Single nucleotide polymorphisms in IL-10-mediated signalling pathways in Korean patients with Behçet's disease

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ABSTRACT

Objective. *Previous genome-wide association studies have demonstrated an association between the IL-10 region and Behçet's disease (BD) in Turkish and Japanese populations. Our aim was to fully examine the relationship between IL-10 and BD, the associations between BD and single nucleotide polymorphisms (SNPs) in IL-10-mediated signalling pathways (JAK1, TYK2, and STAT3) were examined in Korean patients with BD.*

Methods. *DNA samples were obtained from 223 patients who met the international study group criteria for BD and from 222 age- and sex-matched healthy controls. Twenty-four tag SNPs in JAK1, STAT3, and TYK2 were selected for genotyping based on the Japanese panel of international HapMap data with a minor allele frequency >5% and r^2 >0.8.*

Results. *The allele-based analysis showed that the T allele of rs310245 in JAK1 was associated with BD (odds ratio [95% confidence interval] = 1.34 [1.03-1.76], p=0.031), which lost statistical significance after permutation-based correction for multiple testing. In the genotype-based analysis, the JAK1 rs310245 TT homozygote (1.79 [1.14-2.82], p=0.012) and the STAT3 rs2293152 GG homozygote (2.01 [1.16-3.47], p=0.011) showed associations with BD. However, these associations did not achieve significance after correction for multiple testing. We did not observe any genetic interaction between the JAK1 rs310245 TT homozygote and the STAT3 rs2293152 GG homozygote. In the haplotype analysis, GT haplotype at rs17127024 and rs310245 (1.34 [1.03-1.74], p=0.032), and the AA haplotype at rs2256298 and rs3818753 (1.41 [1.03-1.92], p=0.034) in JAK1 were associated with BD, but lost statistical significance after correction for multiple testing.*

Conclusion. *There was no significant association between BD and SNPs in IL-10-mediated intracellular signalling in Korean patients.*

Introduction

Behçet's disease (BD) is a chronic relapsing inflammatory disease characterised by orogenital ulcers, cutaneous inflammation, and uveitis. In addition to its typical muco-cutaneous and ocular manifestations, BD targets the musculoskeletal, vascular, nervous, and gastrointestinal systems (1). Since Ohno *et al.* first reported a strong association between BD and *HL-A*5* in a Japanese population in 1973 (2), the association between BD and *HLA-B*51* has been repeatedly replicated across different ethnic groups (3). Subsequent studies, including genome-wide association studies (GWASs), have identified a number of genetic susceptibility loci other than *HLA-B*51* (4, 5). Additionally, two recent GWASs performed on Turkish and Japanese populations have identified significant associations at *IL-10* and *IL23R-IL12RB2* loci (4, 5). In both studies, the most significant association was observed at single nucleotide polymorphisms (SNPs) in the *IL-10* promoter region, and decreased IL-10 synthesis has been thought to be one of pathogenic mechanisms of BD. However, a subsequent GWAS result obtained from a Korean population failed to replicate this finding (6), suggesting a substantial ethnic difference in genetic susceptibility to BD.

IL-10 is best known for its anti-inflammatory activity (7). The biological effect of IL-10 is mediated by IL-10 receptors that utilise the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signalling pathways. Janus kinases, JAK1 and tyrosine kinase 2 (TYK2), are phosphorylated upon IL-10 receptor engagement (8, 9) and they induce activation of STAT 1,

STAT3, and, occasionally, STAT5 (10, 11). In particular, the presence of JAK1 and STAT3 is essentially required for anti-inflammatory response by IL-10 (9, 12-14). TYK2 is activated by a broader range of cytokines including not only IL-10 but also IL-12, IL-23, and type I interferons (15, 16). To fully clarify the relationship between an IL-10-related mechanism and BD, we examined the associations between BD and SNPs in IL-10-mediated signalling pathways (*JAK1, TYK2,* and *STAT3*) in Korean patients with BD.

Methods

Patients and samples

Genomic DNA samples were obtained from 223 Korean BD patients who met the classification criteria of the International Study Group and from 222 age- and sex-matched healthy controls as previously described (17). A significant number of the patient samples (n=189) used in this study had been also used for the previous Korean GWAS (6). Due to lack of consent and approval to release GWAS control data, we used a different set of controls. This study was approved by the Institutional Review Board of Seoul National University Hospital (no. 0408-131-010), and patient consent was obtained.

SNP selection and genotyping

Tag SNPs to be genotyped were selected in *JAK1* (GenBank accession number, BC132729) on chromosome 1p31.3, *TYK2* (AY549314) on chromosome 19p13.2, and *STAT3* (AY572796) on chromosome 17q21.3 within 2kb upstream and downstream of the gene position and with a minor allele frequency >5% and r^2 >0.8 according to the Japanese panel of international HapMap data. Additional SNPs reportedly associated with inflammatory diseases were also included. Finally, 24 tag SNPs were selected (Table I). Affymetrix genome-wide human SNP array 6.0 (Santa Clara, CA, USA) used in the Korean GWAS (6) had also contained SNPs for *JAK1, TYK2,* and *STAT3*. We intended to include all tag SNPs selected whether they had been genotyped in the previous GWAS or not. Genotyping was performed using TaqMan® predesigned primers and probes (Applied Biosystems, CA, USA) according to the manufacturer's protocol. The genotyping call rate for each SNP was 97.8–100%. Linkage disequilibrium (LD) blocks were structured for each gene, and the associations between potential haplotypes and BD were examined using Haploview software version 4.2 (Broad Institute, MA, USA).

Statistical analysis

The observed genotype frequencies were compared with expected genotype frequencies in control subjects to test the Hardy-Weinberg equilibrium with a cut-off *p*-value of 0.01. Allele and genotype distributions were compared between patients and controls using the chi-square test. Three inheritance models were applied to the genotype-based analysis and the best-fit model was chosen. Corrections for multiple testing were done by permutation testing. The SNP data from the previous Korean GWAS (6) in relation to *JAK1*, *TYK2*, and *STAT3* were also analysed in the same manner. Statistical calculations were performed using SPSS version 17.0 software (SPSS Inc., IL, USA). A *p*-value <0.05 was considered significant. Odds ratios (ORs) with 95% confidence intervals (CI) were estimated whenever applicable.

Results

The baseline clinical characteristics of the 223 BD patients had been previously described (17). No differences in age or gender distribution were observed between patients and controls. All examined SNPs were in Hardy–Weinberg equilibrium. Two SNP loci (rs34536443 and rs12720356) in *TYK2* did not show any polymorphism in study subjects and were excluded from analysis.

The allele-based analysis (Table II) showed that the T allele of rs310245 in *JAK1* (OR [95% CI] = 1.34 [1.03–1.76], *p*=0.031) was associated with BD but lost statistical significance after correction for multiple testing. In the genotype-based analysis (Table II), the *JAK1* rs310245 TT homozygote (1.79 [1.14-2.82], *p*=0.012) and the *STAT3* rs2293152 GG homozygote (2.01 [1.16-3.47], *p*=0.011) showed associations with BD. However, these associations did not achieve significance after correction for multiple testing. We did not observe any genetic interaction between the *JAK1* rs310245 TT homozygote and the *STAT3* rs2293152 GG homozygote (TT/GG combination; 14/223 in patients *vs*. 9/222 in controls, *p*>0.05). When we analysed the SNPs in *JAK1, TYK2,* and *STAT3* using the GWAS data (n=379 for patients, n=800 for controls), no allele or genotype including rs310245 showed a significant association with BD after permutation-based correction for multiple testing.

The haplotype analysis identified potential haplotypes in *JAK1* and *STAT3* (Fig. 1). The GT haplotype at rs17127024 and rs310245 (1.34 [1.03–1.74], *p*=0.032), and the AA haplotype at rs2256298 and rs3818753 (1.41 [1.03–1.92], *p*=0.034) in *JAK1* were associated with BD (Table III) but lost statistical significance after correction for multiple testing.

Table I. Tag SNPs selected for genotyping.

Gene	SNP	Chromosomal position
JAK1	rs17127024†	65303131
	rs310245†	65306182
	rs2230587†	65311262
	rs2780898†	65313945
	rs2274948	65321409
	rs2256298	65330682
	rs3818753	65339232
	rs10889502†	65379982
	rs11208537†	65383632
	rs11208538†	65389289
	rs1353595	65405070
	rs17127171	65412260
	rs7553101†	65420190
	rs4244165†	65421071
TYK2	rs34536443	10463118
	rs12720356	10469975
	rs2304256†	10475652
	rs280523	10477206
	rs12720217	10491339
STAT3	rs3744483	40466438
	rs2293152	40481529
	rs6503695	40499533
	rs744166†	40514201
	rs12948909	40570602

SNP: single nucleotide polymorphism.
†SNPs included both in the previous GWAS (6) and in the present study.

Table II. Allele distribution of 22 SNPs in JAK1, TYK2, and STAT3 between patients with BD and controls.

Gene	SNPs	Allele	Number (%) of alleles		*p*-value	Genotype	Number (%) of alleles		*p*-value
			Patients	Controls			Patients	Controls	
JAK1	rs17127024	G	329 (73.8)	305 (68.7)	NS	G/G	119 (53.4)	105 (47.3)	NS
		T	117 (31.2)	139 (31.3)		G/T	91 (40.8)	95 (42.8)	
						T/T	13 (5.8)	22 (9.9)	
	rs310245	T	258 (57.8)	224 (50.4)	0.027	T/T	74 (33.2)	63 (28.4)	0.012
		C	188 (42.2)	220 (49.6)		T/C	110 (49.3)	98 (44.1)	
						C/C	39 (17.5)	61 (27.5)	
	rs2230587	G	330 (74.3)	327 (73.6)	NS	G/G	121 (54.5)	124 (55.9)	NS
		A	114 (25.7)	117 (26.4)		G/A	88 (39.6)	79 (35.6)	
						A/A	13 (5.9)	19 (8.6)	
	rs2780898	A	209 (47.1)	205 (46.2)	NS	A/A	43 (19.4)	47 (21.2)	NS
		G	235 (52.9)	239 (53.8)		A/G	123 (55.4)	111 (50.0)	
						G/G	56 (25.2)	64 (28.8)	
	rs2274948	T	402 (90.1)	401 (90.7)	NS	T/T	180 (80.7)	183 (82.8)	NS
		C	44 (9.9)	41 (9.3)		T/C	42 (18.8)	35 (15.8)	
						C/C	1 (0.4)	3 (1.4)	
	rs2256298	A	148 (33.3)	126 (28.4)	NS	A/A	23 (10.4)	21 (9.5)	NS
		G	296 (66.7)	318 (71.6)		A/G	102 (45.9)	84 (37.8)	
						G/G	97 (43.7)	117 (52.7)	
	rs3818753	A	418 (93.7)	409 (92.5)	NS	A/A	197 (88.3)	191 (86.4)	NS
		G	28 (6.3)	33 (7.5)		A/G	24 (10.8)	27 (12.2)	
						G/G	2 (0.9)	3 (1.4)	
	rs10889502	G	206 (46.4)	204 (45.9)	NS	G/G	54 (24.3)	49 (22.1)	NS
		C	238 (53.6)	240 (54.1)		G/C	98 (44.1)	106 (49.1)	
						C/C	70 (31.5)	67 (20.3)	
	rs11208537	A	259 (58.1)	245 (55.2)	NS	A/A	82 (36.8)	68 (30.6)	NS
		T	187 (41.9)	199 (44.8)		A/T	95 (42.6)	109 (49.1)	
						T/T	46 (20.6)	45 (20.3)	
	rs11208538	C	341 (76.5)	323 (72.7)	NS	C/C	134 (60.1)	124 (55.9)	NS
		T	105 (23.5)	121 (27.3)		C/T	73 (32.7)	75 (33.8)	
						T/T	16 (7.2)	23 (10.4)	
	rs1353595	A	207 (46.6)	203 (45.7)	NS	A/A	49 (22.1)	40 (18.0)	NS
		C	237 (53.4)	241 (54.3)		A/C	109 (49.1)	123 (55.4)	
						C/C	64 (28.8)	59 (26.6)	
	rs17127171	A	148 (33.2)	130 (29.3)	NS	A/A	28 (12.6)	21 (9.5)	NS
		G	298 (66.8)	314 (70.7)		A/G	92 (41.3)	88 (39.6)	
						G/G	103 (46.2)	113 (50.9)	
	rs7553101	C	188 (42.3)	186 (41.9)	NS	C/C	39 (17.6)	39 (17.6)	NS
		T	256 (57.7)	258 (58.1)		C/T	110 (49.5)	108 (48.6)	
						T/T	73 (32.9)	75 (33.8)	
	rs4244165	G	311 (69.7)	294 (66.2)	NS	G/G	108 (48.4)	98 (44.1)	NS
		T	135 (30.3)	150 (33.8)		G/T	95 (42.6)	98 (44.1)	
						T/T	20 (9.0)	26 (11.7)	
TYK2	rs2304256	C	257 (59.8)	253 (57.5)	NS	C/C	77 (35.8)	73 (33.2)	NS
		T	173 (40.2)	187 (42.5)		C/T	103 (47.9)	107 (48.6)	
						T/T	35 (16.3)	40 (18.2)	
	rs280523	G	414 (94.5)	419 (94.4)	NS	G/G	0	3 (1.4)	NS
		A	24 (5.5)	25 (5.6)		G/A	24 (11.0)	19 (8.6)	
						A/A	195 (89.0)	200 (90.1)	
	rs12720217	T	9 (2.0)	8 (1.8)	NS	T/T	0	0	NS
		A	431 (98.0)	436 (98.2)		T/A	9 (4.1)	8 (3.6)	
						A/A	211 (95.9)	214 (96.4)	
STAT3	rs3744483	T	313 (70.5)	302 (68.0)	NS	T/T	110 (49.6)	100 (45.0)	NS
		C	131 (29.5)	142 (32.0)		T/C	93 (41.9)	102 (45.9)	
						C/C	19 (8.6)	20 (9.0)	
	rs2293152	G	178 (39.9)	163 (36.7)	NS	G/G	42 (18.8)	23 (10.4)	0.011
		C	268 (60.1)	281 (63.3)		G/C	94 (42.2)	117 (52.7)	
						C/C	87 (39.0)	82 (36.9)	
	rs6503695	T	293 (66.0)	289 (65.1)	NS	T/T	98 (44.1)	95 (42.8)	NS
		C	151 (34.0)	155 (34.9)		T/C	97 (43.7)	99 (44.6)	
						C/C	27 (12.2)	28 (12.6)	
	rs744166	A	283 (63.5)	275 (61.9)	NS	A/A	92 (41.3)	83 (37.4)	NS
		G	163 (36.5)	169 (38.1)		A/G	99 (44.4)	109 (49.1)	
						G/G	32 (14.3)	30 (13.5)	
	rs12948909	C	65 (14.7)	53 (12.0)	NS	C/C	5 (2.3)	2 (0.9)	NS
		A	377 (85.3)	389 (88.0)		C/A	55 (24.9)	49 (22.2)	
						A/A	161 (72.9)	170 (76.9)	

BD: Behçet's disease; JAK: Janus kinase; SNP: single nucleotide polymorphism; STAT: signal transducer and activator of transcription; TYK: tyrosine kinase.

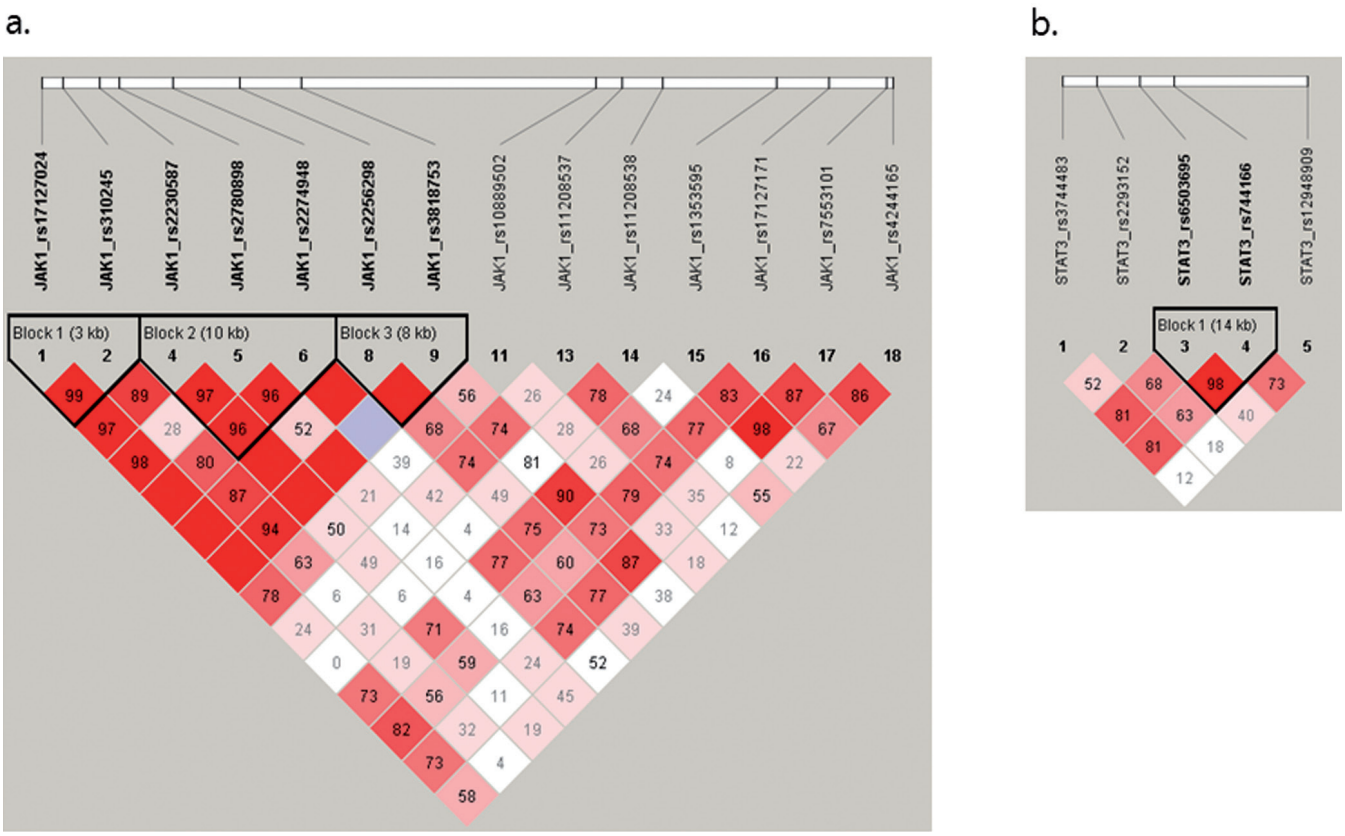

Fig. 1. Linkage disequilibrium (LD) structure and haplotypes in Janus kinase 1 (*JAK1*) (**a**) and signal transducer and activator of transcription 3 (*STAT3*) (**b**). Locations of genotyped tag single nucleotide polymorphisms (SNPs) in relation to LD and haplotypes are shown using Haploview software version 4.0. Inverted triangles represent haplotypes and the numbers in each square represent LD.

Discussion

The significance of IL-10 in the anti-inflammatory response is easily shown in IL-10 and IL-10R2 knock-out (KO) mice, in which inflammatory bowel disease develops in association with markedly elevated pro-inflammatory cytokines such as TNF-α (18, 19). Moreover, these mice are susceptible to a lethal immune response when challenged by lipopolysaccharide or micro-organisms (20, 21). The mechanism of the IL-10-induced anti-inflammatory response critically involves STAT3, which mediates selective regulation of toll-like receptor-inducible pro-inflammatory genes at the transcriptional level (22). TYK2 is recruited to the IL-10R2 subunit in the presence of IL-10 and trans-phosphorylates IL-10R1 and JAK1 (23). The *TYK2*-related risk allele has been identified in systemic lupus erythematosus by candidate gene studies (24, 25) and in inflammatory bowel disease by a meta-analysis (26), which underlies the functional relevance of TYK2 genetic dysregulation in inflammatory diseases. Two recent GWASs and subsequent candidate gene studies have shown that genetic polymorphisms in *IL-10* are associated with decreased IL-10 mRNA expression in BD (4, 5, 27). Considering the functional relevance of IL-10, IL-10 deficiency seems to play a role in the pathogenesis of BD.

The association between BD and SNPs within the *IL-10* promoter region has been investigated in a small number of Korean patients as a replication cohort in the afore-mentioned two GWASs (4, 5). However, only one of the GWASs showed narrowly accepted statistical significance without correction for multiple testing (5). Later, an independent Korean GWAS has not only failed to replicate the association between BD and the *IL-10* region SNPs but also showed novel suscepti-

Table III. Haplotype distribution between patients with BD and controls.

Gene	LD block	SNPs		Number (%) of haplotypes in Patients	Controls	*p*
JAK1	1	rs17127024	rs310245			
		G	T	257 (57.6)	224 (50.5)	0.032
		T	C	116 (26.0)	139 (31.3)	0.080
		G	C	72 (16.1)	81 (18.2)	0.41
	3	rs2256298	rs3818753			
		G	A	298 (66.8)	318 (71.3)	0.11
		A	A	121 (27.1)	93 (20.9)	0.034
		A	G	28 (6.3)	33 (7.4)	0.50

BD: Behçet's disease; JAK: Janus kinase; LD: linkage disequilibrium; SNP: single nucleotide polymorphism.

bility loci, the *GIMAP* gene cluster, in this ethnic group (6). In this GWAS (6), SNPs showing the most significant association in *IL-10* region were rs3024490 (1.51 [1.24-1.84] for A allele, p=4.05x10^{-5}) and rs1554286 (1.43 [1.17-1.74] for A allele, p=4.0x10^{-4}). Both of these two SNPs are in strong LD with the previously reported BD-associated SNPs in *IL-10* promoter region (4, 5), but did not reach genome-wide significance (cut-off p-value, 4.61x10^{-6}) (6). The lack of association between *IL-10* region SNPs and BD in Korean patients might be due to limited power of the GWAS because of a small number of patients included (n = 367). Nevertheless, in terms of robustness, the association of *IL-10* region SNPs with BD was far surpassed by that of *GIMAP* region SNPs. The present study aimed to examine if there is any other mechanism of IL-10 dysregulation than transcriptional decline by promoter variants, such as, genetic variants in IL-10 signalling pathways. We found no significant association between BD and SNPs in *JAK1, TYK2,* or *STAT3*. Few studies have investigated Janus kinase genetic polymorphisms in patients with BD. Hu *et al*. examined the *JAK2* polymorphism in BD but obtained negative results (28). Lately, the G allele at rs2780815, C allele at rs310241, and A allele at rs3790532 in *JAK1* were found to be associated with ocular BD in Chinese Han patients (29). However, none of the alleles at rs2780815 and rs310241 were found to be associated with BD after correction for multiple testing (allele-based, uncorrected p values, 0.0195 and 0.0089, respectively) in the Korean GWAS data (these SNPs were included in the Korean GWAS (6) but not in this study). In addition, a significantly increased frequency of the *STAT3* rs2293152 GG genotype has been reported in 503 Chinese Han patients with BD when compared with 615 healthy controls (99/503, 19.7% in patients versus 77/615, 12.5% in controls, uncorrected p=0.001, corrected p=0.021) (28), but the association did not achieve statistical significance after correction for multiple testing in the present study (42/223, 18.8% in patients *vs*. 23/222, 10.4% in controls, uncorrected p=0.011). Because the prevalence and distribution of *STAT3* rs2293152 GG genotype in our study were not much different from those in the Chinese Han study (28), the lack of association in our study might be due to the limited power of this study. Alternatively, this discrepancy could be attributed to different study populations as all the Chinese Han patients had BD-related uveitis. Lastly, the relevance of *STAT3* rs2293152 in BD might not be as strong in other ethnic groups as in the Chinese Han population, because a lack of association between *STAT3* rs2293152 and BD has been reported in a Spanish population (30/214, 14.02% in BD patients *vs*. 314/1843, 17.04% in healthy controls) (30).

On the other hand, *STAT4* polymorphism has been found to be associated with BD in a Chinese as well as in a Turkish GWAS (31). STAT4 operates with JAK2/TYK2 under IL-12 or IL-23 receptors to modulate transcription of *IFN-γ* or *IL-17* (15). The epistatic interaction between *IL-17A, IL-23R,* and *STAT4* has been reported in 141 Korean patients with intestinal BD (32).

In conclusion, no significant association was found between BD and SNPs in *JAK1, TYK2,* or *STAT3* in this Korean population. Our findings indicate that genetic dysregulation of IL-10-mediated signalling is not a predominant pathogenic mechanism in Korean patients with BD. However, replication studies adopting a larger sample size together with an appropriate meta-analysis (33) or more fine mapping of the target gene with or without imputation method (34, 35) will help conclusively clarify the association between genetic variants in JAK-STAT axis and BD in Korean patients.

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