African-Derived Genetic Polymorphisms in TNFAIP3 Mediate Risk for Autoimmunity


J Immunol published online 7 May 2010
http://www.jimmunol.org/content/early/2010/05/07/jimmunol.1000324

Supplementary Material
http://www.jimmunol.org/content/suppl/2010/05/07/jimmunol.1000324
4.DC1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
African-Derived Genetic Polymorphisms in TNFAIP3 Mediate Risk for Autoimmunity

James P. Lodolce,*1 Lauren E. Kolodziej,*1 Lesley Rhee,* Silvia N. Kariuki,* Beverly S. Franek,* Nancy M. McGreal,*2 Mark F. Logsdon,* Sarah J. Bartulis,* Minoli A. Perera,* Nathan A. Ellis,* Erin J. Adams,† Stephen B. Hanauer,* Meenakshi Jolly,‡ Timothy B. Niewold,* and David L. Boone*

The Journal of Immunology, 2010, 184: 000–000

The TNF α-induced protein 3 (TNFAIP3) is an ubiquitin-modifying enzyme and an essential negative regulator of inflammation. Genome-wide association studies have implicated the TNFAIP3 locus in susceptibility to autoimmune disorders in European cohorts, including rheumatoid arthritis, coronary artery disease, psoriasis, celiac disease, type I diabetes, inflammatory bowel disease, and systemic lupus erythematosus (SLE). There are two nonsynonymous coding polymorphisms in the deubiquitinating (DUB) domain of TNFAIP3: F127C, which is in high-linkage disequilibrium with reported SLE-risk variants, and A125V, which has not been previously studied. We conducted a case–control study in African-American SLE patients using these coding variants, along with tagging polymorphisms in TNFAIP3, and identified a novel African-derived risk haplotype that is distinct from previously reported risk variants (odds ratio = 1.6, p = 0.006). In addition, a rare protective haplotype was defined by A125V (odds ratio = 0.31, p = 0.027). Although A125V was associated with protection from SLE, surprisingly the same allele was associated with increased risk of inflammatory bowel disease. We tested the functional activity of nonsynonymous coding polymorphisms within TNFAIP3, and found that the A125V coding-change variant alters the DUB activity of the protein. Finally, we used computer modeling to depict how the A125V amino acid change in TNFAIP3 may affect the three-dimensional structure of the DUB domain to a greater extent than F127C. This is the first report of an association between TNFAIP3 polymorphisms and autoimmunity in African-Americans. The Journal of Immunology, 2010, 184: 000–000.

*Department of Medicine and †Department of Biochemistry and Molecular Biophysics, University of Chicago; and ‡Rush University Medical Center, Chicago, IL 60612

1Current address: Duke University, Durham, NC.

Received for publication February 2, 2010. Accepted for publication April 8, 2010.

This work was supported by National Institutes of Health Grants DK42086 (to D.L.B.), 1F32AI077235-01A1 (to J.P.L.), T32AI-07090 (to J.P.L.), K01AR083790 (to T.B.N.), and AI071651 (to T.B.N.), Crohn’s and Colitis Foundation of America (to D.L.B.), International Organization for the Study of Inflammatory Bowel Disease (to D.L.B. and S.B.H.), Arthritis National Research Foundation Eng Tan Scholar Award (to T.B.N.), Lupus Research Institute Novel Research Grant (to T.B.N.), University of Chicago Clinical and Translational Science Award (to T.B.N.), and Collaborative University of Chicago/Northshore University Health System Translational Research Pilot Grant ULI 1R032499 (to T.B.N.)

Address correspondence and reprint requests to Dr. Timothy B. Niewold, 924 East 57th Street, JFK K420, Chicago, IL 60637, or Dr. David L. Boone, 900 East 57th Street, Mailbox 9, Chicago, IL 60637. E-mail addresses: tniewold@medicine.bsd.uchicago.edu or dboone@medicine.bsd.uchicago.edu

The online version of this article contains supplemental material.

Abbreviations used in this paper: CAD, coronary artery disease; CD, Crohn’s disease; CeD, celiac disease; CI, confidence interval; dbSNP, Single Nucleotide Polymorphism Database; DUB, deubiquitinating; IB, immunoblotted; IB, inflammatory bowel disease; IP, immunoprecipitation; K48R-Ub, K48-ubiquitin; LD, linkage disequilibrium; OR, odds ratio; PVDF, polyvinylidene fluoride; RA, rheumatoid arthritis; RIP1, receptor-interacting protein 1; SLE, systemic lupus erythematosus; SNP, single nucleotide polymorphism; T1D, type I diabetes; TNFAIP3, TNF α-induced protein 3; TRAF, TNF receptor-associated factor; YRI, Yoruba population in Ibadan, Nigeria; WT, wild-type.

Copyright © 2010 by The American Association of Immunologists, Inc. 0022-1767/10/$16.00 (RIP1) and TNF receptor-associated factor (TRAF) 6, respectively (2, 3). TNFAIP3 controls inflammation by removing the signaling form of K63-linked polyubiquitin (deubiquitinating [DUB] activity) and attaching degradative K48-linked polyubiquitin (ubiquitin ligase activity) to cell signaling molecules like RIP1 and TRAF6. The combined actions of TNFAIP3 result in the elimination of key activated signaling proteins in inflammatory pathways illustrating the importance of TNFAIP3 in the termination of inflammation downstream of multiple stimuli. Mice deficient in TNFAIP3 develop severe multiorgan inflammation that leads to premature death (4). Systemic inflammation occurs spontaneously and resembles many features of human autoimmune disease.

The multiple potential roles of TNFAIP3 in regulating inflammation are not fully understood, and are likely to be cell and context dependent. For example, TNFAIP3 mutations/deletions are frequently associated with the development of B cell lymphomas, suggesting that loss of TNFAIP3 in this cell type is oncogenic, possibly due to increased NF-κB signals that are antiapoptotic/proliferative in B lymphocytes (5–7). Conversely, TNFAIP3 is required to protect thymocytes or fibroblasts from TNF-induced apoptosis, despite prolonged NF-κB activation (4). TNFAIP3 appears to be a central regulator of inflammatory responses, and it is likely that multiple distinct variants of TNFAIP3 could differentially modulate risk of autoimmunity in different diseases and different ancestral backgrounds.

Systemic lupus erythematosus (SLE) is a complex autoimmune disease that is frequently characterized by inappropriate type I IFN responses driven by small nuclear self-Ags (8). Humoral autoimmunity is a hallmark of disease, and the formation of immune complexes containing self-Ags leads to local and systemic inflammation and subsequent clinical symptoms. The pathogenesis of SLE likely involves both environmental and genetic triggers, the
latter highlighted by the observation that siblings of affected individuals have a 20- to 30-fold increased risk of developing SLE (9). In addition, compared with European-Americans the incidence of SLE is three to four times higher in African-Americans (10, 11). Genome-wide association studies have begun to identify genes that contribute to SLE susceptibility (12–17). Most of these studies have been conducted exclusively in European cohorts; thus, little is known about whether variants in these genes are risk factors in other ancestral backgrounds. Differences in autoimmune disease risk variants exist across different continental populations. For example, the autoimmune disease risk variant of the protein tyrosine phosphatase type 22 gene (PTPN22) is almost absent in African-derived populations (18). The TNFAIP3 gene region has been implicated in susceptibility to multiple autoimmune diseases in populations of European ancestry. Single nucleotide polymorphisms (SNPs) ~185 kb upstream of the TNFAIP3 gene are associated with risk for rheumatoid arthritis (RA) (19, 20), type 1 diabetes (T1D) (21), and celiac disease (CeD) (22). The Wellcome Trust Case Control Consortium identified a SNP upstream of TNFAIP3 in 6q23 that is associated with risk for Crohn’s disease (CD) (23). In addition, this same chromosomal region was found as a candidate for susceptibility in a study of 260 inflammatory bowel disease (IBD)-affected relative pairs (24). Intronic TNFAIP3 SNPs are associated with psoriasis (25) and coronary artery disease (CAD) in type 2 diabetes patients (26).

Two independent studies have found an association between TNFAIP3 and SLE in European ancestry. The same SNP identified in RA and CeD that falls ~185 kb upstream of TNFAIP3 was also associated with SLE risk, and a second SLE risk haplotype was identified within the TNFAIP3 gene (15). Another study suggested a complex composition of three individual variants, including a nonsynonymous F127C coding SNP (rs2230926) (17). Meta-analysis and imputation has narrowed down the SLE-associated region of TNFAIP3 in patients of European ancestry to a single 109 kb haplotype centered on the TNFAIP3 gene, which includes the F127C SNP (27). In functional studies, Musone et al. demonstrated decreased activity of the TNFAIP3 protein with the SLE-associated F127C amino acid change (rs2230926) (17). The identification of a functionally defective risk variant may explain how TNFAIP3 contributes to SLE pathogenesis. In people of European ancestry, the minor allele frequency of rs2230926 is relatively low (0.05 in SLE cases and 0.02 in controls) compared with the reported frequency of 0.447 in the Yoruba population in Ibadan, Nigeria (YRI) (28). In addition, the association between SLE and the TNFAIP3 gene region appears to be complex, and not completely accounted for by the F127C polymorphism. Overall, these studies demonstrate that SNPs within or near TNFAIP3 are associated with autoimmune, and significant differences in TNFAIP3 SNP frequencies between European and African populations suggest that disease-associated haplotypes in these two ancestral backgrounds may be dissimilar.

We therefore assessed the contribution of TNFAIP3 variants to autoimmune risk in African-Americans. To better understand how alterations in TNFAIP3 affect autoimmune susceptibility, we conducted a case-control study to look at the association between haplotype-tagging and coding-change SNPs within the TNFAIP3 gene and SLE in African-Americans. We identified a novel African-derived SLE-risk haplotype and a protective haplotype for SLE defined by the A125V polymorphism. We genotyped the A125V and F127C coding variants in African-American subjects with IBD and found that the A125V variant was associated with risk for IBD. Next, we tested the functional effect of four nonsynonymous coding polymorphisms in TNFAIP3. The A125V SNP demonstrated a dramatic functional effect, greatly decreasing TNFAIP3-mediated deubiquitination. Finally, we used computer modeling to examine how these amino acid changes may be affecting the predicted three-dimensional structure of the TNFAIP3 DUB domain.

Materials and Methods

Patient samples

Patient genomic DNA samples were obtained from the Translational Research Initiative in the Department of Medicine registry at the University of Chicago (n = 1222) and from the Rush University Medical Center (n = 33). A total of 217 self-identified African-American SLE patients, 24 African-American IBD patients, 272 African-American nonautoimmune controls, and 615 self-identified white or European-American samples of mixed disease status were studied. All SLE patients met four or more of the American College of Rheumatology criteria for diagnosis (29). Diagnosis of IBD was made historically using intestinal biopsies. The control population consisted of adults recruited from outpatient clinics at the University of Chicago who were either normal healthy or had an unrelated condition diagnosed, such as cancer, type 2 diabetes, or hypertension. The medical records of the control population were examined to exclude those with autoimmune or inflammatory disease diagnoses, including SLE, CD, sarcoidosis, autoimmunity thyroid disease, psoriasis, CeD, RA, and T1D. Subjects with documented CAD were also excluded given the results of a previous study suggesting an association between TNFAIP3 variants and CAD (26). The institutional review boards at both institutions approved this study, and informed consent was obtained from all participants.

Genotyping

Individual patients were genotyped at the coding SNPs A125V (rs5029941) and F127C (rs2230926) using direct sequencing. The close proximity of these two SNPs made probe-based genotyping difficult, and so direct sequencing was used instead. Briefly, a 303 bp fragment was amplified from genomic DNA using PCR (forward primer 5′-GAAAACCTTTGCTGGGTCTT-ACAT-3′, reverse primer 5′-CCATGGACCTGTGTTAGATATA-5′). The PCR product was treated with Exonuclease I and Shrimp Alkaline Phosphatase (USB, Cleveland, OH) prior to submission for ultra high-throughput sequencing at the University of Chicago Cancer Research Center DNA Sequencing Facility. Sequencing was performed on an Applied Biosystems 3730XL capillary sequencer (Applied Biosystems, Foster City, CA). Genotype calls were determined by visual inspection of individual base pair peaks in each sequence trace file. The haplotype-tagging SNPs rs5029939, rs5029953, rs2230926, rs610804, rs3757173, rs5029938, and rs582757 were chosen by focusing on the TNFAIP3 gene and the regions 10 kb upstream and downstream of the coding sequence. A two and three marker-tagging algorithm was used with a correlation coefficient ($r^2$) threshold of 0.8 to choose SNPs with an allele frequency >10% in the International HapMap Project YRI population. Haplotype-tagging SNPs were genotyped using ABI TaqMan primers and probes on the ABI 7900HT Fast Real-Time PCR System (Applied Biosystems) per manufacturer’s protocol. All scatter plots were examined visually to verify the accuracy of the genotype calls. None of the SNPs significantly deviated from predicted Hardy-Weinberg equilibrium in African-American control subjects (lowest p value = 0.0680) (Table I). Two tagging SNPs (rs5029938 and rs582757) were genotyped in only 25% of cases and 48% of controls due to either low allele frequency and/or low information content, whereas the remaining six SNPs were successfully genotyped in >93% of controls and >99% of cases (Supplemental Table I).

Statistical analysis

Odds ratios with 95% confidence intervals and p values using two-tailed χ² tests were calculated with GraphPad Prism software (GraphPad, San Diego, CA). None of the reported p values were corrected for multiple testing. The odds ratios (ORs) were adjusted to account for the “winner’s curse” using WINNER version 1.1 (http://csg.sph.umich.edu/boehnke/winner/) written by Rui Xiao and Michael Boehnke (30). Ascertainment-corrected ORs are listed in Supplemental Table 1. In addition, the minimum genetic effect that could be significantly (p = 0.05) detected in this study with 80% power was calculated using an additive model in the CaTS Power Calculator written by Andrew Skol (www.sph.umich.edu/csg/abc-ca/t/CaTS/) and Power for Association with Error, version 1.2, written by Derek Gordon was used for the smaller cohorts that cannot be calculated using the CaTS program. Power for Association with Error can be found at http://tango.rockefeller.edu/~ca/abca.CurrentRow.html. In this study with African-American SLE cohort, this study had 80% power to detect an OR of ≥1.53 with a p value of 0.05 using an additive genetic model over the allele frequency range represented by the SNPs studied, except the rare
variant A125V (Supplemental Table I). In the case of A125V, we could detect an OR of 0.33 or lower for A125V using the same parameters as above. In the African-American IBD cohort, we had 75% power to detect an OR of $\geq 3.7$ for association of A125V using the same parameters as above. The degree of pairwise linkage disequilibrium (LD) between SNPs ($r^2$ values) and haplotype frequencies were determined with Haploview 4.1 software. Linkage blocks were defined using the solid spine of LD method implemented in the Haplovie software. Multivariate and conditional logistic regression models were performed using an online tool written by John C. Pezzullo (http://statpages.org/logistic.html).

Expression plasmids

The wild-type (WT) human TNFAIP3 cDNA was PCR cloned from HCT116 epithelial cell cDNA into a pENTR entry vector (Invitrogen, San Diego, CA). The C103A, A125V, F127C, R706Q, and A766P point mutants, as well as the A125V/F127C double mutant and the E3 ligase-deficient mutant (C607A, C612A, C624A, and C627A) were generated from WT TNFAIP3 according to the QuikChange Site-Directed Mutagenesis Kit instructions (Stratagene, La Jolla, CA). The V5-tagged TNFAIP3 constructs were each generated by shuttling the gene into pcDNA3.1/nV5 using the Gateway Clonase LR II enzyme kit (Invitrogen). Cytokine response modifier A and PcmV-Myc-ubiquitin expression plasmids were gifts from M. Peter and C. Maki, respectively (University of Chicago, Chicago, IL). WT Myc-ubiquitin was generated from Myc-K48R (University of San Diego, CA). The C103A, A125V, F127C, R706Q, and A766P point mutants were produced by using an expression tool written by John C. Pezzullo (http://statpages.org/logistic.html).

Cell culture and Abs

HEK293 cells (American Type Culture Collection, Manassas, VA) were maintained in DMEM supplemented with 10% FBS, penicillin 100 U/ml, and streptomycin 100 U/ml (Life Technologies, Rockville, MD) and incubated at 37°C in a humidified, 5% CO2 atmosphere. Polyclonal rabbit anti-HA Ab (Y-11) and goat anti-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA); monoclonal mouse anti-Myc and anti-V5 Abs were purchased from Invitrogen. Species-specific secondary Abs conjugated to IRDye or Alexa Fluor infrared dyes (680 nm or 800 nm) were used at a 1:20,000 dilution (Rockland, Gilbertsville, PA, and Invitrogen).

Transfections, immunoprecipitation, and immunoblot analysis

For transient transfection assays, HEK293 cells were seeded at 2 × 106 cells per 10 cm plate (BD Falcon, Franklin Lakes, NJ) 18 h prior to transfection. Immediately before transfection, cells were rinsed in 1× PBS (Life Technologies) and supplemented with serum-free media. Plasmid DNA (7.5 μg total) and a 2 mg/ml polyethylenimine solution (Polysciences, Warrington, PA) were each diluted in 150 μl cytokine reconstituted HCT116 epithelial cell cDNA into a pENTR entry vector (Invitrogen, San Diego, CA). The C103A, A125V, F127C, R706Q, and A766P point mutants, as well as the A125V/F127C double mutant and the E3 ligase-deficient mutant (C607A, C612A, C624A, and C627A) were generated from WT TNFAIP3 according to the QuikChange Site-Directed Mutagenesis Kit instructions (Stratagene, La Jolla, CA). The V5-tagged TNFAIP3 constructs were each generated by shuttling the gene into pcDNA3.1/nV5 using the Gateway Clonase LR II enzyme kit (Invitrogen). Cytokine response modifier A and PcmV-Myc-ubiquitin expression plasmids were gifts from M. Peter and C. Maki, respectively (University of Chicago, Chicago, IL). WT Myc-ubiquitin was generated from Myc-K48R (University of San Diego, CA). The C103A, A125V, F127C, R706Q, and A766P point mutants were produced by using an expression tool written by John C. Pezzullo (http://statpages.org/logistic.html).

The eight TNFAIP3 SNPs genotyped in this study are listed, along with the identity of the minor allele. Minor allele frequency in cases and controls is shown. The p value (from $\chi^2$ analysis) and OR with 95% confidence interval (CI) was calculated with GraphPad Prism software. SNPs with a p value $<0.10$ were conditioned on rs5029953 using logistic regression. The two predictor variables were the rs5029953 genotype and the genotype of the SNP being tested with the outcome variable being SLE.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Minor Allele</th>
<th>Allele Ratio Counts (Cases, Controls)</th>
<th>Minor Allele Freq (Cases, Controls)</th>
<th>p Value</th>
<th>OR (95% CI)</th>
<th>p Value (Conditioned on rs5029953)</th>
<th>HWE p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3757173</td>
<td>G</td>
<td>210:220, 262:302</td>
<td>0.488, 0.428</td>
<td>0.0621</td>
<td>1.276 (0.9875–1.648)</td>
<td>0.5069</td>
<td>0.0680</td>
</tr>
<tr>
<td>rs5029938</td>
<td>G</td>
<td>15:195, 16:124</td>
<td>0.071, 0.114</td>
<td>0.1668</td>
<td>0.5962 (0.2845–1.249)</td>
<td>0.7609</td>
<td>—</td>
</tr>
<tr>
<td>rs5029939</td>
<td>G</td>
<td>166:268, 172:364</td>
<td>0.382, 0.321</td>
<td>0.0453</td>
<td>1.311 (1.005–1.709)</td>
<td>0.5667</td>
<td>0.2606</td>
</tr>
<tr>
<td>A125V</td>
<td>T</td>
<td>5:429, 15:491</td>
<td>0.0115, 0.0296</td>
<td>0.0549</td>
<td>0.3815 (0.1375–1.059)</td>
<td>—</td>
<td>0.3848</td>
</tr>
<tr>
<td>F127C</td>
<td>T</td>
<td>15:195, 16:124</td>
<td>0.071, 0.114</td>
<td>0.2799</td>
<td>1.345 (1.032–1.753)</td>
<td>0.4489</td>
<td>0.2263</td>
</tr>
<tr>
<td>rs852757</td>
<td>C</td>
<td>66:144, 51:87</td>
<td>0.314, 0.370</td>
<td>0.2856</td>
<td>0.7819 (0.4975–1.229)</td>
<td>—</td>
<td>0.3489</td>
</tr>
<tr>
<td>rs610604</td>
<td>T</td>
<td>156:278, 197:325</td>
<td>0.359, 0.377</td>
<td>0.5670</td>
<td>0.9258 (0.7108–1.206)</td>
<td>—</td>
<td>0.7017</td>
</tr>
<tr>
<td>rs5029953</td>
<td>A</td>
<td>80:354, 66:470</td>
<td>0.184, 0.123</td>
<td>0.0080</td>
<td>1.609 (1.130–2.293)</td>
<td>—</td>
<td>0.1297</td>
</tr>
</tbody>
</table>

The results

Polymorphisms in the TNFAIP3 locus are associated with SLE risk in African-Americans

A case–control study was conducted using a cohort of African-American subjects to examine the association of TNFAIP3 variants with altered SLE risk. SLE cases ($n = 217$) and nonautoimmune controls ($n = 272$) were genotyped with real-time PCR using TaqMan probe chemistry at six tagging SNPs within the TNFAIP3 gene region. In addition, the Single Nucleotide Polymorphism Database (dbSNP) lists two SNPs within the DUB domain of TNFAIP3 (aa 92–263) that result in nonsynonymous coding changes in the protein sequence. These include rs5029941 (A125V) and rs2230926 (F127C), the latter associated with SLE risk in European populations (15, 17). Thus, in addition to the six tagging SNPs, we also genotyped these two coding polymorphisms in our patient samples using sequencing.

We tested each of the eight TNFAIP3 SNPs for association with SLE and found that two tagging SNPs (rs5029939 and rs5029953), along with the F127C coding SNP, were associated with SLE susceptibility (Table I). In Europeans, the tagging SNP rs5029939 ($p = 0.0453$, OR = 1.3) and the coding variant F127C ($p = 0.0279$, OR = 1.3) were identified as SLE risk variants, and similar to the European studies, we find these two variants in very high LD ($r^2 = 0.99$) (15, 17). The most significant SLE risk-associated SNP we observed was not either of these two previously associated SNPs, but was instead the African-derived rs5029953 SNP ($p = 0.008$, OR = 1.6). Conditioning on rs5029953 abolished the association signals from the other SNPs (rs5029939 and F127C), indicating that they are not independently contributing to risk (Table I). No SNP-SNP interactions were observed, and thus the simple model of a single risk variant marked by rs5029953 was the best fit (data not shown). In the dbSNP database the minor allele of rs5029953 has a reported
frequency ranging from 0.077 to 0.161 in African populations, and this SNP has not been identified in Asian or European populations. We genotyped a total of 324 white or European-American samples, and all carried the major allele of rs5029953, supporting the idea that this SNP is indeed of African origin.

Surprisingly, we found that the rare minor allele of A125V was associated with protection from SLE in African-American subjects (p = 0.05, OR = 0.38) (Table I). We did not detect this variant in self-reported white or European-ancestry subjects (n = 291) (Supplemental Table II), and this variant was present in our nonautoimmune African-American controls at a frequency of 0.03 (Table II), which is comparable to the frequency reported in African populations in public databases. The minor allele of A125V is in high LD with the minor allele of F127C, as the two patients identified as homozygous for the minor allele of A125V were also homozygous for the minor allele of F127C, and every A125V heterozygote was either heterozygous or homozygous for the minor allele of F127C. The complete distribution of genotypes by ethnicity and autoimmune disease for both A125V and F127C can be found in Supplemental Table II.

Haplotype analysis in African-American subjects detected eight haplotypes with a frequency of ≥1% in cases and controls (Fig. 1A). The rs5029953 SNP associated with SLE risk is unique to one haplotype, and thus marks the SLE risk-associated haplotype (haplotype 2, OR = 1.6, p = 0.0061). A linkage disequilibrium plot of the SLE cases or controls shows that among the SNPs analyzed, rs5029953 is most strongly associated with rs5029939 and F127C (Fig. 1B, Supplemental Fig. 1). In addition, the A125V rare variant is only found on one haplotype, identifying the protective haplotype (haplotype 8, OR = 0.3, p = 0.027), which does not include the SLE-risk-associated allele. These findings taken together suggest that the F127C polymorphism is not a major determinant of SLE risk in African-Americans, and that instead a model of risk, neutral, and protective haplotypes seems to apply. Interestingly, the protective haplotype contains a rare coding polymorphism (A125V) that could alter TNFAIP3 activity; whereas, the functional implications of the risk haplotype are not currently clear.

A key clinical feature of SLE is the production of autoantibodies against nucleic acids (i.e., anti-dsDNA) and small nuclear proteins that stably interact with RNA (RNA binding proteins or RBPs). Other genes that confer risk to SLE, such as IRF5, are associated with higher interferon responses in patients with either anti-dsDNA or anti-RBP autoantibodies (34). We tested whether any of the TNFAIP3 SNPs studied could predict the production of any SLE-related autoantibodies, and found no significant associations (data not shown).

The F127C and A125V TNFAIP3 coding SNPs are associated with autoimmunity in African-Americans

We next assessed the contribution of the A125V and F127C polymorphisms to inflammatory disease susceptibility. The patient samples were grouped according to diagnosis (IBD, SLE, or nonautoimmune controls), and the A125V and F127C genotypes were analyzed together as predictor variables in a logistic regression model. Strikingly, the minor allele of A125V is significantly associated with susceptibility to IBD (Table II) (OR = 3.73, p = 0.027). As noted previously, this same allele is associated with protection from SLE (OR = 0.33, p = 0.032). Thus, the rare variant A125V has opposite effects on risk of IBD and SLE in African-Americans. Despite the low number of African-American IBD case samples, this study had 75% power to detect the large genetic effect identified (OR = 3.7) with a p value = 0.05 (Supplemental Table I). Furthermore, correcting for the “winner’s curse effect” does not significantly decrease the estimated OR for this association (Supplemental Table I). Although the low number of patients limits our confidence in the IBD data to some degree, these preliminary data suggest a strong effect, which is almost directly opposite of that observed in SLE.

**Diminished functional activity of A125V TNFAIP3**

The observation that coding SNPs in TNFAIP3 are associated with autoimmunity in African-Americans suggests that these amino acid substitutions may be altering the enzymatic function of the protein. Because the A125V and the F127C polymorphisms occur together (A125V is never found without F127C; whereas, F127C is often found without A125V), we tested the functional activity of A125V/F127C TNFAIP3. In addition to the A125V/F127C double mutant, four single nonsynonymous coding mutations (A125V, F127C, R706Q, and A766P) were made in the TNFAIP3 cDNA and cloned into mammalian expression vectors. R706Q and A766P were chosen because they are listed in the dbSNP database as validated, nonsynonymous coding SNPs located near the ubiquitin ligase domain of TNFAIP3. These variants were not studied in the genetic association study, as their probable low frequency in human populations based on HapMap data would likely prevent significant conclusions given our sample size. A previous study demonstrated that mutating the cysteine at amino acid position 103 to alanine inhibits the DUB activity of TNFAIP3 (3); thus, C103A TNFAIP3 was used as a negative control. TRAF2 is a key signaling molecule in several inflammatory pathways, is known to be ubiquitinated with K63-linked chains, and has been shown to be associated with TNFAIP3 (35–37). TNFAIP3 is a dual functioning enzyme that both deubiquitinates and induces the degradation of its targets. The two activities of this enzyme are linked, in that TNFAIP3 must deubiquitinate targets before it can induce their degradation. The ability of these TNFAIP3 mutants to induce the degradation of TRAF2 was tested to evaluate the functional consequence of nonsynonymous coding variants in TNFAIP3. WT TNFAIP3 induces the degradation of TRAF2 in a dose-dependent manner; whereas, the catalytic mutant C103A fails to do so (Fig. 2). We found that the A125V/F127C double mutant TNFAIP3 is less efficient at inducing the degradation of TRAF2 when compared with WT TNFAIP3 (Fig. 2A). Of the four single polymorphisms, the A125V SNP appears to have a diminished ability to
Each box represent the pairwise age between the plot generated from African American SLE cases shows the relative link-
functional defect (compare levels of TRAF2 degradation of however, the A125V/F127C mutant may have a more profound
evasion. Thus, the A125V polymorphism is likely the main cause of
studies will be needed to determine the significance of this obser-
functional activity of TNFAIP3 and future genetic and biochemical
In addition, the A766P mutation may have a subtle effect on the
lead to loss of TRAF2, but not to the same extent as WT TNFAIP3.

To further explore the effect of the A125V mutation, we tested
with A125V TNFAIP3 in Fig. 90% of our samples and therefore
unclear whether this results in increased or decreased risk for de-
this mutation causes a reduction in the activity of TNFAIP3, it is
affected enzymatic activity (38). We found that after making the
alanine to valine acid change would have a greater
effect on protein function compared with a phenylalanine to cyste-
the DUB domain of TNFAIP3 has been crystallized, so

Computer modeling of TNFAIP3 structure
Of the four naturally occurring TNFAIP3 polymorphisms tested, we
were surprised to find such a strong functional defect in the A125V
Although both the A125V and F127C are located within the
terminal DUB domain of TNFAIP3, it was not initially obvious why an alanine to valine amino acid change would have a greater
effect on protein function compared with a phenylalanine to cyste-
the center of protein and potentially causing steric interference with
isoleucines 29 and 202 (Fig. 4A). This could result in a conforma-
tional change that affects the nearby catalytic core of the DUB
domain. The F127C substitution pointed away from the protein
Such a change may not directly interfere with the enzym-
atic activity of the protein, but could influence the ability of target
to bind with TNFAIP3. This could also explain why we failed to see a significant functional consequence of F127C in our
assay; whereas, Musone et al. observed decreased regulation of
NF-κB activation by this variant (17).

Discussion
Our results demonstrate the first association of TNFAIP3 with auto-
toimmunity in African-Americans. We have shown that an African-
derived rare variant in the TNFAIP3 gene (A125V) is associated
with risk for IBD, yet is protective for SLE. However, the role of
TNFAIP3 appears complex in SLE, as we also describe a common
African-derived haplotype associated with risk for SLE, marked by
the tagging SNP rs5029953. Thus, we can identify risk, neutral, and
protective haplotypes in TNFAIP3 in African-Americans, and these
haplotypes are defined by African-derived genetic elements. We
tested the effects of nonsynonymous coding SNPs on the enzymatic
activity of TNFAIP3 and found that the A125V mutation causes a
decrease in the ability of TNFAIP3 to deubiquitinate and degrade
the target protein TRAF2. Although our functional data suggest that
this mutation causes a reduction in the activity of TNFAIP3, it is
unclear whether this results in increased or decreased risk for de-
veloping inflammation. In fact, our genetic data argue that the effect
of this polymorphism in inflammatory disease will be complex, as
the same polymorphism is associated with risk of one condition and
susceptibility to another.
We found that the minor allele of the African-American–specific tagging SNP rs5029953 marks the SLE-risk haplotype in 139 African-American samples (n = 485), but in none of the European ancestry samples (n = 324). Our study covered a range of ~10 kb centered on the TNFAIP3 gene. A closer examination of the linkage age of rs5029953 with nearby SNPs in the International HapMap Project database’s YRI population suggests that this polymorphism lies within a linkage block of ~7 kb (Supplemental Fig. 2). There are nine other SNPs in LD with rs5029953 (r² > 0.50), including SNPs that fall within two LD blocks upstream and one LD block downstream of rs5029953 covering ~68 kb of the genetic region in and around the TNFAIP3 gene. All nine SNPs in high linkage with rs5029953 are of African origin (frequency range in YRI between 9–19%), with none of the minor alleles found in Europeans (Supplemental Table III). Thus, the SLE-risk haplotype we identify is of African origin. The SLE-risk signal in African-Americans is centered on the TNFAIP3 gene in our study, similar to the recent SLE meta-analysis in Europeans (27). The functional element on this African-derived SLE-risk haplotype causing SLE susceptibility remains unclear, and the most strongly associated SNP (rs5029953) has no predicted functional effect. However, it is possible that this intronic SNP affects mRNA splicing, stability, or the binding of small regulatory RNAs. Other candidate functional elements include TNFAIP3 coding variants located within the same LD block as rs5029953 (F127C, R706Q, P765, and A766P). Although we find that F127C is associated with SLE risk, it is not independently contributing to the risk associated with rs5029953. We also found no additive effect between rs2230926 and rs5029953. Because we find limited functional or genetic effects with this SNP, it is possible that the result of this coding change in vivo is negligible or is compensated for in another way. Based on our analysis of the structure of the TNFAIP3 DUB domain, further functional studies that focus on the interaction of TNFAIP3 with various target proteins are needed to elucidate the extent of the F127C amino acid substitution on the enzymatic function of the protein. Another coding region SNP (rs5029956) on the risk haplotype is a synonymous base pair substitution in the proline residue 765 (CCC->CCT) in the ninth exon of TNFAIP3. This is a potential functional element, as protein levels can be modulated by synonymous base pair changes in codons. A variation in protein expression of ~250-fold was found by engineering synonymous mutations in GFP expressed by Escherichia coli which resulted in differential mRNA stability and folding (39). The effect of the synonymous

of each protein band was determined, and the densitometry of the TRAF2 and TNFAIP3 bands are plotted using arbitrary units. One of three experiments is shown. B. The ability of TNFAIP3 single mutants to degrade the protein target TRAF2 was tested. Increasing amounts of either V5-tagged WT or A125V/F127C TNFAIP3 (1, 2.5, 3.5, or 5 µg), or V5-tagged C103A TNFAIP3 (3.5 µg) were cotransfected with HA-tagged TRAF2 (0.5 µg) into HEK293 cells. Whole cell lysates were transferred to PVDF membrane and immunoblotted (IB) for HA, V5, or actin. The A125V/F127C mutant fails to degrade TRAF2 to the same extent as WT TNFAIP3, and is comparable to the known catalytic mutant C103A. Equivalent protein loading was confirmed by immunoblotting for actin. The relative intensity

FIGURE 2. A125V TNFAIP3 mediates diminished degradation of TRAF2. A. The ability of the A125V/F127C double mutant to degrade the protein target TRAF2 was tested. Increasing amounts of either V5-tagged WT or A125V/F127C TNFAIP3 (1, 2.5, or 3.5 µg), or V5-tagged C103A TNFAIP3 (3.5 µg) were cotransfected with HA-tagged TRAF2 (0.5 µg) into HEK293 cells. Whole cell lysates were transferred to PVDF membrane and immunoblotted (IB) for HA, V5, or actin. The A125V/F127C mutant fails to degrade TRAF2 to the same extent as WT TNFAIP3, and is comparable to the known catalytic mutant C103A. Equivalent protein loading was confirmed by immunoblotting for actin. The relative intensity
P765 SNP on the expression of TNFAIP3 in vivo remains to be studied. Finally, the nonsynonymous coding SNPs rs3734553 (R706Q) and rs5029957 (A766P) are found within the SLE-associated LD block; however, the existence of these SNPs has not been fully verified, and these may be extremely rare variants. In addition, we found limited functional effects of these mutations. Further fine-mapping and sequencing along this ancestral risk haplotype, along with in vitro functional assays, will help to identify the precise functional element or elements that determine the association between the TNFAIP3 gene and increased SLE risk in African-Americans.

We demonstrated that the A125V human polymorphism in TNFAIP3 has a strong in vitro effect on protein function and also shows significant and opposing genetic effects in two distinct autoimmune diseases (OR.3 in IBD versus OR.0.33 in SLE). There are precedents for genetic variants that are associated with risk in one disease, but with protection in another. The non-synonymous variant R620W in the PTPN22 gene is associated with risk for RA, T1D, SLE, and autoimmune thyroiditis (40).

**FIGURE 3.** A125V TNFAIP3 has reduced DUB activity on non–K48-linked polyubiquitinated target proteins. The ability of WT TNFAIP3 and the TNFAIP3 mutants C103A and A125V to deubiquitinate the protein target TRAF2 was tested. Increasing amounts (1, 2.5, 5, or 7.5 µg) of V5-tagged WT TNFAIP3, C103A TNFAIP3, or A125V TNFAIP3 were cotransfected with Myc-tagged K48R-ubiquitin (1 µg) and HA-tagged TRAF2 (3 µg). All three TNFAIP3 constructs have point mutations in conserved cysteines within zinc-fingers 3 and 4 in the C-terminal ubiquitin ligase domain (C607A, C612A, C624A, and C627A), rendering them ubiquitin ligase deficient (E3 ligase deficient). This helped to reduce subsequent degradation of TRAF2 by TNFAIP3. Whole cell lysates were either transferred to PVDF membranes and IB for Myc, HA, V5, or actin (pre-IP), or IP with Ab against HA. These immunoprecipitated lysates were then transferred onto PVDF membranes and immunoblotted for Myc, HA, and V5. The A125V/E3 Ligase mutant demonstrates a reduced ability to DUB TRAF2 in the IP samples, as well as total non–K48-linked polyubiquitinated protein in the pre-IP samples. Equivalent protein loading in the pre-IP samples was confirmed by immunoblotting for actin. The relative intensity of each protein band was determined, and the densitometry of the K48R-Ub and TRAF2 bands in the IP samples are presented on the left side; whereas, the K48R-Ub, TNFAIP3, and TRAF2 bands in the pre-IP samples are plotted on the right side in the lower half of the figure using arbitrary units. Furthermore, a plot showing levels of K48R-Ub in the samples immunoprecipitated for TRAF2 that has been normalized for the amount of TRAF2 present in the IP is located in the lower left bar graph. One of three experiments is shown.

**FIGURE 4.** The effect of nonsynonymous coding SNPs on the predicted crystal structure in the DUB domain of TNFAIP3. Using MacPyMOL, the reported crystal structure of the DUB domain was used to make the A125V and F127C amino acid changes. PDB accession number 2VFJ was used for this analysis. A, The R group of the valine group at position 125 (pink) has the potential of a steric clash with the R group of the isoleucine at position 29 and/or the isoleucine at position 202 (green). The relative position of the catalytic cysteine at position 103 is indicated in the background in orange. B, The relative positions of the A125V change (purple) pointing into the protein’s core compared with the F127C change (red) pointing away from the protein. The nearby catalytic DUB domain is marked by the cysteine at position 103 (orange).
However, this same polymorphism is protective in CD and Behcet’s disease (41, 42). The H131R variant in the Fcγ receptor 2A (FCGR2A) gene is associated with risk for SLE, T1D, Guillain-Barre syndrome, and multiple sclerosis; however, it is protective in ulcerative colitis in the Japanese population (12, 43–45). Thus, there are several examples of coding variants that have opposing disease associations in autoimmune.

A central question that arises from this study is how a variant that decreases the function of TNFAIP3 is associated with risk for IBD yet protection from SLE. Much of the previous work on TNFAIP3 suggests that impairing its enzymatic activity results in increased inflammation (1). Although this is in agreement with increased susceptibility to a proinflammatory disease like IBD, it is not consistent with protection from SLE. One hypothetical model that could explain this paradox involves differences in the types of cytokine milieu that are produced during the course of each disease, thus influencing the Th1/2 skewing of T lymphocytes. SLE is generally thought of as a Th2-type disease; whereas, IBD is more Th1-mediated. Loss of TNFAIP3 function results in more inflammation, which might alter the Th skewing of immune responses. Thus, inflammatory stimuli insufficient to initiate IBD (an intermediate Th1/2 response) are made sufficient (more toward a Th1 response) by reduced anti-inflammatory activity of TNFAIP3. Conversely, weaker inflammatory stimuli that might lead to SLE are increased in individuals with less functional TNFAIP3, leading to more inflammation and less susceptibility to SLE. This may involve Th1/2 skewing of T cells, such that a Th2 response that might lead to SLE is skewed toward a Th1 profile, reducing the risk for SLE. Our genetic findings are consistent with the observation that very few people are diagnosed with both SLE and IBD (46). Studies correlating TNFAIP3 SNPs with cytokine profiles in African-Americans with SLE versus IBD are needed to provide a basis for this hypothetical model.

The pathogenesis of inflammatory autoimmune diseases is complex, and likely involves both genetic and environmental factors. The identification of potential genetic associations has helped define possible mechanisms of disease initiation and progression. Genetic variation in the TNFAIP3 gene has been associated with several types of autoimmune disease in patients of European ancestry, and in this study we show novel genetic elements on African-derived chromosomes also modulate autoimmunity. Reconciliation of the differences between European- and African-American genetic studies of TNFAIP3 around functional elements will be a critical step in understanding disease biology. Our finding that the A125V polymorphism confers functional differences is an important advance in this effort. A better understanding of how polymorphisms in TNFAIP3 affect risk and protection in autoimmunity will provide valuable mechanistic insights into the pathogenesis and treatment of autoimmune disease.

Acknowledgments

We thank Ken Ouel and Eugene Chang for helpful comments and suggestions; the Digestive Disease Research Core Center of the University of Chicago, the Gastro-Intestinal Research Foundation, and the Gastro-Intestinal Research Associations Board for support.

Disclosures

The authors have no financial conflicts of interest.

References


African-derived genetic polymorphisms in *TNFAIP3* mediate risk for autoimmunity

Lodolce, et al.

Supplemental Data

Supplemental Tables

Supplemental Table I. *Power to detect and corrected OR for genotyped TNFAIP3 SNPs*\(^a\)

<table>
<thead>
<tr>
<th>SNP</th>
<th>disease</th>
<th>% genotyped(^b)</th>
<th>80% power to detect OR(^c)</th>
<th>“winner’s curse” correction(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>controls cases</td>
<td></td>
<td>naive OR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ascertainment-corrected OR</td>
</tr>
<tr>
<td>rs3757173</td>
<td>SLE</td>
<td>97.1 99.1</td>
<td>1.47</td>
<td>1.2755</td>
</tr>
<tr>
<td>rs5029939</td>
<td>SLE</td>
<td>98.5 100</td>
<td>1.46</td>
<td>1.3108</td>
</tr>
<tr>
<td>A125V</td>
<td>SLE</td>
<td>93.0 100</td>
<td>0.33</td>
<td>0.3800</td>
</tr>
<tr>
<td>A125V</td>
<td>IBD</td>
<td>93.0 100</td>
<td>ND</td>
<td>3.7907</td>
</tr>
<tr>
<td>F127C</td>
<td>SLE</td>
<td>99.6 100</td>
<td>1.46</td>
<td>1.3448</td>
</tr>
<tr>
<td>F127C</td>
<td>IBD</td>
<td>99.6 100</td>
<td>ND</td>
<td>1.1790</td>
</tr>
<tr>
<td>rs610604</td>
<td>SLE</td>
<td>96.0 100</td>
<td>1.46</td>
<td>0.9258</td>
</tr>
<tr>
<td>rs5029953</td>
<td>SLE</td>
<td>98.5 100</td>
<td>1.53</td>
<td>1.6093</td>
</tr>
</tbody>
</table>

\(^a\)The six *TNFAIP3* SNPs genotyped in >90% of total samples in this study are listed, along with the disease population (SLE vs. IBD).

\(^b\)Total number of controls=272, SLE cases=217, and IBD cases=24.

\(^c\)The minimum odds ratio (OR) that could be detected with 80% power with a significance of \(p=0.05\) was calculated for each SNP genotyped based on the size of the SLE population. Values were determined using an additive model with the CaTS Power Calculator written by Andrew Skol (http://www.sph.umich.edu/csg/abecasis/CaTS/). Calculations were not determined (ND) for the IBD cases due to the small sample size.

\(^d\)The adjusted odds ratios (ORs) were calculated with WINNER version 1.1 written by Rui Xiao and Michael Boehnke (http://csg.sph.umich.edu/boehnke/winner/) using a significance level of 0.05.
Supplemental Table II. *Genotypes of A125V and F127C in African-American and White/European-American Populations*"^

<table>
<thead>
<tr>
<th>SNP</th>
<th>genotype</th>
<th>White/ European-American</th>
<th>IBD</th>
<th>freq</th>
<th>SLE</th>
<th>freq</th>
<th>controls</th>
<th>freq</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>#</td>
<td>freq</td>
<td>#</td>
<td>freq</td>
<td>#</td>
<td>freq</td>
</tr>
<tr>
<td>A125V</td>
<td>TT</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
<td>0.0040</td>
</tr>
<tr>
<td></td>
<td>TC</td>
<td>0</td>
<td>5</td>
<td>0.21</td>
<td>5</td>
<td>0.023</td>
<td>13</td>
<td>0.052</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>291</td>
<td>19</td>
<td>0.79</td>
<td>212</td>
<td>0.98</td>
<td>238</td>
<td>0.94</td>
</tr>
<tr>
<td>F127C</td>
<td>GG</td>
<td>1</td>
<td>3</td>
<td>0.13</td>
<td>29</td>
<td>0.13</td>
<td>30</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>GT</td>
<td>21</td>
<td>11</td>
<td>0.46</td>
<td>109</td>
<td>0.50</td>
<td>100</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>269</td>
<td>10</td>
<td>0.42</td>
<td>79</td>
<td>0.36</td>
<td>122</td>
<td>0.48</td>
</tr>
</tbody>
</table>

"^

Both African-American and White/European-American samples were sequenced in an area centered on the A125V and F127C SNPs in *TNFAIP3*. The total number of patients with each genotype and the minor allele frequencies for each of these SNPs in both ancestral populations is shown.
Supplemental Table III. *Population frequencies of SNPs in linkage (r^2>0.50) with rs5029953.a*

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position (Mb)</th>
<th>r^2 w/ rs5029953</th>
<th>YRI freq.</th>
<th>YRI allele counts</th>
<th>CEU freq.</th>
<th>CEU allele counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs7740811</td>
<td>138.194843</td>
<td>0.55</td>
<td>0.117</td>
<td>14/120</td>
<td>0</td>
<td>0/118</td>
</tr>
<tr>
<td>rs9494876</td>
<td>138.199541</td>
<td>0.60</td>
<td>0.125</td>
<td>15/120</td>
<td>0</td>
<td>0/120</td>
</tr>
<tr>
<td>rs6923085</td>
<td>138.208718</td>
<td>0.83</td>
<td>0.146</td>
<td>33/226</td>
<td>0</td>
<td>0/118</td>
</tr>
<tr>
<td>rs10223631</td>
<td>138.209746</td>
<td>0.55</td>
<td>0.112</td>
<td>25/224</td>
<td>0</td>
<td>0/118</td>
</tr>
<tr>
<td>rs10223636</td>
<td>138.209907</td>
<td>0.60</td>
<td>0.107</td>
<td>24/224</td>
<td>0</td>
<td>0/118</td>
</tr>
<tr>
<td>rs9484096</td>
<td>138.215016</td>
<td>0.55</td>
<td>0.117</td>
<td>14/120</td>
<td>0</td>
<td>0/118</td>
</tr>
<tr>
<td>rs5029953</td>
<td>138.242453</td>
<td></td>
<td>0.155</td>
<td>35/226</td>
<td>0</td>
<td>0/118</td>
</tr>
<tr>
<td>rs5029956</td>
<td>138.244071</td>
<td>0.66</td>
<td>0.088</td>
<td>20/226</td>
<td>0</td>
<td>0/114</td>
</tr>
<tr>
<td>rs9484098</td>
<td>138.266866</td>
<td>0.84</td>
<td>0.186</td>
<td>22/118</td>
<td>0</td>
<td>0/120</td>
</tr>
<tr>
<td>rs9484099</td>
<td>138.274376</td>
<td>0.74</td>
<td>0.177</td>
<td>40/226</td>
<td>0</td>
<td>0/120</td>
</tr>
</tbody>
</table>

Data from the YRI population was analyzed with Haploview 4.1 software to identify SNPs within 100kb upstream and downstream of rs5029953 that had r^2 values >0.50. The frequency of the minor allele of the nine highly linked SNPs identified along with rs5029953 is shown for both African ancestry (YRI) and Utah residents with ancestry from northern and western Europe (CEU).
**Supplemental Figures**

*Supplemental Figure 1. LD plot of TNFAIP3 SNPs in African-American non-autoimmune controls.*

An LD plot generated from African-American non-autoimmune controls shows the relative linkage between the TNFAIP3 SNPs genotyped in this study. The numbers in each box represent the pairwise $r^2$ value x100 between SNPs, calculated by the Haploview 4.1 software. Darker shaded boxes represent higher $r^2$ values between SNPs. The location of each SNP within the general structure of the TNFAIP3 locus is indicated above the plot. The 5’ and 3’ untranslated regions are colored dark grey, while the coding regions are colored light grey. The corresponding exon number is indicated above each exonic block.
Supplemental Figure 2. LD plot of a ~68 kb region spanning the TNFAIP3 gene from the International HapMap Project YRI population.

Using Haploview 4.1 software an LD plot was generated that includes the 7 kb LD block containing rs5029953, two LD blocks upstream (20 kb and 19 kb), and one LD block downstream (22 kb). The software defined the LD blocks using a solid spine of LD calculation. The numbers in each box represent the pairwise $r^2$ value x100 between SNPs, calculated by the Haploview 4.1 software. Darker shaded boxes represent higher linkage between SNPs. The nine SNPs with an $r^2$ value >0.50 are marked with an arrow, and the rs5029953 SNP is denoted with an arrow and an asterisk (*).