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The rs4774 *CIITA* missense variant is associated with risk of systemic lupus erythematosus

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Abstract

The major histocompatibility complex (MHC) class II transactivator gene (*CIITA*) encodes an important transcription factor required for HLA class II MHC-restricted antigen presentation. MHC genes, including the HLA class II *DRB1*03:01* allele, are strongly associated with systemic lupus erythematosus (SLE). Recently the rs4774 *CIITA* missense variant (+1632G/C) was reported to be associated with susceptibility to multiple sclerosis. In the current study, we investigated *CIITA*, *DRB1*03:01* and risk of SLE using a multi-stage analysis. In stage 1, 9 *CIITA* variants were tested in 658 cases and 1,363 controls (N = 2,021). In stage 2, rs4774 was tested in 684 cases and 2,938 controls (N = 3,622). We also performed a meta-analysis of the pooled 1,342 cases and 4,301 controls (N = 5,643). In stage 1, rs4774**C* was associated with SLE (odds ratio [OR] = 1.24, 95% confidence interval [95% CI] = 1.07–1.44, $P = 4.2 \times 10^{-3}$). Similar results were observed in stage 2 (OR = 1.16, 95% CI = 1.02–1.33, $P = 8.5\times10^{-3}$) and the meta-analysis of the combined dataset (OR = 1.20, 95% CI = 1.09–1.33, $P_{meta} = 2.5\times10^{-4}$). In all three analyses, the strongest evidence for association between rs4774**C* and SLE was present in individuals who carried at least one copy of *DRB1*03:01* ($P_{meta} = 1.9\times10^{-3}$). Results support a role for *CIITA* in SLE, which appears to be stronger in the presence of *DRB1*03:01*.

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Keywords

systemic lupus erythematosus; autoimmunity; major histocompatibility complex; HLA; *CIITA*; *MHC2TA*

Systemic lupus erythematosus (SLE) is the prototypic systemic autoimmune disease and is characterized by autoantibody production and altered immune complex formation and clearance. SLE has a strong genetic component, as demonstrated by twin and other family studies. Major histocompatibility complex (MHC) genes on chromosome 6p21, particularly the class II *HLA-DRB1*03:01* allele, are strongly associated with increased risk of developing SLE. However, MHC genes only account for a portion of the genetic risk. Several non-MHC genes have recently been associated with risk of SLE, including *PTPN22*, *STAT4* and *TNFAIP3*, and 6 genomewide association (GWA) studies have collectively established more than 20 risk loci.

The MHC class II transactivator gene (*CIITA,* also called *MHC2TA*) encodes the CIITA protein, a transcription factor essential for the expression of HLA class II molecules and involved in the expression of HLA class I molecules.[1,2] *CIITA* spans 47.8 kb on chromosome 16p13, with four alternate first exons in a 12kb promoter region (I–IV).[3] Mutations in *CIITA* cause a rare and severe immunodeficiency characterized by HLA class II deficiency (bare lymphocyte syndrome).[2] Thus, *CIITA* is an attractive candidate for genetic studies of autoimmune diseases for which HLA associations have been well established. Recently, the *CIITA* +1632G/C missense mutation (rs4774, alias +1614G/C) showed strong evidence for association with multiple sclerosis (MS) in the presence of *DRB1*15:01*, a well-established MS risk allele.[4]

The purpose of this study was to investigate association between the *CIITA* rs4774 variant and neighboring SNPs in the region and risk of developing SLE, and to identify interactions between *CIITA* variants and the established SLE *HLA-DRB1*03:01* risk allele. A total of 1,463 patients from a multi-center, well-characterized dataset (University of California, San Francisco; Oklahoma Medical Research Foundation; University of Minnesota) were included in this analysis (Table 1).[5] All individuals were of European ancestry, as determined by ancestry informative genetic markers.

In stage 1, we conducted allelic tests of association for 9 *CIITA* SNPs in 658 cases and 1,363 controls (N = 2,021) and stratified on the presence and absence of *DRB1*03:01. DRB1* data was not available for the controls, so instead SNP r2187668 was used as a proxy to tag the *DRB1*0301* allele as previously described.[6] In stage 2, rs4774, the only associated SNP, was tested in 684 cases and 2,938 controls (N = 3,622). We performed a meta-analysis as well as interaction tests in the pooled dataset comprised of 1,342 cases and 4,301 controls (N = 5,643).

As previously reported, *DRB1*03:01* was strongly associated with SLE in both stages 1 and 2 (odds ratio [OR] = 1.96, 95% confidence interval [95% CI] = 1.64 to 2.34, $P = 1\times10^{-4}$, and OR = 1.61, 95% CI = 1.38 to 1.88, $P = 1\times10^{-6}$, respectively).[5] After correcting for multiple testing in the overall sample, association was observed between rs4774 and SLE (OR = 1.24, 95% CI = 1.07–1.44, $P = 4.2 \times 10^{-3}$; *DRB1*03:01*+: OR = 1.27, 95% CI = 1.06–1.53, $P = 9\times10^{-3}$) (Table 2). In stage 2, rs4774 **C* was associated with SLE (OR = 1.16, 95% CI = 1.02–1.33, $P = 8.5\times10^{-3}$; *DRB1*03:01*+: OR = 1.23, 95% CI = 0.98–1.54, $P = 3.5\times10^{-2}$) (Table 3). In the meta-analysis, rs4774 **C* was associated with SLE (OR = 1.20, 95% CI = 1.09–1.33, $P=2.5\times10^{-4}$; *DRB1*03:01*+: OR = 1.25, 95% CI = 1.09–1.45, $P_{meta} = 1.9\times10^{-3}$) (Table 3). Evidence for interaction between *DRB1*03:01* and rs4774*C was not

observed in either case-control or case-only analyses (*data not shown*) (Supplementary Table 1).

The *CLEC16A* gene is adjacent to *CIITA* on chromosome 16, and *CLEC16A* has been previously established as a risk locus for several autoimmune diseases, including SLE (rs12708716**A*, OR = 1.16, $P = 1.6\times10^{-4}$).[7] Evidence for association between the *CLEC16A* rs12599402**A* allele and SLE was also reported in a Chinese population (GWA study: OR = 1.27, 95% CI = 1.10–1.47, $P = 1.4\times10^{-3}$; replication study: OR = 1.23, 95% CI = 1.14–1.33, $P = 2.5\times10^{-6}$; combined: OR = 1.23, 95% CI = 1.15–1.33, $P = 1.3\times10^{-8}$).[8] To confirm that association observed between rs4774 and SLE in the current study was not due to linkage disequilibrium (LD) with *CLEC16A*, we performed an allelic test of the rs12708716**A* risk allele in stages 1 and 2. We also conducted a meta-analysis of the combined sample. Results from our analyses did not provide evidence for association between *CLEC16A* and SLE (stage 1: OR = 1.06, 95% CI = 0.93–1.22, $P = 0.39$; stage 2: OR = 1.10, 95% CI = 0.97–1.25, $P = 0.13$; pooled OR = 1.08, 95% CI = 0.98–1.19, $P_{meta} = 0.09$). However, our study had limited power to observe extremely modest effects for the *CLEC16A* variant in SLE. We examined LD patterns between 14 *CLEC16A* SNPs and 24 *CIITA* SNPs in the controls from stage 1, 43 *CLEC16A* SNPs and 19 *CIITA* SNPs in the controls from stage 2, and 274 *CLEC16A* SNPs and 40 *CIITA* SNPs in HapMap samples of northern and western European origin (CEU, release 24) (*data not shown*). There was no evidence of LD between *CIITA* and *CLEC16A* in the controls ($r2 \leq 0.10$). The patterns of LD derived from three independent samples support a primary role for *CIITA* variation that is independent of *CLEC16A* in SLE.

The rs4774**C* variant, located in exon 11, causes an amino acid substitution from glycine to alanine at amino acid position 500. The rs4774**C* minor allele is ancestral, based on chimpanzee DNA.[9,10] Based on sequence homology and physical properties of amino acids, this amino acid substitution is predicted to have a tolerable effect on protein function.[11] However, the exact functional consequences that result are not known. Steimle et al. previously tested the functionality of this amino acid substitution and did not observe any difference between *CIITA* G500 and A500.[2] However, their experiment evaluated G500A jointly with a three nucleotide insertion that causes an amino acid substitution from lysine to isoleucine-glutamic acid at amino acid position 120.[2] It would be worthwhile to reassess the functional effect of G500A individually, and also to utilize a more sensitive method that can detect minor differences in the efficiency of *CIITA*.[12] Interestingly, exon 11 contains an exonic splice enhancer (ESE) density (0.27) that is more similar to exons containing splice-affecting variants (SAVs) than randomly sampled exons with a similar size distribution (0.38).[13] The lower ESE density, closer to ESE densities in introns, suggests a weakened exon definition that may be more vulnerable to variants that further modulate ESE density and could potentially lead to exon skipping.[13] Animal models provide some evidence supporting a role for *CIITA* in autoimmune disease pathogenesis. *CIITA* transgenic mice with over-expression of *CIITA* in helper T cells demonstrated aggravated oxazolone-induced colitis compared to wild type mice, due to elevated IL-4 production and Th2 inflammation.[14]

Other studies have examined *CIITA* variation in SLE with conflicting findings. A previous study examining SLE risk and the rs4774 variant did not observe evidence for association (OR = 0.97, 95% CI = 0.77–1.20, $P = 0.7$).[15] However, power was limited due to relatively small sample size (394 cases and 514 controls). In addition, investigators did not stratify by *DRB1*03:01* or restrict samples by genetic ancestry information to reduce the possibility of bias from potential population structure. Association between the *CIITA* –168A/G promoter polymorphism (rs3087456) and SLE risk has been previously reported in a Japanese population.[16] In contrast, studies in Swedish and Spanish populations do not support these

findings.[15,17] Our results, based on association testing for rs12928665 as a tagging SNP in stage 1, do not support a role for rs3087456 in SLE.[4]

It is important to note that results from recent GWA studies in SLE have not provided evidence for association with *CIITA*.[18–25] However, the rs4774 variant identified in this study (or any SNPs that are known to tag it) have not been included in previous investigations.[26] Further, *HLA DRB1*03:01* status was not available for study subjects. There are currently two SNPs (rs4780334, rs7196089) known to be in strong LD with rs4774 ($r^2 = 0.96$); they are both intronic, and therefore, do not yet explain the functional relevance of the rs4774 finding.[26] Our current *CIITA* results would not meet stringent genomewide significance. Nevertheless it is possible that a vast number of modest effects failing the stringent GWA significance tests are responsible for some of the unexplained heritability in SLE.[27] A recent study on the missing heritability of height provided evidence that 45% of the variance of height can be explained by considering at least 300,000 SNPs simultaneously, and that the remaining heritability could be due to incomplete LD between casual variants and the genotyped SNPs.[27] This indicates that candidate gene and pathway-based approaches are a useful complementary strategy to GWA studies.

Interestingly, it appears that genetic variation in *CIITA* may be important for some autoimmune diseases but not others. Despite initial reports indicating otherwise, a meta-analysis of over 16,000 RA cases and controls revealed no evidence for association with the -168A/G polymorphism.[28,29] Furthermore, common variation in *CIITA* does not appear to play a role in RA.[30] In contrast, the *CIITA* gene region has reached genomewide significance in recent studies of both ulcerative colitis (UC) and celiac disease.[31,32] McGovern et al. report evidence for association between the intronic *CIITA* rs4781011**T* variant and risk of UC ($P = 3.2\times10^{-6}$, combined weighted analysis of three GWA screens [GWAS1: OR = 1.15; GWAS2 OR = 1.28; GWAS3 OR = 1.16] and two replication studies [OR = 1.06, OR = 1.18]) ($N = 13{,}073$ individuals of European descent). Through tagging based on strong LD, rs4781011 was also captured in the current study; however, a role for this variant in SLE was not supported. Dubois et al. cite evidence for association between the *CIITA-CLEC16A-SOCS1* gene region and increased risk of celiac disease.[32] The *C16orf75* rs12928822**C* allele demonstrated the strongest effect (OR = 1.16, 95% CI = 1.10 to 1.22, $P = 3.1\times10^{-8}$, combined weighted analysis of GWA screen and replication study) ($N = 25{,}885$ individuals of European descent).[32] Within the *CIITA* region, the strongest effect was for the rs6498114**T* promoter allele (OR = 1.11, 95% CI = 1.06 to 1.16, $P = 1\times10^{-5}$).[32] Results from our analysis of rs6498114 did not support a role for this variant in SLE. Functional relevance for either associated SNP is not known at this time.

In conclusion, this is the largest study of *CIITA* and SLE to date, and the first study of *CIITA* in SLE to examine potential interactions between *CIITA* variants and the established SLE *HLA-DRB1*03:01* risk allele. We are the first to report association between the rs4774 missense mutation and SLE, which appears to be stronger in the presence of *DRB1*03:01*. Future studies are warranted to examine the potential functional significance of the rs4774 variant, and consider the contribution of rare *CIITA* variants to SLE risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

SLE study cohorts used for *CIITA* analyses

	SLE1	Stage 1 Controls	SLE2	Stage 2 Controls
N	658	1363	684	2938
Number of *CIITA* SNPs analyzed in study	9	9	1	1
Site, *N*(%)				
University of California, San Francisco	370 (56.2)	607 (44.5)[1]	379 (55.4)	
Oklahoma Medical Research Foundation	185 (28.1)		181 (26.5)	
University of Minnesota	103 (15.7)		124 (18.1)	
Wellcome Trust Case Control Consortium		756 (55.5)		2938 (100)
Mean age (years)	53.8	49.9	53.8	N/A[2]
Age range (years)	20–90	25–86	16–95	21–72
Female, *N*(%)	610 (92.7)	890 (65.3)	630 (92.1)	1492 (50.8)
Mean age at onset (years)	32.8		33.3	
Arthritis, *N*(%)	381 (69.8)		393 (71.2)	
Double strand DNA autoantibody production, *N*(%)	293 (54.0)		267 (49.0)	
Lupus nephritis, *N*(%)	100 (27.3)		87 (24.8)	
Serositis, *N*(%)	169 (31.0)		198 (35.9)	
History of neurological involvement, *N*(%)	29 (11.3)		15 (6.5)	
Sm autoantibody production, n (%)	51 (10.3)		64 (13.2)	
Ro autoantibody production, n (%)	131 (28.2)		116 (25.3)	

[1] Includes 8 controls from Brigham and Women's Hospital, Boston

[2] Not available.

SLE cases met the American College of Rheumatology classification criteria for SLE, and data were collected by medical record review. Estimates of European ancestry in the cases were based on 112 European and 246 Northern European ancestry informative markers.[5,33,34] Stage 1 controls were a subset of controls available from the International Multiple Sclerosis Genetic Consortium, and were of ≥90% European ancestry.[4] European ancestry was estimated using a Bayesian clustering algorithm (Structure v2.3.1, Oxford, UK).[4,35] Stage 2 controls were a subset of controls available from the Wellcome Trust Case Control Consortium, and were frequency matched by geographical region and gender to the 1958 Birth cohort (which included all births in England, Wales and Scotland, during one week in 1958) so as to be nationally representative.[36] European ancestry was estimated by principal components analysis.[36]

HLA-DRB1 genotypes with four-digit resolution, obtained through PCR-SSO methodology, were available for all patients.[5] Coding and tagging variants (identified in Haploview v4.1 through linkage disequilibrium (LD) patterns in publicly available HapMap samples of northern and western European origin (CEU)) were selected for genotyping.[37,38] Nine *CIITA* and one *CLEC16A* single nucleotide polymorphism (SNP) variants were genotyped in the patient dataset using the Sequenom iPLEX platform (San Diego, CA, USA). Previous studies provided genotypes for these 10 variants in the stage 1 and stage 2 controls, derived from a custom Illumina iSelect 48K chip (San Diego, CA, USA) and the Affymetrix GeneChips Mapping 500 K Array Set (Santa Clara, CA, USA), respectively.[4,36] Rs2187668 genotype data were available for all controls in this study to characterize *DRB1*03:01* status, due to very strong correlation between rs2187668 and *DRB1*03:01* as previously described ($r^2 = 0.87$ in HapMap CEU).[6]

Deviation from Hardy-Weinberg equilibrium was examined in SLE1 cases, SLE2 cases, stage 1 controls and stage 2 controls separately using the exact test (PLINK v1.05, Boston, MA, USA).[39] There were no variants with evidence for deviation from Hardy-Weinberg equilibrium ($P < 0.01$).

Table 2

Location, minor allele frequencies (MAF), odds ratios (OR), 95% confidence intervals (95% CI), and *P*-values from allelic tests in stage 1.

Location (bp)	Marker	Allele	SLE MAF	Controls MAF	OR (95% CI)	*P*
10871619	rs6498114	*G*	0.223	0.254	0.85 (0.72–0.99)	0.04
10879381	rs12932187	*G*	0.056	0.066	0.85 (0.64–1.12)	0.24
10889846	rs8043545	*C*	0.242	0.280	0.82 (0.71–0.96)	0.01
10899453	rs4781015	*A*	0.184	0.219	0.81 (0.68–0.95)	0.01
10900989	rs7189406	*G*	0.062	0.069	0.90 (0.69–1.17)	0.46
10903900	rs4781016	*A*	0.289	0.270	1.10 (0.95–1.27)	0.20
10908349	rs4774	*C*	0.313	0.268	**1.24 (1.07–1.44)**	**4.2×10^{-3}**
10911864	rs6498126	*G*	0.204	0.207	0.98 (0.84–1.16)	0.81
10924559	rs4781024	*A*	0.429	0.413	1.07 (0.93–1.22)	0.34

We tested allelic association by creating *2x2* contingency tables and estimating odds ratios (OR) with Fisher's exact test (PLINK). A significance threshold was set in stage 1 ($P = 5.56\times10^{-3}$) using a Bonferroni correction for the number of independent tests. Analyses were stratified by *DRB1*03:01. P*-values reported for the allelic association tests were empirically based on 10,000 permutations and were two-tailed. Empirical *P*-values were estimated by permuting tests, counting the number of times the permuted test was greater than the observed test, and dividing by the total number of simulations (10,000). Interaction between *DRB1*03:01* risk alleles and *CIITA* variants were assessed in PLINK using logistic regression. The predictor was the *CIITA* variant and the outcome was presence of the *DRB1*03:01* allele. The case-only *gene x gene* interaction test was applied to improve power, and the assumption of linkage equilibrium between *DRB1*03:01* and *CIITA* was fulfilled.[40,41] PLINK (maxT permutation procedure) was used to permute the ordering of the outcome status. In stage 1 of this study in the overall sample there was 80% power to detect an OR ranging from 1.4 to 1.5, based on power estimations in PGA v2.0 (Bethesda, MD, USA; minor allele frequency 0.1–0.5, two-sided $\alpha = 5.56\times10^{-3}$).

In the current study, genotypes for the-168A/G promoter polymorphism (rs3087456) were not available. However, strong LD ($r^2 > 0.90$) exists between rs3087456 and nearby rs12928665, as reported for a sample of independent healthy controls.[4] Genotypes for rs12928665 were available for all cases and the stage 1 controls, and did not provide evidence of association.

Table 3

Results from allelic tests of the rs4774 missense mutation in stages 1 and 2 and the combined sample, stratified by the presence of *DRB1*03:01*[1].

	Stage 1		Stage 2		Combined (Meta-analysis)	
Sample	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
Overall	1.24 (1.07–1.44)	**4.2×10^{-3}**	1.16 (1.02–1.33)	**0.02**	1.20 (1.09–1.33)	**2.5×10^{-4}**
*DRB1*03:01+*	1.27 (1.06–1.53)	**9×10^{-3}**	**1.23 (0.98–1.54)**	**0.07**	1.25 (1.09–1.45)	**1.9×10^{-3}**
*DRB1*03:01−*	1.18 (0.90–1.55)	0.23	1.14 (0.96–1.34)	0.12	1.15 (1.00–1.32)	0.05

[1]Characterized by r2187668 genotyping.

The meta-analysis was performed using a fixed effects model. Between-study heterogeneity was assessed with the Cochran's Q test statistic and was not present in the samples (*data not shown*).