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Evaluation of TRAF6 in a Large Multi-Ancestral Lupus Cohort

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Abstract

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Objective—Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease with significant immune system aberrations resulting from complex heritable genetics as well as environmental factors. *TRAF6* is a candidate gene for SLE, which has a major role in several signaling pathways that are important for immunity and organ development.

Methods—Fifteen single-nucleotide polymorphisms (SNPs), across *TRAF6* were evaluated in 7,490 SLE and 6,780 control subjects from different ancestries. Population-based case-control association analyses and meta-analyses were performed. P values, false discovery rate q values, and odds ratios with 95% confidence intervals were calculated.

Results—Evidence of associations in multiple SNPs was detected. The best overall p values were obtained for SNPs rs5030437 and rs4755453 ($p=7.85\times 10^{-5}$ and $p=4.73\times 10^{-5}$, respectively) without significant heterogeneity among populations ($p=0.67$ and $p=0.50$ in Q-statistic). In addition, rs540386 previously reported to be associated with RA was found to be in LD with these two SNPs ($r^2=0.95$) and demonstrated evidence of association with SLE in the same direction (meta-analysis $p=9.15\times 10^{-4}$, OR=0.89, 95% CI=0.83–0.95). Thrombocytopenia improved the overall results in different populations (meta-analysis $p=1.99\times 10^{-6}$, OR=0.57, 95% CI=0.45–0.72, for rs5030470). Finally evidence of family based association in 34 African-American pedigrees with the presence of thrombocytopenia were detected in one available SNP rs5030437 with Z score magnitude of 2.28 ($p=0.02$) under a dominant model.

Conclusion—Our data indicate the presence of association of *TRAF6* with SLE in agreement with the previous report of association with RA. These data provide further support for the involvement of *TRAF6* in the pathogenesis of autoimmunity.

Keywords

TRAF6; polymorphism; systemic lupus erythematosus

The genetic etiology of systemic lupus erythematosus (SLE), the prototypical systemic autoimmune disease, is complex and includes many genetic loci interacting with environmental factors. There have been a number of SLE genome-wide association studies (GWAS) confirming known associations and identifying novel susceptibility genes (1–5).

In this case-control study, we selected and genotyped single nucleotide polymorphisms (SNPs) in *TRAF6* (tumor necrosis factor (TNF) receptor associated factor 6), a candidate SLE susceptibility locus. As an adaptor molecule that has a central role in the nuclear factor NF- κ B activation pathway, *TRAF6* is an important candidate gene for SLE. This pathway, which also includes mitogen-activated protein (MAP) kinases, is of critical importance to survival and activation of multiple immune cell subsets. It regulates inflammation, dendritic cell (DC) development, thymic selection and regulatory T cell production as well as osteoclast formation. The role of *TRAF6* in this pathway is essential, as it transduces signals from the TNF receptor (TNFR) superfamily, TLR/IL-1R family and CD40 to activate the transcription factors NF κ B and activator protein-1 (AP1) (6). Unlike other *TRAF* family members, *TRAF6* also participates in TLR/IL-1R family signaling. The association of TLR-MyD88 activates interleukin-1 receptor-associated kinase (*IRAK*), which in turn leads to TRAF6-mediated activation of the NF κ B and MAPK cascades (7). Therefore, *TRAF6* functions at the central point where signals induced by the TLR and TNFR families converge (7).

TRAF6 maps to chromosome 11p12 and it covers approximately ~22 kb. It resides on the reverse strand of genomic DNA and encodes at least two reference transcripts. The protein has a mass of 59 kDa and 522 amino-acids. According to Aceview (NCBI), the gene is well-expressed in many tissues; however, immunohistochemical analyses suggest that the cell

type-specific patterns of expression of TRAF family members are strikingly different, indicating that they are independently regulated (8).

Evidence of genetic linkage to the chromosomal region harboring *TRAF6* has been previously detected in different autoimmune diseases (9,12). A SNP based genome-wide linkage scan for rheumatoid arthritis (RA) has identified evidence of linkage at chromosome 11p12 (9), where the *TRAF6* gene lies. Indeed, in a recent candidate gene study for RA, evidence of association of a *TRAF6* were reported for an intronic SNP (rs540386) at the level of $p = 3.9 \times 10^{-6}$ in a combined analysis consisting of more than 30,000 individuals (10).

In lupus GWAS studies, TRAF6 has been mainly under-represented in the early GWAS platforms with no report of associations (1, 2). On the other hand, in a single candidate gene study that includes type I interferon-related genes, a couple of SNPs in *TRAF6* (rs5030472 and rs5030482) produced promising results in the homogenous Swedish population ($P=0.009$) (11).

We have previously identified evidence of linkage at 11p13 in African-American multiplex families especially among those with thrombocytopenia manifestations (12–14). In this study, we evaluated common SNPs in *TRAF6* as a candidate gene in this genomic interval, in a large population of SLE cases and controls.

Materials and Methods

Recruitment and Biological Sample Collection

The participants were enrolled in the Lupus Family Registry and Repository and Lupus Genetics Studies at the Oklahoma Medical Research Foundation (OMRF) as previously described (15) and by several collaborators worldwide included in the Large Lupus Association Study 2 (LLAS2) (1, 16–18). LLAS2 is a joint project investigating genetic associations in SLE through candidate gene approach in which individual investigators share their samples, but each investigator select the candidate gene and individually perform the study. A total of 14,270 study participants were included in the current study (Table 1). Protocols were approved by the Institutional Review Boards at each respective institution. Patients met at least four of the 11 revised 1997 ACR criteria for the classification of SLE (19). Ethnicity was self-reported and verified by principal component and admixture proportion calculations.

Genotyping

To determine if TRAF6 is associated with SLE, we genotyped 15 tag SNPs that capture most of the variation in this region. Data were generated using the Illumina iSelect technology at the OMRF. Genotype calls were made using all samples to maximize the accuracy of the cluster plots. Following genotype scoring, SNP clusters were evaluated electronically using the Illumina BEADSTUDIO(r) software package (<http://www.illumina.com>). Ambiguous SNP clusters were evaluated manually and SNPs with poor cluster characteristics were flagged. Genotypic data were only used from samples with a call rate >90% (average sample call rate=99.1%) and from SNPs with a call frequency >90% (average SNP call rate=99.0%). Initial QC analyses were performed by plate, by lot of reagents, and by date genotyped to be certain that systematic error was not introduced. A sample report was generated for every sample attempted in the project, including sample barcode, ethnicity, gender, pedigree information, no calls, calls, call rate, genotype frequency and Gencall score. Any sample with previous genotype data was analyzed for concordance. A summary SNP report was also generated containing chromosome and location, call rate, genotype frequency, and Gencall score.

Statistical Analyses

Testing for association, meta-analyses and Cochran's Q statistic (for analyzing difference in effects) were completed using PLINK (20). In meta-analyses, individual estimates and standard errors from association results of each ethnicity were extracted and then combined using a fixed or random effects model. Cochran's Q is calculated as the weighted sum of squared differences between individual study effects and the overall effect across studies. Q is distributed as a chi-square statistic with k (number of studies) minus one degree of freedom. Haploview version 4.2 (21) was used to estimate the linkage disequilibrium (LD) between markers and haplotypes in the different racial groups. Family based association was performed using the FBAT program (22). Conditional haplotype analyses were conducted using the WHAP program version 2.09 (23). To correct for multiple testing, false discovery rate (FDR) methods were used and q values were calculated using PLINK (20). Q values correspond to the proportion of false positives among the results. Thus, q values < 0.05 signify less than a 5% false positive rate and are taken as a measure of significance. For each SNP, missing data proportions for cases and controls, minor allele frequencies, ORs, 95% CI intervals, P values and exact tests for departures from Hardy-Weinberg expectations were calculated. SNPs needed to pass stringent quality control criteria that included: Hardy-Weinberg proportions (HWP) with a $p > 0.01$ in the controls and > 0.0001 for cases, total proportion missing < 5%, and $p > 0.05$ for differential missingness between cases and controls. The remaining samples were then evaluated for duplicates or related individuals. Genetic outliers were removed from further analysis as determined by principal components analysis (24) and admixture proportions calculated using ADMIXMAP. Principal components were calculated using all SNPs and admixture proportions were calculated using 347 AIMs.

Results

After removing the outliers and correcting for population stratification, the demographic distribution of the population under study is shown in Table 1. Five SNPs were not polymorphic or did not pass the quality controls and were excluded. The remaining SNPs were in Hardy-Weinberg Equilibrium (HWE) and passed the quality controls. First, in order to determine the haplotype structure of this genomic region, HapMap genotyping data for CEPH (Utah residents with ancestry from northern and western Europe) (CEU) was imported and the linkage disequilibrium and correlation coefficient (R^2) calculated. Supplementary Figure 1 shows the overall haplotype structure of this genomic region including *FLJ14213*, *TRAF6*, and *RAG1* haplotype blocks.

The results of the case-control study among different ancestry backgrounds revealed suggestive ($0.05 > p > 0.001$) and consistent evidence of association in multiple SNPs in all ancestries (Supplementary Table 1). Therefore, we subsequently performed a meta-analysis across these four populations, as shown in Figure 2 and Table 2. The best overall p values were obtained for SNPs rs5030437 and rs4755453 ($p = 7.85 \times 10^{-5}$, OR=0.89, 95% CI=0.83–0.95 and $p = 4.73 \times 10^{-5}$, OR=0.88, 95% CI=0.83–0.94, respectively) without significant heterogeneity among populations ($p = 0.67$ and $p = 0.50$ in Q-statistic) (Table 2 and Figure 1). In addition, rs540386, the SNP previously reported to be associated with RA, was found to be in LD with these two SNPs in Europeans ($r^2 = 0.95$) and demonstrated evidence of association with SLE in the same direction (meta-analysis $p = 9.15 \times 10^{-4}$, OR=0.89, 95% CI=0.83–0.95) (Table 2, Figure 2). The minor allele frequency for this SNP in our European control population was similar to European controls used in the RA study and was consistent with the HapMap CEU data (MAF~14%) (10).

In haplotype analyses, our selected SNPs in *TRAF6* span 18 kb as a single haplotypic block regardless of ethnicity. Figure 2 shows this haplotype in our European and African-

American population, respectively. Haplotype analyses revealed a risk haplotype present in the African-American population (GGAAGGGAGA) with a frequency of 30% in cases compared to 26% in controls ($p=2.00 \times 10^{-4}$, OR=1.23, 95% CI=1.10–1.37) (Supplementary Table 2). The same haplotype was also detected in Europeans and other ancestries. Importantly, in these populations, this haplotype displayed evidence of association consistent with that observed in the African-American population, providing further evidence supporting this association (Supplementary Table 2).

Previously we detected evidence of linkage at 11p13 in multiplex African-American families (12–14). This linkage interval was originally ascertained through a stratified analysis using those multiplex pedigrees containing at least one SLE affected individual for which the presence of thrombocytopenia had been confirmed according to ACR criteria (platelets, $<10 \times 10^9/L$ [$<100,000/\mu L$]) (12). Therefore, we tested the hypothesis that SLE patients with thrombocytopenia might be enriched for risk alleles in the *TRAF6* gene. Indeed, we found a general improvement in association results in both African-American as well as European ancestries when we considered this sub-phenotype.

In the tested African-American ancestry population, when 160 SLE cases with presence of thrombocytopenia were compared with healthy controls, we found a stronger association in all of the selected SNPs (Table 3), with the best SNP being rs5030445 ($p=0.0017$, FDR $q=0.029$, OR=0.68, 95% CI 0.53–0.87), with a minor allele frequency of 35% in cases compared to 44% in controls. Similar improvements in the strength of association were obtained in Europeans when 396 European cases with presence of thrombocytopenia were compared to corresponding controls (Table 3) (best $p=0.00018$, FDR $q=0.002$, for SNP rs5030470 with OR=0.55, 95% CI=0.40–0.76). In addition, the directions of allele frequencies were consistent in both populations (Table 3).

In addition, the imported SNP data from the HapMap CEU population and imputation analyses support the notion that the observed effect for SLE in this genomic region is most likely limited to the *TRAF6* haplotype block (Figure 2), although we cannot conclusively confirm this.

In order to further explore this enrichment and potentially shed light on the biology underlying the observed association, we assessed the potential association of these markers with other related ACR criteria. Indeed, in the analysis of other criteria associated with severe manifestations of SLE, some degree of improvement was found with the presence of nephritis and anti-dsDNA in different populations. In addition, since thrombocytopenia is part of the hematologic ACR criteria we also evaluated patients that fulfill any of the criteria of thrombocytopenia, leukopenia or lymphopenia. A similar trend was observed in the association results for these manifestations, although the greatest increase in risk was observed in individuals in whom thrombocytopenia was observed. A summary of the meta-analysis results is shown in Table 4, using these four subphenotypes evaluated in European, African-American, Hispanic and Korean populations. Furthermore, we assessed the potential association with the presence of anti-phospholipid (aPL) antibodies, as these are related to thrombocytopenia. In the Korean population, the presence of aPL antibodies also produced multiple significant results when 223 cases with positive aPL were compared to 740 healthy Korean controls. The best association results were obtained for SNP rs5030445 in this population ($p=0.002$, FDR $q=0.009$, OR=0.58 95% CI=0.40–0.83).

Similar results were obtained in case-only analyses. In particular, when 160 African-American SLE patients with thrombocytopenia were compared to 766 cases with no history of thrombocytopenia, similar association results were observed for SNP rs5030445 ($p=0.01$, FDR $q=0.06$, OR=0.72, 95% CI=0.56–0.93) with a minor allele frequency of 35% in cases

compared to 43% in controls. In Europeans, the best result was obtained for SNP rs5030470 when 396 SLE patients with thrombocytopenia were compared to 1,512 SLE patients without thrombocytopenia ($p=0.001$, FDR $q=0.01$, OR=0.59, 95% CI=0.43–0.82). In addition, for Koreans, when 224 cases with aPL antibodies were compared to 416 SLE cases without aPL, the association of SNP rs5030445 observed in the case-control study was again supported ($p=0.01$, FDR $q=0.05$, OR=0.62, 95% CI=0.42–0.92).

Finally, one of the SNPs (rs5030437) inside *TRAF6* (Table 2), which had previously been genotyped in all family members of African-American pedigrees in the LFRR collection and passed the quality control with no Mendelian inconsistency, was analyzed for family-based association. Out of a total of 121 informative multiplex African-American pedigrees, 34 pedigrees contained at least one SLE patient with thrombocytopenia. Family-based association analysis (FBAT) using these 34 available pedigrees produced a Z score of -2.28 ($p=0.02$) under a dominant model for the minor allele of this SNP. This finding concurs with the case-control association results (Table 3). In addition, 21 of these 34 pedigrees were also informative for the Transmission Disequilibrium Test (TDT) which produced a $p=0.04$. When all 121 multiplex families were analyzed under the same model, no significant associations were shown ($p=0.11$). FBAT results for other sub-phenotypes were suggestive or not significant (data not shown).

Discussion

In this study, we selected *TRAF6* as a candidate gene for SLE and evaluated 15 common tag SNPs, including the SNP rs540386, which was previously reported to confer risk for RA (10). The large multi-ancestral SLE cohort assessed in this study provides us with enough power to detect small association effects. In addition to confirming the previously reported association of SNP rs540386 with autoimmune disease phenotypes in our SLE case-control study (meta-analysis $p=9.15 \times 10^{-4}$), we found further evidence of association of multiple SNPs spanning 18 kb within *TRAF6* and with relatively high LD between SNPs (Table 2). We also found that some sub-phenotypes of SLE, in particular thrombocytopenia, can improve the observed association effect for *TRAF6* SNPs.

In African-American population, we have previously shown evidence of significant parametric linkage effect at the same region with SLE and thrombocytopenia (12). In this case-control study, only one affected case per family was genotyped and therefore we were not able to perform a family-based association study. However, in a separate SNP-based linkage study, in which one of the SNPs in *TRAF6*, rs5030437, had been genotyped in all African-American family members, the family-based association result (FBAT) suggested that *TRAF6* might be responsible for the observed linkage at 11p13 (Z score =2.28, FBAT $p=0.02$). In addition, the case-control study of multiplex African-American pedigrees with thrombocytopenia involving only one affected case per family further supports this association, as the odds ratio for SNPs such as rs5030445 was further improved in this limited set of 34 cases in comparison to the corresponding controls (OR=0.50, 95% CI=0.28–0.90, $p=0.01$), with a minor allele frequency of 28% in cases and 44% in controls.

In a previous GWAS from SLEGEN (1), only one SNP in *TRAF6* gene was available (rs1046864) with no overlapping SNPs with our study. There were 800 samples in our study overlapping with SLEGEN which is 75% of the cases (526/706) and 12% (274/2314) of the controls of SLEGEN samples. In agreement with SLEGEN result, when we reanalyzed this SNP, no significant association was detected ($p=0.13$) (1). This SNP is also modestly correlated with one of our selected SNP, rs5030472, ($r^2=0.79$). In fact, the SNP rs5030472 also showed a weak association in Europeans ($p=0.05$, Table 3) which is consistent with the report from Swedish cohort (11). However, only when meta-analysis of all ethnicities was

performed, this SNP revealed a significance result ($p=4.75\times 10^{-4}$) and with OR in all ethnicities facing the same direction (Table 2, Suppl Table 1).

The SNPs that we put to the test in our study captured variation in *TRAF6* gene comprehensively and our data included the largest number of patients and controls investigating the *TRAF6* in four ethnic groups which can allude to its significance in a sufficiently powered cohort. It is important to mention that the significant results observed in this study were adjusted and corrected based on independent candidate gene approach in which each investigator only has information on the SNPs that they have proposed. While, this large lupus study (LLAS2) provide us enough power to detect small association, these results need to be interpreted cautiously since in theory the significance of observed effects can be lost when more stringent Bonferroni correction for over 32,000 SNPs were considered. Further studies are necessary to confirm these new findings.

It is not clear how common variants in *TRAF6* might influence protein function and hence the observed phenotypes. *TRAF6* is ubiquitously expressed and, as an important adaptor molecule within the Toll-like receptor (TLR) signaling pathway, can activate downstream proteins such as (NF κ B), which is of critical importance to survival and activation of immunocytes. Consistent with our findings concerning *TRAF6*, genetic variants in/near other members of the TLR signaling pathway both upstream and downstream of *TRAF6* have been associated with SLE, including *TNFAIP3*, *IRF5*, *IRF7*, and *IRAK1* (25). A previous report indicated that overexpression of *TRAF6* in mouse bone marrow resulted in thrombocytosis, mild neutropenia and megakaryocytic dysplasia. The bone marrow of these *TRAF6*-chimeric mice showed increased megakaryopoiesis, and after a few months a subset of mice progressed either to marrow failure or acute myeloid leukemia (26). The bone marrow failure in these chimeras was characterized by severe anemia and thrombocytopenia despite normocellular marrow. Altered hematopoiesis in *TRAF6*-deficient chimeric mice also has been shown in another study especially with a decrease in the number of B cells in the bone marrow and spleen of chimeras (27). *TRAF6* also possesses ubiquitin ligase activity and plays an important role in ubiquitination and DNA repair. Common variations in *TRAF6* such as rs331457 have been reported to modify the risk for cutaneous malignant melanomas in which ultraviolet radiation can directly cause DNA damage and influence the expression of apoptosis-related molecules (28). This SNP is also in complete LD with our selected SNPs such as rs5030437 or rs5030470 ($r^2=1$). *TRAF6* is also a critical mediator of signal transduction by viral oncogenes such as latent membrane protein 1 (*LMP1*) of the Epstein Barr virus (29, 30). Overexpression of dominant-negative *TRAF6*^{-/-} mutant cells (which lack the typical RING finger domain) can inhibit EBV *LMP1* signaling in human embryonic kidney cells (29). *LMP1* is critical for effective immortalization and proliferation of B-cells, a function that is essential for viral persistence and pathogenesis. Further studies are necessary to explore how these common variations in *TRAF6* might influence SLE-specific alterations in humoral and cellular immunity to EBV that have been implicated in the development of SLE.

In summary, in our multi-ancestral SLE case-control study we have been able to further support and expand the reported association of *TRAF6* with RA. In agreement with that report, we found that a large number of samples are required to detect these associations. In addition, the observed association at this locus appeared to be driven, at least in part, by SLE subphenotypes, in particular thrombocytopenia, for which evidence of linkage has been previously reported in the same genomic region. Overall, our results indicate that *TRAF6* is an important gene in the pathogenesis of SLE. Further studies will be necessary to confirm this association, define the causal variant(s), and elucidate their mechanism of action.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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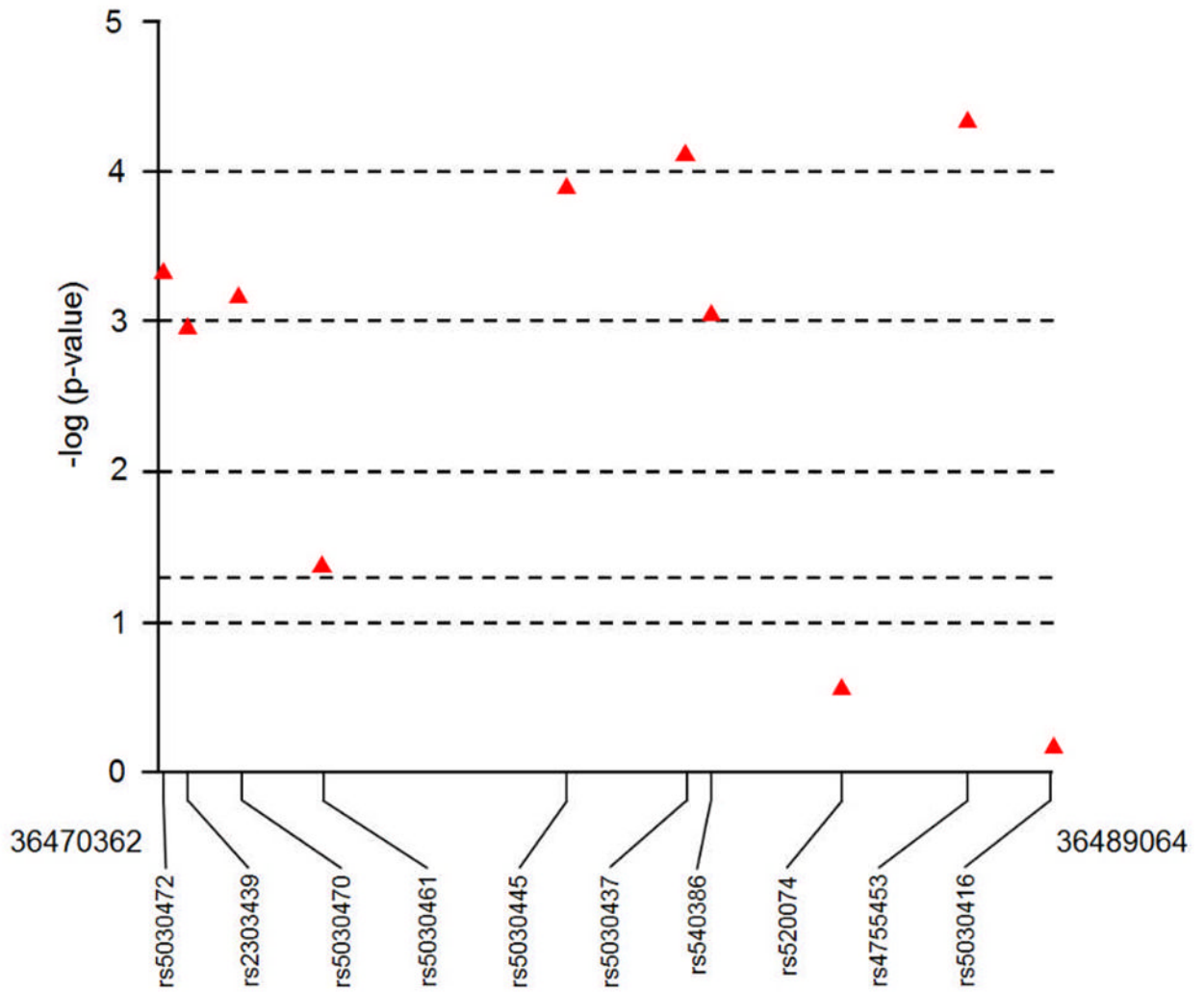


Figure 1. Meta-analyses results using four different populations of Europeans, African-Americans, Koreans, and Hispanics.

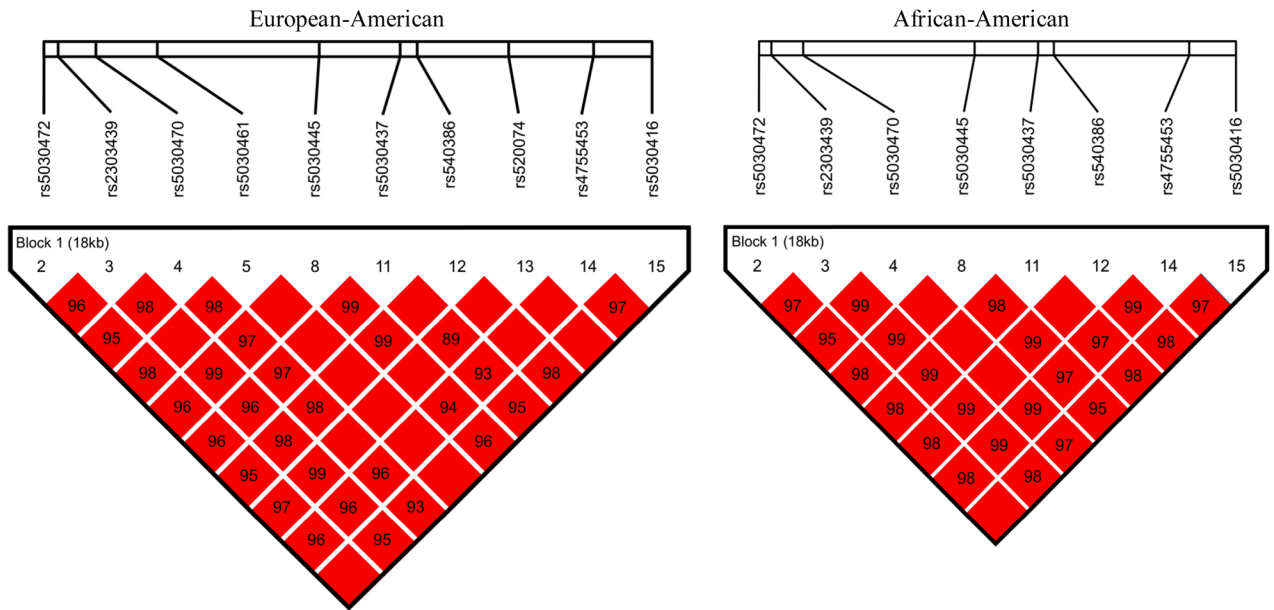


Figure 2. European-American and African-American haplotype block structure. Blocks connecting SNP pairs are shaded according to the strength of the linkage disequilibrium between the SNPs, from 0.0 (white) to 1.0 (bright red), as measured by the disequilibrium coefficient D.

Table 1

Demographic distribution of individuals in study.

	European-American Case/Control	Korean Case/Control	African-American Case/Control	Gullah * Case/Control	Hispanic Case/Control
Total	3936/3491	640/740	1527/1811	152/123	1235/615
Male:	344/1151	37/44	121/574	15/18	117/75
Female	3592/2340	603/696	1406/1237	137/105	1118/540

* The Gullah are African-Americans who live in the Low Country of South Carolina and genetically show a much lower admixture rate with non-African populations than other African-Americans.

Table 2

Meta-analyses results using four populations; Q= Cochran's Q that measures the population heterogeneity and differences in effect size ($P < 0.05$ means significant heterogeneity among population).

Position	SNP	Minor Allele	Major Allele	Meta-P	OR	I ²	R ²	Q
36470362	rs5030472	A	G	4.75×10^{-4}	0.85	0.77	0.92	0.38
36470866	rs2303439	A	G	1.12×10^{-3}	0.90	0.84	0.96	0.68
36472022	rs5030470	G	A	7.13×10^{-4}	0.85	0.77	0.93	0.21
36473732	rs5030461	G	A	4.38×10^{-2}	0.84	0.77	0.99	0.99
36478836	rs5030445	A	G	1.31×10^{-4}	0.88	0.83	0.94	0.54
36481331	rs5030437	A	G	7.85×10^{-5}	0.88	0.83	0.94	0.67
36481869	rs540386*	A	G	9.15×10^{-4}	0.89	0.83	0.95	0.42
36484601	rs520074	C	A	2.85×10^{-1}	0.90	0.79	1.10	0.01
36487220	rs4755453	C	G	4.73×10^{-5}	0.88	0.83	0.94	0.50
36489064	rs5030416	C	A	7.18×10^{-1}	0.98	0.92	1.05	0.65

* SNP associated with Rheumatoid Arthritis.

Table 3

Case-control association results in African-American and European SLE cases with presence of thrombocytopenia in compare to corresponding controls respectively.

SNP	BP	Minor Allele	Major Allele	Case	African-American			P	FDR q	OR (95% CI)
					Control	CHISQ	P			
rs5030472	36470362	A	G	0.038	0.067	3.984	0.04593	0.066	0.55 (0.31–1.00)	
rs2303439	36470866	A	G	0.345	0.426	7.639	0.005711	0.038	0.71 (0.56–0.91)	
rs5030470	36472022	G	A	0.042	0.063	2.059	0.1513	0.247	0.66 (0.37–1.17)	
rs5030461	36473732	G	A	0.107	0.100	0.116	0.7336	0.862	1.08 (0.70–1.65)	
rs5030445	36478836	A	G	0.350	0.442	9.816	0.00173	0.029	0.68 (0.53–0.87)	
rs5030437	36481331	A	G	0.306	0.387	8.088	0.004456	0.038	0.70 (0.54–0.90)	
rs540386	36481869	A	G	0.209	0.240	1.512	0.2188	0.324	0.84 (0.63–1.11)	
rs520074	36484601	C	A	0.053	0.078	2.575	0.1085	0.224	0.67 (0.40–1.10)	
rs4755453	36487220	G	C	0.494	0.422	6.156	0.01309	0.048	1.34 (1.06–1.68)	
European										
rs5030472	36470362	A	G	0.072	0.106	8.611	0.003342	0.017	0.65 (0.49–0.87)	
rs2303439	36470866	A	G	0.115	0.147	5.883	0.01529	0.023	0.75 (0.59–0.95)	
rs5030470	36472022	G	A	0.069	0.119	14.04	0.000179	0.002	0.55 (0.40–0.76)	
rs5030445	36478836	A	G	0.119	0.153	6.34	0.01181	0.023	0.74 (0.59–0.94)	
rs5030437	36481331	A	G	0.125	0.159	5.756	0.01643	0.024	0.76 (0.60–0.95)	
rs540386	36481869	A	G	0.104	0.139	7.58	0.005901	0.020	0.72 (0.56–0.91)	
rs4755453	36487220	C	G	0.123	0.159	6.668	0.009816	0.023	0.74 (0.59–0.93)	

Table 4

Summary of meta-analyses results for individual ACR classification criteria.

Thrombocytopenia						
SNP	BP	Minor Allele	Major Allele	Meta-P	OR (95% CI)	Q
rs5030472	36470362	A	G	3.24×10^{-4}	0.68 (0.55–0.84)	0.54
rs2303439	36470866	A	G	7.02×10^{-4}	0.79 (0.68–0.90)	0.67
rs5030470	36472022	G	A	1.99×10^{-6}	0.57 (0.45–0.72)	0.93
rs5030445	36478836	A	G	1.20×10^{-4}	0.76 (0.66–0.88)	0.61
rs5030437	36481331	A	G	4.25×10^{-4}	0.78 (0.68–0.90)	0.65
rs540386	36481869	A	G	1.16×10^{-3}	0.77 (0.66–0.90)	0.65
rs4755453	36487220	C	G	5.07×10^{-4}	0.79 (0.69–0.90)	0.61
rs5030416	36489064	C	A	1.93×10^{-1}	0.91 (0.79–1.05)	0.05
Renal disorder						
rs5030472	36470362	A	G	2.46×10^{-4}	0.79 (0.70–0.90)	0.34
rs2303439	36470866	A	G	1.21×10^{-2}	0.90 (0.83–0.98)	0.25
rs5030470	36472022	G	A	1.93×10^{-5}	0.75 (0.66–0.86)	0.73
rs5030445	36478836	A	G	5.70×10^{-3}	0.89 (0.82–0.97)	0.23
rs5030437	36481331	A	G	1.81×10^{-3}	0.88 (0.81–0.95)	0.58
rs540386	36481869	A	G	8.34×10^{-4}	0.85 (0.78–0.94)	0.72
rs4755453	36487220	C	G	8.48×10^{-4}	0.87 (0.80–0.94)	0.75
rs5030416	36489064	C	A	5.07×10^{-1}	1.03 (0.94–1.13)	0.84
Anti-dsDNA						
rs5030472	36470362	A	G	2.46×10^{-4}	0.79 (0.70–0.90)	0.34
rs2303439	36470866	A	G	1.21×10^{-2}	0.90 (0.83–0.98)	0.25
rs5030470	36472022	G	A	1.93×10^{-5}	0.75 (0.66–0.86)	0.73
rs5030445	36478836	A	G	5.70×10^{-3}	0.89 (0.82–0.97)	0.23
rs5030437	36481331	A	G	1.81×10^{-3}	0.88 (0.81–0.95)	0.58
rs540386	36481869	A	G	8.34×10^{-4}	0.85 (0.78–0.94)	0.72

Thrombocytopenia						
SNP	BP	Minor Allele	Major Allele	Meta-P	OR (95% CI)	Q
rs4755453	36487220	C	G	8.48 × 10 ⁻⁴	0.87 (0.80–0.94)	0.75
rs5030416	36489064	C	A	5.07 × 10 ⁻¹	1.03 (0.94–1.13)	0.84
Hematologic disorder						
rs5030472	36470362	A	G	6.31 × 10 ⁻⁴	0.80 (0.70–0.91)	0.61
rs2303439	36470866	A	G	2.68 × 10 ⁻²	0.91 (0.83–0.99)	0.39
rs5030470	36472022	G	A	1.72 × 10 ⁻⁵	0.74 (0.65–0.85)	0.55
rs5030445	36478836	A	G	2.14 × 10 ⁻³	0.87 (0.80–0.95)	0.45
rs5030437	36481331	A	G	4.77 × 10 ⁻³	0.88 (0.81–0.96)	0.61
rs540386	36481869	A	G	1.07 × 10 ⁻³	0.85 (0.77–0.94)	0.48
rs4755453	36487220	C	G	3.06 × 10 ⁻³	0.88 (0.81–0.96)	0.47
rs5030416	36489064	C	A	2.89 × 10 ⁻¹	0.95 (0.87–1.04)	0.61

The number of cases and controls for each sub-phenotypes are: Europeans: Thrombocytopenia=396 SLE and 3,491 controls, Renal disorder=1,103 SLE and 3,491 controls, Anti-dsDNA: 1,234 SLE and 3,491 controls, Hematologic disorder (Presence of either leukopenia, thrombocytopenia or lymphopenia)=1,116 SLE and 3,491 controls, African-Americans: Thrombocytopenia=160 SLE and 1,934 controls, Renal disorder=728 SLE and 1,934 controls, anti-dsDNA=741 SLE and 1,934 controls, Hematologic disorder=519 SLE and 1,934 controls, Koreans: Thrombocytopenia=147 SLE and 740 controls, Renal disorder=280 SLE and 740 controls, anti-dsDNA=504 SLE and 740 controls, Hematologic disorder=523 SLE and 740 controls, Hispanic: Thrombocytopenia=246 SLE and 615 controls, Renal disorder=545 SLE and 615 controls, anti-dsDNA=619 SLE and 615 controls, Hematologic disorder=520 SLE and 615 controls.