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EXTENDED REPORT

Genome-wide association study identifies GIMAP as a novel susceptibility locus for Behçet's disease

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ABSTRACT

Objectives To identify non-major histocompatibility complex susceptible genes that might contribute to Behçet's disease (BD).

Methods We performed a genome-wide association study using DNA samples from a Korean population consisting of 379 BD patients and 800 controls. A replication study was performed in a Japanese population (363 BD patients and 272 controls). To evaluate the functional implication of the target single nucleotide polymorphisms (SNP), gene expression levels in peripheral T cells, allele-specific modulation of promoter activity and biological effect of mRNA knockdown were investigated.

Results We found a novel association of BD to the *GIMAP* locus, mapped to chromosome 7q36.1 (rs1608157, $p=6.01\times10^{-8}$ in a minor allele dominant model; rs11769828, allele based $p=1.60\times10^{-6}$). A fine mapping study identified an association with four additional SNP: rs1522596 (OR=1.45, $p=7.70\times10^{-6}$) in *GIMAP4*; rs10266069 (OR=1.32, $p=2.67\times10^{-4}$) and rs10256482 (OR=1.27, $p=5.27\times10^{-4}$) in *GIMAP2*; and rs2286900 (OR=1.61, $p=3.53\times10^{-5}$) in *GIMAP1* areas. Replication study using DNA samples from the Japanese population validated the significant association between BD and the *GIMAP* locus. The *GIMAP4* promoter construct plasmid with the minor allele of rs1608157 displayed significantly lower activity than one with the major allele. Moreover, CD4 T cells from BD patients showed a lower level of *GIMAP4* mRNA, and *GIMAP4* knockdown was protective against Fas-mediated apoptosis.

Conclusions These results suggest that a *GIMAP* cluster is a novel susceptibility locus for BD, which is involved in T-cell survival, and T-cell aberration can contribute to the development of BD.

INTRODUCTION

Behçet's disease (BD) is a chronic remitting systemic inflammatory disorder, characterised by a high prevalence in the area of the Mediterranean and east Asia and a symptom triad of oral ulcers, genital ulcers and uveitis. Although the aetiology remains elusive, a plausible hypothesis is that an environmental factor such as an infectious agent may trigger dysregulation of the immune cells in genetically susceptible individuals, resulting in the inflammation of each affected organ.[1 2] Therefore, complex interactions of genetic, environmental and immunological factors are believed to contribute to the pathogenesis of BD.

Genetic studies have provided evidence that major histocompatibility complex (MHC) class I genes, including human leukocyte antigen B51 (*HLA-B51*), are strongly associated with the disease, and recent genome-wide association studies (GWAS) have confirmed these associations.[3 4] However, a recent meta-analysis reported that the HLA-B51 contribution varies from 32 to 52%, according to the geographical area.[5] Common variations of BD-related genes have been searched for outside of the MHC region,[1 6] and two non-MHC loci were recently reported to be related to BD using a large GWAS study: Mizuki *et al*[3] and Remmers *et al*[4] reported the associations of *IL23R-IL12RB2* and *IL-10* regions to BD.

However, novel BD-related genetic loci are still to be searched for because previous studies were based on sparsely packed 500 k chips. Therefore we conducted a GWAS in 379 Korean BD cases and 800 unaffected controls, using densely packed 1000 k chips.

METHODS

Population samples

The BD patient samples analysed for the GWAS (n=379, 188 women and 191 men) were collected at outpatient rheumatology clinics of five tertiary referral hospitals in Korea (Seoul National University Hospital, Seoul National University Bundang Hospital, Chungnam National University Hospital, Kyungpook National University Hospital and Gacheon University Hospital) and they fulfilled the international study group criteria of BD diagnosis.[7] Their demographic and clinical characteristics are summarised in table 1. A panel of 800 sex and age-matched individuals (408 women and 392 men) with the same ethnic background was recruited from the DNA biobank of the Korea National Institute of Health. Written informed consent was obtained from all participants and the study was approved by the institutional review board of the Seoul National University Hospital (IRB no. H-0812-012-264).

The replication study samples were taken from a collection of Japanese BD cases (n=363) and controls (n=272) collected at the Uveitis Survey

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Table 1 Demographic and clinical characteristics of BD patients

	Korean BD (n=379)	Japanese BD (n=363)
Gender (male : female)	191 : 188	204 : 160
Age (years)	41.6±10.1*	33.7±10.8*
Disease duration since diagnosis (years)	10.9±8.3*	ND
HLA-B51 positivity	40.1%	46.3%
Clinical manifestation		
Recurrent oral ulcer	100%	100%
Recurrent genital ulcer	74.4%	68.6%
Skin lesions	89.2%	93.9%
Eye lesions	43.5%	84.6%
Positive pathergy test	54.6% (130/238)	ND
Vascular involvement	16.4%	ND
Central nerve system involvement	11.6%	ND
Joint involvement	41.4%	ND

*Mean±SD.
BD, Behçet's disease; ND, not determined.

Clinic of Hokkaido University Hospital and Yokohama City University Hospital (table 1). The Japanese cases were diagnosed according to the international study group criteria.

Genome-wide association genotyping

Samples were genotyped using the Affymetrix genome-wide human single nucleotide polymorphism (SNP) array 6.0, which can identify 906 600 SNP (Santa Clara, California, USA). Samples were quality controlled using the following criteria: greater than 95% call rate of both cases and controls, more than 1% minor allele frequency of cases or controls and Hardy–Weinberg equilibrium of 0.0001 or greater. After cluster image quality control (QC), 367 cases and 800 control samples remained for final analysis with a total of 594 591 SNP data points.

Fine map genotyping and HLA-B51 type analysis

An additional 29 SNP were selected for fine map genotyping from the BD-associated regions that were identified in the discovery stage of the GWAS. The additional SNP were selected with $0.3<R^2<1$ threshold and a minor allele frequency over 10% from HapMAP Japanese panel data using Haploview V.4 (http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview). *HLA-B51* types were determined from DNA samples using two-step PCR amplification with nested sequence-specific primers.[8]

Replication sample genotyping

Replication samples were genotyped for rs1916012, rs1522596, rs1608157, rs10266069, rs10256482 and rs2286900 with TaqMan SNP genotyping assays (Applied Biosystems, Foster City, California, USA) using the manufacturer's protocol and recommended QC measures.

Isolation of peripheral CD4 or CD8 T lymphocytes

Peripheral blood mononuclear cells from heparinised blood samples of 31 BD patients (mean age±SEM 48.0±2.0 years; 12 men) and 31 age and gender-matched healthy donors (mean age 47.8±2.3 years) were separated by the use of Ficoll–Hypaque density gradient centrifugation (GE Healthcare, New Jersey, USA). For magnetic-activated cell sorting (MACS) separation (Miltenyi Biotec, Bergisch Gladbach, Germany), isolated peripheral blood mononuclear cells were labelled with MACS CD4 or CD8 microbeads and passed through a positive selection column type MS^+.

GIMAP mRNA expression in peripheral T lymphocytes

Total RNA was extracted from purified CD4 or CD8 T-cell fractions using an RNeasy mini kit (Qiagen, Valencia, California, USA). The expressions of *GIMAP1*, *GIMAP2* and *GIMAP4* messenger RNA transcripts in CD4 or CD8 T cells were analysed using a Qiagen QuantiTect SYBR Green PCR kit (Qiagen) in duplicate with β-actin as the housekeeping gene. The specific primer pairs and annealing temperatures used in the quantitative real-time PCR are summarised in supplementary table S1 (available online only). The relative standard curve method was used to measure the amount of relative GIMAP in patients and controls. The expression amount was expressed as a relative ratio of GIMAP to β-actin.

Reporter gene assays

Because the rs1608157 polymorphic site, the one with the highest p value in the Korean population, is located far upstream of the site of initiation of *GIMAP4* transcription (approximately 19.9 kb) and long-range chromatin interactions can explain its functional activity, we explored the interaction between polymorphic sites and the *GIMAP4* promoter region.[9] The promoter fragment of *GIMAP4*, located from position −3570 to −2408 from the translation start codon, was amplified from human genomic DNA by PCR with a forward primer containing *Nhe* I (Life Technologies, Invitrogen, Carlsbad, California, USA) and a reverse primer containing *Bgl* II (Promega, Madison, Wisconsin, USA) restriction sites.[10] The 1163-bp amplicon was cloned in pGL3 luciferase reporter vectors (Promega). In addition, the 478-bp fragment including the rs1608157 polymorphic site, located from position −20 181 to −19 704, and the 587-bp fragment including the rs1522596 polymorphic site, located from position −31 508 to −30 922, were amplified and then cloned in pGL3-*GIMAP4* promoter vectors (see supplementary figure S1, available online only). The primer sequences are shown in supplementary table S1 (available online only). Cloned allele-specific plasmids were transfected into Jurkat cells at 250 V and 960 uF using the Gene Pulser Xcell system (Bio-Rad Laboratories, Richmond, California, USA). Forty-eight hours after transfection, luciferase activity was determined in three different clones using luciferase assay kits (Promega).

GIMAP4 knockdown and Fas-mediated apoptosis in Jurkat T cells

Human Jurkat cells (ATCC, Rockville, USA; 1×10^{-6} cells) were transfected with 100 nM of human *GIMAP4*-specific small interfering RNA (sc-89794; Santa Cruz Biotechnology, Santa Cruz, California, USA) or control siRNA (sc-37007; Santa Cruz Biotechnology) using an electroporator. Electroporation was performed at 300 V and 150 uF and *GIMAP4* knockdown was confirmed through RT-PCR and immunoblot using rabbit anti-human GIMAP4 antibody (Novus Biologicals, Littleton, Colorado, USA). The forward and reverse primers for human *GIMAP4* mRNA were 5′–TTTCTCAACATCCTGGCTTAG–3′ and 5′–GTGGCTTTGTGCTCTTCCTC–3′.[11]

One day after siRNA transfection, Jurkat cells were stimulated by 50 ng/ml of phorbol myristate acetate (Sigma-Aldrich, Louis, Missouri, USA) and 0.5 μg/ml of ionomycin (Sigma-Aldrich) for 24 h and then transferred to 48-well plates (5×10^{-4} cells/well). For Fas-mediated apoptosis induction, Jurkat cells were incubated in serum-free RPMI 1640 media

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with histidine-tagged soluble Fas ligand (PeproTech, Rocky Hill, New Jersey, USA; 100 ng/ml) and anti-histidine antibody (AbFrontier, Seoul, Korea; 1 ug/ml) for 24 h. Cell survival was determined using the MTT assay kit. The absorbance was measured at 450 nm.

Statistical analysis

A χ^2 test was applied to evaluate the association for dominant, recessive and allele models and a Cochran–Armitage trend test was applied for the additive model. For top hits, OR and 95% CI were calculated. Multiple comparisons in GWAS were corrected by the false discovery rate (FDR) for a single SNP marker, according to the method of Benjamini and Hochberg.[12] A genome-wide p value of 4.61×10^{-6} was selected as the cut-off for genome-wide significance, which was set at FDR less than 5% (no more than 5% of discoveries to be false). For comparison of continuous variables, the Mann–Whitney U test or Kruskal–Wallis test was used as applicable. PLINK[13] and SAS V.9.1.3 were used for the statistical analysis.

RESULTS

Genome-wide association study

After sample and SNP QC, we could analyse a total of 594 591 SNP in 367 cases and 800 controls. Among them, 55 SNP reached statistical significance using a 5% FDR rate. The most significantly associated SNP (rs4947296, allele-based χ^2 p value=4.01×10^{-13}) was located on chromosome 6p21.3. In the allele-based genetic model, 65.2% of the 23 significant SNP resided on chromosome 6p21.3, where the *HLA-B* locus is mapped (figure 1 and see supplementary table S2, available online only). Among the SNP outside of chromosome 6p21.3, the top five were located on the following chromosomes: 5q34 (rs1965673, allele-based χ^2 p=4.91×10^{-7}; rs1465400, p=1.58×10^{-6}), 7q36.1 (rs11769828, p=1.60×10^{-6}), 20p12 (rs4239774, p=2.07×10^{-6}) and 4q13.2 (rs10033058, p=2.38×10^{-6}), respectively. The SNP rs11769828 is located in the intergenic region between the GTPases of immunity-associated protein 7 (*GIMAP7*) and the GTPases of immunity-associated protein 4 (*GIMAP4*), and four other SNP (rs1608157, rs1916012, rs10236188 and rs10277380) belonging to the *GIMAP* cluster were in a significant range under a dominant model (p=6.01×10^{-8}, p=2.62×10^{-7}, p=3.98×10^{-7}, and p=3.98×10^{-7}, respectively). Moreover, rs1608157 showed genome-wide significance after Bonferroni's correction (corrected p=0.0357). These five SNP were found to be in strong linkage disequilibrium (LD) with each other, except for rs1608157 (figure 2). The total SNP with an allelic χ^2 p value less than 4.61×10^{-6} in the GWAS are summarised in supplementary table S2 (available online only). The *IL23R-IL12RB2* and *IL-10* regions, which were reported to be significantly associated with BD, were not associated with BD patients in this study.[3 4]

Fine mapping and replication studies

Among the top five SNP outside of chromosome 6p21.3, rs1965673 and rs1465400 are located in the intron region of the WW domain-containing protein 1 (*WWC1*) or kidney and brain-expressed protein (*KIBRA*). WWC1 is mainly expressed in memory-related brain regions and common *WWC1* alleles are reported to be associated with memory performance.[14] Furthermore, adjacent SNP of both target SNP have no significant association with BD. However, the GIMAP family is mainly expressed in the lymphocytes and is known to play a role in T-cell biology.[15–18] In addition, *GIMAP4* SNP rs1608157 (p=6.01×10^{-8}) retained genome-wide significance following both Bonferroni's and FDR correction. In such a context, we fine mapped the association between the *GIMAP* cluster and BD using an additional 29 SNP. All SNP were genotyped successfully. Ten SNP in this fine mapping stage were significantly

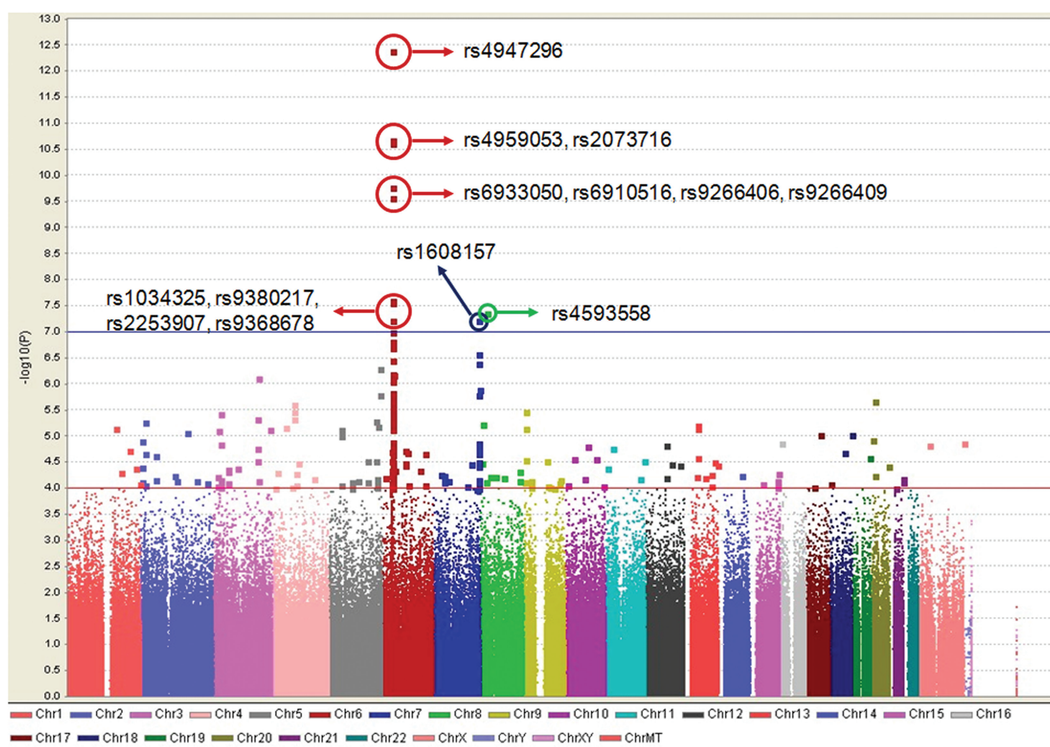

Figure 1 Scatterplot of chromosomal position against $-\log_{10}$ genome-wide association study p value. The 594 591 single nucleotide polymorphisms, sorted according to chromosomal position, and the $-\log_{10}(p)$ values of 367 Behçet's disease cases and 800 controls from Korea are plotted.

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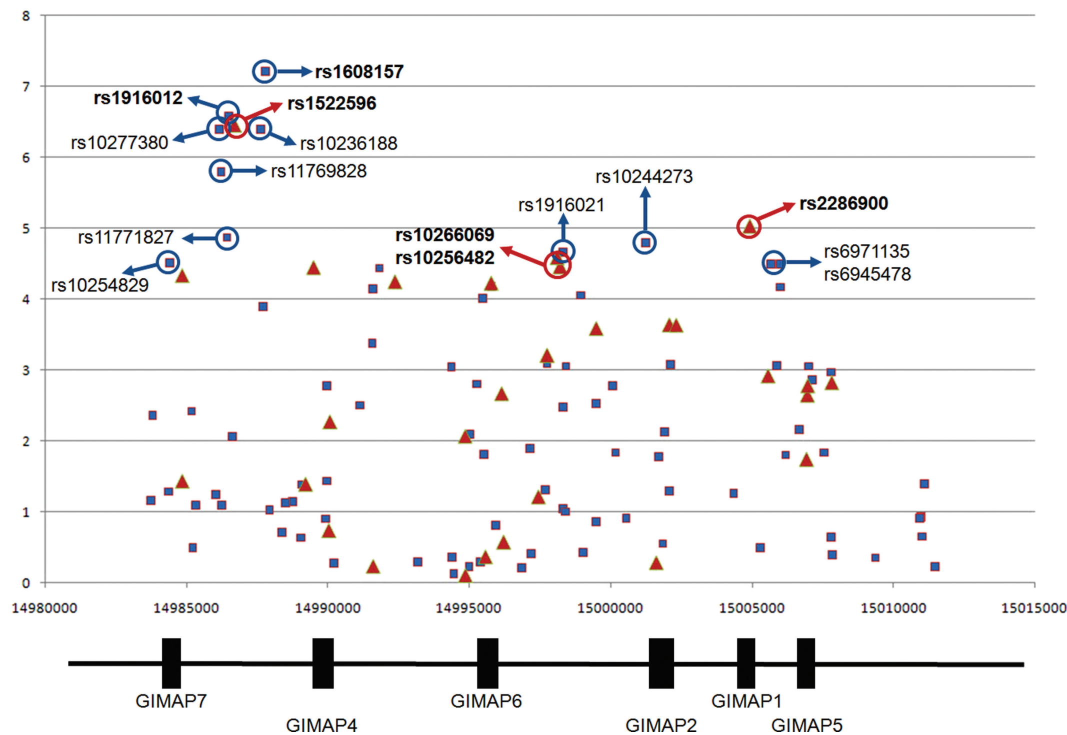

Figure 2 Log_{10}(p) values of the *GIMAP* cluster single nucleotide polymorphisms (SNP). Genotyped SNP in the genome-wide association study are indicated with a blue square, and genotyped SNP for the fine mapping are indicated with a red triangle.

related to BD (allele-based $p<1.0\times10^{-3}$) and the most significant association was observed with rs1522596 ($p=7.7\times10^{-6}$, see supplementary table S3, available online only). These 10 SNP lie within a *GIMAP* region of approximately 200 kb and are schematically summarised by three LD blocks across the genes (figure 3). The results from two stages are summarised in table 2. The results remained statistically significant even after stratification by the presence of an *HLA-B51* allele in BD patients.

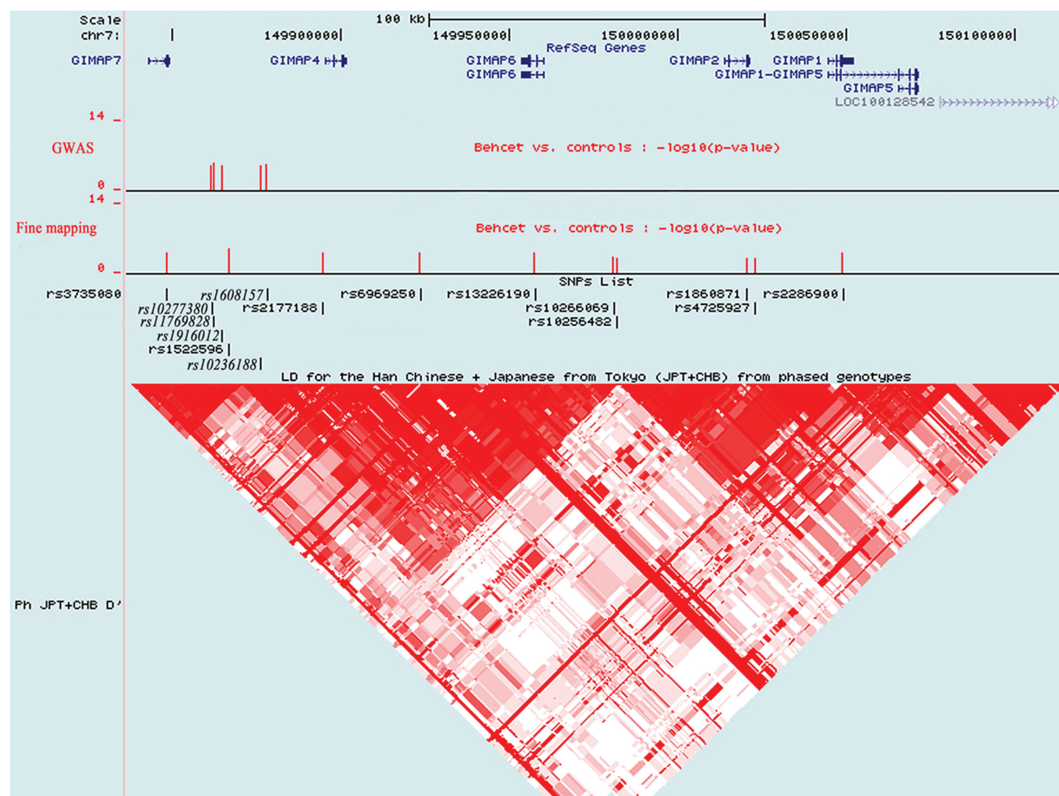

Figure 3 Linkage disequilibrium (LD) structure in the *GIMAP* cluster. The single nucleotide polymorphisms (SNP) genotyped in the genome-wide association study are shown in italic letters. The genotyped SNP are roughly summarised by three LD blocks across the target gene region.

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Table 2 Associations between *GIMAP* SNP and BD in the Korean (367 cases and 800 controls) and Japanese (363 cases and 272 controls) populations

			Risk allele frequency			Allelic model		Risk allele dominant model	
SNP ID	Gene symbol	Risk allele	Population	Cases	Controls	p Value	OR (95% CI)	p Value	OR (95% CI)
rs1916012	GIMAP4	A	Korean	0.584	0.484	6.19×10^{-6}	1.50 (1.26 to 1.79)	2.62×10^{-7}	2.38 (1.70 to 3.33)
			Japanese	0.474	0.410	0.0234	1.30 (1.04 to 1.62)	0.0331	1.45 (1.03 to 2.04)
rs1522596	GIMAP4	A	Korean	0.585	0.484	7.70×10^{-6}	1.45 (1.09 to 1.92)	3.47×10^{-7}	2.38 (1.69 to 3.33)
			Japanese	0.474	0.410	0.0234	1.30 (1.04 to 1.62)	0.0331	1.45 (1.03 to 2.04)
rs1608157	GIMAP4	C	Korean	0.589	0.484	2.83×10^{-6}	1.52 (1.28 to 1.82)	6.01×10^{-8}	2.53 (1.80 to 3.57)
			Japanese	0.474	0.408	0.0197	1.30 (1.04 to 1.64)	0.0331	1.45 (1.03 to 2.04)
rs10266069	GIMAP2	A	Korean	0.493	0.411	2.67×10^{-4}	1.32 (1.08 to 1.61)	2.57×10^{-5}	1.83 (1.38 to 2.43)
			Japanese	0.461	0.403	0.0373	1.27 (1.01 to 1.59)	0.0256	1.47 (1.05 to 2.06)
rs10256482	GIMAP2	T	Korean	0.503	0.425	5.27×10^{-4}	1.27 (1.04 to 1.55)	2.82×10^{-5}	1.83 (1.38 to 2.43)
			Japanese	0.464	0.403	0.0289	1.29 (1.03 to 1.61)	0.0204	1.49 (1.06 to 2.10)
rs2286900	GIMAP1	A	Korean	0.210	0.141	3.53×10^{-5}	1.61 (1.28 to 2.03)	9.22×10^{-6}	1.81 (1.39 to 2.37)
			Japanese	0.182	0.196	0.550	0.92 (0.69 to 1.22)	0.338	0.86 (0.62 to 1.20)

BD, Behçet's disease; SNP, single-nucleotide polymorphism.

To replicate our results, we evaluated the three SNP (rs1916012, rs1522596 and rs1608157) located upstream to *GIMAP4*, two SNP (rs10266069 and rs10256482) located upstream of *GIMAP2*, and one SNP (rs2286900) located in the 3′-untranslated region of *GIMAP1* in 363 Japanese BD patients and 272 controls. In this independent study, the five SNP in the *GIMAP2* and *GIMAP4* regions showed a significant association with the disease in Japanese BD patients (table 2, see supplementary table S4, available online only, for combined analysis).

GIMAP expression in peripheral T lymphocytes

Peripheral CD4 T cells from 31 patients with BD showed significantly lower levels of *GIMAP1* ($p<0.05$ by Mann–Whitney test) and *GIMAP4* ($p<0.05$) transcripts than age and gender-matched healthy controls (figure 4A,C). Moreover, CD8 T cells in BD patients had significantly lower *GIMAP2* ($p<0.01$) mRNA levels (figure 4B). The usage of glucocorticoids or immunosuppressants did not affect the levels of GIMAP in BD patients. In healthy controls, we could not find any association between *GIMAP4* mRNA levels and its genetic polymorphisms genotyped.

The effect of rs1608157 polymorphisms on GIMAP4 promoter activity

In a luciferase reporter assay to test whether rs1608157 affects *GIMAP4* promoter activity, the construct containing the C allele of rs1608157 showed a significant decrease in luciferase activity than one containing the G allele (by approximately 50%, $p<0.01$ by Mann–Whitney test; figure 4D). However, allele-specific plasmids of rs1522596, neighbouring rs1608157, revealed no difference in luciferase activity. These findings imply that *GIMAP4* promoter activity could depend on the allele status of rs1608157, when the DNA segment containing SNP rs1608157 is spatially colocalised with the promoter.

The effect of GIMAP4 knockdown on Fas-mediated apoptosis in Jurkat T cells

After human *GIMAP4*-specific siRNA was transfected into Jurkat T cells, the silencing of *GIMAP4* expression showed significantly enhanced cell survival to Fas-mediated apoptosis ($p<0.05$ by Kruskal–Wallis test, see supplementary figure S2, available online only). On the contrary, non-transfected and scramble siRNA transfected Jurkat cells had significantly decreased survival ($p<0.05$ by Mann–Whitney test) after Fas stimulation.

DISCUSSION

For the first time, we observed a significant association between *GIMAP* gene cluster and BD using genome-wide association scans and found that peripheral CD4 T cells from BD patients had a significantly lower expression of *GIMAP1* and *GIMAP4* than those from healthy controls. Furthermore, *GIMAP4* knockdown Jurkat cells showed significantly enhanced survival following Fas–Fas ligand interaction. Even though the present study failed to discover any statistically significant difference in the *GIMAP4* mRNA levels in the peripheral T cells of control subjects according to SNP genotypes, results from our luciferase activity assay suggested that the *GIMAP4* promoter activity can

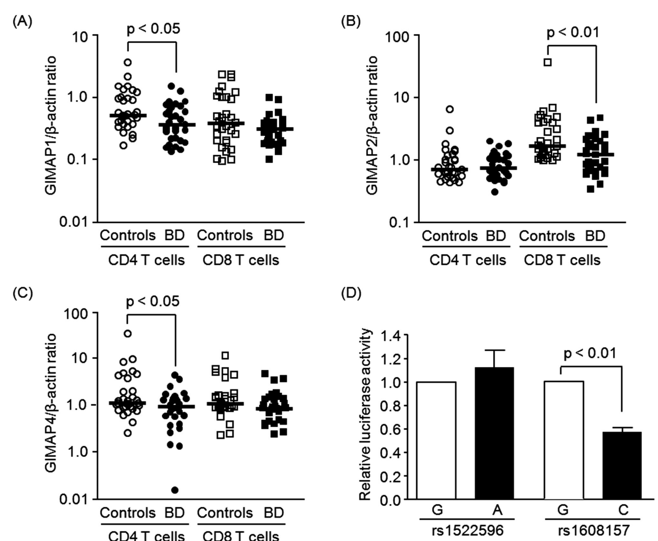

Figure 4 The mRNA expression levels of *GIMAP1* (A), *GIMA2* (B) and *GIMAP4* (C) were significantly decreased in peripheral CD4 or CD8 T cells from patients with Behçet's disease (BD). Thick lines indicate median. (D) The relative luciferase activity driven by non-coding DNA segments containing rs1608157 or rs1522596. Relative luciferase activity (firefly luciferase activity/renilla luciferase activity) was calculated and that for the G allele, a major allele of each single nucleotide polymorphism, was arbitrarily defined as 1.0. Data represent the mean values of the relative luciferase activities±SE of three (for rs1522596) or five (for rs1608157) independent experiments.

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be under the control of rs1608157. Therefore, *GIMAP4* SNP may confer the risk of BD in the Asian population. Recently, Mizuki *et al*[3] and Remmers *et al*[4] reported that BD is associated with the *IL23R-IL12RB2* and *IL-10* regions, but our study did not reproduce this finding. In fact, 119 and 77 Korean BD patients, who were included in two aforementioned studies, respectively, also did not show a significant association with *IL23R-IL12RB2* and *IL-10* regions except rs1800872 (p=0.038) and rs1800871 (p=0.044).

Among the 23 SNP with an allelic χ^2 p value falling below the cut-off ($<4.61\times10^{-6}$), the SNP located outside chromosome 6p21 were eight (34.8%) in our GWAS (see supplementary table S2, available online only) and the top five significant non-MHC-related SNP included *WWC1* (rs1965673, rs1465400), *PLCB1* (rs4239774) and *YTHDC1* (rs10033058), other than *GIMAP4*. Among them, only *GIMAP4* showed a significant association between their genotype and BD in a dominant model (rs1608157, rs1916012, rs10236188 and rs10277380). Furthermore, multiple SNP in the GIMAP region showed association with BD while only one or two SNP in *WWC1* (rs1965673 and rs1465400), *PLCB1* (rs4239774) and *YTHDC1* (rs10033058) showed significant association with BD.

Several studies have demonstrated that GIMAP play a role in peripheral T-cell function as well as T-cell development and selection. *GIMAP1* knockout mice have shown a severe reduction of mature T and B cells without any effect on immature thymocytes,[16] and *GIMAP1* was upregulated by p53 during apoptosis in a mouse myeloid leukaemia cell line.[19] The study using *GIMAP4* knockout mice revealed that GIMAP4 appears to promote apoptosis of T lymphocytes via interaction with a pro-apoptotic protein, Bax.[17] T lineage cells from inbred brown Norway rats, which carry a natural hypomorphic variant of *GIMAP4*, display slower kinetics in the induced cell death programme.[20] In addition, overexpression of *GIMAP4* led to apoptosis of mouse thymocytes.[21] On the contrary, a mutation study on the *GIMAP5* gene in rats showed that GIMAP5 protects T cells from spontaneous cell death through anti-apoptotic Bcl-2 family members.[22] Such regulation mechanisms of T-cell apoptosis play a critical role in the resolution of immune and inflammatory responses and any defect in this physiological process may lead to an increased susceptibility to autoimmune disorders.

We showed that *GIMAP4* silencing is able to decrease Fas-mediated cell death of Jurkat T cells. It was reported that peripheral lymphocytes from BD patients are resistant to Fas-induced apoptosis.[23 24] In addition, the expression of anti-apoptotic protein Bcl-2 increased in the T lymphocytes in inflammatory sites of BD patients.[25 26] The present study first discovered that *GIMAP4* transcript levels in T lymphocytes of BD patients were significantly decreased when compared to their levels in healthy controls. Therefore, when taking into consideration the above-mentioned data of the previous studies, the lower expression of GIMAP4 could contribute resistance to T-cell apoptosis in BD.

In BD patients, the involved tissues are infiltrated mainly by T cells and the disease activity is in parallel with the percentage of interferon-γ producing T-helper (Th) type 1 cells.[27] In addition, serum levels of Th1 cytokines such as interleukin (IL)-12 and IL-18 are shown to be elevated in patients with active BD.[28] Furthermore, the expression of the Th1-specific T-box transcription factor, T-bet, is upregulated in peripheral blood mononuclear cells of BD patients with active uveitis.[29] Therefore, BD is considered a Th1-driven disease. Interestingly, both GIMAP1 and GIMAP4 are upregulated by IL-12 during Th1 differentiation, whereas they are downregulated by IL-4 during early Th2 differentiation.[18] However, because *GIMAP1* and *GIMAP4* transcript levels in BD patients were significantly decreased when compared to their levels in healthy controls, GIMAP could not play a major role in the imbalance of helper T cell subsets in BD pathogenesis.

Because many disease-associated SNP in GWAS are non-coding, unravelling their functional implication has been challenging. A long-range regulation by enhancer, repressor or insulator can account for an association between non-coding SNP and polygenic diseases.[30] Because the distance between the rs1608157 polymorphic site and *GIMAP4* transcription unit is ≈20 kb, we tested whether the polymorphic segment can influence *GIMAP4* promoter activity through a long-range regulatory interaction. Our luciferase reporter assays suggest that the DNA segment containing SNP rs1608157 can act as a long-range enhancer to regulate the *GIMAP4* promoter activity and its activity depends on the allele status of rs1608157. Potential mechanisms have been proposed to explain such long-distance gene regulation, including chromatin loop formation, tracking, facilitated tracking and linking.[31] Although it remains to be elucidated which mechanism is involved in the dynamic interaction between the distant DNA segment and *GIMAP4* promoter, SNP rs1608157 has a functional relevance and represents a novel genetic variant of long-range enhancer for BD susceptibility, when taking into account a higher prevalence of the C allele in BD patients and lower expression levels of *GIMAP4* mRNA in BD CD4 T cells. However, no difference in the levels of *GIMAP4* mRNA according to the SNP genotypes was observed in controls. Such a discrepancy may be attributed to the multiple transcriptional regulatory pathways of GIMAP expression.

Even though polymorphism studies on *GIMAP* gene clusters are very limited, a common polyadenlylation polymorphism in the *GIMAP5* gene has previously been reported to be associated with systemic lupus erythematosus and anti-IA-2 antibody in patients with type I diabetes.[32–34] Therefore, the contribution of *GIMAP* to disease susceptibility does not seem to be unique to BD. When more physiological roles of GIMAP are elucidated in future studies, we will be able to understand better how GIMAP are involved in the pathogenesis of T-cell-mediated diseases.

The present study is the first genetic investigation of an association between *GIMAP* gene cluster and BD. However, there are some limitations. First, this study was performed in BD patients from eastern Asia. Despite similar clinical manifestations in different ethnic groups,[35] the results of candidate gene analysis, including HLA-B51, has shown different results according to ethnicities.[5 36] Therefore, our result may not be applicable to other ethnic groups. Second, the sample size was not large and the association between BD and the *GIMAP* gene could remain inconclusive. Because BD is an uncommon disease, further large collaborative studies should be carried out to clarify the issue. Third, all non-MHC-related SNP including *GIMAP4* did not reach genome-wide statistical significance in an allele-based genetic model, when the Bonferroni method was applied in the discovery phase of our GWAS (p value cut-off of 8.4×10^{-8}). Controlling the probability of type I and type II errors is an important issue in GWAS because of multiple comparisons. Bonferroni's correction, the most stringent method, provides a safeguard against false positives but it can reduce the number of true discoveries. On the contrary, the FDR method is less conservative and provides greater power than the Bonferroni method at a cost of increasing the number of false positives.[37] Therefore, the occurrence of a type I error cannot be excluded in our study. Fourth, we did not identify

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the long-range regulatory mechanisms. All *GIMPA4* non-coding SNP in our study may be tightly linked with another functional polymorphism in the *GIMAP4* gene.

In conclusion, we report a GWAS uncovering a *GIMAP* cluster as a novel susceptibility locus for BD, which is involved in T-cell survival, at least in eastern Asia. This finding could shed new light on the role of GIMAP in T-cell-mediated diseases and could be helpful for understanding the contribution of T-cell aberration in the pathophysiology of BD.

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Contributors YJL, YH, YMK, SWK, HJB, NK, AM, NM, KN, SI, EN, EYL, YWS, SO and EBL provided patient samples and clinical information. YJL, EYL, YWS and EBL designed the GWAS. GRW, EN and EBL performed quality control and preliminary association analyses of the GWAS data. YJL performed the genetic matching, quantile–quantile, and conditional analyses of the GWAS data under the supervision of EBL. EBL defined the 'best region' SNP among SNP with GWAS $p<0.0001$. YMK, YH, SWK, HJB, NK, AM, NM, SI, EYL, SO and EBL designed and performed the replication study. JAP and IC analysed clinical information and replication data under the supervision of EBL. JC and YSC performed the functional study. The paper was written by YJL and YH with contributions from SO. GRW, SO and EBL coordinated the genotyping, analysis, and manuscript writing efforts for this study. GRW, SO and EBL discussed the results and helped with manuscript preparation. The first two authors contributed equally.

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REFERENCES

1. **Mendes D,** Correia M, Barbedo M, *et al*. Behçet's disease – a contemporary review. *J Autoimmun* 2009;**32**:178–88.
2. **Gül A.** Behçet's disease: an update on the pathogenesis. *Clin Exp Rheumatol* 2001;**19**:S6–12.
3. **Mizuki N,** Meguro A, Ota M, *et al*. Genome-wide association studies identify IL23R-IL12RB2 and IL10 as Behçet's disease susceptibility loci. *Nat Genet* 2010;**42**:703–6.
4. **Remmers EF,** Cosan F, Kirino Y, *et al*. Genome-wide association study identifies variants in the MHC class I, IL10, and IL23R-IL12RB2 regions associated with Behçet's disease. *Nat Genet* 2010;**42**:698–702.
5. **de Menthon M,** Lavalley MP, Maldini C, *et al*. HLA-B51/B5 and the risk of Behçet's disease: a systematic review and meta-analysis of case-control genetic association studies. *Arthritis Rheum* 2009;**61**:1287–96.
6. **Fei Y,** Webb R, Cobb BL, *et al*. Identification of novel genetic susceptibility loci for Behçet's disease using a genome-wide association study. *Arthritis Res Ther* 2009;**11**:R66.
7. **Criteria for diagnosis of Behçet's disease.** International Study Group for Behçet's Disease. *Lancet* 1990;**335**:1078–80.
8. **Hein J,** Böttcher K, Grundmann R, *et al*. Low resolution DNA typing of the HLA-B5 cross-reactive group by nested PCR-SSP. *Tissue Antigens* 1995;**45**:27–35.
9. **Kleinjan DA,** van Heyningen V. Long-range control of gene expression: emerging mechanisms and disruption in disease. *Am J Hum Genet* 2005;**76**:8–32.
10. **Stamm O,** Krücken J, Schmitt-Wrede HP, *et al*. Human ortholog to mouse gene imap38 encoding an ER-localizable G-protein belongs to a gene family clustered on chromosome 7q32–36. *Gene* 2002;**282**:159–67.
11. **Schwefel D.** *Structural and functional analysis of immunity-associated GTPases* (dissertation). Berlin, Freie Universität Berlin, 2010.
12. **Benjamini Y,** Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B* 1995;**57**:289–300.
13. **Purcell S,** Neale B, Todd-Brown K, *et al*. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007;**81**:559–75.
14. **Papassotiropoulos A,** Stephan DA, Huentelman MJ, *et al*. Common Kibra alleles are associated with human memory performance. *Science* 2006;**314**:475–8.
15. **Krücken J,** Schroetel RM, Müller IU, *et al*. Comparative analysis of the human gimap gene cluster encoding a novel GTPase family. *Gene* 2004;**341**:291–304.
16. **Saunders A,** Webb LM, Janas ML, *et al*. Putative GTPase GIMAP1 is critical for the development of mature B and T lymphocytes. *Blood* 2010;**115**:3249–57.
17. **Schnell S,** Démollière C, van den Berk P, *et al*. Gimap4 accelerates T-cell death. *Blood* 2006;**108**:591–9.
18. **Filén JJ,** Filén S, Moulder R, *et al*. Quantitative proteomics reveals GIMAP family proteins 1 and 4 to be differentially regulated during human T helper cell differentiation. *Mol Cell Proteomics* 2009;**8**:32–44.
19. **Kannan K,** Kaminski N, Rechavi G, *et al*. DNA microarray analysis of genes involved in p53 mediated apoptosis: activation of Apaf-1. *Oncogene* 2001;**20**:3449–55.
20. **Carter C,** Dion C, Schnell S, *et al*. A natural hypomorphic variant of the apoptosis regulator Gimap4/IAN1. *J Immunol* 2007;**179**:1784–95.
21. **Nitta T,** Nasreen M, Seike T, *et al*. IAN family critically regulates survival and development of T lymphocytes. *PLoS Biol* 2006;**4**:e103.
22. **Keita M,** Leblanc C, Andrews D, *et al*. GIMAP5 regulates mitochondrial integrity from a distinct subcellular compartment. *Biochem Biophys Res Commun* 2007;**361**:481–6.
23. **Todaro M,** Zerilli M, Triolo G, *et al*. NF-kappaB protects Behçet's disease T cells against CD95-induced apoptosis up-regulating antiapoptotic proteins. *Arthritis Rheum* 2005;**52**:2179–91.
24. **Yang P,** Chen L, Zhou H, *et al*. Resistance of lymphocytes to Fas-mediated apoptosis in Behçet's disease and Vogt–Koyangi–Harada syndrome. *Ocul Immunol Inflamm* 2002;**10**:47–52.
25. **Hamzaoui K,** Hamzaoui A, Zakraoui L, *et al*. Expression of Bcl-2 in inflammatory sites from patients with active Behçet's disease. *Mediators Inflamm* 1999;**8**:101–6.
26. **Bariş YS,** Yildiz L, Sentürk N, *et al*. Fas (CD95) and bcl-2 expression in active skin lesions of Behçet's disease. *J Eur Acad Dermatol Venereol* 2005;**19**:569–72.
27. **Ben Ahmed M,** Houman H, Miled M, *et al*. Involvement of chemokines and Th1 cytokines in the pathogenesis of mucocutaneous lesions of Behçet's disease. *Arthritis Rheum* 2004;**50**:2291–5.
28. **Frassanito MA,** Dammacco R, Cafforio P, *et al*. Th1 polarization of the immune response in Behçet's disease: a putative pathogenetic role of interleukin-12. *Arthritis Rheum* 1999;**42**:1967–74.
29. **Li B,** Yang P, Zhou H, *et al*. T-bet expression is upregulated in active Behçet's disease. *Br J Ophthalmol* 2003;**87**:1264–7.
30. **Noonan JP,** McCallion AS. Genomics of long-range regulatory elements. *Annu Rev Genomics Hum Genet* 2010;**11**:1–23.
31. **Dean A.** On a chromosome far, far away: LCRs and gene expression. *Trends Genet* 2006;**22**:38–45.
32. **Hellquist A,** Zucchelli M, Kivinen K, *et al*. The human GIMAP5 gene has a common polyadenylation polymorphism increasing risk to systemic lupus erythematosus. *J Med Genet* 2007;**44**:314–21.
33. **Lim MK,** Sheen DH, Kim SA, *et al*. IAN5 polymorphisms are associated with systemic lupus erythematosus. *Lupus* 2009;**18**:1045–52.
34. **Shin JH,** Janer M, McNeney B, *et al*. IA-2 autoantibodies in incident type I diabetes patients are associated with a polyadenylation signal polymorphism in GIMAP5. *Genes Immun* 2007;**8**:503–12.
35. **Lewis KA,** Graham EM, Stanford MR. Systematic review of ethnic variation in the phenotype of Behcet's disease. *Scand J Rheumatol* 2007;**36**:1–6.
36. **Touma Z,** Farra C, Hamdan A, *et al*. TNF polymorphisms in patients with Behçet disease: a meta-analysis. *Arch Med Res* 2010;**41**:142–6.
37. **Rice TK,** Schork NJ, Rao DC. Methods for handling multiple testing. *Adv Genet* 2008;**60**:293–30.

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