Comprehensive analysis of the association between *UBAC2* polymorphisms and Behçet’s disease in a Japanese population

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Behçet’s disease (BD) is reportedly associated with polymorphisms of the ubiquitin-associated domain containing 2 (*UBAC2*) gene in Turkish, Italian, and Chinese populations. Here we investigated whether *UBAC2* polymorphisms were associated with BD in a Japanese population. Using data from 611 Japanese BD patients and 737 Japanese controls who participated in our previous genome-wide association study, we analyzed the 58 genotyped single-nucleotide polymorphisms (SNPs) in the region 100 kb upstream and downstream of *UBAC2*. We also performed imputation analysis in the region, with 562 imputed SNPs included in the statistical analyses. Association testing revealed that the T allele of rs9517723 in the lncRNA LOC107984558 was significantly associated with ocular and central nervous system (CNS) lesions and showed the strongest association under the recessive model (TT vs. CT+CC: ocular lesion, $P_c=0.0099$, OR = 1.56; CNS lesion, $P_c=0.0052$, OR = 3.42). Expression analysis revealed that rs9517723 TT homozygotes showed significantly increased *UBAC2* expression ($P<0.05$). Our findings suggest that enhanced *UBAC2* expression associated with the homozygous risk allele (TT) of rs9517723 could induce overactivation of ubiquitination-related pathway, resulting in the development of ocular and CNS lesions in BD.

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are an important risk factor for BD susceptibility in multiple populations. However, the association between UBAC2 variants and BD has not yet been assessed in a Japanese population.

The aim of the present study was to investigate whether genetic variants in the UBAC2 region are associated with BD in a Japanese population. We performed a comprehensive association analysis of SNPs in the UBAC2 region among Japanese patients with BD.

Results

Comprehensive allelic association analysis. Our allelic association analysis in a Japanese population included a total of 620 SNPs (58 genotyped and 562 imputed). Of these SNPs, 100 SNPs showed an association with BD with a $P$ value of $< 0.05$ (Fig. 1). The strongest association was observed for rs9517723 located on LOC107984558, which showed an increased frequency of its major allele (T) in cases compared to controls ($P = 0.0024$; odds ratio (OR) = 1.27). However, this increase did not reach significance after correcting for multiple testing ($P_c = 0.13$) (Table 1). While the SNPs rs3825427, rs9513584 and rs7999348 were previously reported to be associated with BD, we found no such associations in our present population.

LD analysis. We observed long-range LD across the UBAC2 gene region. The strongest signal was for rs9517723, which exhibited a strong or moderate LD ($r^2 \geq 0.8$) with many of the other 99 SNPs that showed a significant $P$ value of $< 0.05$ (Fig. 1). However, rs9517723 exhibited very moderate or low LD ($r^2 < 0.2$) with many of the remaining 520 SNPs with $P$ values of $> 0.05$. Among the 100 SNPs showing a significant $P$ value of $< 0.05$, we performed stepwise regression analysis to test the independence of multiple possible associations in the region. Conditioning by rs9517723 eliminated the association of the other 99 SNPs ($P > 0.05$), indicating that rs9517723 could account for most of the association of these SNPs with BD in the Japanese population. On the other hand, rs9517723 was in very moderate or low LD with the previously reported BD-associated SNPs (rs3825427: $r^2 = 0.07$; rs9513584: $r^2 = 0.15$; rs7999348: $r^2 = 0.10$).

Figure 1. In-depth SNP analysis of the UBAC2 region. The lead SNP (rs9517723) is depicted as a purple diamond. The color coding of all other SNPs indicates linkage disequilibrium (LD) with the lead SNP: red, $r^2 \geq 0.8$; yellow, $0.6 \leq r^2 < 0.8$; green, $0.4 \leq r^2 < 0.6$; cyan, $0.2 \leq r^2 < 0.4$; blue, $r^2 < 0.2$; and gray, $r^2$ is unknown. The left y axis represents the $-\log_{10} P$ values for allelic association with Behçet’s disease, and the right y axis represents the estimated recombination rate. The horizontal red line indicates the significance level of $P = 0.05$. Gene annotations are shown below the figure. The plot was created using LocusZoom.

| SNP      | Position on Chr. 13 (GRCh37) | Alleles (1 > 2)$^b$ | Risk Allele | Allele Frequency, % | Controls (N = 737) | $P$ | $P_c$ | OR (95% CI)$^d$ |
|----------|------------------------------|---------------------|-------------|---------------------|---------------------|-----|-------|-----------------|
| rs3825427| 99,848,971                   | C>A                 | A           | 38.7                | 36.4                | 0.24|        | 1.10 (0.94–1.29) |
| rs9513584| 99,876,281                   | G>A                 | G           | 59.2                | 56.3                | 0.12|        | 1.13 (0.97–1.32) |
| rs7999348| 99,932,922                   | G>A                 | G           | 63.0                | 61.5                | 0.41|        | 1.07 (0.91–1.25) |
| rs9517723| 100,084,679                  | T>C                 | T           | 63.3                | 57.5                | 0.0024| 0.13 | 1.27 (1.09–1.49) |

Table 1. Allelic association results for rs3825427, rs9513584, rs7999348, and rs9517723 in the UBAC2 region. $^a$rs9513584, rs7999348, and rs9517723 were genotyped on the GWAS panel while rs3825427 was imputed with the 1000 Genomes reference panel. $^b$1, major allele; 2, minor allele. $^cP_c$, corrected $P$; if the $P_c$ value is greater than 1, it is set to 1. $^d$OR, odds ratio; CI, confidence interval.
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**Expression analysis.**  The SNP rs9517723 is located on the first exon of LOC107984558, which encodes a long non-coding RNA (lncRNA) and is located between the protein coding genes, UBAC2 (43 kb downstream) and TM9SF2 (68 kb upstream) (Fig. 1). Through a variety of mechanisms, lncRNA can regulate gene expression in cis or in trans. Thus, we investigated whether rs9517723 affected the expression level of UBAC2 and/or TM9SF2. UBAC2 expression was significantly increased in the rs9517723 TT homozygotes (TT vs. CT, OR = 1.26; TT vs. CC, OR = 1.36; CNS lesion: OR = 2.78). Table 3 shows the genotypic association results for rs9517723. In both additive and recessive models, we found an association with the disease at \( P < 0.05 \) among all entire Japanese patients; however, this association did not reach significance after correction (\( P_c > 0.05 \)). On the other hand, the T allele of rs9517723 was significantly associated with increased risk of ocular and CNS lesions under the additive and recessive models. The OR was stronger among patients with CNS lesions, and the associations with both ocular and CNS lesions were stronger in the recessive model (ocular lesion: OR = 2.78; CNS lesion: OR = 1.56; CNS lesion: OR = 2.78). Moreover, the T allele of rs9517723 was associated with 1.33-fold to 1.42-fold increased risks of oral ulcer, skin lesion, genital ulcer, and arthritis; however, these risks were not significant (\( P < 0.05, P_c > 0.05 \)).

**Discussion**

In our present study, we aimed to assess whether genetic variants in the UBAC2 region affected BD development in a Japanese population. We performed comprehensive association analysis of SNPs in the region 100 kb upstream and 100 kb downstream of UBAC2 among Japanese patients with BD. This study is the first comprehensive investigation of the UBAC2 region for association with BD. We found that SNP rs9517723 in the lncRNA LOC107984558 was recessively associated with the risks of ocular and CNS lesions (showing a stronger association with CNS lesions than with BD itself in the Japanese population). This suggests that rs9517723 contributes to the development of ocular and CNS lesions, especially CNS lesions, with a recessive effect.

**Association between rs9517723 and clinical symptoms of BD.**  We also analyzed the relationships between rs9517723 and clinical symptoms of BD (Table 2). An allelic association test in the Japanese population revealed that the T allele of rs9517723 was significantly associated with increased risk of ocular and central nervous system (CNS) lesions, with a stronger association with CNS lesions: ocular lesion, \( P_c = 0.018, OR = 1.36; \) CNS lesion, \( P_c = 0.0066, OR = 2.78 \). Table 3 shows the genotypic association results for rs9517723. In both additive and recessive models, we found an association with the disease at \( P < 0.05 \) among all entire Japanese patients; however, this association did not reach significance after correction (\( P_c > 0.05 \)). On the other hand, the T allele of rs9517723 was significantly associated with increased risk of ocular and CNS lesions under the additive and recessive models. The OR was stronger among patients with CNS lesions, and the associations with both ocular and CNS lesions were stronger in the recessive model (ocular lesion: \( P_c = 0.0099, OR = 1.56; \) CNS lesion: \( P_c = 0.0052, OR = 3.42 \)) than in the additive model (ocular lesion, \( P_c = 0.023, OR = 1.34; \) CNS lesion, \( P_c = 0.0078, OR = 2.94 \)). Moreover, the T allele of rs9517723 was associated with 1.33-fold to 1.42-fold increased risks of oral ulcer, skin lesion, genital ulcer, and arthritis; however, these risks were not significant (\( P < 0.05, P_c > 0.05 \)).

| Phenotype       | N  | Risk Allele (T) Freq., % | \( P \)  | \( P_c \)  | OR (95% CI)  |
|-----------------|----|-------------------------|---------|-----------|--------------|
| Controls        | 737| 57.9                    |         |           |              |
| Cases ALL       | 611| 63.0                    | 0.0024  | 0.13      | 1.27 (1.09–1.49) |
| Oral ulcer      | 589| 63.4                    | 0.0037  | 0.19      | 1.26 (1.08–1.48) |
| Skin lesion     | 510| 62.7                    | 0.015   | 0.76      | 1.23 (1.04–1.44) |
| Ocular lesion   | 491| 65.1                    | 0.00034 | 0.018     | 1.36 (1.15–1.60) |
| Genital ulcer   | 372| 64.5                    | 0.0026  | 0.13      | 1.32 (1.10–1.59) |
| Arthritis       | 229| 62.7                    | 0.069   |           | 1.22 (0.98–1.52) |
| Epididymitis    | 38 | 61.8                    | 0.49    |           | 1.18 (0.73–1.90) |
| GI lesion\(^a\) | 100| 61.5                    | 0.33    |           | 1.16 (0.86–1.57) |
| Vascular lesion | 29 | 65.5                    | 0.25    |           | 1.38 (0.80–2.40) |
| CNS lesion\(^b\) | 41 | 79.3                    | 0.00013 | 0.0066    | 2.78 (1.62–4.80) |

**Table 2.** Allelic association results between rs9517723 and clinical symptoms of Behçet's disease. \(^a\)OR, odds ratio; CI, confidence interval. \(^b\)GI, gastrointestinal. \(^c\)CNS, central nervous system.
UBAC2 expression, suggesting that decreased UBAC2 expression also contributes to BD risk. These contradictory findings indicate a need for further functional studies to clarify how UBAC2 contributes to BD pathophysiology.

LncRNAs are defined as non-coding RNA transcripts of more than 200 nucleotides in length. Recent evidence shows that lncRNAs play key functional roles in diverse biological processes, including chromatin remodeling, transcriptional, posttranscriptional, and epigenetic regulation. They also contribute to the pathophysiology of various diseases, including cancers and neurological, autoimmune, and ocular diseases. Genetic variants in lncRNAs can modulate the structure and expression of localized lncRNAs, leading to functional alterations of their interacting partners.

Table 3. Genotypic association results between rs9517723 and clinical symptoms of Behçet's disease. *Pc*, corrected P; If the Pc value is greater than 1, it is set to 1. **OR, odds ratio; CI, confidence interval. GI, gastrointestinal. CNS, central nervous system.

Figure 2. Expression analysis of UBAC2 (a) and TM9SF2 (b) stratified by rs9517723 genotype.
pathophysiology. In general, lncRNA expression is more tissue-specific than the expression of protein-coding genes, and most lncRNAs are highly expressed in the CNS with low expression in other tissues \(^{21,35}\). This characteristic expression pattern of lncRNAs may explain why rs9517723 in the lncRNA LOC107984558 is more strongly associated with CNS lesions than with other BD symptoms. However, we did not assess the expression pattern of LOC107984558 in our current study, nor did we find public databases containing LOC107984558 expression data.

In conclusion, our present findings indicate that rs9517723 in the lncRNA LOC107984558 was significantly associated with increased risks of ocular and CNS lesions within a Japanese population. Our results further suggest that rs9517723 is associated with enhanced UBAC2 expression, which contributes to the development of those lesions. Further validation studies in other ethnic populations are needed to confirm these findings. Additionally, further expression analyses using RNA isolated from cells of ocular and CNS lesions in patients with BD are needed to more clearly elucidate the effect of 9517723 on UBAC2 expression. In the future, rs9517723 may serve as a useful genetic marker for BD diagnosis, especially in cases with CNS lesions.

**Methods**

**Subjects.** Our previous GWAS enrolled 611 unrelated Japanese individuals with BD, and 737 unrelated Japanese controls\(^{26}\). Here we used genotype data from that study, specifically for the 58 SNPs found from 100 kb upstream to 100 kb downstream of the UBAC2. All 58 SNPs satisfied the following quality control criteria: a call rate >98%, Hardy-Weinberg equilibrium (HWE) \(P > 0.001\), and minor allele frequency >1%. The Japanese patients were diagnosed with BD according to the standard criteria\(^{27}\) proposed by the Japan Behçet's Disease Research Committee. All control participants were healthy volunteers, who were unrelated to each other or to the patients. All participants gave their written informed consent. The study methodology adhered to the tenets of the Declaration of Helsinki, and was approved by the Ethics Committee of Yokohama City University School of Medicine.

**Imputation analysis of the UBAC2 gene region.** To evaluate potential associations with un-genotyped SNPs within the region encompassing the UBAC2 gene, we performed imputation analysis. The genotypes of our Japanese GWAS set were imputed based on the 58 genotyped SNPs using MACH v1.0 (http://www.sph.umich.edu/csg/abecasis/MACH/index.html)\(^{38,39}\). The reference panel comprised the 1000 Genomes Phase 3 datasets of 315 East Asian samples, which included a set of Japanese samples from Tokyo (JPT, \(N = 104\), Han Chinese samples from Beijing (CHB, \(N = 103\)), and Southern Han Chinese samples (CHS, \(N = 108\)) (http://www.1000genomes.org/)\(^{39}\). All imputed SNPs were filtered with the following quality control settings: HWE \(P > 0.001\), minor allele frequency >1%, and a squared correlation between imputed and true genotypes (\(R^2\)) of >0.90. A total of 562 imputed SNPs were included in further analysis.

**Expression analysis.** From our genome-wide expression (GWE) dataset, we obtained expression data for the UBAC2 and the transmembrane 9 superfamily member 2 (TM9SF2) genes from 313 Japanese healthy volunteers (Meguro et al., unpublished data). The GWE analysis was performed using the Illumina HumanHT-12 v4 Expression BeadChip Kit. First, whole blood was collected from subjects in PAXgene Blood RNA tubes (Becton Dickinson, Heidelberg, Germany), and total RNA was extracted from whole blood using the PAXgene Blood RNA Kit (Qiagen) following the manufacturers’ protocols. Next, the total RNA samples were processed using the TargetAmp-Nano Labeling Kit for the Illumina Expression BeadChip (Epicentre, Wisconsin, USA) and hybridized to the BeadChips following the manufacturers’ protocols.

**Statistical analysis.** We performed allelic and genotypic association analyses and stepwise regression analyses, and calculated linkage disequilibrium (LD) using SNP & Variation Suite software version 8.6.0 (Golden Helix, Inc., Bozeman, MT, USA). A correlation/trend test was used to assess differences in allele and genotype frequencies between cases and controls. We generated a regional association plot for the UBAC2 region using LocusZoom (http://csg.sph.umich.edu/locuszoom/)\(^{31}\). Tagging SNPs were selected from the genotype data of the Japanese GWAS set (611 BD patients and 737 controls) with an \(r^2\) threshold of 0.80, using PLINK version 1.07 (http://pngu.mgh.harvard.edu/purcell/plink/)\(^{37}\). This identified 52 tagging SNPs, capturing all 620 SNPs (58 genotyped and 562 imputed SNPs) within the UBAC2 region. The obtained \(P\) values were corrected for multiple testing using Bonferroni correction based on the number of tagging SNPs. A corrected \(P\) (\(P_c\) value of <0.05 was considered significant. Differences in the expression levels of UBAC2 and TM9SF2 were analyzed using the Mann-Whitney U test.

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Author Contributions
A.M., S.O. and N.M. designed the study. S.O. and N.M. contributed study samples. K.Y., A.M., M.T. and E.S. conducted the experiments and analyzed data. K.Y. and A.M. wrote the manuscript. All authors reviewed the manuscript.

Additional Information

Competing Interests: The authors declare that they have no competing interests.

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