Genetic variants near TNFAIP3 on 6q23 are associated with systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is an autoimmune disease influenced by genetic and environmental factors. We carried out a genome-wide association scan and replication study and found an association between SLE and a variant in TNFAIP3 (rs5029939, meta-analysis P = 2.89 × 10⁻¹², OR = 2.29). We also found evidence of two independent signals near TNFAIP3 associated with SLE, including one previously associated with rheumatoid arthritis (RA). These results establish that variants near TNFAIP3 contribute to differential risk of SLE and RA.

Systemic lupus erythematosus (SLE, MIM152700) is an autoimmune disease characterized by dysregulated interferon responses and loss of self-tolerance to cellular antigens. Autoantibody production leads to immune complex formation, resulting in local and systemic inflammation and organ failure. The prevalence of SLE is estimated to be between 40 and 400 cases per 100,000 individuals, with higher prevalence rates and more severe complications occurring in persons of Hispanic or African ancestry. SLE affects women at a rate nine times that of men and most often presents during the years between menarche and menopause. The etiology of SLE is complex and poorly defined, requiring interplay between genetic predisposition and environmental triggers.

To identify genetic variants contributing to SLE, we tested association of 311,238 successfully genotyped SNPs in 431 unrelated SLE cases and 2,155 controls (see Supplementary Methods online). We compared the distribution of the observed P values to the expected distribution under the null hypothesis, and deviation of the tail of the observed P value distribution was evident, suggesting the presence of significant genetic effects (Supplementary Fig. 1 online). Three regions met a pre-specified threshold for genome-wide significance (P < 5 × 10⁻⁸): the previously defined HLA and IRF5-TNPO3 regions, and a previously unreported SLE locus at chromosome 6q23 within the TNFAIP3 gene (Fig. 1a).

TNFAIP3, also known as A20, functions as key regulator of NF-kB signaling through ubiquitin modification of adaptor proteins RIP and TRAF6 downstream of TNFα and Toll-like receptors, respectively. We observed evidence for association with a variant in TNFAIP3 (rs5029939, GWAS P = 2.55 × 10⁻⁸, Fig. 1b) and two flanking SNPs (rs10499197, GWAS P = 2.11 × 10⁻⁸; rs7749323, GWAS P = 9.63 × 10⁻⁷) in strong LD with rs5029939 (r² > 0.95) (Fig. 1b). A SNP located ~185 kb upstream of TNFAIP3 recently reported to be associated with risk of RA5,6 (rs6920220) showed modest association in the SLE GWAS dataset (GWAS P = 0.01; Fig. 1b).

We tested 134 loci (excluding HLA and IRF5) for association with SLE in 740 independent trios (Supplementary Table 1 online) and calculated meta-analysis P values to determine the overall evidence for association from the GWAS and trio replication datasets. Association with rs5029939 in TNFAIP3 replicated in the trios (trio P = 2.47 × 10⁻⁵), which together with the GWAS data produced a convincing meta-analysis P = 2.89 × 10⁻¹² (Table 1). Variants in ST4AT and BLK regions also showed association with SLE in both the GWAS and trio datasets and together produced meta-analysis P values exceeding genome-wide significance (rs3821236 in ST4AT, meta P = 8.49 × 10⁻¹¹; rs2618476 in BLK, meta P = 1.7 × 10⁻⁸; Table 1). These two loci as well as ITGAM have recently been identified in two other SLE GWA scans7–9. In our study, a variant in ITGAM also showed convincing evidence of replication (rs11150610, meta P = 1.72 × 10⁻⁶; Supplementary Table 1).

Variants in the TNFAIP3 region have recently been reported to independently influence risk of RA5,6. The minor allele of rs6920220 is associated with increased risk of RA (MAF cases > controls), whereas the minor allele of rs10499194 confers protection (MAF controls > cases) (Supplementary Fig. 2 online). To evaluate the locus in more detail in SLE, we genotyped a panel of eight SNPs that tag the RA and SLE alleles in our complete set of trio families (n = 991). We also included a putative causal missense variant (rs2230926) in exon 3 of TNFAIP3 (Supplementary Table 2 online). We found that...
the minor allele of rs6920220 that contributes to RA risk was also associated with susceptibility to SLE ($P = 8.92 \times 10^{-5}$); however, no evidence for association was seen for the rs10499194 RA protective variant ($P = 0.72$) (Supplementary Table 2 and Supplementary Fig. 2).

We then examined the haplotypic association for the five most common haplotypes (frequency >1%) formed by the associated markers rs6920220, rs10499197, rs5029939, rs2230926 and rs7749323 in the SLE pedigrees (~224-kb span; Supplementary Fig. 3 online). Haplotype 2, present on 19.3% of the chromosomes tested, is associated with SLE and carries only the minor allele of rs6920220 (haplotypic $P = 0.0064$). Haplotype 4, present on 1.1% of chromosomes tested, is also associated with SLE and carries the minor alleles of rs10499197, rs5029939, rs2230926 and rs7749323 but not rs6920220 (haplotypic $P = 3.67 \times 10^{-5}$). These results suggested the possibility of two independent genetic effects, one marked by the minor allele of rs6920220 (haplotype 2) and one marked by the minor alleles of rs10499197, rs5029939, rs2230926 and rs7749323 (haplotype 4).

Haplotype 3, also associated with SLE, carries the minor alleles of all five markers (haplotypic $P = 1 \times 10^{-4}$).

We performed conditional association analyses to test for independent effects at this locus. The epidemiological and genetic similarities between SLE and RA suggest that not only the same genes, but also identical variants, might affect risk of each disease. We therefore sought to test the contribution in SLE of variants previously shown to modulate risk of RA. Using logistic regression, we conditioned on the signal we identified in TNFAIP3 to determine whether association persisted at the RA-associated markers (Supplementary Table 3 online). Conditioning on either rs7749323 or rs10499197, we observed residual association at the RA risk marker rs6920220 ($P = 0.0024$ conditional on rs10499197; $P = 0.0017$ conditional on rs7749323). The converse analysis also supports independent signals, as adjustment for the effect at rs6920220 did not eliminate signal at any of the four markers in the region surrounding TNFAIP3 (rs10499197).
\[ P = 4.51 \times 10^{-5}, \text{rs5029939} \quad P = 9.00 \times 10^{-5}, \text{rs2230926} \]
\[ P = 0.0012, \text{rs7749323} \quad P = 1.75 \times 10^{-5} \]. These results suggest that two independent variants in the TNFAIP3 region contribute to SLE risk, although we are unable to exclude the possibility that a single variant with multiple risk alleles (for example, the INS VNTR in type 1 diabetes) or one causal allele partially captured by rs6920220 and rs10499197-rs7749323 underlies the signal.

The haplotype and conditional analyses also suggest that the missense SNP in exon 3 of TNFAIP3 (rs2230926) may not be the only putative causal variant. Haplotype 5 carries the minor allele of rs2230926 but is not associated with SLE (Supplementary Fig. 3). In addition, the association at rs2230926 can be completely explained by the association at rs10499197 and rs7749323 (Supplementary Table 3). Taken together, these results suggest that an untyped variant(s) carried on the haplotype tagged by the minor alleles of rs10499197 and rs7749323 is responsible for this SLE association effect. However, additional genotyping and resequencing will be necessary to identify the causal allele(s) and definitively determine the contribution of the TNFAIP3 locus to SLE.

This genome-wide association study identifies TNFAIP3 as a new susceptibility locus in SLE. Through its action as a negative regulator of the NF-kB pathway, TNFAIP3 plays a key role in modulating a broad range of cellular functions, including cell activation, cytokine signaling and apoptosis\(^{10}\). Mechanistically, TNFAIP3 catalyzes the ubiquitin modification of adaptor proteins downstream of TNFR, TLR and IL1R\(^{3,4,11}\). These pro-inflammatory pathways contribute to the pathogenesis of RA and SLE.

Data for RA\(^{5,6}\) and our results for SLE suggest a genetic model of three alleles with independent effects: (i) a risk allele \( \sim 185 \text{ kb upstream of TNFAIP3} \) at rs6920220 for RA and SLE, (ii) a nearby protective allele at rs10499194 for RA but not apparent in SLE, and (iii) a risk haplotype for SLE marked by a haplotype comprised of the minor alleles of rs10499197 and rs7749323 that directly spans TNFAIP3.

In addition to our study, three other genome-wide scans in SLE have been performed\(^{7,8,12}\). New and convincing genetic associations present in two or more of these scans include variants in HLA, IRF5, STAT4, ITGAM and BLK. Other genes, including BANK1, PAX5 and TNFAIP3, achieved genome-wide significance in only one study. The sensitivity to detect association in a GWAS can be influenced by a variety of factors, including sample size, cohort demographics, study design, analytical strategies, environmental factors, phenotype definition, variation in the estimation of control allele frequencies and, in the case of TNFAIP3, SNP selection. Our ability to recognize the TNFAIP3 association was facilitated, in part, because SNPs that capture the associated haplotype were well represented on the Affymetrix 5.0 array. In contrast, the other studies used genotyping arrays with SNPs that fail to capture the TNFAIP3 risk haplotype effectively, thus making the signal virtually impossible to detect.

In summary, we have identified a previously unreported association between variants in the TNFAIP3 region and SLE. Our data support the presence of genetic effects that are both shared and distinct from the recently described association with RA. TNFAIP3, through its role as a key regulator of NF-kB signaling, represents a compelling candidate locus for further studies of autoimmune pathogenesis.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS


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