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Identification of a systemic lupus erythematosus risk locus spanning *ATG16L2, FCHSD2*, and *P2RY2* in Koreans

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Competing Financial Interests:
The authors declare no competing financial interests.

Supplemental Data Summary
Supplemental data includes 16 tables and 14 figures.

Web-based Resources:
OMIM, www.omim.org/
SNPGWA version 4.0, www.phs.wfubmc.edu
SHAPEIT, www.shapeit.fr/

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Abstract

Objective—Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder whose etiology is incompletely understood, but likely involves environmental triggers in genetically susceptible individuals. We sought to identify the genetic loci associated with SLE in a Korean population by performing an unbiased genome-wide association scan.

Methods—A total of 1,174 Korean SLE cases and 4,248 population controls were genotyped with strict quality control measures and analyzed for association. For select variants, replication was tested in an independent set of 1,412 SLE cases and 1,163 population controls of Korean and Chinese ancestries.

Results—Eleven regions outside the HLA exceeded genome-wide significance ($P<5\times10^{-8}$). A novel SNP-SLE association was identified between *FCHSD2* and *P2RY2* peaking at rs11235667 ($P = 1.0\times10^{-8}$, odds ratio (OR) = 0.59) on a 33kb haplotype upstream to *ATG16L2*. Replication for rs11235667 resulted in $P_{meta\text{-}rep}=0.001$ and $P_{meta\text{-}overall}=6.67\times10^{-11}$ (OR=0.63). Within the HLA region, association peaked in the Class II region at rs116727542 with multiple independent effects. Classical HLA allele imputation identified HLA-DRB1*1501 and HLA-DQB1*0602, both highly correlated, as most strongly associated with SLE. We replicated ten previously established SLE risk loci: STAT1-STAT4, TNFSF4, TNFAIP3, IKZF1, HIP1, IRF5, BLK, WDFY4, ETS1 and *IRAK1-MECP2*. Of these loci, we identified previously unreported independent second effects in *TNFAIP3* and *TNFSF4* as well as differences in the association for a putative causal variant in the *WDFY4* region.

Conclusions—Further studies are needed to identify true SLE risk effects in other suggestive loci and to identify the causal variant(s) in the regions of *ATG16L2, FCHSD2,* and *P2RY2.*

Systemic lupus erythematosus (SLE; [MIM152700]) is a chronic, heterogeneous autoimmune disease characterized by the loss of tolerance to self-antigens, dysregulated type I interferon responses, and inflammation, often resulting in systemic end-organ damage(1). Immune dysfunction of SLE involves both B and T lymphocytes of the adaptive immune system, together with elements of the innate immune system, including dendritic cells and the complement system(1). The clinical manifestations of SLE can be quite variable and can involve virtually any organ system. Although the precise etiology of SLE is largely unknown, the pathogenic mechanism likely involves environmental triggers in a genetically susceptible host(2). Few effective treatment options exist, largely due to an incomplete understanding of the pathophysiological basis of the disease.

Genetic predisposition leading to increased risk of SLE is supported by high heritability (>66%), increased risk among siblings of affected patients ($\lambda_s\approx30$), and an ~25% monozygotic twin concordance(3). Today, associations of more than 50 loci with SLE susceptibility have been identified and confirmed(4). Many of these genes fall into known pathways that are key to innate and adaptive immune responses, lymphocyte activation and/or function, and immune complex clearance(4). However, a significant proportion of heritable risk to SLE has yet to be explained(5). The identification of SLE-associated genes and their pathogenic mechanisms will greatly enhance our understanding of lupus pathophysiology and facilitate the development of effective diagnostic, prognostic, and therapeutic tools. To date, large-scale genome-wide genetic studies of Asian SLE populations have focused on Han Chinese(6-8) and Japanese(9). Moreover, several reports have shown that transracial mapping of SLE loci can aid in the dissection of risk effects(4). In this study, we performed a genome-wide association (GWA) scan to identify genes associated with SLE in an East Asian population from Korea.

Methods

Subjects

A total of 1,174 patients with SLE were recruited from the Hanyang University Hospital for Rheumatic Diseases (HUHRD) and six other university hospitals in Korea(10). In addition, 552 ethnically matched healthy controls were recruited from HUHRD. The 3,700 ethnically matched out-of-study population controls were recruited from the Korean National Institutes of Health(10). In addition, an independent cohort of 1,412 SLE cases and 1,163 population controls were used for the replication studies(11, 12). This sample set consisted of 739 Korean SLE cases and 436 Korean controls as well as 677 Chinese SLE cases and 709 Chinese controls (Supplementary Table 1).

Written, informed consent from each participant was obtained by each participant following protocols approved by the Institutional Review Boards of participating institutes. All cases used in this study fulfilled at least 4 of the 11 American College of Rheumatology criteria for SLE(13), while healthy, population-based controls were without family history of SLE or any other autoimmune disease.

GWA scan Genotyping, Sample Quality Control, and Ascertainment of Populations Stratification

Samples were genotyped using the Illumina HumanOmni1-Quad or HumanOmniExpress arrays using Infinium chemistry at Oklahoma Medical Research Foundation (OMRF) following the manufacturer's protocol (Illumina, Inc., San Diego, CA). The out-of-study GWA controls were genotyped on the HumanOmni1-Quad arrays by the Korea National Institutes of Health. Strict quality control standards were implemented for SNPs retained in the association analysis, including requirements for well-defined cluster scatter plots. Samples were excluded if they had a SNP call rate <90%. SNPs were considered high quality SNPs if they had call rates >95%, no evidence of differential missingness between cases and controls ($P < 0.05$) and no evidence of a departure from expected Hardy-Weinberg proportions (controls $P < 0.01$, cases $P < 0.000001$). Inference is primarily based on those SNPs with minor allele frequency (MAF) greater than 1%.

Based on the SNPs that passed the above quality control thresholds, samples were removed if there were inconsistencies between recorded and genotype-inferred gender or excess heterozygosity on the autosomes. Duplicates and first- or second-degree relatives were removed based on identity-by-descent statistics computed by the program KING(14). Principal components (PCs) were computed with the samples and merged with HapMap phase 3 individuals (CEU, YRI, and CHB) as reference populations(15) using EIGENSOFT(16). Principal component analysis (PCA) was performed on a subset of autosomal SNPs that were selected by removing regions of known high linkage disequilibrium (LD), removing variants with MAF < 0.05, and pruning markers to reduce extended pairwise LD. The PCs were used to remove genetic outliers (Supplementary Figure 1). The dataset that passed laboratory and statistical quality control was composed of 1174 SLE cases (1096 females and 78 males) and 548 within-study controls (547 females and 1

male). In addition, 3698 out-of-study controls (2330 females and 1368 males) were merged into the within-study genotype data.

Statistical Analysis

To test for an association between a SNP and SLE status, a logistic regression analysis was computed including PC 3 as a covariate since no additional PC significantly changed the inflation factor (λ). Primary inference was based on the additive genetic model unless there was significant lack-of-fit ($P < 0.05$). If there was evidence of a departure from an additive model, then inference was based on the most significant value from the dominant, additive or recessive genetic models. The additive and recessive models were computed only if there were at least 10 and 30 individuals homozygous for the minor allele, respectively. The analyses were completed using the program SNPGWA version 4.0. For the analysis of chromosome X SNPs, the samples were stratified by gender and then meta-analyzed across gender using the program METAL(17).

To determine the number of independent associations within each SLE-risk locus exceeding the genome-wide significance threshold, a manual stepwise model or conditional analysis was computed. The stepwise modeling or conditional analysis was implemented using forward selection with backward elimination using the entry and exit criteria of $P < 0.0001$, which accounted for approximately 500 independent variants within a given genomic region. Specifically, for each region of interest, the top SNP was included as a covariate and the association statistics were re-calculated. SNPs were allowed to enter and exit models in this stepwise fashion until no additional SNPs met a significance threshold of $P < 0.0001$.

Replication Genotyping, Sample Quality Control, and Ascertainment of Populations Stratification

Genotypes were obtained using TaqMan assays (Life Technologies, Grand Island, NY) for four SNPs: rs2267828, rs10901656, rs11235667, and rs1048257. Analysis was conducted for these cohorts independently to allow for PC analysis using previously collected data. Ancestry adjustments for the Koreans were described previously in Lessard et al., (11). For the Chinese subjects, the PCA was done with slight modification from what was reported in Kaiser et al. (12). In this study, 7,918 randomly selected autosomal ImmunoChip SNPs with MAF>1%, low pairwise LD ($r^2 < 0.1$), and no evidence of association with SLE ($P > 0.01$) were used to perform PC analysis using EIGENSOFT. PC analysis plots of the CHB and CHS subjects in the 1000 Genomes Project along with our subjects were used to select and remove genetic outliers. The first PC (Chinese cohort) and PCs 1, 2, and 3 (OMRF and UCLA Korean datasets) were included as covariates in the logistic regression models based on the variance explained in each dataset. These dataset were than meta-analyzed using the program METAL(17). To test for heterogeneity among the individual association results in the meta-analysis, we utilized both the Cochran's Q test statistic(18) and I^2 index(19).

Imputation

To help localize the associations in the genome-wide significant regions, ungenotyped variants were imputed based on the reference panel from the 1000 Genomes Project(20). Specifically, the program SHAPEIT was used to pre-phase the genotype data(21). After

phasing the data, IMPUTE2 was used for the imputation with the 1000 Genomes Phase I integrated reference panel(22). The imputed data was filtered using standard post-imputation quality control based on IMPUTE2 information scores >0.5 and confidence scores >0.9 for subsequent association tests. Post-association analysis required genotyped SNPs in LD with imputed variants to support the inferred alleles as true signals. The program SNPTESTv2 was used to test for association of the imputed variants(23).

Imputation of the HLA classical alleles in the genes HLA-A, -B, -C, -DPB1, -DQA1, -DQB1, and -DRB1 was done using the program HiBAG(24) and the Asian reference panel. In this sample, ~21% of the reference SNPs used by HiBAG were missing genotype data. To address this issue, HLA imputation was repeated after filling in the missing genotype data with the "best guess" imputed SNP data from the 1000 Genomes imputation described above. By using the "best guess" genotype data with a posterior probability >0.90, the percent of missing variants in the reference set was reduced to 0.36%.

Results

Summary of the genome-wide association phase

We observed modest inflation in the test statistic (λ=1.09) with only slight deviation from expected once the HLA and other known SLE loci were removed (Supplementary Figure 2). A total of eleven regions surpassed the genome-wide significance threshold of $P < 5\times10^{-8}$ with *STAT4* (MIM600558) yielding the most significant genotyped association with SLE at rs11889341 ($P = 8.02\times10^{-19}$; Figure 1A and Table 1). Of the non-HLA regions, 10 risk loci had been previously identified and confirmed as risk loci for SLE, including *STAT1* (MIM600555)-*STAT4*, *IKZF1* (MIM603023), *TNFAIP3* (MIM191163), *TNFSF4* (MIM603594), *HIP1* (MIM601767), *IRF5* (MIM607218), *ETS1* (MIM164740), *BLK* (MIM191305), *WDFY4* (MIM613316), and *IRAK1* (MIM300283)-*MECP2* (MIM300005). In addition, association not previously described for SLE risk was observed at 11q14 (Figure 1A).

Association at 11q14 with SLE

This SNP-SLE association was observed with a single variant located between *FCHSD2* (MIM not available) and *P2RY2* (MIM600041) (rs11235667; $P=1.03\times10^{-8}$; odds ratio (OR) = 0.59; 95% confidence interval (CI) = 0.50-0.71; Figure 1B, Table 2, and Supplementary Table 2). Moreover, additional support was observed with genotyped variants in the region (Supplementary Table 2). After imputation of the 11q14 region showing association with SLE, rs11235667 remained the most significant association (Figure 1B and Supplementary Table 2). However, a haplotype was identified with 8 variants exceeding the genome-wide significance threshold that spanned from *ATG16L2* (MIM not available) through *FCHSD2* to the shared promoter region with *P2RY2*. Stepwise logistic regression analysis adjusting for rs11235667 indicated the presence of only a single effect (Supplementary Figure 3).

Replication analysis for the primary signal in the region of *FCHSD2-P2RY2* was done using independent cohorts from Korea and China (Supplementary Table 1). The SNP rs11235667 between *FCHSD2* and *P2RY2* continued to show significant SLE association and similar

effect size ($P_{meta\text{-}rep}$ = 0.001; OR = 0.71, 95% CI = 0.57-0.87). The overall meta-analysis between GWA and replication studies yielded a $P_{meta\text{-}overall} = 6.67\times10^{-11}$ (OR = 0.63, 95% CI = 0.55-0.72; Table 2). No evidence of heterogeneity was observed in the meta-analysis (Table 2).

Bioinformatics analysis using Haploreg v2(25) revealed that the region around rs11235667 was hypersensitive to DNase 1 in B cells by the ENCODE project(26). This variant has been shown to be located within an enhancer element in multiple immunological cell types based on the Epigenetic Roadmap data (Supplementary Table 3)(27). Chromatin immunoprecipitation followed by sequencing (ChIP-seq) experiments carried out by the ENCODE project found POL2 and YY1 proteins cross-linked to this region. Moreover, sequence prediction methods indicate that rs11235667 can alter the binding motif for the FOXa family of transcription factors using sequence prediction methods according to Haploreg v2(25). These data suggest the likely functional mechanism involves regulation of expression of *ATG16L2, FCHSD2* and/or *P2RY2*. However, current eQTL databases do not suggest that rs11235667 influences the expression of these loci(25, 28). This could be due to the lack of data in the correct cell and/or tissue type and/or that some databases do not interrogate this SNP in their studies.

Of the other 8 variants that exceeded genome-wide significance on the haplotype, several findings make rs11235604 an intriguing potential causal variant (Supplemental Table 3). This variant is a missense allele (R220W) that resides in the coding region of *ATG16L2*. Although it is predicted to be benign by PolyPhen-2(29), it is possible this variant my still impact SLE. Haploreg v2(25) does report that rs11235604 alters 8 predicted regulatory motifs, and this variant is thought to be an active enhancer in several immunologically relevant cell types (Supplementary Table 3). Further work is needed to conclusively identify the polymorphism(s) responsible for this association signal. These studies would include evaluating the potential impact of rs11235667 on the expression of *ATG16L2, FCHSD2,* and/or *P2RY2.* In addition, experiments are needed toassess the impact on *ATG16L2* of the missense allele arising from rs11235604 and/or any other variant(s) within this haplotype.

Association in the HLA region in Koreans with SLE

One of the most consistent associations with SLE has been with the human leukocyte antigen (HLA) region. Although the HLA was not the most statistically significant genotyped region, the SNP rs116727542 ($P = 6.15\times10^{-24}$; Table 3), which is located in a broad peak of association that was observed spanning HLA-DR (MIM142860) through -DQ (MIM146880), showed the strongest SNP-SLE association after imputation (Figure 2A, Table 3, and Supplementary Table 4). The interval between HLA-DR and -DQ has previously been implicated Koreans(30). In an attempt to identify the number of independent effects in this complex region, we used the stepwise approach described above and found ten independent effects (see Supplementary Figure 4A for results of the stepwise regression analysis). The first four variants identified in the stepwise regression analysis, rs116727542, rs9273371, rs114653103, and rs115253455, are all located in the HLA Class II region (Figure 2A, Supplementary Figure 4A, and Supplementary Table 4).

To better understand the relationship between the variants reported in this study and the classical HLA alleles, we imputed alleles at HLA A, B, C, DPB1, DQA1, DQB1 and DRB1. The peak statistical significance was observed at $P = 5.55\times10^{-16}$ for the two tightly linked alleles, HLA-DRB1*1501 (OR = 1.85; 95% CI = 1.59-2.14) and HLA-DQB1*0602 (OR = 1.90; 95% CI = 1.62-2.21; Figure 2A, Table 4, and Supplementary Table 5). Stepwise logistic regression modeling of the HiBAG-imputed HLA alleles identified 13 independent effects (Table 4). To better relate the classical alleles to the variants identified in this GWA scan, stepwise modeling was done with both SNPs and classical HLA alleles. The peak effect after 1000 Genomes imputation was rs116727542, which accounted for by HLA-DQB1*0602 and HLA-DRB1*0803 (see Supplementary Figure 4B for results of the additional 8 rounds of the stepwise regression analysis).

Non-HLA SLE associations previously reported and identification of novel independent effects

Several previously identified non-HLA SLE loci were also replicated in this study, including: STAT1-STAT4, TNFSF4, TNFAIP3, IKZF1, HIP1, IRF5, BLK, WDFY4, ETS1, and *IRAK1-MECP2* (Table 1, Figure 2B-D, Supplementary Figures 5 to 11, and Supplementary Tables 6 to 15. Of these loci, the associations in the region of *TNFAIP3, TNFSF4*, and *WDFY4* have notable differences from previous studies.

After imputation of the *TNFAIP3* region, the primary independent effect in the stepwise model was observed at rs5029937 located within the second intron of *TNFAIP3* (Table 1 and Figure 2B). The second independent effect was identified at rs9373203 3' of the *TNFAIP3* coding region. A previous SLE study in Han Chinese(6) reported rs2230926 as associated with disease, and another SLE transracial mapping study in Koreans (with partial overlap of subjects with the current study) and Europeans(31) identified risk of SLE with rs7749323. Both variants (rs2230926 and rs7749323) are highly correlated with rs5029937, with D' = 1.0 and $r^2 > 0.98$, indicating consistency between our results and these previous reports (Supplementary Figure 12). In addition, the second effect tagged by rs9373203 was not identified in either the Han et al or Adrianto et al. studies (Figure 2B). Musone et al. (32) identified multiple effects, some of which spanned even further 3' of *TNFAIP3* than rs9373203. After their stepwise analysis, they identified rs6922466 as the tagging variant accounting for this association; however, this variant is not associated with SLE in the current study of Koreans. Moreover, the LD between these variants is very weak in Koreans ($r^2 = 0.00$; D' = 0.43), giving additional evidence that rs9373203 may be an independent effect warranting further study (Supplementary Figure 12).

In the region of *TNFSF4* after imputation, two independent effects were observed in the stepwise model. The first effect, peaking at rs76413021, is located in the first intron of the *TNFSF4* coding region (Table 1 and Figure 2C). This variant is in LD with rs2205960 (D'=0.98, r^2=0.94) and rs1234315 (D' = 0.97, $r^2 = 0.48$) and was previously identified from the Han Chinese GWA scan(6) (Supplementary Figure 13). Moreover, this effect is consistent with the results reported in Europeans(33). The second independent effect, which is distinct from previous studies, peaks at rs4916342, which is located in an intron LOC100506023 just 5' of *TNFSF4* (Figure 2C). Neither Han et al. (6) nor Cunninghame

Graham et al. (33) reported association signals as far 5' of *TNFSF4* as our observation of the second independent effect tagged by rs4916342. In a transracial mapping study of this region by Manku et al. (34) that included subjects from East Asia, a second independent effect (tagged by rs1234314) was identified that is. In our current study of Koreans, we found that the first effect, tagged by rs76413021, accounted for rs1234314 in the stepwise model. Although rs1234314 and rs4916342 are located in the same general genomic location, the LD structure further supports the observation that they are not the same genetic effect; however, all the risk variants are located on a single risk haplotype (Supplementary Figure 13). This suggests that risk alleles for both rs76413021 and rs4916342 are needed to confer susceptibility to disease.

In the region of *WDFY4*, the current study did not replicate rs877819, which has been previously reported to result in a downregulation of *WDFY4* through modification of a YY1 binding site (Table 1)(35). However, the results for *WDFY4* in Koreans are consistent with two previous studies. First, the most statistically significant association within this region, the coding variant rs7097397 leading to an amino acid substitution R1816Q ($P = 2.10\times10^{-9}$), was previously reported by Yang et al. (8). Second, we also demonstrate association for rs1913517, which was identified previously by Han et al. (6) ($P = 2.54\times10^{-5}$; Table 1 and Figure 2D). Our haplotype and stepwise regression analysis indicated that there were two independent effects in the region, with rs7097397 accounting for the association observed at rs1913517, and rs10857631 tagging the second independent effect (Figure 2D and Supplementary Figures 14 and 15).

Suggestive association with SLE identified in the genome-wide phase

In total, 15 genotyped variants surpassed the suggestive threshold of $P < 2\times10^{-6}$ and were considered for further replication (Supplementary Table 16). Replication was attempted for three additional variants located within *GTF2IRD1, DOCK1*, and *AHNAK2,* all of which have multiple genotyped variants showing suggestive significance and/or have been previously implicated in other related phenotypes (Table 2). Only rs2267828 near *GTF2IRD1* yielded a $P_{meta\text{-}rep} < 0.05$, but this variant did not surpass genome-wide significance after meta-analysis with the GWA scan (Table 2). The variant in the region of *AHNAK2,* rs1048257, was trending towards significance, while rs10901656 near *DOCK1* showed association in one replication cohort with the opposite allele (Table 2). Outside of the 10 regions previously reported SLE loci described above, we observed only eight additional loci with $5\times10^{-8} < P < 5\times10^{-5}$ on the list of ~50 that have been described previously (Supplemental Table 17). This is likely due to the limited power of this study and/or results from population-specific differences from the studies in which these discoveries were originally identified.

Discussion

The association in this region peaks between three candidate genes, *ATG16L2*, *FCHSD2*, and *P2RY2*, all of which have the biological potential to impact SLE pathophysiology. While this locus has not been reported in other systemic autoimmune diseases, variants in this region are associated with Crohn's disease (MIM266600) in Korean subjects(36).

Moreover, the peak variant identified in Crohn's disease, rs11235667, was also the variant discovered in this current study with SLE. The missense variant, rs11235604, was also reported to be associated in Crohn's disease(36). GWA studies conducted in SLE in Europeans did not identify this locus since it is monomorphic in that population. Moreover, GWA scans in Han Chinese both used the Illumina Human 610-Quad bead chip, which did not contain rs11235667(6-8).

ATG16L2 (autophagy related 16-like 2) is a ubiquitously expressed homologue of the gene *ATG16L1* (MIM610767) that has been implicated as a risk locus for Crohn's disease in patients of European descent(37, 38). Both loci are involved in autophagy; however, little is known about the role *ATG16L2* plays in the process. Interestingly, this pathway has been previously implicated in SLE. The gene *ATG5* (MIM604261) has also been implicated as a risk locus for lupus(6, 39). Studies in the mouse have shown that Apg16l (the mouse equivalent of human ATG16L) interacts with Apg5 (the mouse equivalent of human ATG5) suggesting that it is possible that ATG16L2 and ATG5 may also interact humans(40). More studies are needed to understand the function *ATG16L2* and if it is involved in the association with SLE.

FCHSD2 (FCH and double SH3 domains 2) has been described as regulator of F-actin assembly through interactions with WAS (also known as WASP) and WASL (also known as N-WASP)(41). *FCHSD2* is primarily expressed in CD19+ B cells, dendritic cells, myeloid cells, CD4+ T cells, and CD8+ T cells(38). Previous studies have shown that WAS plays an important role in the migration of T cells through reorganization of the actin cytoskeleton subsequent to interactions with dendritic or B cells(42).

P2RY2 (purinergic receptor P2Y, G-protein coupled, 2) is known to be involved in many cellular functions and is expressed in myeloid cells including monocytes(38). P2RY2 is a receptor for ATP and UTP that acts as a sensor for the release of nucleotides by apoptotic cells(43). Mice null for P2RY2 showed a decreased ability to recruit monocytes and macrophages upon activation of nucleotides from apoptotic cells(43). P2RY2 is also known to induce CCL2 secretion in macrophages, and coding variants in the receptor have been shown to influence secretion of this proinflammatory chemokine(44).

Although the HLA region has been implicated in SLE susceptibility since the 1970s, the precise loci responsible for risk have not been fully characterized. A further cross comparison of populations will be beneficial to take advantage of differences in linkage disequilibrium and will likely help further refine association signals seen in the GWA studies. For the classical alleles, previous studies have identified associations with alleles in the HLA-DR locus in Europeans, Chinese, Japanese, and Koreans, but HLA-DQB1*0602 has not been implicated in Koreans before this current study(45-48). Two prominent Classical HLA alleles identified in Europeans with SLE showed differences in association in Koreans. While HLA-DRB1*1501 was among the most significantly associated with SLE, HLA-DRB1*0301 was found to be at low frequency in this population and is not associated with SLE (Supplementary Table 5). These results are consistent with a recent study in Koreans that evaluated the role of HLA in this population(49). Moreover, this study report that amino acid changes to HLA-DRB1 at positions 11, 13, and 26 account for the HLA

association in SLE(49). However, it is possible that there are other amino acid changing variants, non-coding RNAs, and/or transcriptional changes for other HLA loci that are co-inherited with HLA-DRB1 on these haplotypes may also contributing to SLE risk.

This GWA scan replicated several loci that have been identified by prior studies, including STAT1-STAT4, TNFSF4, TNFAIP3, IKZF1, HIP1, IRF5, BLK, WDFY4, ETS1, and *IRAK1-MECP2*. It is important to note that a previous GWA scan of Korean women with SLE has also reported replication of *STAT4* and *BLK* at a genome-wide significant level(30). Of these replicated regions, *TNFSF4, TNFAIP3, IKZF1, HIP1, IRF5, BLK,* and *ETS1* have functional effects that have been previously described (see details in Supplementary Table 18). Although most of the signals in these loci are identical, we did describe notable differences with independent effects in *TNFSF4* and *TNFAIP3*. Moreover, we did not observe association with rs877819, which had been proposed as putative causal variant leading to expression differences of *WDFY4(35)*.

In conclusion, we performed a GWA scan of Korean SLE cases and population controls in which we identified 12 regions that surpassed genome-wide significance. The region from *ATG16L2* through *FCHSD2* to the promoter region of the *P2RY2* locus was identified and confirmed as an SLE-associated region. Here, we also observed strong associations in the HLA region and showed the relationship between the classical HLA alleles and the variants reported within this GWA study in Koreans. The ten additional regions, STAT1-STAT4, TNFSF4, TNFAIP3, IKZF1, HIP1, IRF5, BLK, WDFY4, ETS1, and *IRAK1-MECP2* have previously been implicated in SLE. Additional replication is needed for the suggestive loci identified in this study to determine their relationship with SLE. Although GWA approaches have been very successful in the identification of risk loci, continued efforts are need to narrow association signals to the causal variant(s) and to determine the functional causal mechanism(s) contributing to SLE pathogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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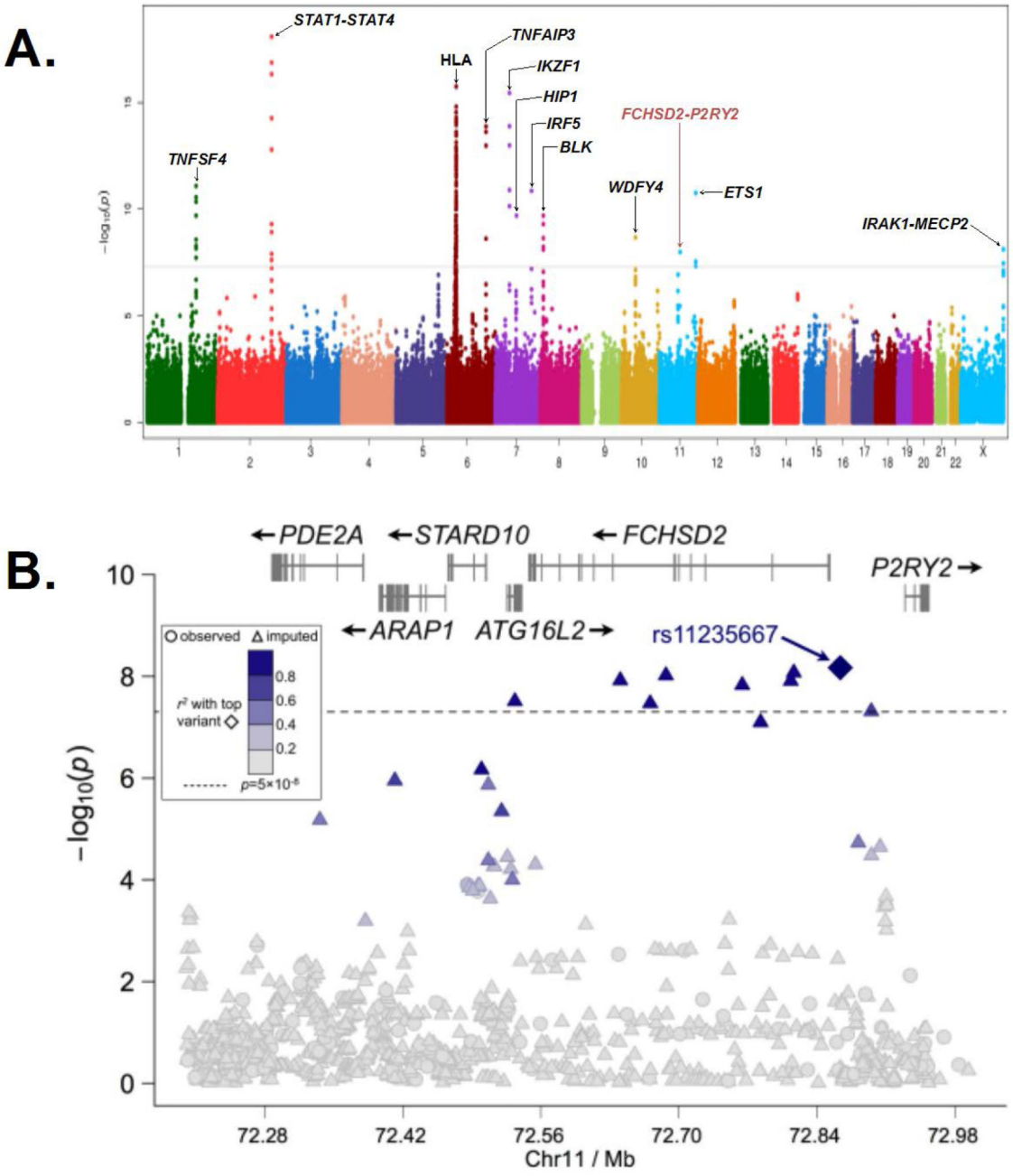

Figure 1. Summary of the genome-wide association results for 1174 SLE cases and 3698 controls of Korean ancestry and zoomed plot of the region associated with SLE at 11q14

(A) The -$\log_{10}$(P-value) for each genotyped variant is plotted along the Y-axis with the chromosome and chromosomal position along the X-axis. The gray line indicates the genome-wide significance threshold of $P = 5\times10^{-8}$. (B) The $-\log_{10}$(P-value) is plotted for each genotyped (shown as circles) and imputed (shown as triangles) variants, and with the peak association, rs11235667, is plotted as a diamond. The linkage disequilibrium with rs11235667 is given by the scale on the figure. The genome-wide significance threshold is displayed as a dashed line at $P = 5\times10^{-8}$. Association exceeding this threshold was found extending from *ATG16L2* through *FCHSD2* to the shared promoter region with *P2RY2*.

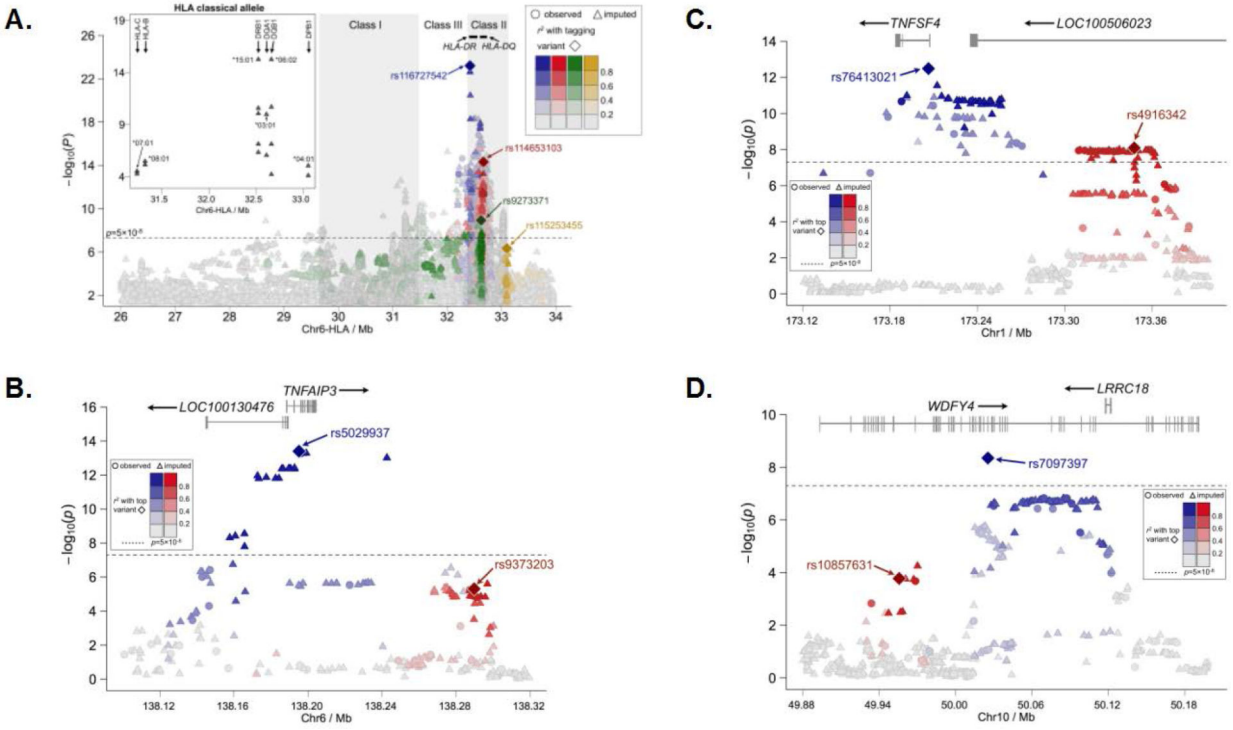

Figure 2. Expanded view of the association between SLE and the HLA, *TNFAIP3, TNFSF4,* and *WDFY4* regions

The $-\log_{10}$(P-value) is plotted for each observed (shown as circles) and imputed (shown as triangles) variant in the MHC region according to base-pair position from 26Mb to 34Mb on chromosome 6 (A). Linkage disequilibrium with the first four variants included in the stepwise logistic regression analysis is shown with rs116727542 (blue diamond), rs114653103 (red diamond), rs9273371 (green diamond), and rs115253455 (gold diamond) all located within the HLA Class II region. The insert on the left shows the $-\log_{10}$(P-value) of the imputed classical alleles plotted according to base-pair position from 31Mb to 33.5Mb. The additional plots show results for Chromosome 6 in the region of *TNFAIP3* (B), Chromosome 1 for the *TNFSF4* (C) effects, and Chromosome 10 for *WDFY4* (D). For each independent effect, the peak associations are represented by a diamond (blue for the first effect and red for the second, if applicable), and the correlation of variants accounted for by each effect is given in their respective color according the legends present in each plot. The genome-wide significance threshold is displayed as a dashed line on each plot at $P = 5 \times 10^{-8}$.

Table 1

Single locus analysis of previous SLE associations outside of HLA region

Marker	Chr[A]	Position	Upstream Gene	Downstream Gene	Within Gene	MAF[B]	P_{GWAS}	Model	Obs/Imp[C]	OR (95% CI[D])	Maj/Min[E]
rs1234314	1	173177392	1kb from *TNFSF4*	269kb from *PRDX6*	-	0.37	9.25×10^{-11}	Add	Imp	1.37 (1.25-1.51)	C/G
rs2205960	1	173191475	15kb from *TNFSF4*	255kb from *PRDX6*	-	0.25	1.03×10^{-11}	Add	Imp	1.44 (1.30-1.60)	G/T
rs76413021	1	173206297	30kb from *TNFSF4*	240kb from *PRDX6*	-	0.23	3.26×10^{-13}	Add	Imp	1.52 (1.36-1.71)	G/A
rs844644	1	173209495	33kb from *TNFSF4*	237kb from *PRDX6*	-	0.4	4.47×10^{-11}	Add	Obs	1.37 (1.25-1.50)	A/C
rs10489265	1	173236065	60kb from *TNFSF4*	211kb from *PRDX6*	-	0.25	8.19×10^{-12}	Add	Obs	1.43 (1.29-1.58)	A/C
rs4916342	1	173347837	172kb from *TNFSF4*	99kb from *PRDX6*	-	0.3	8.22×10^{-9}	Add	Imp	0.75 (0.67-0.82)	A/G
rs16833239	2	191940260	-	-	*STAT4*	0.15	9.69×10^{-10}	Add	Imp	0.67 (0.59-0.76)	G/A
rs11889341	2	191943742	-	-	*STAT4*	0.32	8.02×10^{-19}	Add	Obs	1.53 (1.40-1.69)	C/T
rs12612769	2	191953998	-	-	*STAT4*	0.31	2.37×10^{-19}	Add	Imp	1.59 (1.43-1.75)	A/C
rs13192841	6	137967214	152kb from *OLIG3*	221kb from *TNFAIP3*	-	0.13	7.50×10^{-3}	Dom	Obs	1.23 (1.06-1.43)	G/A
rs5029937	6	138195151	-	-	*TNFAIP3*	0.07	3.98×10^{-14}	Dom	Imp	2.11 (1.74-2.55)	G/T
rs5029939	6	138195723	-	-	*TNFAIP3*	0.07	4.09×10^{-14}	Dom	Imp	2.10 (1.74-2.55)	C/G
rs2230926	6	138196066	-	-	*TNFAIP3*	0.07	2.34×10^{-14}	Dom	Obs	1.93 (1.63-2.28)	T/G
rs9373203	6	138289848	86kb from *TNFAIP3*	120kb from *PERP*	-	0.37	4.89×10^{-6}	Add	Imp	1.25 (1.14-1.37)	C/T
rs6922466	6	138444930	16kb from *PERP*	38kb from *KIAA1244*	-	0.18	0.297	Dom	Obs	1.08 (0.94-1.24)	A/G
rs11185602	7	50299077	100kb from *C7orf72*	45kb from *IKZF1*	-	0.33	1.53×10^{-16}	Add	Imp	0.66 (0.60-0.73)	A/G
rs17552904	7	50318308	120kb from *C7of72*	26kb from IKZF1	-	0.33	3.51×10^{-16}	Add	Obs	0.65 (0.59-0.72)	G/T
rs6964720	7	75180344	-	-	*HIP1*	0.24	2.00×10^{-10}	Add	Obs	1.40 (1.26-1.56)	A/G
rs139110493	7	75209951	-	-	*HIP1*	0.06	1.21×10^{-12}	Dom	Imp	2.48 (1.93-3.19)	G/C
rs4728142	7	128573967	11kb from *LOC392787*	4kb from *IRF5*	-	0.14	1.38×10^{-11}	Add	Obs	1.53 (1.35-1.73)	G/A
rs113478424	7	128575797	13kb from *LOC392787*	2kb from *IRF5*	-	0.14	3.97×10^{-12}	Add	Imp	1.59 (1.39-1.81)	15-mer */T
rs922483	8	11351912	-	-	*BLK*	0.25	2.00×10^{-10}	Add	Obs	0.71 (0.64-0.79)	T/C
rs2736345	8	11352485	-	-	*BLK*	0.24	7.88×10^{-11}	Add	Imp	0.70 (0.63-0.78)	G/A
rs10857631	10	49955821	-	-	*WDFY4*	0.13	1.67×10^{-4}	Add	Imp	1.30 (1.13-1.48)	A/G
rs7097397	10	50025396	-	-	*WDFY4*	0.37	2.10×10^{-9}	Add	Obs	1.33 (1.21-1.46)	A/G
rs877819	10	50042951	-	-	*WDFY4*	0.16	0.0558	Add	Imp	1.13 (1.00-1.28)	G/A

Marker	Chr[A]	Position	Upstream Gene	Downstream Gene	Within Gene	MAF[B]	P_{GWAS}	Model	Obs/Imp[C]	OR (95% CI[D])	Maj/Min[E]
rs10776651	10	50084526	-	-	*WDFY4*	0.34	1.54×10^{-7}	Add	Imp	1.29 (1.18-1.43)	C/T
rs1913517	10	50119054	-	-	*WDFY4*	0.31	2.54×10^{-5}	Add	Obs	1.24 (1.12-1.37)	G/A
rs12576753	11	128304141	None within 500kb	25kb from *ETS1*	-	0.39	1.74×10^{-11}	Add	Obs	1.37 (1.25-1.56)	C/A
rs1128334	11	128328959	-	-	*ETS1*	0.38	7.17×10^{-12}	Add	Imp	1.39 (1.26-1.52)	G/A
rs5986948	X	153266172	17.3kb from *TMEM187*	9.8kb from *IRAK1*	-	0.24	4.36×10^{-10}	Rec	Imp	0.64 (0.56-0.74)	C/T
rs1059702	X	153284192	-	-	*IRAK1*	0.26	5.14×10^{-10}	Rec	Imp	0.65 (0.57-0.74)	A/G
rs2734647	X	153292180	-	-	*MECP2*	0.25	7.54×10^{-9}	Dom	Obs	0.62 (0.51-0.75)	T/C

[A] Chr = Chromosome

[B] MAF = Minor allele frequency

[C] Obs/Imp = observed/imputed

[D] CI = Confidence interval

[E] Maj/Min = Major/Minor allele

[*] 15-mer= CTTAGCTATTGCTC

Table 2

Single locus analysis results for regions genotyped in the replication study.

Marker	Region Name	Maj/Min[A]	MAF[B] Case / Ctrl[C]	P_{GWAS}	Model	OR (95% CI[D])	P Meta Rep[E]	Q / I^2 Meta Rep[E]	OR (95% CI[D]) Meta Rep[E]	P Meta Overall[F]	Q / I^2 Meta Overall[F]	OR (95%CI[D]) Meta Overall
rs2267828	GTF2IRD1	A/G	0.40 / 0.45	7.02×10^{-7}	Add	0.79 (0.72-0.87)	0.02	0.56 / 0	0.87 (0.77-0.98)	6.46×10^{-8}	0.41 / 0	0.81 (0.76-0.88)
rs10901656	DOCK1	C/T	0.27 / 0.23	6.91×10^{-7}	Dom	1.39 (1.22-1.58)	0.095	0.6 / 0	1.14 (0.98-1.31)	9.56×10^{-6}	0.23 / 28.72	1.21 (1.12-1.32)
rs11235667	FCHSD2-P2RY2	A/G	0.07 / 0.11	1.03×10^{-8}	Add	0.59 (0.50-0.71)	0.0014	0.29 / 18.43	0.71 (0.57-0.87)	6.67×10^{-11}	0.14 / 44.37	0.63 (0.55-0.72)
rs1048257	AHNAK2	T/C	0.34 / 0.39	1.67×10^{-6}	Add	0.79 (0.72-0.87)	0.086	0.29 / 17.06	0.90 (0.80-1.01)	8.66×10^{-7}	0.12 / 47.82	0.82 (0.76-0.89)

[A] Maj/Min = Major/Minor allele

[B] MAF = Minor allele frequency

[C] Case/Ctrl = Case/Control

[D] CI = Confidence interval

[E] Meta Rep = Meta-analysis for the replication

Table 3

Single locus and stepwise results for top ten independent HLA associations

Marker[A]	Position	Upstream Gene	Downstream Gene	Within Gene	MAF[B]	P-value	OR (95%CI[C])	Maj/Min[D]	Stepwise P-value	Stepwise OR (95%CI[C])
rs116727542	32421227	8.4kb from *HLA-DRA*	64kb from *HLA-DRB5*	-	0.1700	6.15×10^{-24}	0.53 (0.47-0.60)	G/A	1.96×10^{-18}	1.74 (1.54-1.97)
rs9273371	32626565	14kb from *HLA-DQA1*	675bp from *HLA-DQB1*	-	0.1000	1.18×10^{-9}	1.61 (1.38-1.87)	C/T	8.43×10^{-5}	1.38 (1.18-1.63)
rs114653103	32668846	34kb from *HLA-DQB1*	40kb from *HLA-DQA2*	-	0.1200	7.31×10^{-15}	0.57 (0.49-0.66)	G/T	1.81×10^{-13}	0.50 (0.41-0.60)
rs115253455	33100021	43kb from *HLA-DPB1*	30kb from *COL11A2*	-	0.1300	4.68×10^{-7}	0.70 (0.61-0.80)	T/A	4.51×10^{-7}	0.66 (0.56-0.77)
chr6:31996524	31996524	-	-	*C4B*	0.1700	4.95×10^{-8}	0.71 (0.63-0.80)	C/A	4.87×10^{-10}	0.64 (0.55-0.73)
rs113833333	32594898	37kb from *HLA-DRB1*	10kb from *HLA-DQA1*	-	0.4100	2.30×10^{-5}	0.82 (0.74-0.90)	C/T	3.09×10^{-8}	0.74 (0.67-0.82)
rs116427960	31319226	79kb from *HLA-C*	2.4kb from *HLA-B*	-	0.0081	8.96×10^{-7}	4.57 (2.49-8.38)	C/T	1.69×10^{-5}	3.17 (1.87-5.35)
rs114904515	29362756	20kb from *OR12D3*	1.7kb from *OR12D2*	-	0.1000	1.55×10^{-6}	0.69 (0.60-0.80)	C/T	5.70×10^{-6}	0.67 (0.56-0.79)
rs118044183	30954150	-	-	*MUC21*	0.1200	3.61×10^{-6}	1.50 (1.27-1.79)	C/T	1.39×10^{-4}	1.39 (1.17-1.64)
rs2736191	31560910	~150bp from *NCR3*	22kb from *AIF1*	-	0.3800	7.53×10^{-7}	0.79 (0.71-0.86)	C/G	1.01×10^{-4}	0.81 (0.73-0.90)

Note: Tables are in the order they were identified in the stepwise model. The stepwise results presented in this table are for adjusting for all other variants in the table. For complete results in the HLA region, please refer to Supplementary Table 4.

[A] All variants within this table have been imputed.

[B] CI = Confidence interval

[C] MAF = Minor allele frequency

[D] Maj/Min = Major allele/Minor allele

Table 4

Multi-locus model of HIBAG-imputed HLA dosages, single locus and stepwise results

HLA Allele	Dosage Frequency		Best Guess Count		OR (95% CI[A])	Single Locus P-value	Stepwise P-value	Stepwise OR (95%CI[A])
	Cases	Controls	Cases	Controls				
DQB1*0602[B]	0.25	0.15	301	636	1.90 (1.62 - 2.21)	5.55×10^{-16}	1.93×10^{-23}	2.35 (1.99 – 2.78)
DRB1*0803	0.20	0.13	251	613	1.59 (1.34 - 1.88)	7.37×10^{-8}	7.63×10^{-16}	2.14 (1.78 - 2.58)
DQB1*0202	0.18	0.12	212	502	1.60 (1.35 - 1.90)	7.57×10^{-8}	2.19×10^{-18}	2.50 (2.04 - 3.07)
DQA1*0302	0.19	0.16	286	847	1.41 (1.15 - 1.75)	1.27×10^{-3}	4.92×10^{-9}	1.98 (1.58 - 2.50)
B*0801	0.02	0.004	20	14	5.43 (2.66 - 11.08)	3.42×10^{-6}	5.15×10^{-6}	5.71 (2.70 -12.07)
DQA1*0401	0.04	0.03	54	133	1.73 (1.17 - 2.57)	5.94×10^{-3}	3.11×10^{-5}	2.36 (1.58 - 3.54)
C*0702	0.22	0.17	261	711	1.37 (1.17 - 1.59)	5.45×10^{-5}	2.06×10^{-3}	1.30 (1.10 - 1.54)
DRB1*0406	0.03	0.07	41	354	0.15 (0.09 - 0.26)	2.63×10^{-11}	1.11×10^{-4}	0.32 (0.18 - 0.57)
DPB1*0501	0.79	0.72	930	3092	1.16 (1.05 - 1.28)	2.87×10^{-3}	1.06×10^{-4}	1.23 (1.11 - 1.36)
DRB1*1602	0.03	0.02	38	87	1.78 (1.16 - 2.72)	7.73×10^{-3}	1.53×10^{-3}	2.05 (1.32 - 3.20)
DPB1*1701	0.03	0.04	41	160	0.90 (0.62 - 1.30)	5.66×10^{-1}	1.07×10^{-3}	0.5 (0.33 - 0.76)
C*0102	0.29	0.32	359	1429	0.88 (0.77 - 1.01)	6.51×10^{-2}	5.82×10^{-3}	0.82 (0.71 - 0.94)
DRB1*1202	0.04	0.07	39	266	0.52 (0.37 - 0.74)	2.98×10^{-4}	7.07×10^{-3}	0.61 (0.42 - 0.87)

Note: Tables are in the order they were identified in the stepwise model. The stepwise results presented in this table are for adjusting for all other variants in the table

[A] CI = Confidence interval

[B] The allele HLA-DRB1*1501 had the same P-value as HLA-DQB1*0602, but the later was selected by the stepwise modeling procedure. For complete results, please refer to Supplementary Table 5.