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Fine mapping and trans-ethnic genotyping establish *IL2/IL21* genetic association with lupus and localize this genetic effect to *IL21*

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Abstract

Objective—Genetic association of the *IL2*/*IL21* region at 4q27 has been previously reported in lupus and a number of autoimmune and inflammatory diseases. Herein, using a very large cohort of lupus patients and controls, we localize this genetic effect to the *IL21* gene.

Methods—We genotyped 45 tag SNPs across the *IL2*/*IL21* locus in two large independent lupus sample sets. We studied a European-derived set consisting of 4,248 lupus patients and 3,818 healthy controls, and an African-American set of 1,569 patients and 1,893 healthy controls. Imputation in 3,004 WTCCC additional control individuals was also performed. Genetic association between the genotyped markers was determined, and pair-wise conditional analysis was performed to localize the independent genetic effect in the *IL2*/*IL21* locus in lupus.

Results—We established and confirmed the genetic association between *IL2*/*IL21* and lupus. Using conditional analysis and trans-ethnic mapping, we localized the genetic effect in this locus to two SNPs in high linkage disequilibrium; rs907715 located within *IL21* (OR=1.16 (1.10–1.22), $P = 2.17 \times 10^{-8}$), and rs6835457 located in the 3'-UTR flanking region of *IL21* (OR= 1.11 (1.05–1.17), $P = 9.35 \times 10^{-5}$).

Conclusion—We have established the genetic association between lupus and *IL2*/*IL21* with a genome-wide level of significance. Further, we localized this genetic association within the *IL2*/*IL21* linkage disequilibrium block to *IL21*. If other autoimmune *IL2*/*IL21* genetic associations are similarly localized, then the *IL21* risk alleles would be predicted to operate in a fundamental mechanism that influences the course of a number of autoimmune disease processes.

Introduction

Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease characterized by the production of autoantibodies against nuclear self-antigens. While the etiology of lupus is not certain, a number of genetic susceptibility loci have been identified. (1–2) We have previously reported genetic association between common variants within the *IL21* gene and lupus. (3) The *IL2*/*IL21* region at 4q27 has been identified as a genetic susceptibility locus in a number of autoimmune disorders.(4–8) While these data suggest that the *IL2*/*IL21* region is a genetic susceptibility locus for human autoimmunity, the localization of this genetic effect at this locus has not been established. Indeed, both *IL2* and *IL21* are equally attractive biological candidates.

Aberrant regulation of both IL-2 and IL-21 has been implicated as a potential driver of autoimmunity in human and murine lupus. (9–11) IL-2 plays an important role in regulatory T cell homeostasis and survival but is not essential for Treg proliferation or suppression. (12–13) However, reduced survival of regulatory T cells in response to reduced IL-2 expression has been observed, leading to autoimmunity in NOD mice. (14) IL-21 is a cytokine that plays a central role in the antibody mediated immune response. Produced primarily by CD4+ T cells, it acts on NK cells, CD4+ T cells, and B lymphocytes to induce and sustain antibody production and mediate antibody class switching. (15) IL-21 also induces Th17 differentiation through a pathway distinct from IL-6 involving STAT3 signaling and activation of RORγt.(16–17) Th17 cells are mediators of inflammation and have been shown to possess a pathogenic role in autoimmunity.(18) While IL-21 producing CD4+ T cells readily arise under Th17 polarizing conditions, not all CD4+ T cells that produce IL-21 produce IL-17A or IL-17F. (19) IL-6, IL-21 and STAT3 signaling have also been shown to be important in T follicular helper proliferation. (20) An uncontrolled T

follicular helper response has been shown to induce systemic autoimmunity through excess germinal center formation and high affinity antibody production. (21–22)

Herein we confirm, replicate, and fine map the genetic association with the *IL2/IL21* locus in lupus using two large European-derived and African-American lupus sample sets. We localize the genetic association in this locus, and demonstrate that the main genetic effect in this locus is explained by the genetic association with *IL21*.

Materials and Methods

Study participants and genotyping

A total of 4,248 lupus cases and 3,818 controls of European descent and 1,569 African-American lupus cases and 1,893 African-American controls were included in this study. 45 haplotype tagging SNPs (tag $r^2 \geq 0.8$) were genotyped in the *IL2/IL21* locus at 4q27 to cover the entire LD block in that locus spanning from *KIAA1109* to *BBS12*. Tag SNPs were selected using HapMap CEU data as our European-derived population represents the larger sample set included in this study, and capture all HapMap SNPs in the LD block examined with a mean r^2 value of 0.96. In addition, 347 ancestry informative markers (AIMs) were genotyped (23–26). All lupus cases fulfilled the ACR lupus classification criteria (27–28). Genotyping was performed using Illumina Custom Bead system on the iSCAN instrument, as part of a large multi-investigator candidate gene association study for lupus that included a total of 32,216 SNPs in a number of candidate genes. This collaborative genotyping approach helped us to maximize our sample size and reduce genotyping costs.

Data Analysis

Individuals with a genotype success rate of <90% were excluded from the analysis. A total of 326 and 17 European-derived and African-American samples, respectively, were removed due to low genotype success rate. The remaining samples were then evaluated for duplicates or related individuals and one individual from each pair was removed if the proportion of alleles shared identical by descent (IBD) > 0.4. Samples with increased heterozygosity (>5 standard deviation around the mean) were then removed from the analysis. Finally, genetic outliers were removed from further analysis as determined by principal components analysis (PCA) and admixture proportions calculated using ADMIXMAP. A total of 10 principal components were calculated and 2 principal components that explain the majority of variation in samples included in this study were used to identify outliers in the European-derived and African-American sample sets. Outliers were defined by 4 standard deviations from the mean of each of the 2 principal components. Addition outliers identified using ADMIXMAP were also removed. A total of 51 genetic outliers were removed from the European-derived samples (44 outliers identified by PCA and 7 additional outliers identified by ADMIXMAP). In the African-American sample, a total of 30 outliers were removed (25 outliers identified by PCA and 5 additional outliers identified by ADMIXMAP). After filtering based on the aforementioned criteria, a total 3,980 cases and 3,546 control individuals of European descent and 1,414 cases and 1,767 controls of African-American descent were included in subsequent analyses. All study participants singed an informed consent, and all protocols were approved by the institutional review boards of our institutions.

For each sample set analyzed, markers with a genotype success rate (GSR) below 0.90, Hardy-Weinberg equilibrium (HWE) P value below 0.001, or minor allele frequency (MAF) less than 0.01 were excluded from further analysis. Of the 45 markers genotyped, 35 passed the inclusion threshold in European-derived participants and were subsequently analyzed (10 SNPs were excluded: 3 SNPs due to HWE, GSR, and MAF; 3 due to HWE only; 3 due

to GSR only; 1 due to MAF only). 35 markers were analyzed in African-Americans (10 SNPs were excluded: 3 due to HWE, GSR, and MAF; 1 due to HWE only; 1 due to GSR only; 5 due to MAF only).

Allele frequencies in patients and controls, odds ratios and corresponding 95 percent confidence intervals, and χ^2 with corresponding P values were determined for each SNP using PLINK (29). Hardy-Weinberg equilibrium P values were calculated and LD plots generated using Haploview 4.2 (30).

Pairwise conditional analysis was performed in the European-derived sample set to test for associations independent of the haplotypic background. Two-marker haplotypes were constructed using all associated SNPs. Total haplotypic association was calculated for each two-marker haplotype constructed. Each two-marker haplotype was subsequently conditioned on each of its constituent markers. Markers maintaining significance upon conditioning were said to have an effect independent of the alternate marker in the two-marker haplotype.

Meta-analysis was performed on the single SNP associations obtained for rs6835457 and rs907715 using the European-derived and African-American participants using PLINK. Meta-analysis odds-ratios and corresponding 95 percent confidence intervals, P values, and heterogeneity p values were calculated for both markers.

Imputation analysis was performed using Impute version 2 (31).

Results

In the European-derived participants, a number of SNPs contained throughout the *IL2*/*IL21* region are significantly associated with lupus susceptibility ($P<0.05$). (Table 1) Markers in and around *IL21* show the most significant association with lupus susceptibility in individuals of European ancestry. (Table 1, Figure 1A) The two markers with the highest association (rs6835457, $P= 6.76\times10^{-5}$; rs907715, $P= 7.21\times10^{-5}$) are located in the *IL21* 3'-UTR flanking region, and within *IL21*, respectively. (Figure 1A, 2A) Two SNPs in *IL2* show significant association with lupus susceptibility in the European-derived sample set (rs2069779, P=0.0071; rs6814718, P= 0.00034).

Pairwise conditional analyses were performed on significantly associated markers in the European-derived sample set. The strongest genetic effect observed in rs6835457 maintained significance when conditioned against all other associated markers. However, rs6835457 located in the 3'-UTR flanking region of *IL21* is in almost complete LD with rs907715, a SNP located within *IL21* ($r^2=0.98$). This made it impossible to distinguish an independent effect of these SNPs using conditional analysis, and therefore either of the two *IL21* SNPs or both can explain the genetic association between lupus and the *IL2*/*IL21* LD block. (Table 2)

There is, however, a smaller effect in *IL2* that is not entirely accounted for by the effect in rs6835457 and rs907715 in lupus patients of European descent. Specifically, rs2069779 has an effect that is independent of the most significant marker rs6835457, as rs2069779 and rs6835457 are not in strong LD ($r^2=0.02$).

Genetic association in the African-American sample set reveals a total of four associated markers (Table 3). Notably, all significant markers are contained in the *IL21* region (Figure 1B, 2B), and the two most significantly lupus-associated SNPs in the European-derived participants are also associated with lupus in African-Americans. Furthermore, the only markers found to possess significant association in both ethnicities are rs6835457 and

rs907715, located in the 3'UTR flanking regions of *IL21* and within *IL21*, respectively. Similar to the European-derived participants, rs6835457 and rs907715 have a high degree of LD in the African-Americans, suggesting that they represent the same genetic effect (r^2= 0.90).

To establish the genetic association detected in *IL21* with a genome-wide significance ($P<5\times 10^{-8}$), we attempted to increase the sample size by including control samples from the Wellcome Trust Case-Control Consortium (WTCCC). Since neither rs6835457 nor rs907715 were genotyped in WTCCC, we imputed genotype calls for rs907715 and rs683545 in the 3,004 WTCCC control individuals from a reference panel consisting of phased HapMap3 and 1000 Genomes haplotypes. Genotype calls were made on the basis of a 0.75 imputation threshold. Imputation success rate was >90% for both imputed SNPs. Imputed calls for each marker were combined with the non-imputed genotypes obtained in our European-derived study participants, and the genetic association test was repeated. Meta-analysis of associations obtained in our European-derived participants combined with WTCCC controls and our African-American study participants was performed. The genetic association between lupus and rs907715 within *IL21* was validated and with a genome-wide significance ($P= 2.17\times10^{-8}$) establishing the genetic association between the *IL21* locus with lupus (Table 4).

Discussion

Our data confirmed, replicated and localized the genetic association between the *IL2*/*IL21* haplotype block and susceptibility to lupus. We observed genetic association in both *IL2* and *IL21* genes in the European-derived participants with the most significant association residing in and around *IL21*. In the European-derived sample set there is also a smaller but independent association with one marker contained within the *IL2* gene. Analysis in the African-American participants revealed far fewer significant SNPs associated with lupus than in the European-derived sample set, likely reflecting smaller haplotype blocks in African-Americans. Interestingly, the two most significantly associated markers in the European-derived sample set (contained in and around *IL21*) were also associated with lupus in the African-American participants, while polymorphisms in *IL2* were not associated with lupus in African-Americans (Table 2). Although rs2069779 located in *IL2*, did not meet the inclusion criterion for minor allele frequency in African-Americans (rs2069779, MAF= 0.9%), no difference in allele frequency was observed between cases and controls (MAF_{Case}= 0.009, $MAF_{Control}$= 0.009). While the genetic association with rs2069779 in *IL2* appears to be independent of the main genetic association in this locus in *IL21* in the European-derived sample set, this effect was not reproducible in the African-American lupus patients. The failure to confirm this *IL2* association in African-Americans further supports a stronger role for *IL21* polymorphisms in lupus susceptibility at the *IL2*/*IL21* locus.

We and others have demonstrated the genetic association of polymorphisms within the *IL2*/*IL21* locus at 4q27 with lupus and multiple other autoimmune and inflammatory disorders. (3–7,32–33) Our data show a modest association between lupus and the commonly studied *IL2*/*IL21* inter-genic marker rs6822844 in the European-derived but not the African-American lupus sample set (P= 0.014, 0.41, respectively). These data, together with conditional analysis in the European-derived participants, suggest that the observed association between lupus and rs6822844 is explained by the association with rs6835457 and rs907715, which shows an independent genetic effect in our studies. Neither rs6835457 nor rs907715 were included in commonly used genome-wide association platforms and therefore were not evaluated in genome-wide associated studies of other autoimmune or inflammatory diseases.

The association of polymorphisms at 4q27 has been demonstrated in type I diabetes in human studies.(4,32) Significant evidence for involvement of *IL2* and *IL21* in type 1 diabetes also comes from the NOD mouse in which the most highly associated non-MHC locus is the *Idd3* region of the murine genome which contains the *IL2* and *IL21* genes. In one study, the overexpression of IL-21 was observed to correlate with the number of *Idd3* alleles, and this change in expression was shown to occur in response to polymorphisms which establish an Sp1 binding site upstream of *IL21*. (34) This study observed no difference in the expression levels of IL-2. In contrast to these findings, a previous study reported the underexpression of IL-2 at the NOD *Idd3* locus, leading to the loss of stability in peripheral Treg cell cohorts, and these changes were unaccompanied by changes in IL-21 expression. (14) This reduction in Treg cell numbers was in turn shown to lead to an increase in presentation of beta cell antigens by dendritic cells.

While these studies and others present compelling evidence for the involvement of both *IL2* and *IL21* in type 1 diabetes, the associations observed in other autoimmune diseases are illustrative as well. Significant epistasis has been reported between rs6822844 and polymorphisms in *IL23R* in ulcerative colitis.(35) The association of these distal variants suggests a role for polymorphisms at 4q27 in the establishment of a Th17 defect.(36) Furthermore, these data suggest that IL-21, a driver of Th17 differentiation (37), likely accounts for the genetic effect observed at 4q27 in ulcerative colitis.

We have previously reported the association of polymorphisms in *IL21R* with lupus susceptibility (rs3093301, P_{meta}=0.0001, OR=1.16 [95% confidence interval 1.08–1.25]) (38). The association of polymorphisms in the *IL21R* gene located at 16p11 further implicates the IL-21/IL-21R pathway signaling in lupus risk. IL-21/IL-21R signaling plays a pathogenic role in multiple models of murine lupus. Blockade of IL-21R signaling with IL-21R.Fc attenuates the severity of disease in the MRL/*lpr* lupus mouse model. (39) The deletion of IL-21R in BXSB-*Yaa* mice ameliorates antibody-mediated disease manifestations, while IL-21R competent BXSB-*Yaa* mice produce high levels of IgG1, IgG2b, and IgG3.(10) These data further support a role for an IL-21/IL-21R signaling defect in lupus pathogenesis.

In conclusion, using two large ethnically-diverse lupus sample sets, conditional analysis, and trans-ethnic genotyping, we fine-mapped and localized the genetic association with lupus in the *IL2*/*IL21* LD block to *IL21*. These data might be relevant to a number of other autoimmune and inflammatory diseases with a reported genetic association in the same region.

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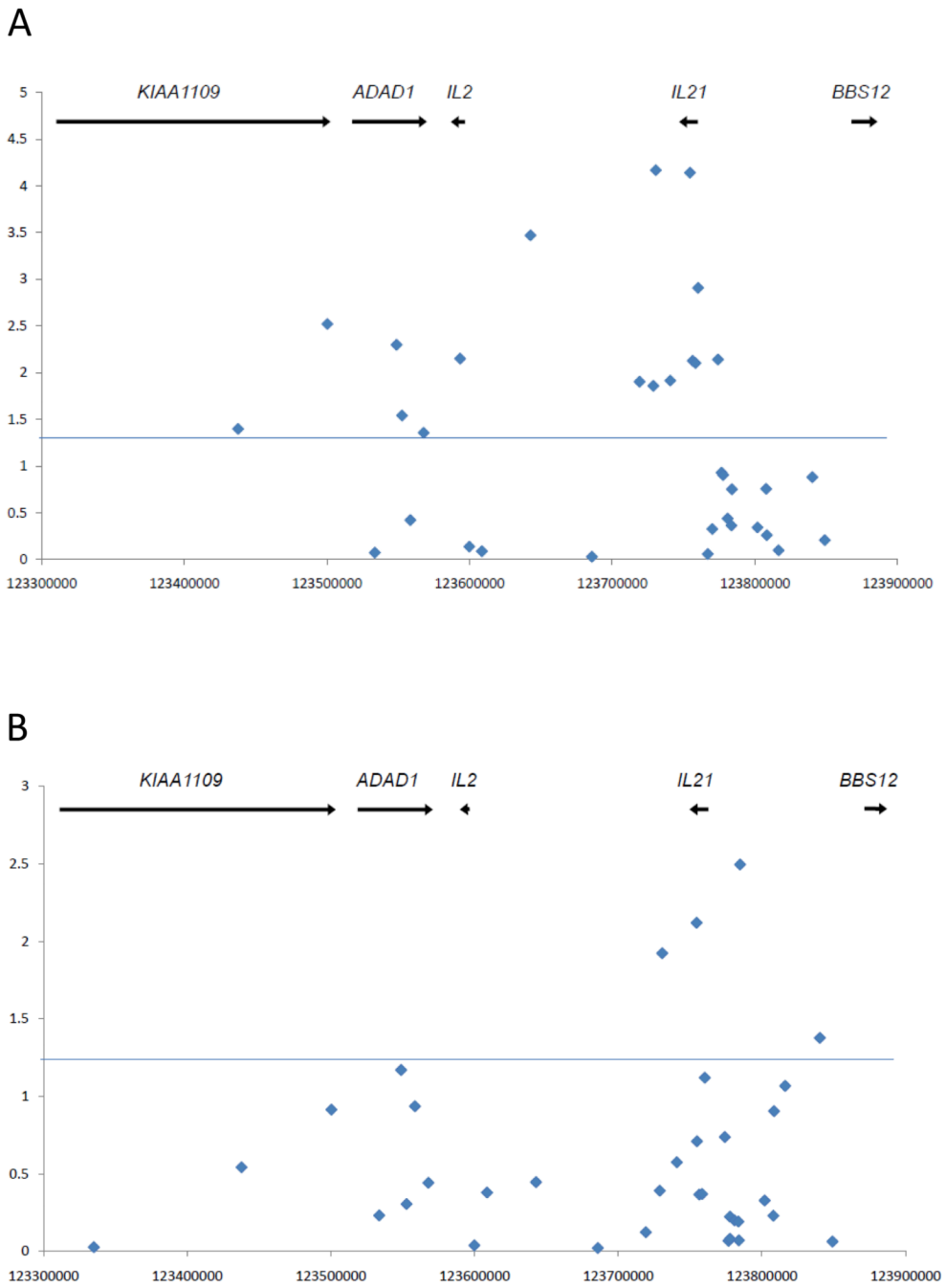

Figure 1.

Genetic association at the *IL2*/*IL21* locus in the European-derived (A), and the African-American (B) sample sets included in this study. Y-axis values represent −log(10)-p-values for individual markers genotyped, while x-axis values represent chromosomal coordinates for each marker.

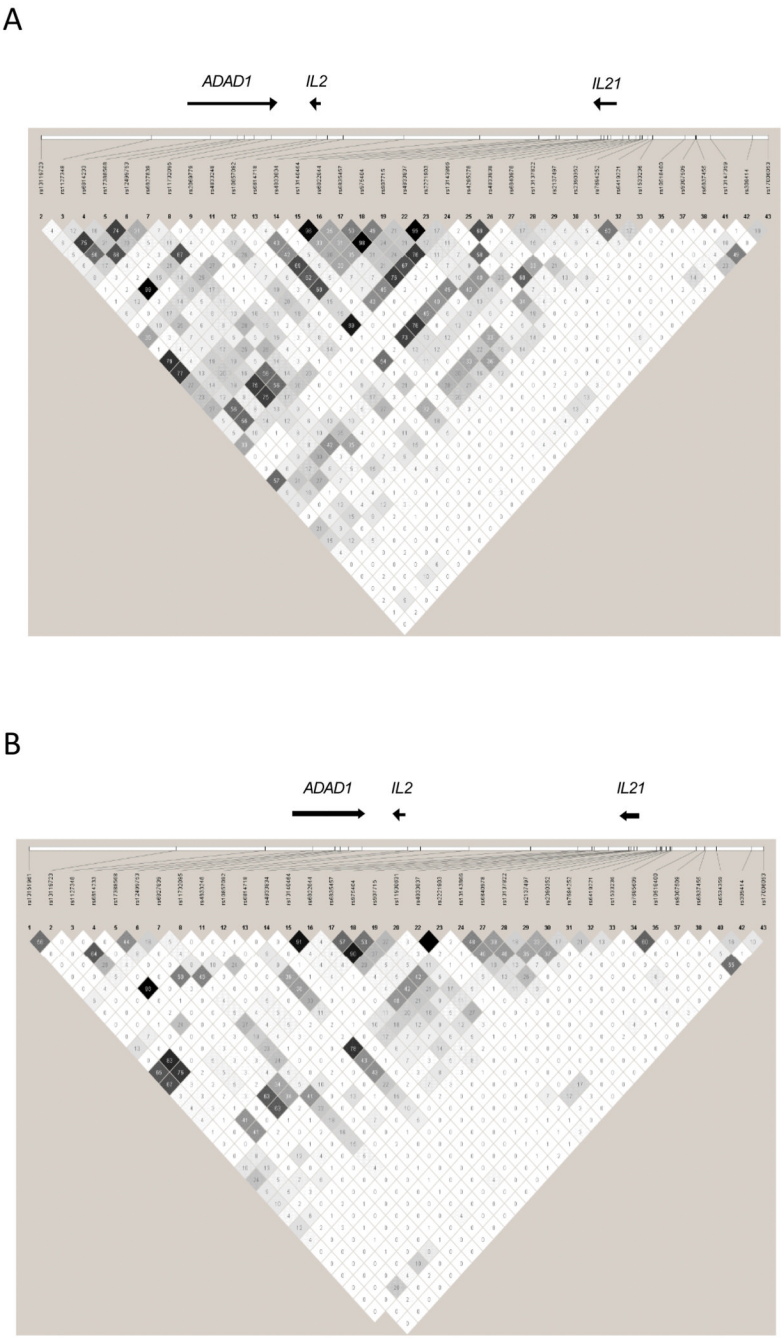

Figure 2.
LD plots depicting genetic markers analyzed in the European-derived (A) and the African-American (B) participants included in this study. Pairwsie r^2 values are shown.

Table 1

Genetic association between *IL2/IL21* in European-derived lupus patients and controls.

				Associated	Frequency			95% CI		
Marker	**Position**	**Gene**	**Function**	**Allele**	**Cases**	**Controls**	**OR**	**LL**	**UL**	***P* Value**
rs13119723	123437763	*KIAA1109*	Intron	A	0.88 (6633:935)	0.87 (5840:912)	1.11	1.01	1.22	0.04
rs1127348	123500310	*KIAA1109*	Coding	G	0.23 (1843:6073)	0.21 (1507:5581)	1.12	1.04	1.21	0.003
rs6814233	123533582	*ADAD1*	Intron	C	0.70 (5421:2367)	0.70 (4826:2122)	1.01	0.94	1.08	0.85
rs17388568	123548812	*ADAD1*	Intron	A	0.28 (2168:5610)	0.26 (1795:5157)	1.11	1.03	1.19	0.005
rs12499753	123552627	*ADAD1*	Intron	G	0.34 (2587:5145)	0.32 (2205:4737)	1.08	1.01	1.16	0.029
rs6827839	123558465	*ADAD1*	Intron	A	0.40 (3056:4536)	0.40 (2678:4096)	1.03	0.96	1.10	0.38
rs11732095	123567795	*ADAD1*	Intron	A	0.91 (7205:725)	0.90 (6346:714)	1.12	1.00	1.25	0.044
rs2069779	123593347	*IL2*	Intron	A	0.08 (586:7002)	0.07 (444:6324)	1.19	1.05	1.36	0.0071
rs4833248	123599855	*IL2*	Flanking 5' UTR	G	0.70 (5355:2345)	0.69 (4754:2108)	1.01	0.94	1.09	0.73
rs10857092	123608669	*IL2*	Flanking 5' UTR	A	0.07 (519:7433)	0.06 (456:6632)	1.02	0.89	1.16	0.82
rs6814718	123642766	*IL2*	Flanking 5' UTR	G	0.73 (5779:2113)	0.71 (4975:2073)	1.14	1.06	1.22	0.0003
rs4833834	123685801	*IL21*	Flanking 3' UTR	G	0.10 (816:7070)	0.10 (725:6309)	1.00	0.90	1.12	0.94
rs13140464	123719195	*IL21*	Flanking 3' UTR	C	0.86 (6770:1108)	0.85 (5917:1087)	1.12	1.03	1.22	0.013
rs6822844	123728871	*IL21*	Flanking 3' UTR	C	0.86 (6829:1121)	0.85 (5984:1100)	1.12	1.02	1.23	0.014
rs6835457	123730576	*IL21*	Flanking 3' UTR	A	0.68 (5433:2507)	0.65 (4629:2453)	1.15	1.07	1.23	6.8×10^{-5}
rs975404	123740742	*IL21*	Flanking 3' UTR	A	0.65 (5169:2745)	0.63 (4443:2571)	1.09	1.02	1.17	0.012
rs907715	123754503	*IL21*	Intron	G	0.69 (5374:2462)	0.66 (4590:2416)	1.15	1.07	1.23	7.2×10^{-5}
rs4833837	123756413	*IL21*	Coding	G	0.32 (2508:5412)	0.30 (2093:4967)	1.10	1.03	1.18	0.0074
rs2221903	123758362	*IL21*	Intron	G	0.32 (2474:5382)	0.30 (2063:4935)	1.10	1.03	1.18	0.0079
rs13143866	123760208	*IL21*	Intron	G	0.74 (5523:1985)	0.71 (4733:1921)	1.13	1.05	1.22	0.0012
rs4295278	123766991	*IL21*	Flanking 5' UTR	A	0.95 (7563:375)	0.95 (6734:338)	1.01	0.87	1.18	0.87
rs4833838	123770148	*IL21*	Flanking 5' UTR	A	0.97 (7690:258)	0.97 (6841:245)	1.07	0.89	1.28	0.47
rs6840978	123774157	*IL21*	Flanking 5' UTR	G	0.82 (6553:1399)	0.81 (5714:1366)	1.12	1.03	1.22	0.0072
rs13137822	123776686	*IL21*	Flanking 5' UTR	C	0.45 (3498:4334)	0.43 (3043:3971)	1.05	0.99	1.12	0.12
rs2137497	123777704	*IL21*	Flanking 5' UTR	C	0.58 (4564:3252)	0.58 (4035:2917)	1.01	0.95	1.08	0.66
rs2390352	123777780	*IL21*	Flanking 5' UTR	A	0.96 (7530:336)	0.95 (6691:337)	1.13	0.97	1.32	0.13
rs7694252	123780886	*IL21*	Flanking 5' UTR	A	0.71 (5543:2277)	0.70 (4879:2071)	1.03	0.96	1.11	0.37

Marker	Position	Gene	Function	Associated Allele	Frequency Cases	Frequency Controls	95% CI OR	95% CI LL	95% CI UL	*P* Value
rs6419221	123783569	*IL21*	Flanking 5' UTR	G	0.64 (5048:2806)	0.64 (4503:2571)	1.03	0.96	1.10	0.43
rs1533236	123783908	*IL21*	Flanking 5' UTR	G	0.51 (4060:3896)	0.50 (3541:3551)	1.04	0.98	1.11	0.18
rs10518400	123801876	*IL21*	Flanking 5' UTR	A	0.08 (600:7354)	0.07 (512:6576)	1.05	0.93	1.18	0.45
rs9307509	123807941	*IL21*	Flanking 5' UTR	T	0.91 (6673:639)	0.91 (5947:617)	1.08	0.96	1.22	0.18
rs6837455	123808392	*IL21*	Flanking 5' UTR	C	0.15 (1189:6755)	0.15 (1036:6050)	1.03	0.94	1.13	0.55
rs13147359	123816621	*IL21*	Flanking 5' UTR	A	0.97 (7691:229)	0.97 (6847:209)	1.02	0.85	1.24	0.80
rs309414	123840362	*BBS12*	Flanking 3' UTR	A	0.39 (2974:4606)	0.38 (2619:4271)	1.05	0.98	1.13	0.13
rs17006053	123849111	*BBS12*	Flanking 3' UTR	C	0.76 (5958:1836)	0.76 (5275:1657)	1.02	0.94	1.10	0.62

OR, odds ratio; LL, lower limit; UL, upper limit; CI, confidence interval. The numbers between parenthesis represent the number of risk (associated) and protective (non-associated) alleles, respectively.

Table 2

Pairwise two-SNP haplotype conditional analysis of rs6835457 with all other associated SNPs in the *IL2*/*IL21* locus in the European-derived lupus patients and controls.

	Single SNP	Haplotype	Conditional Marker *P* Value	
Marker	***P* Value**	***P* Value**	**Haplotype SNP**	**rs6835457**
rs13119723	0.04	0.00071	0.0038	0.60
rs1127348	0.003	0.0001	0.0016	0.12
rs17388568	0.005	0.00016	0.0024	0.18
rs12499753	0.029	0.00059	0.0019	0.44
rs11732095	0.044	0.00036	0.00042	0.95
rs2069779	0.0071	0.000044	0.00055	0.034
rs6814718	0.0003	0.00090	0.19	0.63
rs13140464	0.013	0.00035	0.0037	0.58
rs6822844	0.014	0.00035	0.0018	0.84
rs6835457	0.000068	--	--	--
rs975404	0.012	0.00086	0.0052	0.64
rs907715	0.000072	0.00011	NA	NA
rs4833837	0.0074	0.00024	0.0016	0.39
rs2221903	0.0079	0.00023	0.0020	0.33
rs13143866	0.0012	0.00064	0.10	0.85
rs6840978	0.0072	0.00036	0.0042	0.89

Table 3

Genetic association between *IL2/IL21* in African-American lupus patients and controls.

				Associated	Frequency		95% CI			
Marker	**Position**	**Gene**	**Function**	**Allele**	**Cases**	**Controls**	**OR**	**LL**	**UL**	***P* Value**
rs13151961	123334952	*KIAA1109*	Intron	A	0.96 (2475:103)	0.96 (3188:134)	1.01	0.78	1.31	0.94
rs13119723	123437763	*KIAA1109*	Intron	G	0.03 (75:2735)	0.02 (79:3427)	1.19	0.86	1.64	0.29
rs1127348	123500310	*KIAA1109*	Coding	A	0.97 (2744:82)	0.96 (3403:127)	1.25	0.62	1.66	0.12
rs6814233	123533582	*ADAD1*	Intron	A	0.08 (212:2602)	0.07 (251:3247)	1.05	0.87	1.27	0.59
rs17388568	123548812	*ADAD1*	Intron	G	0.96 (2700:124)	0.95 (3334:190)	1.24	0.98	1.56	0.068
rs12499753	123552627	*ADAD1*	Intron	A	0.90 (2541:283)	0.90 (3156:372)	1.06	0.90	1.25	0.50
rs6827839	123558465	*ADAD1*	Intron	A	0.63 (1769:1035)	0.61 (2117:1345)	1.09	0.98	1.20	0.12
rs11732095	123567795	*ADAD1*	Intron	A	0.97 (2732:82)	0.97 (3411:117)	1.14	0.86	1.52	0.36
rs4833248	123599855	*IL2*	Flanking 5' UTR	A	0.07 (208:2598)	0.07 (252:3180)	1.01	0.83	1.22	0.92
rs10857092	123608669	*IL2*	Flanking 5' UTR	A	0.06 (162:2666)	0.05 (186:3348)	1.09	0.88	1.36	0.42
rs6814718	123642766	*IL2*	Flanking 5' UTR	G	0.77 (2179:643)	0.76 (2691:839)	1.06	0.94	1.19	0.36
rs4833834	123685801	*IL21*	Flanking 3' UTR	G	0.02 (55:2773)	0.02 (68:3466)	1.01	0.71	1.45	0.95
rs13140464	123719195	*IL21*	Flanking 3' UTR	C	0.97 (2726:80)	0.97 (3380:104)	1.05	0.78	1.41	0.75
rs6822844	123728871	*IL21*	Flanking 3' UTR	C	0.97 (2745:81)	0.97 (3418:114)	1.13	0.85	1.51	0.41
rs6835457	123730576	*IL21*	Flanking 3' UTR	A	0.61 (1730:1096)	0.58 (2051:1479)	1.14	1.03	1.26	0.012
rs975404	123740742	*IL21*	Flanking 3' UTR	A	0.62 (1743:1081)	0.60 (2128:1398)	1.06	0.96	1.17	0.27
rs907715	123754503	*IL21*	Intron	G	0.62 (1762:1060)	0.59 (2083:1439)	1.15	1.04	1.27	0.0076

Marker	Position	Gene	Function	Associated Allele	Frequency Cases	Frequency Controls	OR	95% CI LL	95% CI UL	*P* Value
rs11930631	123754745	*IL21*	Intron	A	0.31 (861:1949)	0.29 (1014:2466)	1.07	0.96	1.20	0.20
rs4833837	123756413	*IL21*	Coding	A	0.93 (2629:199)	0.92 (3267:267)	1.08	0.89	1.31	0.43
rs2221903	123758362	*IL21*	Intron	A	0.93 (2629:199)	0.92 (3265:267)	1.08	0.89	1.31	0.43
rs13143866	123760208	*IL21*	Intron	G	0.79 (2211:579)	0.77 (2665:779)	1.12	0.99	1.26	0.076
rs6840978	123774157	*IL21*	Flanking 5' UTR	G	0.86 (2430:394)	0.85 (2999:535)	1.10	0.96	1.27	0.18
rs13137822	123776686	*IL21*	Flanking 5' UTR	C	0.70 (1963:851)	0.70 (2437:1067)	1.01	0.91	1.12	0.86
rs2137497	123777704	*IL21*	Flanking 5' UTR	A	0.71 (2002:812)	0.71 (2466:1030)	1.03	0.92	1.15	0.60
rs2390352	123777780	*IL21*	Flanking 5' UTR	G	0.53 (1500:1326)	0.53 (1860:1662)	1.01	0.92	1.12	0.83
rs7694252	123780886	*IL21*	Flanking 5' UTR	G	0.22 615:2197)	0.21 (751:2763)	1.03	0.91	1.16	0.63
rs6419221	123783569	*IL21*	Flanking 5' UTR	G	0.61 (1727:1093)	0.61 (2138:1386)	1.02	0.93	1.13	0.64
rs1533236	123783908	*IL21*	Flanking 5' UTR	G	0.38 (1070:1758)	0.38 (1329:2205)	1.01	0.91	1.12	0.85
rs7685609	123784752	*IL21*	Flanking 5' UTR	G	0.98 (2694:68)	0.96 (3302:130)	1.56	1.16	2.10	0.0032
rs10518400	123801876	*IL21*	Flanking 5' UTR	G	0.95 (2668:156)	0.94 (3220:210)	1.08	0.87	1.34	0.47
rs9307509	123807941	*IL21*	Flanking 5' UTR	T	0.97 (2599:91)	0.96 (3122:118)	1.08	0.82	1.43	0.59
rs6837455	123808392	*IL21*	Flanking 5' UTR	C	0.22 (612:2212)	0.20 (707:2811)	1.10	0.97	1.24	0.13
rs6534359	123816144	*IL21*	Flanking 5' UTR	A	0.94 (2651:157)	0.93 (3277:233)	1.20	0.97	1.48	0.086
rs309414	123840362	*BBS12*	Flanking 3' UTR	C	0.76 (2147:673)	0.74 (2600:918)	1.13	1.00	1.26	0.042
rs17006053	123849111	*BBS12*	Flanking 3' UTR	C	0.75 (2099:689)	0.75 (2600:862)	1.01	0.90	1.13	0.87

OR, odds ratio; LL, lower limit; UL, upper limit, CI, confidence interval. The numbers between parenthesis represent the number of risk (associated) and protective (non-associated) alleles, respectively.

Table 4

Meta-analysis of genetic associations in the European-derived sample set combined with WTCCC controls and the African- American sample set for markers in the *IL21* gene.

	Associated Allele	European-derived with WTCCC Controls		African-American		Meta-analysis		
SNP		**OR (95% CI)**	***P* Value**	**OR (95% CI)**	***P* Value**	**OR (95% CI)**	***P* Value**	$P_{heterogeneity}$
rs6835457	A	1.10 (1.03–1.17)	2.3×10^{-3}	1.14 (1.03 1.26)	0.012	1.11 (1.05–1.17)	9.35×10^{-5}	0.55
rs907715	G	1.16 (1.04–1.27)	8.41×10^{-7}	1.15 (1.04 1.27)	0.0076	1.16 (1.10–1.22)	2.17×10^{-8}	0.83