E alleles with BD in Japanese cohort as well as other cohorts, we performed sequencing-based typing method in 382 BD cases and healthy controls. Then, we analyzed the association of these alleles with BD. Our results indicated that HLA-B*51 is strongly associated with BD in Japanese cohort (Allelic model’s P value and OR are 4.59x10^-26 and 4.59, respectively). Dominant model’s P value and OR are 2.1x10^-24 and 5.32, respectively, while HLA-A*26 is also significantly associated with BD (Allelic model’s P value and OR are 5.3x10^-2 and 1.80, respectively. Dominant model’s P value and OR are 8.6x10^-2 and 2.06, respectively.). HLA-E*01:01 was not observed to have an significant association with BD. We also obtained the available data sets of Chinese and Japanese genome sequence from public data base and then, analyzed each Linkage disequilibrium (LD) structure using SNPs in HLA-A, -B and -E region. The pattern of LD including HLA-E was different between two cohorts. Collectively, we suggested that HLA-B*51 and HLA-A*26 are associated with BD in Japanese cohort as well as another cohort and that HLA-E*01:01 is not associated with BD. Our data suggest that LD structure of the region including HLA-E depends on a type of ethnic group even among Asian ethnic group.

Keywords
Behcet’s disease; Human leukocyte antigen (HLA)-A; HLA-B; HLA-E; Polymorphism; Association study

Abstract
Behcet’s disease (BD) is a chronic inflammatory autoimmune disease characterized by recurrent oral and genital ulcers, skin lesions and uveitis [1]. Helper 1 (Th1) associated cytokines such as interferon-gamma and interleukin-12 were enhanced in BD patients [2]. Indeed, many studies have revealed the dominance of Th1 in the development of BD [3]. Recently, it’s been thought that another T cell subsets, Th17 plays an important role in the pathogenesis and development of BD [4]. The incidence of BD is clearly high in the population who lives in from Middle East to Asia, along the “Old Silk Road”, and rare in the USA especially in African Americans [5,6].

The patient’s family members have a predisposition of BD including genetic and environmental factors. Indeed, the incidence of BD is clearly higher in those peoples than in general peoples [7]. Thus, epidemiological evidence indicates genetic factors contribute to the pathogenesis of BD. Several association studies have reported that human leukocyte antigen (HLA)-B*51 is strongly associated with BD among various ethnic groups including Japanese and Korean [8-11], while HLA-A*26 is also associated with BD [12,13]. Among Korean cohort, HLA-E*01:01 was shown to reduce the risk of BD [14]. Except for HLA, we identified TRIM39 gene as novel susceptible gene of BD independently of HLA-B*51 and -A*26 [15]. Recent Genome-wide association studies (GWAS) including our group identified that IL-23R-IL12RB2 and IL-10 as novel BD susceptible loci [16,17]. More recent study identified CCR1, STAT4 and KLRC4 [18]. These results support that Th1 and Th17 type inflammation have an important role in the pathogenesis and development of BD.

As well as in Korean cohort, whether or not HLA-E*01:01 is associated with BD in Japanese cohort has remained to be identified. Thus, in this study, we performed sequencing-based typing of HLA-A, -B and -E using large amounts of genome sample from Japanese BD patients and healthy subjects. And then, we analyzed the polymorphism of each HLA and validated its association with BD susceptibility. We also obtained the data sets of genomic sequence including HLA-A, -B and -E in Japanese and Chinese cohort from public database and then, analyzed the difference of LD structure between two cohorts.

Abbreviations
BD: Behcet’s Disease; LD: Linkage Disequilibrium; HLA: Human Leukocyte Antigen; GWAS: Genome-Wide Association Studies; QC: Quality Control; OR: Odds Ratio

Introduction
Behcet’s disease (BD) is a chronic inflammatory autoimmune disease characterized by recurrent oral and genital ulcers, skin lesions and uveitis [1]. Helper 1 (Th1) associated cytokines such as interferon-gamma and interleukin-12 were enhanced in BD patients [2]. Indeed, many studies have revealed the dominance of Th1 in the development of BD [3]. Recently, it’s been thought that another T cell subsets, Th17 plays an important role in the pathogenesis and development of BD [4]. The incidence of BD is clearly high in the population who lives in from Middle East to Asia, along the “Old Silk Road”, and rare in the USA especially in African Americans [5,6].

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Materials

Patients and controls

We used a cohort of 384 Japanese unrelated BD patients and ethnically matched 384 unrelated healthy controls. All of the BD patients were diagnosed according to standard criteria [19] by the Japan Behcet’s disease Research Committee at the Yokohama City University, Hokkaido University, Kurume University, Yuasa Eye Clinic and Fujioka Eye Hospital. These are included in our previous study [20] and [16]. The study methodology compiled with the guidelines of the Declaration of Helsinki. The study details were explained to all case and controls before obtaining their consent for genetic screening.

Methods

Quality control filtering

At first, quality control (QC) filtering of genotype data sets was performed to avoid false positive finding. We excluded two cases and two controls whose missing genotypes rates were more than 5%. After that, we could not exclude any SNP’s showing departure from Hardy-Weinberg equilibrium the data sets consisted of 382 BD cases and 382 healthy controls were available. The QC filtering was performed by PLINK [21].

Genotype analysis of the HLA variants

Genomic DNA was extracted from peripheral blood lymphocytes using QIAamp DNA Maxi Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. To determine haplotypes of each HLA gene, we did sequencing-based typing method using primers previously reported in [22-24]. Determined alleles were confirmed to be consistent with WHO Nomenclature 2006 ImmunoGeneTics/HLA Database (URL: http://www.ebi.ac.uk/ipd/immt/hla/).

Haplotype analysis

We obtained data sets of Han Chinese in Beijing (CHB) and Japanese of Tokyo (JPT) genome from International HapMap Project (URL: http://hapmap.ncbi.nlm.nih.gov) and then, visualized LD structure between two ethnic groups showing association signals Haploview [25]. D’ and R2 were used for measurement of LD. The significant threshold was |D’| > 0.8 or R2>0.5.

Statistical analysis

The statistical significance of the difference in each HLA allelic frequencies between BD patients and healthy controls was determined by the chi-square test and Fisher’s exact test using PLINK. The levels of significance was set to that P value is less than 0.05, and odds ratios (OR) with 95% confidence intervals (CI) were calculated. We fit the data to three genetic models such as allelic, dominant and recessive.

Results and Discussion

To examine whether or not HLA-A, -B or -E are associated with BD in Japanese cohort, we performed sequencing-based typing using large amount of BD patient and healthy control. In coincidence with previous reports [8-11], HLA-B*51 was strongly associated with BD in Japanese cohort when we calculated in allelic model and dominant model (Table 1, P values are 4.59x10^-6 and 2.1x10^-4, respectively. OR are 4.59 and 5.32, respectively), although we did not in recessive model. HLA-A*26 is also significantly associated with BD in both allelic model and dominant model (Table 1, P values are 5.3x10^-5 and 8.6x10^-6, respectively. OR are 1.80 and 2.06, respectively.). However, HLA-E*01:01 was not shown to have any significant association with BD even in three genetic models (Table 1). Park et al. [14] reported that HLA-E*01:01, which is one of the two major haplotypes, is significantly associated with BD in Korean cohort (P values and OR are 2.0x10^-4 and 0.7, respectively). They also reported that the polymorphism of CD94/NKG2A, which are an inhibitory receptor complex binding to HLA-E, are associated with BD in Korean cohort [26]. As such different susceptibilities may be dependent on different genetic background of each cohort. To examine that, using available data sets from public database of human genome sequence, International HapMap Project, we also analyzed the difference of LD structure between JPT and CHB in the genomic region covering HLA-A, -B and -E (Figure 1). Our result indicates that LD pattern of the region containing HLA-E gene is different between JPT (Figure 1A & 1B) and CHB (Figure 1C & 1D). The pattern of LD in JPT was more associated with HLA-A region than in CHB (Figure 1 light green and yellowish line). We hypothesized that susceptibility of HLA-E in JPT may be much higher than in CHB because JPT has stronger LD with HLA-A (Figure 1 light green and yellowish line). Indeed, any HLA-A allele is not significantly associated with BD in Han Chinese cohort [27], while it’s associated in Japanese cohort [13]. In contrast to HLA-A region, those of HLA-B were quite similar LD pattern between JPT and Chinese (Figure 1 Light blue line). These regions may be quite conserved among ethic groups. Taken together, we concluded that HLA-B*51 and -A*26 are associated with BD in Japanese cohort as well as another cohorts and that HLA-E*01:01 is not associated with BD in Japanese cohort although previous

Table 1: Association and analysis of HLA alleles with Behcet’s disease.

| HLA Allele | Frequency in Cases | Frequency in Controls | Allelic Model | Dominant Model | Recessive Model |
|------------|--------------------|-----------------------|--------------|----------------|----------------|
|            | OR (95% CI)        |                       |              | OR (95% CI)   | OR (95% CI)   |
|            | P-value            |                       |              | P-value        | P-value        |
| HLA-A*26   | 19.2               | 11.7                  | 1.80 (1.35-2.39) | 2.06 (1.50-2.83) | 1.21 (0.37-4.00) |
|            | 5.3x10^-4          |                       |              | 8.6x10^-6      | 0.77           |
| HLA-B*51   | 28.4               | 7.9                   | 4.59 (3.38-6.23) | 5.32 (3.79-7.48) | NA             |
|            | 4.7x10^-4          |                       |              | 2.1x10^-4      | 1.7x10^-6      |
| HLA-E*01:01| 28.1               | 31.6                  | 0.85 (0.68-1.06) | 0.14            | 0.78 (0.59-1.04) |
|            |                    |                       |              | 0.11             | 0.90 (0.56-1.46) |

*OR: Odds Ratio; CI: Confidence Interval

‡The P-value was calculated using the Fisher’s exact test

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Figure 1: Regional association plot of HLA-A, -B and -E region between JPT (A and B) and CHB (C and D). A and C indicate the measurement of LD using D', while B and D indicate that of LD using R2. Light green line indicates HLA-A gene and its LD block, which possibly can affect other genome region. Light yellowish line indicates HLA-E gene. Light blue line indicates HLA-B and its LD block, which possibly can affect other genome region.
study reported that it’s associated with BD in Korean cohort. Our data suggest that LD structure of the region depends on a type of ethnic group even among Asian ethnic group, resulting in differences with susceptibility of HLA haplotype with BD.

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