Evaluation of TRAF6 in a Large Multiancestral Lupus Cohort


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. We undertook to study the role of TRAF6 as a candidate gene for SLE, since it plays 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

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SLE patients 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 (ORs) with 95% confidence intervals (95% CIs) were calculated.

**Results.** Evidence of associations was detected in multiple SNPs. 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 $P = 0.50$, respectively, in Q statistic). In addition, SNP rs540386, which was previously reported to be associated with rheumatoid arthritis (RA), was found to be in linkage disequilibrium with these 2 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]). The presence of 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 was detected in 1 available SNP (rs5030437) with a Z score magnitude of 2.28 ($P = 0.02$) under a dominant model.

**Conclusion.** Our data indicate the presence of 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 was detected in 1 available SNP (rs5030437) with a Z score magnitude of 2.28 ($P = 0.02$) under a dominant model.

The genetic etiology of systemic lupus erythematosus (SLE), the prototypic 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 receptor [TNFR]–associated factor 6), a candidate SLE susceptibility locus. As an adaptor molecule that plays a central role in the NF-κB activation pathway, TRAF6 is an important candidate gene for SLE. This pathway, which also includes MAPKs, is of critical importance to survival and activation of multiple immune cell subsets. It regulates inflammation, dendritic cell development, thymic selection, and Treg cell production as well as osteoclast formation. The role of TRAF6 in this pathway is essential, as it transduces signals from the TNFR superfamily, the Toll-like receptor (TLR)/interleukin-1 receptor (IL-1R) family, and CD40 to activate the transcription factors NF-κB and activator protein 1 (6). Unlike other TRAF family members, TRAF6 also participates in TLR/IL-1R family signaling. The association of TLR with myeloid differentiation factor 88 activates IL-1R–associated kinase, which in turn, leads to TRAF6-mediated activation of the NF-κB 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 ~22 kb. It resides on the reverse strand of genomic DNA and encodes at least 2 reference transcripts. The protein has a mass of 59 kd and a total of 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 previously been detected in different autoimmune diseases (9). 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 TRAF6 was 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 underrepresented in the early GWAS platforms, with no report of associations (1,2). On the other hand, in a single candidate gene study that included type I...
interferon–related genes, a couple of SNPs in TRAF6 (rs5030472 and rs5030482) produced promising results in a homogeneous 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 the present study, we evaluated common SNPs in TRAF6 as a candidate gene in this genomic interval in a large population of SLE patients and controls.

**PATIENTS AND METHODS**

**Recruitment and biologic sample collection.** The participants were enrolled in the Lupus Family Registry and Repository (LFRR) and the Lupus Genetics Studies program 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 selects the candidate gene and individually performs the study. A list of the members of the BIO Lupus Network and the Genoma en Lupus (GENLES) Network who provided samples used in this study is provided in Appendix A. 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 4 of the 11 revised 1997 American College of Rheumatology (ACR) criteria for the classification of SLE (19). Ethnicity was self-reported and verified by principal components analysis 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 software package (http://www.illumina.com). Ambiguous SNP clusters were evaluated manually, and SNPs with poor cluster characteristics were flagged. Genotype data were only used from samples with thrombocytopenia manifestations (12–14). In the present study, we evaluated common SNPs in TRAF6 as a candidate gene in this genomic interval in a large population of SLE patients and controls.

| Table 1. Demographic distribution of the individuals in the study* |
|----------------------|------------------|------------------|------------------|
|                      | European American | Korean           | African American | Gullah†          | Hispanic         |
| Total                | 3,936/3,491       | 640/740          | 1,527/1,811      | 152/123          | 1,235/615        |
| Male                 | 344/1,151         | 37/44            | 121/574          | 15/18            | 117/75           |
| Female               | 3,592/2,340       | 603/696          | 1,406/1,237      | 137/105          | 1,118/540        |
| * Values are the number of patients/number of controls.
| † 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.|

**RESULTS**

The demographic distribution of the population under study, after removing the outliers and correcting for population stratification, 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 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) were imported, and the LD and correlation coefficient ($r^2$) were calculated. The overall haplotype structure of this genomic region, including \textbf{FLJ14213}, \textbf{TRAF6}, and \textbf{RAG1} haplotype blocks, is shown in Supplementary Figure 1 (available on the \textit{Arthritis & Rheumatism} Web site at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1529-0131).

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 (see Supplementary Table 1 and Supplementary Figure 2, available on the \textit{Arthritis & Rheumatism} Web site at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1529-0131).

Therefore, we subsequently performed a meta-analysis across these 4 populations, as shown in Figure 1 and Table 2. The best overall $P$ values were obtained for SNPs rs5030437 ($P = 7.85 \times 10^{-5}$, OR 0.88 [95% CI 0.83–0.94]) and rs4755453 ($P = 4.73 \times 10^{-5}$, OR 0.88 [95% CI 0.83–0.94]) without significant heterogeneity among populations ($P = 0.67$ and $P = 0.50$, respectively, in Q statistic) (Figure 1 and Table 2). In addition, rs540386, the SNP previously reported to be associated with RA, was found to be in LD with these 2 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]) (Figure 2 and Table 2). The MAF for this SNP in our European control population was similar to that in European controls used in the RA study and was consistent with the HapMap CEU data ($\sim 14\%$) (10).

In haplotype analyses, our selected SNPs in \textit{TRAF6} spanned 18 kb as a single haplotype block regardless of ethnicity. Figure 2 shows this haplotype in our European American and African American populations, 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]) (see Supplementary Table 2, available on the \textit{Arthritis & Rheumatism} Web site at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1529-0131). 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 associ-

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**Figure 1.** Meta-analysis results using 4 different populations of Europeans, African Americans, Koreans, and Hispanics. Triangles indicate $-\log P$ value for such corresponding single-nucleotide polymorphism.

**Table 2.** Meta-analyses results using 4 populations

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position</th>
<th>Minor allele</th>
<th>Major allele</th>
<th>$P$, for meta-analysis</th>
<th>OR (95% CI)*</th>
<th>$P$, for Cochran’s Q†</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs5030472</td>
<td>36470362</td>
<td>A</td>
<td>G</td>
<td>$4.75 \times 10^{-4}$</td>
<td>0.85 (0.77–0.92)</td>
<td>0.38</td>
</tr>
<tr>
<td>rs2303439</td>
<td>36470866</td>
<td>A</td>
<td>G</td>
<td>$1.12 \times 10^{-3}$</td>
<td>0.90 (0.84–0.96)</td>
<td>0.68</td>
</tr>
<tr>
<td>rs5030470</td>
<td>36472022</td>
<td>G</td>
<td>A</td>
<td>$7.13 \times 10^{-4}$</td>
<td>0.85 (0.77–0.93)</td>
<td>0.21</td>
</tr>
<tr>
<td>rs5030461</td>
<td>36473732</td>
<td>G</td>
<td>A</td>
<td>$4.38 \times 10^{-2}$</td>
<td>0.84 (0.77–0.99)</td>
<td>0.99</td>
</tr>
<tr>
<td>rs5030445</td>
<td>36478836</td>
<td>A</td>
<td>G</td>
<td>$1.31 \times 10^{-4}$</td>
<td>0.88 (0.83–0.94)</td>
<td>0.54</td>
</tr>
<tr>
<td>rs5030437</td>
<td>36481331</td>
<td>A</td>
<td>G</td>
<td>$7.85 \times 10^{-5}$</td>
<td>0.88 (0.83–0.94)</td>
<td>0.67</td>
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<td>rs540386‡</td>
<td>36481869</td>
<td>A</td>
<td>G</td>
<td>$9.15 \times 10^{-4}$</td>
<td>0.89 (0.83–0.95)</td>
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<td>rs520074</td>
<td>36484601</td>
<td>C</td>
<td>A</td>
<td>$2.85 \times 10^{-1}$</td>
<td>0.90 (0.79–1.10)</td>
<td>0.01</td>
</tr>
<tr>
<td>rs4755453</td>
<td>36487220</td>
<td>C</td>
<td>G</td>
<td>$4.73 \times 10^{-5}$</td>
<td>0.88 (0.83–0.94)</td>
<td>0.50</td>
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<td>rs5030416</td>
<td>36489064</td>
<td>C</td>
<td>A</td>
<td>$7.18 \times 10^{-1}$</td>
<td>0.98 (0.92–1.05)</td>
<td>0.65</td>
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</table>

* OR = odds ratio; 95% CI = 95% confidence interval.
† Measures population heterogeneity and differences in effect size ($P < 0.05$ means significant heterogeneity among population).
‡ Single-nucleotide polymorphism (SNP) associated with rheumatoid arthritis.
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 1 SLE-affected individual in whom the presence of thrombocytopenia had been confirmed according to ACR criteria (platelet count $< 100,000/\mu l$) (12). We therefore 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 subphenotype.

Table 3. Case–control association results in African American and European patients with systemic lupus erythematosus and thrombocytopenia compared with healthy control subjects* 

<table>
<thead>
<tr>
<th>Ethnic group, SNP</th>
<th>Position</th>
<th>Minor allele</th>
<th>Major allele</th>
<th>Case MAF</th>
<th>Control MAF</th>
<th>$\chi^2$</th>
<th>$P$</th>
<th>FDR q</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
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<td>African American</td>
<td>rs5030472</td>
<td>36470362 A</td>
<td>G</td>
<td>0.038</td>
<td>0.067</td>
<td>3.984</td>
<td>0.04593</td>
<td>0.066</td>
<td>0.55 (0.31–1.00)</td>
</tr>
<tr>
<td></td>
<td>rs2303439</td>
<td>36470866 A</td>
<td>G</td>
<td>0.345</td>
<td>0.426</td>
<td>7.639</td>
<td>0.005711</td>
<td>0.038</td>
<td>0.71 (0.56–0.91)</td>
</tr>
<tr>
<td></td>
<td>rs5030470</td>
<td>36472022 G</td>
<td>A</td>
<td>0.042</td>
<td>0.063</td>
<td>2.059</td>
<td>0.1513</td>
<td>0.247</td>
<td>0.66 (0.37–1.17)</td>
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<td></td>
<td>rs5030461</td>
<td>36473732 G</td>
<td>A</td>
<td>0.107</td>
<td>0.100</td>
<td>0.116</td>
<td>0.7363</td>
<td>0.862</td>
<td>1.08 (0.70–1.65)</td>
</tr>
<tr>
<td></td>
<td>rs5030445</td>
<td>36478836 A</td>
<td>G</td>
<td>0.350</td>
<td>0.442</td>
<td>9.816</td>
<td>0.00173</td>
<td>0.029</td>
<td>0.68 (0.53–0.87)</td>
</tr>
<tr>
<td></td>
<td>rs5030437</td>
<td>36481331 A</td>
<td>G</td>
<td>0.306</td>
<td>0.387</td>
<td>8.088</td>
<td>0.004456</td>
<td>0.038</td>
<td>0.70 (0.54–0.90)</td>
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<tr>
<td></td>
<td>rs540386</td>
<td>36481869 A</td>
<td>G</td>
<td>0.209</td>
<td>0.240</td>
<td>1.512</td>
<td>0.2188</td>
<td>0.324</td>
<td>0.84 (0.63–1.11)</td>
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<tr>
<td></td>
<td>rs520074</td>
<td>36484601 C</td>
<td>A</td>
<td>0.053</td>
<td>0.078</td>
<td>2.575</td>
<td>0.1085</td>
<td>0.224</td>
<td>0.67 (0.40–1.10)</td>
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<td></td>
<td>rs4755453</td>
<td>36487220 G</td>
<td>C</td>
<td>0.494</td>
<td>0.422</td>
<td>6.156</td>
<td>0.01309</td>
<td>0.048</td>
<td>1.34 (1.06–1.68)</td>
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<td>European</td>
<td>rs5030472</td>
<td>36470362 A</td>
<td>G</td>
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<td>0.003342</td>
<td>0.017</td>
<td>0.65 (0.49–0.87)</td>
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<td>G</td>
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<td>0.023</td>
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<td>0.002</td>
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<td>G</td>
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<td>0.153</td>
<td>6.34</td>
<td>0.01181</td>
<td>0.023</td>
<td>0.74 (0.59–0.94)</td>
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<td></td>
<td>rs5030437</td>
<td>36481331 A</td>
<td>G</td>
<td>0.125</td>
<td>0.159</td>
<td>5.756</td>
<td>0.01643</td>
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<td>0.76 (0.60–0.95)</td>
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<td>rs540386</td>
<td>36481869 A</td>
<td>G</td>
<td>0.104</td>
<td>0.139</td>
<td>7.58</td>
<td>0.005901</td>
<td>0.020</td>
<td>0.72 (0.56–0.91)</td>
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<tr>
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<td>G</td>
<td>0.123</td>
<td>0.159</td>
<td>6.668</td>
<td>0.009816</td>
<td>0.023</td>
<td>0.74 (0.59–0.93)</td>
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</tbody>
</table>

* SNP = single-nucleotide polymorphism; MAF = minor allele frequency; FDR = false discovery rate; OR = odds ratio; 95% CI = 95% confidence interval.
the best SNP being rs5030445 \( (P = 0.0017, \text{FDR} \ q = 0.029, \text{OR} \ 0.68 \ [95\% \ CI \ 0.53–0.87]) \), with a MAF of 35% in cases compared to 44% in controls. Similar improvements in the strength of association were obtained in Europeans when 396 European patients with thrombocytopenia were compared to corresponding controls (best \( P = 0.00018, \text{FDR} \ q = 0.002, \text{for SNP rs5030470 with OR} \ 0.55 \ [95\% \ CI \ 0.40–0.76]) \) (Table 3). In addition, the directions of allele frequencies were consistent in both populations (Table 3). Furthermore, the imported SNP data from the HapMap CEU population and imputation analyses supported the notion that the observed effect for SLE in this genomic region was most likely limited to the TRAF6 haplotype block (Figure 2) (see Supplementary Figure 3, available on the Arthritis & Rheumatism Web site at http://onlinelibrary.wiley.com/ journal/10.1002/(ISSN)1529-0131, 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–double-stranded DNA in different populations. In addition, since thrombocytopenia is part of the hematologic ACR criteria, we also evaluated patients who fulfilled any of the criteria for 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 with thrombocytopenia. A summary of the meta-analysis results is shown in Table 4, using these 4 subphenotypes evaluated in European, African American, Hispanic, and Korean populations. Furthermore, we assessed the potential association with the presence of antiphospholipid antibodies (aPL), as these are related to thrombocytopenia. In the Korean population, the presence of aPL also produced multiple significant results when 223 aPL-positive patients were compared to 740 healthy 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 SLE patients 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 MAF of 35% in cases compared to 43% in controls. In Europeans, the best result was obtained for SNP rs5030445 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 SLE patients with aPL were compared to 416 SLE patients 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. Of a total of 121 informative multiplex African American pedigrees, 34 pedigrees contained at least 1 SLE patient with thrombocytopenia. 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 is consistent with the case–control association results (Table 3). In addition, 21 of these 34 pedigrees were also informative for the transmission disequilibrium test, which produced a $P$ value of 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 subphenotypes 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 multiancestral 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 subphenotypes of SLE, particularly thrombocytopenia, can improve the observed association effect for TRAF6 SNPs.

In the 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 1 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 1 of the SNPs in TRAF6, rs5030437, had been genotyped in all African American family members, the FBAT result suggested that TRAF6 might be responsible for the observed linkage at 11p13 ($Z$ score of 2.28, FBAT $P = 0.02$). In addition, the case–control study of multiplex African American pedigrees with thrombocytopenia involving only 1 affected patient per family further supports this association, as the OR 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 0.28–0.89], $P = 0.01$), with a MAF of 28% in cases compared to 44% in controls.

In a previous GWAS from the International Consortium on the Genetics of Systemic Lupus Erythematosus (SLEGEN) (1), only 1 SNP in the TRAF6
The SNPs that we examined in our study captured variation in the TRAF6 gene comprehensively, and our data included the largest number of patients and controls used to investigate the TRAF6 gene in 4 ethnic groups, which can indicate 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 an independent candidate gene approach in which each investigator had information on only the SNPs that he or she had proposed. While this large lupus study (LLAS2) provides us with enough power to detect small associations, these results need to be interpreted cautiously, since in theory, the significance of observed effects can be lost when more stringent Bonferroni corrections for >32,000 SNPs are 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 TLR signaling pathway, can activate downstream proteins such as NF-κB, which is of critical importance to the survival and activation of immunocytes. Consistent with our findings concerning TRAF6, genetic variants in or 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-deficient chimeric mice showed increased megakaryopoiesis, and after a few months, a subset of mice progressed either to marrow failure or to 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 of 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 (r² = 1). TRAF6 is also a critical mediator of signal transduction by viral oncogenes such as LMP1 of the Epstein-Barr virus (EBV) (29,30). Overexpression of dominant-negative TRAF6ΔR3 mutant cells (which lack the typical ring-finger domain) can inhibit EBV latent membrane protein 1 (LMP-1) signaling in human embryonic kidney cells (29). LMP-1 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 this multiancestral SLE case-control study, we have been able to further support and expand the reported association of TRAF6 with RA. As was noted for the RA studies, 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, particularly 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 the mechanism of action.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Bae had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.


ADDITIONAL DISCLOSURE

Author Alarcón-Riquelme is an employee of the Center for Genomics and Oncological Research Pfizer–University of Granada–Junta de Andalucía, Granada, Spain.

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APPENDIX A: MEMBERS OF BIOLUPUS NETWORK 
AND GENLES NETWORK WHO PROVIDED SAMPLES 
USED IN THIS STUDY

The members of the BIOLUPUS Network who provided 
samples used in this study are Bernard R. Lauwerys (Belgium); Peter 
Junker and Helle Laustrup (Denmark); Emoke Endreffy and László 
Kovács (Hungary); Sandra D’Alfonso, M. D. Danieli, Mauro Gal- 
leazzi, Sergio Miglioresi, Rafaela Scorza, and Gian Domenico Sebas- 
tiani (Italy); Marc Bijl and Cees Kallenberg (The Netherlands); Berta 
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Gutierrez, Norberto Ortego, Julio Sanchez Román, Mario Sabio, and 
Ana Suárez (Spain). Dr. Lennart Truedsson provided controls from 
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Cristina Drenkard, Alicia Eimon, Susana Gamron, Mercedes A. 
Garcia, Cesar E. Graf, Sebastian Grimaudo, Carolina Guillérón, 
Marisa Jorfen, Jorge Manni, Ana I. Marcos, Juan C. Marcos, Pilar C. 
Marino, Emilia Menso, Estela L. Motta, Sandra M. Navarro, Sergio 
Paira, Simon A. Palatnik, Carlos E. Perandones, Jose L. Presas, 
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