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Genome-wide association analysis identifies new susceptibility loci for Behçet's disease and epistasis between *HLA-B*51* and *ERAP1*

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Abstract

Patients with Behçet's disease (BD) suffer from episodic inflammation often affecting the orogenital mucosa, skin, and eyes. To discover new BD-susceptibility loci, we performed a genome-wide association study (GWAS) of 779,465 SNPs with imputed genotypes in 1,209 Turkish BD patients and 1,278 controls. We identified novel associations at *CCR1, STAT4*, and *KLRC4*. Additionally, two SNPs in *ERAP1*, encoding ERAP1 p.Asp575Asn and p.Arg725Gln, recessively conferred disease risk. These findings replicated in 1,468 independent Turkish and/or 1,352 Japanese samples (combined meta-analysis $p < 2 \times 10^{-9}$). We also found evidence for interaction between *HLA-B*51* and *ERAP1* ($p = 9 \times 10^{-4}$). The *CCR1* and *STAT4* variants were associated with gene expression differences. Three risk loci shared with ankylosing spondylitis and psoriasis (*MHC-I, ERAP1*, and *IL23R*, and the *MHC-I-ERAP1* interaction), as well as two loci shared with inflammatory bowel disease (*IL23R* and *IL10*) implicate shared pathogenic pathways in the spondyloarthritides and BD.

Behçet's disease (BD) is a form of vasculitis that manifests with orogenital ulcers, uveitis, skin inflammation, arthritis, enterocolitis, and inflammation in other organs[1,2]. BD is relatively common in Turkey, Japan, and modern-day countries that fall on Marco Polo's ancient Silk Routes, and is an important cause of vision loss in these countries[2]. Genetic risk factors contribute to disease-susceptibility. *HLA-B*51* is the most strongly associated risk factor for BD, confirmed in multiple populations[3-5]. Although its association was established more than three decades ago, the role of *HLA-B*51* in disease pathogenesis remains elusive[5]. In addition to *HLA-B*51*, two recent independent GWASs identified variants in regions encompassing *MHC-I, IL10*, and *IL23R* associated with BD in both the Turkish and Japanese populations[6,7]. However, the combined effects of these genetic factors do not fully explain the observed disease heritability.

The pathobiology of BD is also largely unknown. In 1974, based on clinical features, Moll et al. proposed the concept of “seronegative spondylarthritides”, and included BD along with ankylosing spondylitis (AS), psoriatic arthritis, reactive arthritis, and inflammatory bowel disease (IBD)[8]. Since then, the inclusion of BD within the spondyloarthropathy (SpA) category has been debated as BD patients rarely exhibit sacroiliitis, and BD is associated with *HLA-B*51* rather than *HLA-B*27*[9-11]. On the other hand, overlapping extra-articular clinical manifestations (inflammation in the eyes, skin, and intestine), genetic associations at *MHC-I* and *IL23R*[6,7,12-14], and the effectiveness of tumor necrosis factor (TNF)-α blockade[15,16], suggest shared pathogenesis between BD and SpA. Furthermore, *IL10* and *IL23R* variants were found associated with both BD and IBD (Crohn's disease and ulcerative

colitis), implicating common inflammatory pathways between BD and these other members of the SpA group.[17,18]

To identify novel genetic variants associated with BD, we imputed genotypes of autosomal SNPs in our GWAS collection of 1,209 cases and 1,278 controls using the previously genotyped SNPs[6] and a reference panel of 96 Turkish controls genotyped for 814,474 SNPs (**Online Methods**). After quality control filtering, 779,465 autosomal imputed SNPs were subjected to statistical analysis by basic allele test using the best-guess imputed genotypes. In addition to the previously reported *MHC-I*, *IL10*, and *IL23R* discovery signals, we observed a strong signal in the *CCR1* (C-C chemokine receptor type 1)-*CCR3* locus, with a p-value that exceeded genome-wide significance, $p<5 \times 10^{-8}$ (Figure 1, rs7616215, $p=1.29 \times 10^{-8}$). Markers with $p<3 \times 10^{-5}$ are listed in Supplementary Table 1.

We selected 21 SNPs from the novel loci identified by imputation and one SNP from the previously reported Japanese GWAS[7] for genotyping in a Turkish replication collection, comprising newly collected 838 Turkish cases and 630 controls (Supplementary Table 2). Four promising loci, *STAT4* (signal transducer and activator of transcription 4), *KLRC4* (killer cell lectin-like receptor subfamily C, member 4), the *CCR1-CCR3* locus, and *IL12A* (interleukin-12 alpha chain) were then selected for validation of the imputed data (by direct genotyping) and fine-mapping studies in the original Turkish GWAS samples. As shown in Figure 2a-c, we found the strongest signals at rs7616215 3′ of *CCR1*, at rs7574070 in intron 3 of *STAT4*, and at nonsynonymous SNPs rs2617170 and rs1841958, encoding KLRC4 p.Asn104Ser and p.Ile129Ser. In a meta-analysis, we combined the Turkish GWAS and replication data, and if polymorphic, the Japanese replication data (from the reported GWAS collection with 612 cases and 740 controls[7]) using Cochran-Mantel-Haenszel tests (Table 1 and Figure 2). Three loci (*CCR1-CCR3, STAT4*, and *KLRK1-KLRC1)* were associated with BD at genome-wide significance ($p=1.34 \times 10^{-9}$ to 4.30×10^{-13}). Additionally, the *IL12A* locus exhibited suggestive association ($p=6 \times 10^{-7}$). Of the 612 Japanese cases ascertained using the Japanese diagnostic criteria[7], 496 also fulfilled the International Study Group Criteria[19]. An analysis including only cases that met the International Study Group criteria revealed genome-wide significance for the same three loci despite the reduced numbers (Supplementary Table 3).

In an attempt to reduce genetic heterogeneity, we performed genome-wide association tests in the subset of GWAS discovery patients with uveitis (435 cases, 1,278 controls)[6]. Neither the basic allelic test nor the dominant model test showed associations outside of the MHC. However, when we applied a recessive model, one SNP in the *ERAP1-ERAP2* locus exhibited association with a p-value close to genome-wide significance level (rs2927615, $p=1.02 \times 10^{-7}$). We performed fine-mapping of this region in the uveitis subset of the GWAS discovery collection and identified rs10050860 and rs17482078, encoding ERAP1 p.Asp575Asn and p.Arg725Gln, which conferred risk for BD with uveitis in a recessive model (Figure 2d). A meta-analysis of p.Arg725Gln combining the Turkish discovery collection and the Turkish replication collection (with 370 BD cases with uveitis and 630 controls) exceeded the three model threshold for genome-wide significance and revealed a large effect size of the homozygous p.Arg725Gln genotype on BD with uveitis (odds ratio=4.56, $p=4.73 \times 10^{-11}$, Table 1 and Figure 2d).

Because a recessive model was required to detect the *ERAP1* association in BD patients with uveitis, we tested whether the recessive model would reveal the ERAP1 p.Arg725Gln association with BD susceptibility in the combined uveitis and non-uveitis samples. A meta-analysis of the GWAS and replication collections found significant association of the homozygous p.Arg725Gln genotype with BD susceptibility ($p=4.35 \times 10^{-8}$, Supplementary Table 4). The minor allele frequency of rs2927615 (a variant in strong linkage disequilibrium [LD] with p.Arg725Gln) was too low in the Japanese population (1.8% in BD cases, 2.0% in controls) to evaluate recessive effects. Furthermore, none of the Japanese GWAS SNPs from the regions encompassing *ERAP1* or *IL12A* (rs17810546 was not polymorphic in the Japanese population) were associated with BD (Supplementary Table 5).

ERAP1 is an endoplasmic reticulum expressed amino peptidase that functions to trim peptides for loading onto MHC Class I[20]. Previous GWASs have established associations of *ERAP1* variants in psoriasis[21, 22] and AS[12]. ERAP1 p.Asp575Asn and p.Arg725Gln, which are in strong LD, confer protection against these diseases through reduced peptide trimming and antigen presentation by MHC-Class I[12,22-24]. Of note, recent reports have shown that these *ERAP1* variants confer protection preferentially in *HLA-B*27* positive individuals in AS[23] and *HLA-C*06* positive individuals in psoriasis[22], suggesting that peptide processing and binding/presentation mechanisms contribute to the pathogenesis of these diseases.

We therefore tested for an interaction between *HLA-B*51* and *ERAP1* in BD. The *ERAP1* variants preferentially conferred risk for BD in *HLA-B*51* positive individuals (p-value for interaction=0.0009 from a logistic likelihood ratio test comparing the full model including a multiplicative interaction term with the reduced model without interaction term) in the combined Turkish GWAS and replication (including uveitis and non-uveitis) samples (Figure 3). Furthermore, ERAP1 p.Arg725Gln homozygosity was associated with an odds ratio for BD of 3.78 [95% CI 1.94-7.35] in the *HLA-B*51* positive individuals versus an odds ratio of 1.48 [95% CI 0.78-2.80] in the *HLA-B*51* negative individuals. This finding indicates that the disease-associated peptidase variant contributes to disease susceptibility through an interaction with the HLA-B*51 protein.

Homozygosity for the *ERAP1* variants is associated with increased risk for BD, but decreased risk for AS[23] and psoriasis[22]. The difference between risk and protection among these three diseases may depend on the variability and binding affinities of peptides loaded onto the respective MHC Class I molecules, which can affect their stability and function. Indeed, repertoires of MHC-bound peptides are altered in ERAP1 deficient mice[25]. ERAP1 p.Arg725Gln-related alterations might affect the repertoire of peptides that bind to HLA-B*51, which is known for its promiscuous peptide binding features[5,26]. The recessive nature of the *ERAP1* effect in BD (one wild type copy in heterozygotes is sufficient to obscure the risk effect of the mutant allele) suggests that homozygotes fail to produce one or more disease-protective peptides.

The *KLRC4* BD-associated SNP (Table 1) is within a haplotype block containing five natural killer (NK) cell receptor genes (*KLRK1, KLRC1-4*) (Figure 2c). Two non-synonymous variants in *KLRC4*, rs1841958 and rs2617170, encoding KLRC4 p.Ile29Ser and Asn104Ser, are found on the BD-protective haplotype of this LD block. This haplotype

has been associated with reduced peripheral blood leukocyte cytotoxicity and increased incidence of cancer[27]. Conditional logistic regression analysis, conditioning on the KLRC4 Asn104Ser variant, showed no additional independent association signals within this LD block (Supplementary Figure 1a). KLRC4, also called NKG2F, encodes a c-type lectin receptor whose function is largely unknown. A possible clue to its function may be found in a related family member, NKG2D, encoded by *KLRK1* and also located within the disease-associated haplotype block. NKG2D is expressed on NK cells and $\gamma\delta$ T cells, and can act as a co-stimulatory molecule for $CD4^+$ and $CD8^+$ T cells[28,29]. Interestingly, a ligand of the NKG2D receptor is MICA (the MHC Class I chain-related protein A)[28]. The *MICA* gene is located within the MHC region and SNPs within the *MICA* locus are in linkage disequilibrium with *HLA-B*51*[6]. The importance of NK receptors in BD pathogenesis is also supported by the observation that the strongest linkage peak in Turkish familial BD (LOD score of 3.94) is found at chr12p12-13, which includes the *KLRK* region locus[30].

The disease-associated variants in the 3′ flanking region of *CCR1-CCR3* (rs7616215) and within the third intron of *STAT4* (rs7574070) are noncoding and are not in strong LD with any coding variants (Figure 2a-b**)**. The *CCR1-CCR3* locus contains a cluster of chemokine receptor genes within the LD block. Logistic regression analysis conditioning on covariate rs7616215 revealed only a single association signal in the region (Supplementary Figure 1b). ENCODE data indicated that rs7616215 and rs7574070 are located within DNase I hypersensitivity and histone 3 lysine 4 methylation sites, suggesting effects on transcription.

Indeed, *CCR1* mRNA expression was higher in primary human monocytes from healthy donors with the disease protective C allele (Figure 4a, Zeller et al.[31], $p = 9.5 \times 10^{-6}$, and 4b, $p = 0.017$). The BD-associated variant was not, however, found associated with expression of the nearby gene *CCR3*.[31] Concordant with expression data, migration of monocytes in response to a gradient of the *CCR1* ligand MIP1-α was higher in C allele individuals (Figure 4c, $p = 0.015$). Comparison between *CCR1* mRNA expression level and migration index within matched samples (n=34) showed significant correlation (Spearman's $\rho = 0.46$, p= 0.007, Figure 4d). Thus, *CCR1* expression and monocyte chemotaxis were reduced in individuals with the disease risk allele suggesting that impaired clearance of pathogens may contribute to BD pathogenesis. Future experiments will be required to further elucidate the importance of the observed differences.

STAT4 mRNA expression was higher in individuals with the risk allele A (Figure 4e and 4f). The BD-associated variant rs7574070 (and its surrogate rs7572482) is in poor LD with the previously reported autoimmune disease-associated *STAT4* variant, rs7574865[32]; in fact it is located two LD blocks away, suggesting the associations are independent, although both variants are located within the large third intron of *STAT4* (Figure 2b). Both variants are associated with increased expression of *STAT4* (ref 33 and data presented here), but the genetically distinct disease-associations suggest different *STAT4* regulatory mechanisms in BD compared with rheumatoid arthritis, systemic lupus erythematosus, and other autoimmune diseases. Smaller effect sizes observed for the associations of the *CCR1* and *STAT4* variants in the Japanese replication (Table 1) could be explained by “the winner's curse”[34].

In conclusion, this study adds substantially to the understanding of genetic factors that contribute to BD susceptibility (the new loci are *CCR1-CCR3*, *STAT4*, *KLRK1-KLRC4*, and *ERAP1*). Furthermore, the results support an emerging concept delineating common pathogenic mechanisms for BD and the SpA. BD, AS, and psoriasis are inflammatory disorders affecting the skin, eyes, and joints, with significant MHC Class I associations (B*51 for BD, B*27 for AS, and C*06 for psoriasis). Recent genetic studies implicate variants of *IL23R*, encoding an upstream molecule in Th17 activation, in susceptibility to all three disorders. The present work adds *ERAP1* to the list of shared genetic factors and furthermore, interactions between *MHC Class I* and *ERAP1* are also found in all three of these diseases. ERAP1 trims peptides for proper loading onto Class I antigens, thus suggesting that peptide-MHC Class I interactions contribute to all three of these diseases. These data suggest the existence of shared inflammatory pathways among these diseases leading to the possibility of common therapeutic strategies, while raising questions about the specific disease characteristics, which may be related to their different MHC Class I associations.

Online methods

Patients

1,215 Turkish Behçet's disease (BD) cases and 1,278 genetically matched controls used in previous GWAS were studied[6]. Individuals who also met the Tel-Hashomer clinical criteria for the diagnosis of familial Mediterranean fever (FMF)[35] were excluded (n=6). For replication, an additional similarly collected 838 Turkish cases (none fulfilled FMF criteria) and 630 controls, and 612 Japanese BD cases and 740 control samples enrolled in the previous GWAS[7] were included. Turkish BD patients fulfilled the International Study Group diagnostic criteria for Behçet's disease[19]. All Japanese BD patients fulfilled the Japanese BD diagnostic criteria[7] and 496 of them also fulfilled the International Study Group criteria. All study participants provided written informed consent, and the study was approved by the Ethics Committees of each investigative institution.

Genotype imputation

We imputed genotypes of 1,209 BD cases and 1,278 controls using MACH v1.0. For a reference panel, we used 96 Turkish healthy controls who participated in the previous BD GWAS using the HumanHap370CNV chip (Illumina) and additionally genotyped on the Human OMNI 1M chip (Illumina, San Diego, CA). For quality control, we excluded SNPs from the reference panel if they had a minor allele frequency less than 5%, deviated from Hardy–Weinberg equilibrium ($P<0.0001$), or had a call rate below 95%, yielding 814,474 SNPs for the imputation. Quality scores for the imputation are shown in Supplementary Table 6. SNPs with Rsq<0.3 were excluded from the association analysis. A total of 779,465 imputed SNPs were included in the genome-wide association analysis.

Validation and fine-mapping

The Turkish GWAS imputation provided the discovery data. Twenty-two SNPs from the novel genetic loci were selected for evaluation in the Turkish replication samples. Three of these SNPs with $P < 0.001$ and one SNP from the phenotypic subset analysis identified four

regions for validation and fine-mapping by i-PLEX assays (TOF-MS, Sequenom, San Diego, CA) using the original Turkish GWAS samples[6]. The *IL12A* locus with two SNPs with $P < 0.05$ in the replication samples was also investigated, but failed to reach genome-wide significance. For variants that failed TOF-MS design or reaction, TaqMan genotyping was performed (Applied Biosystems, Foster City, CA). Genotyping was performed in an unbiased fashion by masking the phenotype of the samples. For the fine-mapping, we used the Tagger SNP selection tool from HapMap to select SNPs to augment the coverage of the GWAS SNPs with the intent to obtain 100% coverage of the HapMap Phase III SNPs with greater than 5% minor allele frequency in the CEU HapMap population with pairwise $r^2 > 0.8$. Although already tagged, additional SNPs with $r^2 > 0.8$ with the most significantly associated SNP of the region were also included. Genotyping of the same samples used for the Turkish GWAS discovery collection was performed[6]. After quality control, the resulting coverage for the *CCR1* locus (chr3:45441901-46908964, hg18) was 92%, the STAT4 locus (chr2:191602386-191769025) was 84%, the *KLRK1-KLRC1* locus (chr12:10329925-10557292) was 92%, and the *ERAP1-2* locus (chr5:96026703-96305246) was 94%. The most significantly associated marker from each region was used for the replications. The Japanese replication collection genotypes were from the Japanese GWAS[7]. The Turkish replication collection samples were genotyped by TOF-MS or TaqMan assay.

mRNA expression data and migration assay

CCR1 mRNA expression data were extracted from the report by Zeller and colleagues, which includes genome-wide SNP data along with mRNA expression array data from monocytes of n = 1490 European ancestry individuals[31]. SNP rs7616215 showed association with *CCR1* mRNA expression ($p=9.54\times10^{-6}$), whereas *CCR3* data were not reported, indicating that the association of rs7616215 with *CCR3* is less significant ($p>5\times10^{-5}$). *STAT4* mRNA expression data in lymphoblastoid samples were obtained from Gene Expression Omnibus (GEO) datasets[36, 37]. Primary human monocytes from unrelated healthy volunteers were isolated from peripheral blood mononuclear cells by MACS Human Monocyte Isolation Kit (Miltenyi Biotec, Gladbach, Germany). RNA was isolated from PBMCs by RNeasy kit (Qiagen, Valencia, CA) and preserved at −80 C° until used. cDNAs were prepared from DNase I (Invitrogen, Carlsbad, CA)-pretreated-RNA using SuperScript II according to manufacturer's protocol (Invitrogen). Q-PCR gene expression assay for human *CCR1* (Hs00174298_m1) was purchased from Applied Biosystems (Foster City, CA). Human GAPDH (4310884E) served as an internal control. Multiplex PCR (*GAPDH* with *CCR1*) was performed in triplicate or quadruplicate as in the manufacturer's protocol (Applied Biosystems). The $\Delta\Delta C_T$ method was used for the analysis (n=93).

A monocyte migration assay was performed with 24-well Transwell 5 μm polycarbonate membrane chambers (Costar, Corning, NY). 1% bovine serum albumin RPMI1640 medium was used for incubation of the cells. 5×10^4 cells were seeded in the upper chamber and the CCR1 chemokine MIP1-α (0 or 10ng/ml) (R&D, Minneapolis, MN) was placed in lower chamber. After incubation for 2 hours, cells that migrated into the membrane were fixed and stained by DiffQuik (Siemens, Newark, DE). Cells were counted in 5 high power fields on membranes from each of two duplicate wells. The relative change in cell migration in

response to the chemokine was determined by dividing the migrated cell count obtained with MIP1-α by the cell count obtained from the same sample without MIP1-α (n=61).

Statistical analysis

Genome-wide SNP association tests were performed based on allelic tests by comparing the allele frequencies between BD cases and controls, using Golden Helix SVS 7.5.2 software (Golden Helix, Bozeman, MT). $P<5\times10^{-8}$ was considered genome-wide significance. For the uveitis subset analysis, as well as the basic allelic test, we applied dominant and recessive genetic model tests and therefore employed 1.67×10^{-8} for genome-wide significance. Conditional analyses were performed by fitting the logistic regression model with SNPs rs2617170 (for *KLRK1-KLRC1*) or rs7616215 (for *CCR1-CCR3*) as covariates. For meta-analysis, Cochran-Mantel-Haenszel tests were performed. To test for the interactive effects, we fit the log likelihood of the full model, including additive terms of the main effects and a multiplicative term of the interaction effect, versus a reduced model of the additive terms of the main effects only. P value was calculated by likelihood ratio tests between full and reduced models based on 1 degree of freedom Chi-square test statistics. For comparisons in expression and chemotaxis data, the Kruskal-Wallis rank sum test as a non-parametric version of one-way ANOVA and Spearman's rank correlation test were performed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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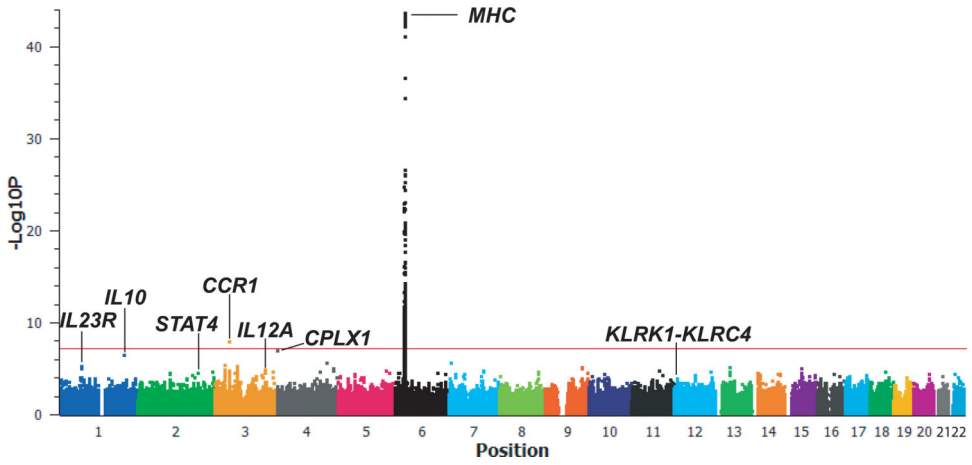

Figure 1. Manhattan plot of imputed SNPs

The $-\log_{10}P$ values for association of 779,465 autosomal imputed SNPs by basic allelic test in 1,209 Turkish BD cases and 1,278 controls. The results were segregated by chromosome and are shown by genomic position. The red horizontal line indicates genome-wide significance, $p=5 \times 10^{-8}$.

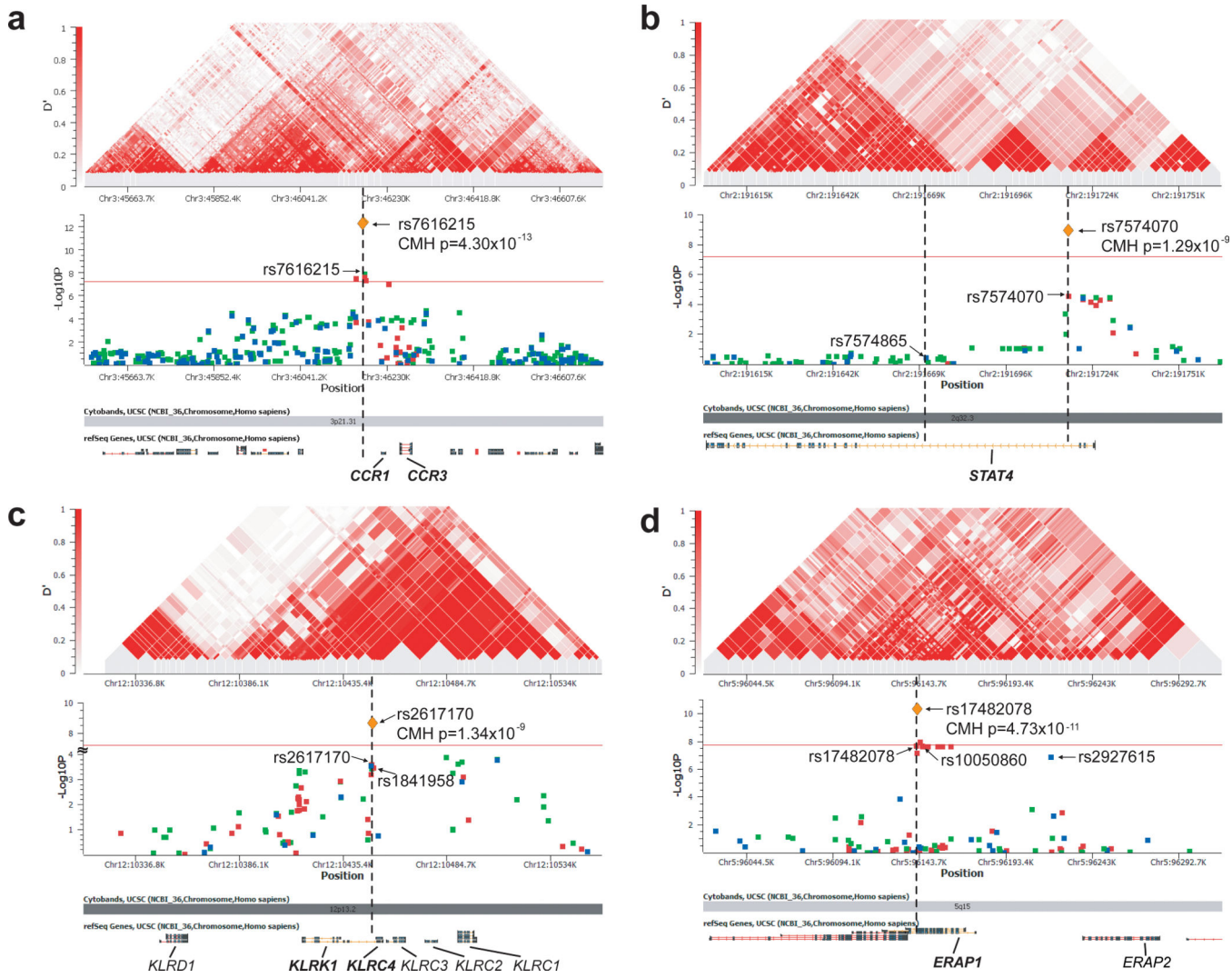

Figure 2. Regional association plots

Regional association plots (-$\log_{10}$ P values) and LD structures of the disease-associated regions surrounding (**a**) *CCR1-CCR3*, (**b**) *STAT4*, (**c**) *KLRK1-KLRC1*, and (**d**) *ERAP1-ERAP2*. The associations in (**a-c**) are for basic allelic tests, whereas the associations in (**d**) are for a recessive model test. Data are from the discovery collection with the genome-wide analysis genotypes (blue), imputed genotypes (green) and the fine-mapping genotypes (red). Orange diamonds show the meta-analyses results described in Table 1. Red horizontal lines in (**a-c**) indicate genome-wide significance ($p=5 \times 10^{-8}$) and in (**d**) indicates genome-wide significance accounting for three genetic models ($p=1.67 \times 10^{-8}$). SNP rs7574865 in (**b**) is a *STAT4* intronic SNP associated with multiple autoimmune diseases. The LD structures of the same regions are shown in the upper panels, with red filled squares linking pairs of markers that indicate the strength of LD by intensity of fill: $D' = 1$ (intense red) to $D' = 0$ (no fill).

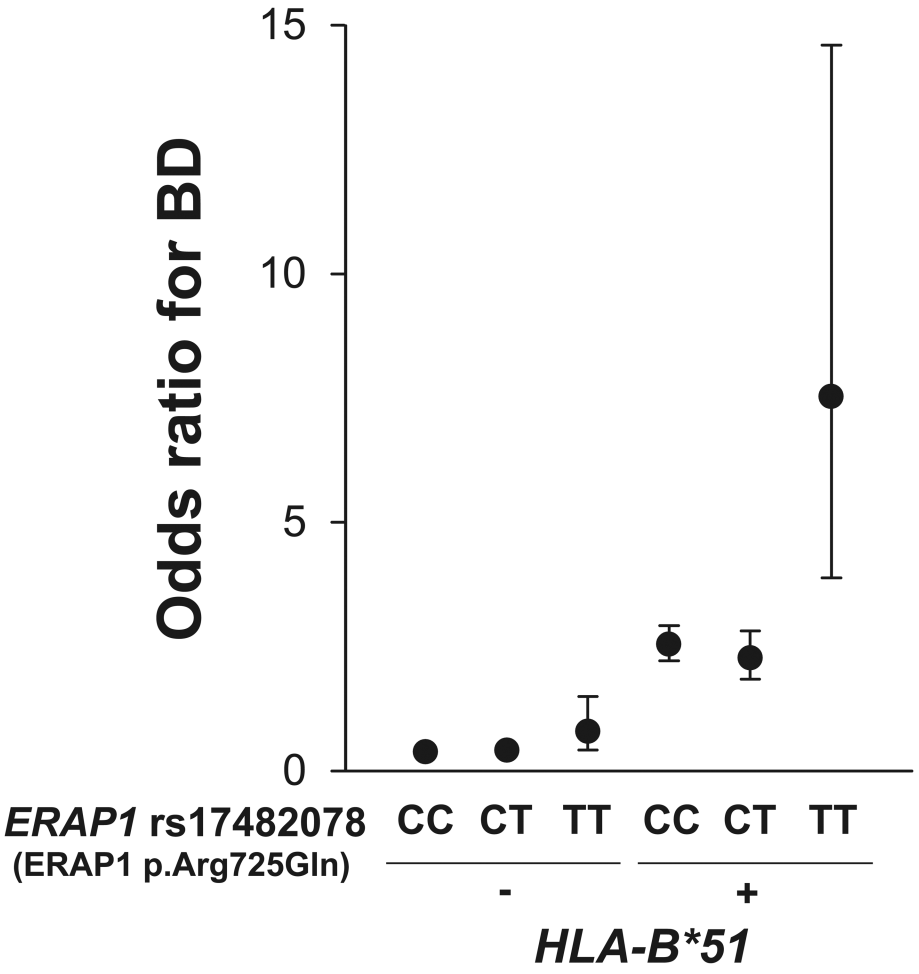

Figure 3. Epistasis between *HLA-B*51* and *ERAP1* rs17482078 (ERAP1 p.Arg725Gln) coding variant in BD

Epistasis was analyzed in the combined Turkish GWAS and replication samples. *HLA-B*51+* indicates individuals either heterozygous or homozygous for *HLA-B*51*. In the replication samples, the surrogate marker rs2848713 (r^2=0.68 with *HLA-B*51* in Turkish GWAS) was used to predict *HLA-B*51* positivity. Odds ratios for BD were determined comparing the frequency of the two marker genotypes in cases versus controls. The odds ratios shown are relative to the lowest disease-risk genotype group (*HLA-B*51* negative and *ERAP1* rs17482078 CC). Error bars represent the 95% confidence intervals. In an analysis restricted to the *HLA-B*51* positive individuals, the *ERAP1* TT genotype had an odds ratio of 3.78 (95% CI: 1.94-7.35), whereas in the *HLA-B*51* negative individuals, the TT genotype odds ratio was 1.48 (95% CI: 0.78-2.80).

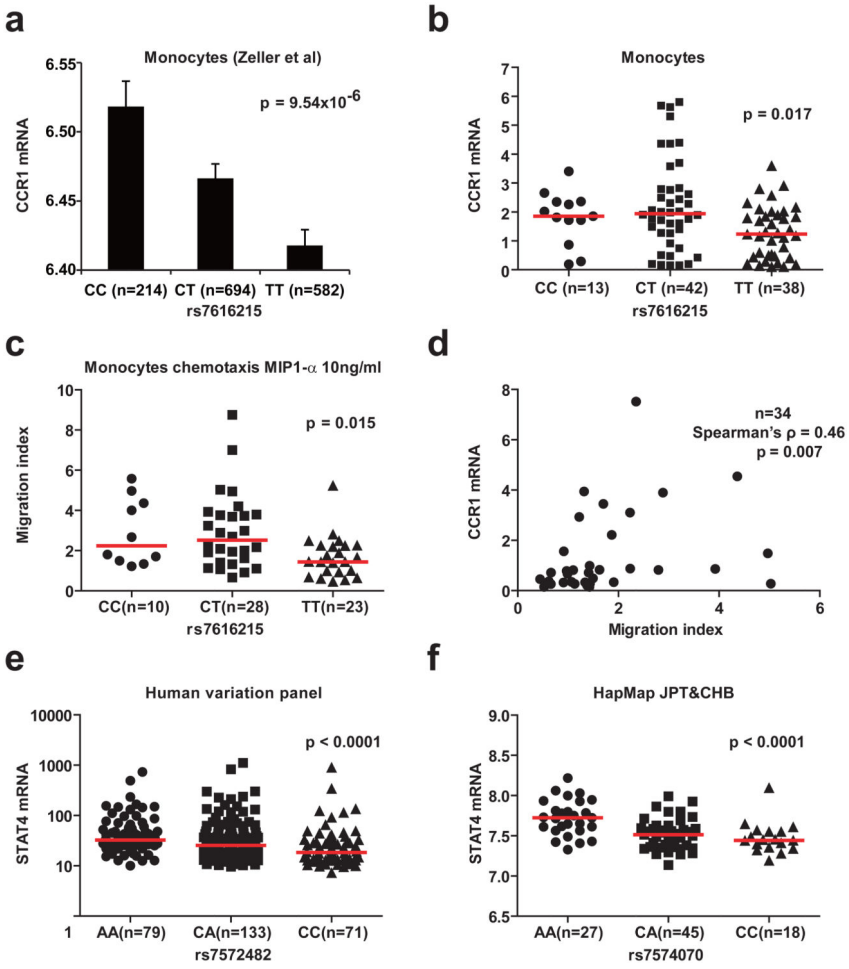

Figure 4. Association of *CCR1* and *STAT4* variants with gene expression levels and function in human cells

(**a**) Mean *CCR1* mRNA expression in human primary monocytes according to rs7616215 genotype (data from Zeller et al[31]). Error bars represent the standard error of the mean. (**b**) *CCR1* mRNA expression in human primary monocytes from our study according to rs7616215 genotype. (**c**) Chemotaxis of human primary monocytes against a gradient of MIP1-α according to rs7616215 genotype. "Migration index" indicates number of migrated cells in the presence of MIP1-α divided by the number of migrated cells in the absence of MIP1-α from the same sample. (**d**) Correlation between monocyte *CCR1* expression and chemotactic activity. Comparison between *CCR1* mRNA level and chemotaxis migration index were determined in monocytes isolated from the same blood sample. Spearman correlation test was performed. (**e**) *STAT4* mRNA expression in Human Variation Panel samples (GEO GSE24277). rs7572482 is a surrogate marker for rs7574070 (r^2=1, D′=1 in HapMap CEU samples). (**f**) *STAT4* mRNA expression in HapMap JPT and CHB samples according to rs7574070 genotype (GEO GSE6536). In panels b, c, e, and f, the horizontal bars indicate median values and the p-values shown are from the Kruskal-Wallis rank sum test.

Table 1

Cochran-Mantel-Haenszel meta-analysis of BD associated loci.

	Series*	Method	Diagnosis	Subject *n*	C	T	MAF(%)	OR (95% CI)	*P* value
***CCR1-CCR3* rs7616215**	Turkish Discovery	TOF-MS	BD	1180	630	1730	27	0.71 (0.63-0.80)	2.17×10^{-8}
			Control	1258	855	1661	34		
	Japanese replication	Affy 500K	BD	610	160	1060	13.1	0.82 (0.66-1.02)	0.079
			Control	692	215	1169	15.5		
	Turkish replication	TOF-MS	BD	816	441	1191	27	0.70 (0.60-0.82)	1.09×10^{-5}
			Control	630	436	824	34.6		
	Combined		**BD**	**2606**				**0.72 (0.66-0.79)**	**4.30×10^{-13}**
			Control	**2580**					
	Series	**Method**	**Diagnosis**	**Subject *n***	**A**	**C**	**MAF(%)**	**OR (95% CI)**	***P* value**
***STAT4* rs7574070**	Turkish Discovery	TOF-MS	BD	1166	1128	1204	48.4	1.28 (1.14-1.43)	1.92×10^{-5}
			Control	1250	1059	1441	42.4		
	Japanese replication	Affy 500K	BD	602	618	586	51.3	1.18 (1.01-1.37)	0.034
			Control	716	676	756	47.2		
	Turkish replication	TOF-MS	BD	815	803	827	49.3	1.34 (1.15-1.55)	1.12×10^{-4}
			Control	630	530	730	42.1		
	Combined		**BD**	**2583**				**1.27 (1.17-1.37)**	**1.29×10^{-9}**
			Control	**2596**					
	Series	**Method**	**Diagnosis**	**Subject *n***	**T**	**C**	**MAF(%)**	**OR (95% CI)**	***P* value**
***KLRC4* rs2617170 (KLRC4p.Asn104Ser)**	Turkish Discovery	TOF-MS	BD	1144	734	1554	32.1	0.80 (0.71-0.90)	2.54×10^{-4}
			Control	1257	933	1581	37.1		
	Japanese replication	Affy 500K	BD	580	361	799	31.1	0.75 (0.64-0.89)	8.36×10^{-4}
			Control	657	493	821	37.5		
	Turkish replication	TaqMan	BD	821	510	1132	31.1	0.77 (0.66-0.89)	7.61×10^{-4}
			Control	628	465	791	37		
	Combined		**BD**	**2545**				**0.78 (0.72-0.85)**	**1.34×10^{-9}**
			Control	**2542**					
	Series	**Method**	**Diagnosis**	**Subject *n***	**A**	**G**	**MAF(%)**	**OR (95% CI)**	***P* value**

	Series*	Method	Diagnosis	Subject *n*	C	T	MAF(%)	OR (95% CI)	*P* value
***IL12A* rs17810546**	Turkish Discovery	Illumina chip	BD	1209	208	2210	8.60	1.64 (1.31-2.04)	1.20×10^{-5}
			Control	1278	139	2417	5.44		
	Turkish replication	TOF-MS	BD	821	136	1504	8.29	1.41 (1.06-1.89)	0.020
			Control	626	75	1173	6.00		
	Combined		**BD**	**2029**				**1.55 (1.30-1.85)**	**6.01×10^{-7}**
			Control	**1902**					
	Series	**Method**	**Diagnosis**	**Subject *n***	**TT**	**CC+CT**	**TT(%)**	**OR (95% CI)**	***P* value**
***ERAP1* rs17482078 (ERAP1 p.Arg725Gln)**	Turkish Discovery	TOF-MS	Uveitis BD	420	32	388	7.62	4.21 (2.45-7.23)	2.03×10^{-8}
			Control	1249	24	1225	1.92		
	Turkish replication	TOF-MS	Uveitis BD	370	21	349	5.67	5.36 (2.46-11.7)	2.44×10^{-5}
			Control	630	7	623	1.11		
	Combined		**Uveitis BD**	**790**				**4.56 (2.88-7.22)**	**4.73×10^{-11}**
			Control	**1879**					

* TOF-MS, time-of-flight mass-spectrometry; MAF, minor allele frequency, OR, odds ratio; 95%CI, 95% confidence interval. Meta-analysis results are denoted in **bold**. Japanese samples were not included in meta-analysis of the *IL12A* rs17810546 and *ERAP1* rs17482078 variants because they are not polymorphic or they demonstrate too low frequency in the Japanese population.