

The Autoimmune Disease Risk Allele of UBE2L3 in African American Patients with Systemic Lupus Erythematosus: A Recessive Effect Upon Subphenotypes

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ABSTRACT. Objective. UBE2L3 is associated with susceptibility to systemic lupus erythematosus (SLE) and rheumatoid arthritis in European ancestry populations, and this locus has not been investigated fully in non-European populations. We studied the UBE2L3 risk allele for association with SLE, interferon- α (IFN- α), and autoantibodies in a predominantly African American SLE cohort.

Methods. We studied 395 patients with SLE and 344 controls. The UBE2L3 rs5754217 polymorphism was genotyped using Taqman primer-probe sets, and IFN- α was measured using a reporter cell assay.

Results. The UBE2L3 rs5754217 T allele was strongly enriched in African American patients with anti-La antibodies as compared to controls, and a recessive model was the best fit for this association (OR 2.55, $p = 0.0061$). Serum IFN- α also demonstrated a recessive association with the rs5754217 genotype in African American patients, and the TT/anti-La-positive patients formed a significantly high IFN- α subgroup ($p = 0.0040$). Similar nonstatistically significant patterns of association were observed in the European American patients with SLE. Case-control analysis did not show large allele frequency differences, supporting the idea that this allele is most strongly associated with anti-La-positive patients.

Conclusion. This pattern of recessive influence within a subgroup of patients may explain why this allele does not produce a strong signal in standard case-control studies, and subphenotypes should be included in future studies of UBE2L3. The interaction we observed between UBE2L3 genotype and autoantibodies upon serum IFN- α suggests a biological role for this locus in patients with SLE *in vivo*. (First Release Nov 1 2011; J Rheumatol 2012;39:73–8; doi:10.3899/jrheum.110590)

Key Indexing Terms:

SYSTEMIC LUPUS ERYTHEMATOSUS

INTERFERON- α

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Recent studies have begun to unravel the genetic architecture of systemic lupus erythematosus (SLE)¹. Despite recent successes, much of the heritability of the disorder remains to be

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described, and most large-scale studies directed at gene discovery to date have been performed exclusively in populations of European ancestry. SLE in African Americans is both more common and more severe than in European Americans^{2,3}, and thus genetic study of SLE in African American populations is a high priority. While some of the genetic risk loci reported in European populations have been replicated in African American populations, there is already clear precedent that some European ancestry genetic risk factors will not be relevant in African Americans⁴. Additionally, emerging evidence supports the existence of genetic risk factors for SLE that are particular to those of African ancestry^{5,6,7,8}.

Genetic variation in the *UBE2L3* gene has been associated with risk of rheumatoid arthritis⁹, Crohn's disease¹⁰, and SLE^{1,11}. These studies have all been performed in people of European ancestry. Interestingly, the autoimmune disease risk allele of UBE2L3 reported in Europeans is more common in African populations in the HapMap dataset (rs5754217 T allele frequency is 0.168 in the European-derived CEU population and 0.496 in the African YRI population). This presents the possibility that this genetic risk factor could be more

important in African Americans, and that studies of this allele will be characterized by greater statistical power in this ancestral background. Ubiquitin-conjugating enzyme E2L3 (UBE2L3, also known as UbcH7) attaches ubiquitin molecules to other proteins, targeting them for destruction¹². UBE2L3 has been shown to attach ubiquitin molecules to nuclear factor- κ B, p53, and Fos¹³. Additionally, UBE2L3 interacts with Triad3A (RNF216), which can regulate the degradation of Toll-like receptors (TLR)¹⁴. In SLE, signaling through the endosomal TLR is thought to be an important pathway for the generation of interferon- α (IFN- α)¹⁵.

Much evidence supports the idea that increased IFN- α pathway signaling is causal in human SLE. Serum IFN- α is high in many patients with SLE¹⁶, and a number of patients treated with recombinant human IFN- α for malignancy and chronic viral hepatitis have developed *de novo* SLE, which typically resolves after the IFN- α is discontinued¹⁷. Many of the confirmed SLE risk genes function within the IFN- α pathway^{1,18,19}. We have shown previously that some established IFN- α pathway SLE risk genes are associated with increased IFN- α signaling in patients with SLE^{20,21,22,23}. Elevated serum IFN- α is clustered within SLE families in a pattern consistent with a complex trait¹⁶, further supporting the idea that a multifactorial heritable tendency toward high IFN- α is a primary pathogenic mediator in SLE²⁴. SLE-associated autoantibodies are strongly associated with increased IFN- α in patients with SLE²⁵, and these autoantibodies may also be primary pathogenic factors in SLE²⁶.

We investigated the UBE2L3 rs5754217 autoimmune disease risk allele in our local predominantly African American SLE cohort. We hypothesized that there may be an association between this allele and serum IFN- α or autoantibody traits in SLE, and that by studying genetic associations with these intermediate phenotypes we may be able to better understand the effect of this locus on human disease.

MATERIALS AND METHODS

Patients and samples. We studied serum and genomic DNA samples from 395 patients with SLE from the University of Chicago Translational Research in the Department of Medicine (TRIDOM) registry and Rush University Medical Center. The SLE cohort consisted of 252 African American and 143 European American patients with SLE. All patients met the revised 1982 American College of Rheumatology criteria for the diagnosis of SLE²⁶. Sex-matched controls were also obtained from the TRIDOM registry, including 239 African American and 105 European American subjects. The control subjects were all screened for absence of autoimmune disease by medical record review. The subjects in this study were not related to each other. Informed consent was obtained from all subjects at each site, and the study was approved by the institutional review board at each institution.

Single-nucleotide polymorphism (SNP) genotyping of UBE2L3 rs5754217. Patients with SLE were genotyped at UBE2L3 rs5754217 using ABI Taqman Assays-by-Design primers (Applied Biosystems, Foster City, CA, USA) and probes on an ABI 7900HT polymerase chain reaction (PCR) machine with > 98% genotyping success. Scatterplots were all reviewed individually for quality, and genotype frequencies did not deviate significantly from the expected Hardy-Weinberg proportions ($p > 0.01$ in each ancestral background).

Reporter cell assay for IFN- α . The reporter cell assay for IFN- α has been

described in detail¹⁶. Reporter cells were used to measure the ability of patient sera to cause IFN-induced gene expression. The reporter cells (WISH cells, American Type Culture Collection CCL-25) were cultured with 50% patient sera for 6 hours, and then lysed. Messenger RNA (mRNA) was purified from cell lysates, and cDNA was made from total cellular mRNA. Then cDNA was quantified using real-time PCR with the SYBR Green fluorophore system. Forward and reverse primers for the genes MX1, PKR, and IFIT1, which are known to be highly and specifically induced by IFN- α , were used in the reaction¹⁶. GAPDH was amplified in the same samples to control for background gene expression.

The amount of PCR product of the IFN- α -induced gene was normalized to the amount of product for the housekeeping gene GAPDH in the same sample. The relative expression of each of the 3 tested IFN-induced genes was calculated as a fold increase compared to its expression in WISH cells cultured with media alone. Results from the IFN- α assay were standardized to a healthy multiethnic reference population as described, and a serum IFN- α activity score was calculated based upon the mean and SD of the reference population¹⁶. This assay has been highly informative when applied to SLE as well as other autoimmune disease populations^{27,28,29}.

Measurement of autoantibodies. Antibodies to anti-Ro, anti-La, anti-Sm, and anti-RNP were measured in all samples by ELISA methods using kits from Inova Diagnostics (San Diego, CA, USA), and anti-dsDNA antibodies were measured using *Crithidia luciliae* immunofluorescence, with detectable fluorescence considered positive. All samples were assayed in a University of Chicago clinical laboratory. Standard cutoff points designated by the manufacturer for a positive test were used to categorize samples as positive or negative.

Statistical analysis. To account for potential differences related to proportional ancestry in admixed populations, we performed a principal component analysis on data from 12 SNP that confer information about genetic ancestry and were genotyped in all cases and controls as described in Kariuki, *et al*¹⁶, similar to the approach outlined in Parra, *et al*³⁰. The first principal component in this analysis provided strong separation of self-reported European versus African American ancestry, and this component was included in logistic regression analyses as a covariate to control for any differences in the degree of admixture between cases and controls that could otherwise potentially confound genetic association analyses.

Logistic regression models were used to detect associations between genotype at rs5754217 and cases versus control status, and the presence or absence of each of the 5 tested autoantibodies in the SLE cases (case-case analysis). The IFN- α data was non-normally distributed, and the nonparametric Mann-Whitney U test was used to compare quantitative IFN- α data in patients with SLE between genotype subgroups. P values shown here are uncorrected for multiple comparisons. To account for multiple comparisons, we used a threshold p value of 0.01 or smaller to control the family-wise type I error rate at 0.05 using a Bonferroni correction when testing differences in allele frequencies between different patient groups defined by autoantibodies. For the serum IFN- α studies, p values < 0.017 would withstand a Bonferroni correction for the number of comparisons possible between the different genotype groups presented on the graphs in Figures 1 and 2.

RESULTS

UBE2L3 rs5754217 T association with SLE in African American patients with anti-La autoantibodies. In logistic regression models, we detected an association between rs5754217 T and anti-La autoantibodies in African Americans, and the recessive model produced the strongest association. Table 1 shows the case-case and case-control analyses examining allele frequencies in anti-La-positive patients versus anti-La-negative patients and anti-La-positive patients versus controls (OR 2.55, $p = 0.0061$). The other SLE-associated autoantibodies did not show evidence for

Table 1. UBE2L3 rs5754217 genotype and allele frequencies in controls and cases stratified by anti-La autoantibody status.

Background	Clinical Category	N	GG	GT	TT	MAF (T)	La+ Cases vs La- Cases, Additive	La+ Cases vs LA- Cases, Recessive	La+ Cases vs Controls, Additive	La+ Cases vs Controls, Recessive
African American	Controls	239	74	127	38	0.425				
	Cases	252	71	129	52	0.462				
	La+ Cases	46	10	21	15	0.554	OR 1.57, p = 0.050	OR 2.21, p = 0.027	OR 1.69, p = 0.022	OR 2.55, p = 0.0061
	La- Cases	206	61	108	37	0.442				
European American	Controls	105	64	36	5	0.219				
	Cases	143	88	51	4	0.206				
	La+ Cases	13	7	5	1	0.269	OR 1.32, p = 0.47	OR 3.52, p = 0.27	OR 1.31, p = 0.46	OR 1.66, p = 0.38
	La- Cases	130	81	46	3	0.200				

La+ indicates patients with a positive test for anti-La antibodies. La- indicates patients who lack anti-La antibodies. MAF: minor allele frequency.

association with rs5754217 genotype. As shown in Table 1, the majority (63%) of excess T alleles in African American cases were contributed by the relatively small anti-La-positive group (18% of patients). Thus, the anti-La-positive subgroup can largely account for the allele frequency difference between African American SLE cases and controls. A similar nonsignificant tendency was observed in the European American patients with SLE, but the lower prevalence of the allele in European ancestry and the low frequency of anti-La antibodies prevents any definitive comment about whether the anti-La association extends to European Americans. In overall case-control analysis in African Americans, we observed a modest effect size (OR 1.16, $p = 0.24$) that is similar to the

effect observed in European ancestry cohorts to date (OR of 1.20–1.22)^{1,11}. Thus, in African Americans the recessive effect within the anti-La-positive group was clearly a stronger genetic model for UBE2L3 rs5754217 than the overall additive case-control model (OR 2.55, $p = 0.0061$, vs OR 1.16, $p = 0.24$).

Serum IFN- α is increased in patients with SLE who have the rs5754217 TT genotype. We next examined serum IFN- α in patients with SLE in the context of the UBE2L3 rs5754217 genotype. As shown in Figure 1A, elevated serum IFN- α is observed only in African American subjects with the TT genotype, and there is no evidence for increased IFN- α in the GT genotype category. This supports a recessive effect of the

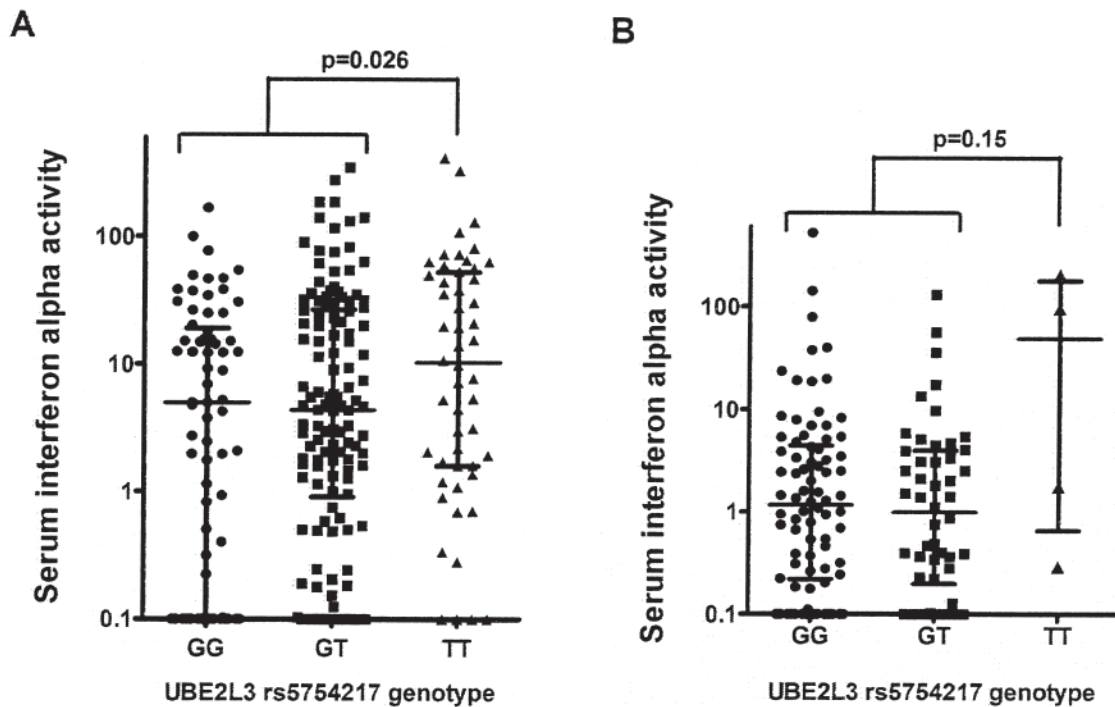


Figure 1. Serum interferon- α in patients with SLE stratified by UBE2L3 rs5754217 genotype. A. African American patients with SLE. B. European American patients with SLE. Serum IFN- α activity is shown on the Y-axis. Lines indicate the median and error bars show the interquartile range. P values were calculated using the Mann-Whitney U test, comparing TT genotype to the GG and GT genotypes (recessive model).

rs5754217 SNP upon serum IFN- α in African Americans, similar to the effect observed upon anti-La antibodies. European American patients with SLE demonstrated a similar nonsignificant tendency toward a recessive effect upon serum IFN- α (Figure 1B).

IFN- α is higher in anti-La positive individuals with the rs5754217 TT genotype. When serum IFN- α was examined in African American subjects stratified by anti-La autoantibodies, the difference in serum IFN- α related to rs5754217 genotype was observed only in anti-La-positive subjects (Figure 2). The data again fit a recessive model, and the recessive effect upon IFN- α observed in African American patients illustrated in Figure 1 is wholly restricted to the anti-La-positive subjects. In European Americans, only 1 subject had both anti-La antibodies and TT genotype, so these data are not shown and it is not clear whether this pattern extends beyond African American patients with SLE.

DISCUSSION

These data demonstrate a recessive influence of the rs5754217 T allele upon important pathogenic subphenotypes in SLE in African American patients. While our European American dataset was more limited and the risk allele is less common in people with this ancestral background, we observed similar

nonsignificant patterns there as well. The UBE2L3 rs5754217 T allele is characterized by a modest overall association with SLE in European populations, with an OR of about 1.2 in additive model case-control studies^{1,11} and 1.30 in a family-based study³¹. A similar effect size was demonstrated in our African American population in overall case-control analysis, while a much greater effect was demonstrated using a recessive model in the anti-La-positive subgroup (OR > 2.5). This recessive effect upon a subgroup of patients would not be strongly detected in case-control study designs. Our findings support the idea that heterogeneity in the pathogenesis of SLE underlies some of the modest effect size and missing heritability observed in case-control genetic studies of SLE to date.

We have shown that a number of genetic polymorphisms are associated with increased serum IFN- α in SLE^{20,21,22,32}. This supports the idea that a number of genetic factors converge upon IFN- α as a common pathogenic mediator. We have also been able to use unbiased genome-wide techniques to identify a number of novel polymorphisms related to both autoantibodies and serum IFN- α in SLE in multiple different ancestral backgrounds⁷. These studies support the relevance of these subphenotypes in genetic studies of SLE, and also suggest that both of these phenotypes are primary pathogenic factors in SLE. We find a strong recessive influence of UBE2L3 rs5754217 T upon the serum IFN- α phenotype, and interestingly this is also dependent upon anti-La autoantibodies. We have observed this pattern with the SLE risk-associated polymorphisms of interferon regulatory factors (IRF)-5 and IRF-7^{20,22}, and both of these IRF proteins function in the IFN- α pathway downstream of endosomal TLR signaling. SLE-associated autoantibodies can activate the endosomal TLR pathway of IFN- α generation³³, and this chronic endogenous stimulation of TLR may emphasize the genetic effects of these polymorphisms upon IFN- α generation in patients with SLE *in vivo*¹⁵. It is possible that the autoantibody-dependent influence of UBE2L3 genotype upon serum IFN- α also indicates a role for the UBE2L3 risk variant downstream of endosomal TLR signals in human SLE.

The exact molecular function of the rs5754217 polymorphism and the reason for a recessive association are not clear. It is possible that this SNP is in linkage disequilibrium with a functional element and is not the true causal allele, and future fine-mapping studies will assist in this determination. Regarding the recessive effect, UBE2L3 functions normally in targeting proteins involved in inflammatory signaling for destruction, and the risk allele may result in decreased function of this protein. In that case, it is possible that 1 nonrisk allele could compensate for the risk allele, and a decrease in UBE2L3 function may only occur when 2 risk alleles coincide. This possibility would have to be addressed in future mechanistic studies.

We do not have data regarding disease activity for the patients in our study, and cannot comment upon whether the

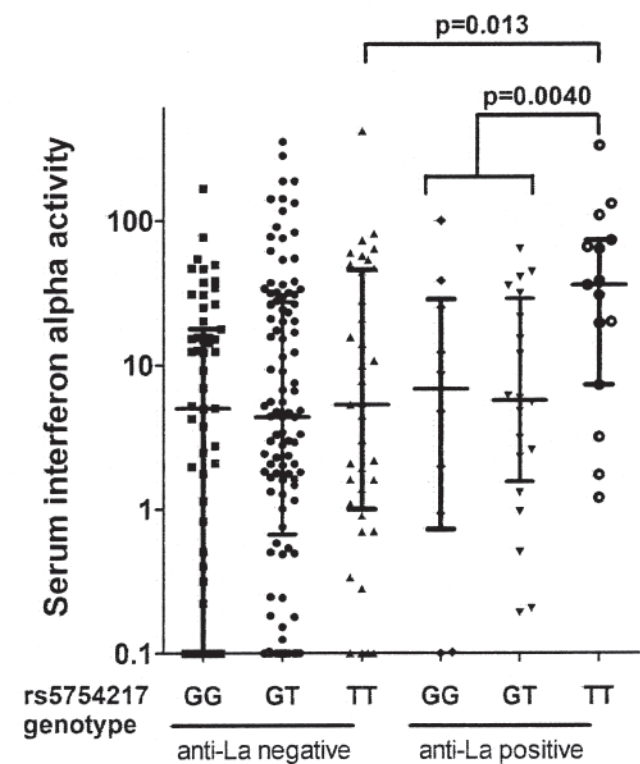


Figure 2. Serum interferon- α (IFN- α) in African American patients with SLE stratified by UBE2L3 rs5754217 genotype and anti-La autoantibodies. Serum IFN- α activity is shown on the Y-axis. Lines indicate the median and error bars show the interquartile range. P values are calculated using the Mann-Whitney U test.

rs5754217 allele is associated with SLE disease activity. While a cross-sectional association between disease activity and serum IFN- α has been observed³⁴, longitudinal studies of IFN- α in SLE to date have not supported a strong relationship between changes in serum IFN- α and fluctuations in disease activity^{35,36}. In previous work, we have shown that serum IFN- α is heritable within families in which SLE is present, in both affected and unaffected members¹⁶, and that serum levels of IFN- α are correlated with fixed genetic factors^{24,37}. These data support the concept of a relatively stable threshold for circulating IFN- α levels in the individual. The potential effect of the UBE2L3 rs5754217 polymorphism upon disease activity, as either a primary or secondary factor, would be an area of interest for future work.

Given our findings, we would predict that larger-scale genetic studies of this locus will confirm an association between rs5754217 T and SLE in African Americans. Additionally, our study will inform future genetic studies of this locus, as we find strong recessive association in the anti-La-positive subgroup that may not be evident in analyses that are not stratified by autoantibodies. The gene-antibody interaction we describe affects pathogenic cytokine levels in SLE, providing some insight into the biological function of this locus in patients with SLE *in vivo*.

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