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Genetic Association of *CD247* (CD3 ζ) with SLE in a Large-Scale Multiethnic Study

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Abstract

A classic T-cell phenotype in Systemic lupus erythematosus (SLE) is the downregulation and replacement of the CD3 ζ chain that alters TCR signaling. However, genetic associations with SLE in the human *CD247* locus that encodes CD3 ζ are not well established and require replication in independent cohorts. Our aim was therefore to examine, localize and validate *CD247*-SLE association in a large multi-ethnic population. We typed 44 contiguous *CD247* SNPs in 8 922 SLE patients and 8 077 controls from four ethnically distinct populations. The strongest associations were found in the Asian population (11 SNPs in intron 1, $4.99 \times 10^{-4} < P < 4.15 \times 10^{-2}$), where we further identified a five-marker haplotype (rs12141731-rs2949655-rs16859085-rs12144621-rs858554; G-G-A-G-A; $P_{\text{hap}} = 2.12 \times 10^{-5}$) that exceeded the most associated single SNP rs858554 ($\text{MAF}_{\text{Controls}} = 13\%$; $P = 4.99 \times 10^{-4}$, OR=1.32) in significance. Imputation and subsequent association analysis showed evidence of association ($P < 0.05$) at 27 additional SNPs within intron 1. Cross-ethnic meta-analysis, assuming an additive genetic model adjusted for population proportions, showed 5 SNPs with significant P -values ($1.40 \times 10^{-3} < P < 3.97 \times 10^{-2}$), with one (rs704848) remaining significant after Bonferroni correction ($P_{\text{meta}} = 2.66 \times 10^{-2}$). Our study independently confirms and extends the association of SLE with *CD247*, which is shared by various autoimmune disorders and supports a common T cell-mediated mechanism.

INTRODUCTION

Systemic lupus erythematosus (SLE; OMIM 152700) is a chronic and potentially fatal autoimmune disorder characterized by the production of autoantibodies that cause widespread tissue damage. T-cells from patients with SLE have a number of phenotypic and functional abnormalities (1,2). Some of the strongest confirmed genetic associations with SLE obviously affect T-cells, including *HLA-DR*, which still exceeds all other associations in significance, as well as *PTPN22*, a TCR signal modifier (3), and *PTTG1* affecting miR146a (4) that appears particularly relevant for regulatory T-cells (5). One of the most characteristic aberrations, likely influential in altering intracellular signaling and subsequent aberrant responses of T-cells, is the specific downregulation of the CD3 ζ component of the T-cell receptor complex (6,7), *CD247*. In SLE T-cells, this molecule is specifically replaced by the Fc receptor γ chain that is coupled with a different intracellular signaling pathway (8). In addition to this demonstrated functional relevance, association of genetic polymorphisms within *CD247* with SLE has been discovered. Two reports have provided evidence for such an association, identifying two 3' UTR SNPs in strong linkage disequilibrium and showing association with differential CD3 ζ expression (9) as well as with SLE (10) in a European population. More recently, several SNPs within *CD247* (particularly in intron 1) were also found associated to SLE in Asian populations (11). Because the epidemiology of SLE has demonstrated that the prevalence of disease differs substantially across ethnic groups, it is logical that there exists significant genetic heterogeneity in the causes of SLE across populations (12,13). This has been supported by the differential findings obtained in

genome-wide association studies (GWAS) performed in different populations (14-19), with novel loci such as *RASGRP3* and *WDFY4* found to be associated with SLE in Asian, but not European populations. In this study, in order to further test the association of *CD247* gene with SLE in different populations, we typed 44 SNPs in a large multi-ethnic sample with total 17 003 individuals.

RESULTS

Association study and imputation analysis in the Asian population

The strongest associations were found in the Asian population (11 SNPs in intron 1, $4.99 \times 10^{-4} < P < 4.15 \times 10^{-2}$) (SNPs 14, 17, 24, 26, 27, 28, 30, 31, 32, 35, 36 as identified in Table 1; also see Figure 1). The most associated rs858554 (SNP 31, $MAF_{\text{Controls}}=13\%$) reached a significance of $P=4.99 \times 10^{-4}$ (OR[95%CI]=1.32[1.13-1.55]) and a corresponding $P=1.50 \times 10^{-2}$ after Bonferroni correction for multiple testing.

Several of the 11 significant SNPs were in very strong LD ($r^2 > 0.75$): 14 and 17; 26, 27, 28 and 30; 32, 35 and 36. SNP 24 had moderate to strong LD with SNPs 26, 27, 28 and 30 ($0.57 < r^2 < 0.67$). The most significant, SNP 31, however showed weak LD with all other SNPs in our dataset ($r^2 < 0.25$) (Figure 2). Four SNPs (SNPs 14, 17, 24, 35) remained nominally associated with SLE after conditional logistic regression analysis based on rs858554 (SNP 31), and one newly gained significance: rs16859085 (SNP 29) (Table 1, Figure 2). This suggests the existence of multiple genetic variants within *CD247* implicated in SLE.

Haplotype association analysis in the Asian population identified a five-marker haplotype containing five SNPs in intron 1 (rs12141731-rs2949655-rs16859085-rs12144621-rs858554; G-G-A-G-A; identified in Figure 2) showing robust association with SLE ($P_{\text{hap}}=2.12 \times 10^{-5}$).

Even though we investigated 42 SNPs in *CD247*, a proportion of the genetic variation in the region was not assessed because of the size of the gene (Figure 1). To evaluate the potential association of unobserved polymorphisms in this gene in the Asian population, we imputed SNPs in chromosome 1 using data from HapMap as well as the genotypes observed at the 30 fully genotyped markers. In the *CD247* gene, we obtained imputed genotypes meeting minimum quality standards (MAF in controls > 0.05 and $SNP\ INFO > 0.8$) for 51 SNPs, including 9 of the genotyped SNPs (Figure 1; identified with the SNP ID in Supplementary Table S1). Previously genotyped SNPs were imputed using the observed genotypes at the other SNPs, and a concordance rate $> 85\%$ between imputed and observed genotypes was obtained (Figure 1).

From the 51 imputed SNPs, 27 (including 7 of the genotyped SNPs) were associated with SLE susceptibility ($P < 0.05$) (Supplementary Table S1, Figure 1), the most significant of which were rs858557, rs858556 and rs858553 (all with: $P=4.82 \times 10^{-4}$, $SNP\ INFO=1.04$). All these polymorphisms are located in intron 1 close to our most strongly associated typed SNP rs858554.

Our most significant findings are consistent with those from a previous report in Asian populations (11) that resulted from the examination of GWAS data (19). In these studies, 14 SNPs in the *CD247* gene locus (including both upstream and downstream regions of the gene) were found to be significantly associated with SLE, five of which were inside the *CD247* gene (personal communication from authors of (19), May 2012), all located in intron 1 (as indicated by the dark blue dots in Figure 1). In our study, the 11 significant SNPs were also all located in intron 1 (although in different variants; as indicated by the green dots in Figure 1).

The plot of pairwise LD of the genotyped SNPs in our Asian samples (Figure 2) showed very similar LD patterns to the plot of CHB HapMap samples (Supplementary Figure S1), supporting the use of this reference dataset to check linkage between the significant SNPs in our Asian cohort and those in *CD247* from the GWAS data (19). We can see that the significant GWAS SNPs and our SNPs (black squares and asterisks, respectively, in Supplementary Figure S1) are physically close but in different LD blocks. Namely, the most significant SNPs in both studies, our rs858554 (SNP 31) and the GWAS rs704853, are in two different blocks located in intron 1. Furthermore, all the significant GWAS SNPs are in weak LD ($r^2 < 0.25$) among themselves and with our associated SNPs (Supplementary Figure S1). Taken together, the results of both studies complement each other, pointing to the existence of different variants in the same gene region that are not in strong LD and were observed independently, which strengthens the general result. Imputation did not return results for the top significant variants in Li *et al.* (11) and GWAS (19).

Non-Asian populations multiethnic association study, and meta-analysis

Five SNPs were significantly associated with SLE in the European ancestry samples ($1.12 \times 10^{-2} < P < 4.51 \times 10^{-2}$) including four SNPs within intron 1 (SNPs 14, 15, 35 and 36) and one downstream of *CD247* (SNP 1). In the other ethnicities, 3 SNPs were associated in African ancestry (SNPs 6, 24 and 35, $5.92 \times 10^{-3} < P < 2.95 \times 10^{-2}$), and 1 SNP in the Hispanic/Amerindian (SNP 36 $P = 3.39 \times 10^{-2}$) populations (Figure 3, Supplementary Table S2). None of these SNPs, however, remained significant upon Bonferroni correction for multiple testing. Nevertheless, several of these significant SNPs were common to the associated SNPs in the Asian cohort, namely SNPs 14, 35 and 36 in the European ancestry, SNPs 24 and 35 in the African ancestry, and SNP 36 in the Hispanic/Amerindian ancestry (Figure 3).

The significant haplotype identified in the Asian population was not associated in these three populations although the LD structures were similar (Supplementary Figure S2).

Cross-ethnic meta-analysis of the four populations, assuming an additive genetic model and adjusted for population proportions, showed 5 SNPs with significant *P*-values ($1.40 \times 10^{-3} < P < 3.97 \times 10^{-2}$), all located in intron 1 of *CD247* (Table 2, Figure 3). One marker was still significant after Bonferroni correction for multiple testing: rs704848 (SNP 36) with $P_{\text{meta}} = 2.66 \times 10^{-2}$.

DISCUSSION

In this multiethnic association study, we independently validated and extended the previous association of *CD247* genetic variants with SLE, primarily in the Asian population.

Two studies have previously found an association of the 3'UTR of this gene with reduced expression of CD3 ζ (9) and SLE (10). In contrast, our discoveries highlight genetic association in Asians in the 5' region (intron1) of *CD247*. This is consistent with recent studies performed in Asian populations (11). Considering the ethnic heterogeneities in the epidemiology of SLE (12,13), these observations suggest a particular association of *CD247* genetic variants in Asian populations. Although pointing to heterogeneity in the genetic association of *CD247* with SLE, most importantly, these results further support and highlight the implication of this gene in SLE.

The *CD247* gene spans 88 kb and has been mapped to chromosome 1q24.2. The first intron spans about 78 kb, followed by seven other exons of the gene. The 11 significant SNPs in the Asian population and 78% of the significant SNPs in the other three populations tested lie in intron 1, suggesting a possible role in the regulation of *CD247* expression (11). This region is further highlighted by the imputation analysis (27 imputed SNPs reached significance) and haplotypic association ($P_{\text{hap}}=2.12\times 10^{-5}$) in the Asians, and by an overall significant meta-analysis of all four populations.

Gorman *et al.* (9) found two SNPs (in high LD), rs1052230 and rs1052231 in 3'UTR of the gene being associated with *CD247* expression levels in both SLE patients and healthy controls. However, only weak association with disease risk was found for haplotypes in the 3'UTR region of the gene. In addition, Warchoř *et al.* (10) found that rs1052231 conferred increased risk of incidence of SLE. In our study, SNP rs1052230 did not show significant disease association ($P=0.2575$), and imputation on rs1052231 was neither significant ($P=0.2950$). These discrepancies from our results suggest an implicit genetic heterogeneity in the different populations while principally providing further evidence of the involvement of *CD247* in SLE susceptibility.

Interestingly, other studies on autoimmune diseases also reported their main findings in intron 1 of *CD247* (20-25), supporting a common mechanism behind the involvement of this gene in the etiology of these autoimmune disorders. A recent GWAS on systemic sclerosis (SSc), an autoimmune disease that shares some autoantibody and clinical features with SLE, identified *CD247* as a major susceptibility gene (rs2056626, located in intron 1, $P=3.39\times 10^{-9}$) (20). This association with SSc was replicated in two other cohorts (21,22). In our study, rs2056626 was not genotyped but was found to be significantly associated when imputed ($P=1.42\times 10^{-2}$). Furthermore, this SNP is in strong LD ($r^2=0.75$) with rs7523907 (SNP 14) (using HapMap data; release 23), which had $P=3.00\times 10^{-2}$ in our study. A meta-analysis of GWAS in celiac disease and rheumatoid arthritis identified several non-HLA shared loci, among which the SNP rs864537 in intron 1 of *CD247* ($P_{\text{combined}}=2.20\times 10^{-11}$) (23). In our study, rs864537 was not genotyped (or imputed) and it is not in LD with any of our SNPs (using HapMap data; release 23). Several GWAS also showed suggestive association of *CD247* with Crohn's disease (summarized in Wang *et al.*, (24)), with the

relevant SNPs being rs704853, rs12061855, rs1799704, rs2988276 and rs870875 ($P=1.80\times 10^{-3}<P<2.40\times 10^{-2}$). The SNP rs870875 was tested in our study but with no association, and rs2988276 had a borderline association using imputed data ($P=3.98\times 10^{-2}$). None of these SNPs is in high LD with any of our variants (using HapMap data; release 23). Recently, a novel association with *CD247* (rs1773560, in intron 1) was also identified for juvenile idiopathic arthritis ($P=2.57\times 10^{-7}$) (25). This SNP showed an imputed association in our study ($P=1.83\times 10^{-2}$) and is in strong LD ($r^2=0.71$) with rs7523907 (SNP 14, significant in our SLE study) and with rs2056626 ($r^2=0.94$) (found associated with SSc) (using HapMap data; release 23).

T cells are considered to be central to the pathogenesis of SLE because aberrations in their functionality are very likely strongly contributing to the altered immune responses and overproduction of pathogenic autoantibodies (26). *CD247* encodes the T-cell receptor zeta chain (CD3 ζ), a component of the T-cell receptor (TCR)-CD3 complex (27). TCR ζ is a pivotal component of the TCR signaling machinery and vital for T cell activation. A defective expression of the CD3 ζ -chain has been associated with autoimmune diseases including SLE (6, 7, 28) and rheumatoid arthritis (29,30), but also other conditions such as tumors and chronic infection (31). It is one established reason for various functional alterations in T cells in these conditions that TCR signaling through CD3 ζ is replaced by FcR γ (8) and its associated Syk pathway that enhances calcium and cytoskeletal reactions (32). This mechanism could be responsible for the shared association of several autoimmune diseases with *CD247*. Another effect that seems particularly relevant for SLE is that CD3 ζ signaling reportedly augments IL-2 production (7), indicating that its loss likely contributes to the defective IL-2 production that characterizes T cells in SLE (33). Potential mechanisms as per how autoimmunity-associated genetic variants exert their effects may include differences in expression, splicing and posttranslational processing, but their relevance is still not clear (34). Our findings confirm the relevance of these effects for SLE pathogenesis and highlight that the development of SLE is influenced by mechanisms shared with other autoimmune diseases, which involve a role of the TCR signaling pathway that should be further characterized. This is a part of several GWAS-identified risk loci shared between SLE and other autoimmune disorders pointing to common immunological mechanisms (35). In this study, we provide a replication establishing *CD247* as a genetic risk factor for SLE, which generates new implications for the pathogenesis of the disease and might lead to new therapeutic targets for disease management.

PATIENTS AND METHODS

Study design

The genotype data used in this study were generated as a part of a joint effort of more than 40 investigators from around the world. These investigators contributed samples, funding, and hypotheses on a combined array containing ~35,000 SNPs (Figure S1 from Lessard et al. (36)). The Oklahoma Medical Research Foundation (OMRF) served as the coordinating center, ran the arrays, and sent the data to a central facility for quality control at Wake Forest Medical Center. These data were then distributed back to the investigators, who requested the SNPs, for final analysis of their own respective hypotheses.

Patient and control samples

A total of 17 003 samples (8 922 SLE patients and 8 077 healthy controls; 4 with unknown disease status) from four main populations with Asian, Hispanic/Amerindian, European and African ancestry were initially enrolled in this multiethnic study. Details regarding the characteristics of the study participants in each dataset were previously described (37). The samples were assembled at the Oklahoma Medical Research Foundation (OMRF) after collection in multiple institutions around the world, following ethics committee approval and informed consent in accordance with the Declaration of Helsinki. Patients were classified with SLE based upon using the American College of Rheumatology criteria (38).

Genotyping

A total of 44 SNPs in the *CD247* region and 347 ancestral-informative markers (AIMs) were genotyped using the Illumina iSelect technology (Illumina, San Diego, CA, USA). Extensive quality control was performed following stringent criteria to select the SNPs to be used in the analysis, namely well-defined cluster scatter plots, >90% call rates across the entire study and in this specific set of SNPs, deviations from Hardy-Weinberg equilibrium with $P > 0.01$ in controls and $P > 0.0001$ in cases (using the PLINK (39) Hardy-Weinberg analysis), total proportion missing <5%, and $P > 0.05$ for differential missingness between cases and controls. Only SNPs with MAF > 5% in both case and controls groups were analysed for association in each population.

Samples with <90% call rate, excess heterozygosity, as well as first-degree relatives, duplicates and individuals with self-reported vs. genetically determined gender inconsistencies were excluded from the analysis as previously described (37).

EIGENSTRAT (40) was used to identify population substructure within the samples based on AIMs. The AIMs were selected to distinguish four continental ancestral populations: Africans, Europeans, American Indians, and East Asians (41,42). Principal components from EIGENSTRAT outputs were used to identify genetic outliers from each population cluster (as described in (37)). After quality control a total of 1 452 samples were excluded. The final meta-dataset used in the analysis consisted of 15 551 subjects (8 214 SLE cases and 7 337 controls): 2 488 Asian, 2 247 Hispanic/Amerindian, 7 248 European and 3 568 African. Characteristics of the study participants in each dataset are described in Table 3.

Two SNPs (rs1214603 and rs10918694) were excluded due to genotyping failure. Of the 42 SNPs with genotyping results, three further were excluded in all the four populations (rs2995087, rs1214604 and rs704855), nine more in the Asians, nine more in the Hispanic/Amerindians, eight more in the Europeans and six more in the African ancestry (African American/Gullah) samples (Table 1, Supplementary Table S2) due to quality control issues previously described (37). A final set of 39 SNPs were successfully genotyped in at least one population (SNP ID 1–39; listed in Table 1 and Supplementary Table S2): 30 in the Asian population; 30 in the Hispanic/Amerindians; 31 in the Europeans; and 33 in the Africans.

Statistical Analysis

Multiple logistic regression (PLINK (39); additive genetic model) was used to test for SLE association. Analysis was adjusted for the first three principal components calculated from AIMs, and gender. Conditional analyses based on the most strongly associated SNP (rs858554) (results expressed as conditional P [P_{cond}] values) were performed with logistic regression using PLINK (39), (additive genetic model; adjusted for the first three principal components and gender). Results were considered significant below the conventional level of $P < 0.05$. Correction for multiple testing was performed using the conservative Bonferroni method.

Haplotypic association was tested using PLINK (39) sliding window analysis. Linkage disequilibrium (LD) plots for each cohort were created using Haploview 4.2 (43). We also used the HapMap CHB (Han Chinese from Beijing, China, $n=84$) reference dataset (downloaded from the International HapMap Project website; HapMap3, release 2; chr1:165663570..165742500) to construct the LD plot of the reference Asian population and check linkage between the significant SNPs in our Asian cohort and those in *CD247* from the GWAS data that we had access to (19).

Meta-analysis of the 19 SNPs with association data for the four populations were calculated using Stouffer's Z_{trend} method implemented in METAP (44), weighted by sample size and taking into account effect directions.

Imputation Analysis

SNPs not directly genotyped in the *CD247* region for the Asian population, where we had the strongest associations, were imputed with PLINK (39) using HapMap Phase II and specific reference panels for the Asian population (Release 23; 161 230 SNPs on chromosome 1, 90 JPT+CHB founders). For every imputed SNP, PLINK provides an information content metric INFO, ranging from 0 to 1 (although it can be greater than 1 occasionally). A higher INFO value generally means a better SNP imputation. All imputed SNPs with MAF smaller than 0.05 and with $\text{INFO} < 0.8$ were excluded. For genotyped SNPs, PLINK calculates the concordance rate among observed and imputed genotypes (Figure 1).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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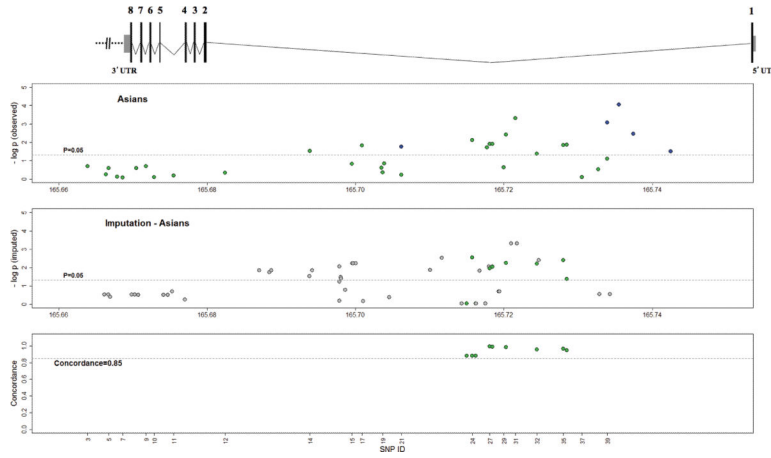


Figure 1. Results of association tests with SLE for observed and imputed single-nucleotide polymorphisms (SNPs) in the *CD247* gene. The Scaled diagram of the *CD247* gene structure is represented above the plots: exons are represented by black boxes and marked with its corresponding number; 5'UTR and 3'UTR are represented by grey boxes; introns are represented by black lines between exons. The top plot shows the negative logarithms of the *P*-values for genotypic association (under the additive model and adjusted for the first three principal components and gender) for the polymorphisms successfully genotyped by us in the Asian population (green dots), and the significant SNPs from GWAS data (19) (dark blue dots; personal communication from authors, May 2012). The second plot displays the negative logarithms of the *P*-values for 51 SNPs in chromosome 1 imputed with high quality (SNPs with a minor allele frequency, MAF 0.05, and SNP INFO 0.80, grey dots), including SNPs that were previously genotyped (green dots). The bottom graph displays the rate of concordance of observed and imputed genotypes. Broken horizontal lines in top and second plots indicate a significance level of $P=0.05$. In all plots, the SNPs that had been initially genotyped are represented with green dots.

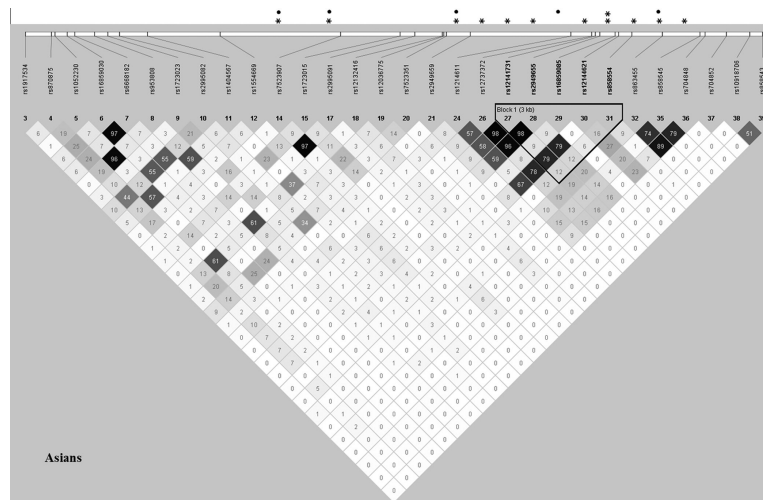


Figure 2. Linkage disequilibrium plot for the 30 genotyped single-nucleotide polymorphisms (SNPs) in *CD27* in the Asian population. This plot was obtained using the genotyping data from our study with Haploview 4.2 using the pairwise R-square color scheme in a grey scale. The position of the most significantly associated haplotype is indicated. *Significant P -value under the additive model and adjusted for the first three principal components and gender ($P_{\text{adj}} < 0.05$); **Significant P -value overpassing Bonferroni correction ($P_{\text{adj}} < 0.0017$); ●Significant P -value from the association analysis conditioned on the most significantly associated SNP, rs858554 ($P_{\text{cond}} < 0.05$).

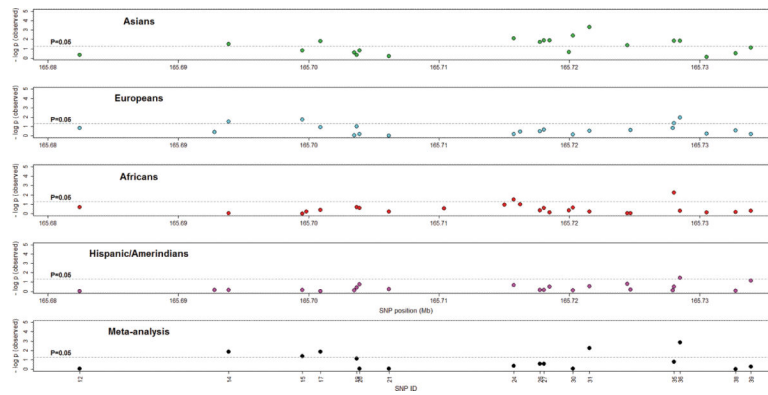


Figure 3.

Results of association tests with SLE and meta-analysis in the four cohorts in our study, specifically in intron 1 of the *CD247* gene. The plots show the negative logarithm of the *P*-value of genotypic association (under the additive model and adjusted for the first three principal components and gender) for the observed polymorphisms genotyped in the: Asians (first plot; 30 SNPs; green dots); Europeans (second plot; 31 SNPs; blue dots); Africans (third plot; 33 SNPs; red dots); and Hispanic/Amerindians (fourth plot; 30 SNPs; pink dots). The bottom plot shows the negative logarithm of the *P*-value for the meta-analysis (under the additive model and adjusted for the first three principal components and gender). Only SNPs with association results in the four study populations were tested (19 SNPs; black dots).

Table 1

Results of SNP association in *CD247* gene at 1q24.2 with SLE in the Asian population

SNP ID	SNP reference	Position (Mb)	Gene region	Alleles (minor/major)	MAF	Genotypes	SLE cases (n = 1265)	Controls (n = 1260)	<i>P</i> _{adj}	OR[95% CI]	<i>P</i> _{cond}	OR[95% CI]
3	rs1917534	165663896	Downstream	A/G	0.34	AA/AG/GG	161/549/527	142/539/553	0.2003	1.08[0.96-1.21]	0.4342	1.05[0.93-1.18]
4	rs870875	165666359	Downstream	C/A	0.45	CC/CA/AA	257/600/389	259/616/367	0.5805	0.97[0.87-1.08]	0.7392	0.98[0.88-1.10]
5	rs1052230	165666706	3' UTR	C/G	0.20	CC/CG/GG	63/399/783	45/401/796	0.2575	1.08[0.94-1.24]	0.1747	1.10[0.96-1.26]
6	rs16859030	165667879	Intron 7	A/G	0.24	AA/AG/GG	77/446/721	74/440/726	0.7608	1.02[0.90-1.16]	0.8690	1.01[0.89-1.15]
7	rs6668182	165668608	Intron 7	A/G	0.23	AA/AG/GG	73/421/716	73/407/713	0.8349	1.01[0.89-1.16]	0.9301	1.01[0.88-1.15]
8	rs953808	165670469	Intron 5	G/C	0.20	GG/GC/CC	60/398/786	41/401/797	0.2582	1.08[0.94-1.25]	0.1710	1.10[0.96-1.27]
9	rs1723023	165671757	Intron 4	A/G	0.11	AA/AG/GG	9/238/954	18/245/939	0.2060	0.89[0.74-1.07]	0.0861	0.85[0.71-1.02]
10	rs2995082	165672870	Intron 4	G/A	0.35	GG/GA/AA	144/560/518	157/539/526	0.8065	0.99[0.88-1.11]	0.5434	0.96[0.86-1.09]
11	rs1404567	165675499	Intron 2	G/A	0.29	GG/GA/AA	108/487/649	100/512/626	0.6690	0.97[0.86-1.10]	0.9901	1.00[0.88-1.13]
12	rs1554669	165682416	Intron 1	G/A	0.15	GG/GA/AA	29/312/905	28/297/917	0.4660	1.06[0.91-1.24]	0.8027	1.02[0.87-1.20]
14	rs7523907	165693872	Intron 1	G/A	0.08	GG/GA/AA	11/166/1068	13/203/1026	3.00E-02	0.80[0.66-0.98]	3.98E-02	0.81[0.66-0.99]
15	rs1723015	165699519	Intron 1	A/G	0.48	AA/AG/GG	310/580/322	279/599/341	0.1485	1.09[0.97-1.21]	0.0791	1.11[0.99-1.24]
17	rs2995091	165700902	Intron 1	G/A	0.08	GG/GA/AA	11/158/1077	13/200/1029	1.50E-02	0.78[0.64-0.95]	1.99E-02	0.79[0.64-0.96]
18	rs12132416	165703507	Intron 1	A/G	0.16	AA/AG/GG	38/346/861	24/342/876	0.2498	1.09[0.94-1.27]	0.1783	1.11[0.95-1.29]
19	rs12036775	165703672	Intron 1	A/G	0.29	AA/AG/GG	100/514/630	111/518/613	0.4529	0.95[0.84-1.08]	0.7899	0.98[0.87-1.11]
20	rs7523351	165703888	Intron 1	G/C	0.26	GG/GC/CC	90/498/658	86/455/700	0.1479	1.10[0.97-1.25]	0.3888	1.06[0.93-1.20]
21	rs2949659	165706163	Intron 1	G/A	0.32	GG/GA/AA	135/535/574	131/528/583	0.6102	1.03[0.92-1.16]	0.3874	1.05[0.94-1.19]
24	rs1214611	165715729	Intron 1	A/G	0.43	AA/AG/GG	258/592/395	199/610/433	7.81E-03	1.17[1.04-1.30]	1.25E-02	1.16[1.03-1.29]
26	rs12737372	165717740	Intron 1	A/G	0.45	AA/AG/GG	240/595/405	268/623/345	1.96E-02	0.88[0.78-0.98]	0.2695	0.93[0.83-1.05]
27	rs12141731	165718065	Intron 1	A/G	0.45	AA/AG/GG	244/596/405	273/629/340	1.25E-02	0.87[0.78-0.97]	0.2091	0.93[0.82-1.04]
28	rs2949655	165718475	Intron 1	A/G	0.46	AA/AG/GG	245/594/381	275/626/319	1.28E-02	0.87[0.77-0.97]	0.2082	0.92[0.82-1.05]
29	rs16859085	165719959	Intron 1	G/A	0.09	GG/GA/AA	5/193/1048	10/206/1025	0.2380	0.89[0.73-1.08]	3.22E-02	0.80[0.65-0.98]
30	rs12144621	165720283	Intron 1	G/C	0.50	GG/GC/CC	341/613/292	278/627/337	3.91E-03	1.18[1.05-1.32]	0.1279	1.10[0.97-1.25]
31	rs858554	165721539	Intron 1	A/G	0.13	AA/AG/GG	48/312/882	19/277/943	4.99E-04	1.32[1.13-1.55]	NA	NA
32	rs863455	165724449	Intron 1	G/A	0.36	GG/GA/AA	173/580/493	136/578/528	4.15E-02	1.13[1.01-1.27]	0.3327	1.06[0.94-1.20]
35	rs858545	165728016	Intron 1	A/C	0.31	AA/AC/CC	122/567/556	97/535/612	1.41E-02	1.17[1.03-1.32]	4.20E-02	1.14[1.01-1.29]
36	rs704848	165728498	Intron 1	G/C	0.35	GG/GC/CC	166/593/485	132/572/536	1.36E-02	1.16[1.03-1.31]	0.1191	1.10[0.98-1.25]

SNP ID	SNP reference	Position (Mb)	Gene region	Alleles (minor/major)	MAF	Genotypes	SLE cases (n = 1265)	Controls (n = 1260)	<i>P</i> _{adj}	OR[95% CI]	<i>P</i> _{cond}	OR[95% CI]	Genotype numbers	
													MAF	Genotypes
37	rs704852	165730541	Intron 1	C/A	0.12	CC/CA/AA	21/250/974	17/260/965	0.7842	0.98[0.82-1.16]	0.5492	0.95[0.80-1.13]		
38	rs10918706	165732746	Intron 1	A/G	0.32	AA/AG/GG	142/533/562	129/523/584	0.3030	1.06[0.95-1.20]	0.4216	1.05[0.93-1.18]		
39	rs858543	165733923	Intron 1	G/A	0.49	GG/GA/AA	314/620/310	295/590/356	0.0797	1.10[0.99-1.23]	0.0881	1.10[0.99-1.23]		

Abbreviations: Mb, Megabases; MAF, minor allele frequency (in controls); OR, odds ratio; 95% CI, 95% confidence interval. The presented genetic association *P*-values are under the additive model and adjusted for the first three principal components and gender (*P*_{adj}). The *P*-values from the association analysis conditioned on the most associated SNP, rs858554 (*P*_{cond}), are also indicated. Significant *P*-values (<0.05) are highlighted in bold.

Table 2

Its of meta-analysis testing in the *CD247* gene with SLE

ID	SNP reference	Position (Mb)	Gene region	Asian			European			African			Hispanic / Amerindian			
				Alleles ^a	P-value	OR[95% CI]	Alleles ^a	P-value	OR[95% CI]	Alleles ^a	P-value	OR[95% CI]	Alleles ^a	P-value	OR[95% CI]	
1	rs1052230	165666706	3' UTR	C/G	0.2575	1.08[0.94-1.24]	C/G	0.9290	1.00[0.90-1.10]	C/G	0.4898	1.05[0.92-1.20]	C/G	0.8993	1.01[0.83-1.23]	0.4442
2	rs1723023	165671757	Intron 4	A/G	0.2060	0.89[0.74-1.07]	A/G	0.5936	0.98[0.92-1.05]	A/G	0.0924	0.86[0.73-1.03]	A/G	0.7990	0.98[0.85-1.13]	0.0771
3	rs1404567	165675499	Intron 2	G/A	0.6690	0.97[0.86-1.10]	G/A	0.2269	0.94[0.85-1.04]	G/A	0.1472	1.14[0.95-1.37]	G/A	0.8441	0.98[0.80-1.20]	0.7039
4	rs1554669	165682416	Intron 1	G/A	0.4660	1.06[0.91-1.24]	G/A	0.1464	0.90[0.79-1.04]	G/A	0.1938	1.08[0.96-1.21]	G/A	0.9670	1.00[0.82-1.21]	0.9154
5	rs7523907	165693872	Intron 1	G/A	3.00E-02	0.80[0.66-0.98]	G/A	2.85E-02	0.93[0.86-0.99]	G/A	0.8992	1.01[0.91-1.11]	G/A	0.6858	0.97[0.85-1.11]	1.40E-02
6	rs1723015	165699519	Intron 1	A/G	0.1485	1.09[0.97-1.21]	G/A	1.83E-02	0.92[0.86-0.99]	G/A	0.9620	1.00[0.91-1.10]	G/A	0.6715	1.03[0.91-1.17]	3.97E-02
7	rs2995091	165700902	Intron 1	G/A	1.50E-02	0.78[0.64-0.95]	G/A	0.1160	0.95[0.88-1.01]	G/A	0.4225	0.93[0.79-1.10]	G/A	0.9499	1.00[0.86-1.15]	1.42E-02
8	rs12036775	165703672	Intron 1	A/G	0.4529	0.95[0.84-1.08]	A/G	0.0931	1.06[0.99-1.14]	A/G	0.1951	1.07[0.97-1.19]	A/G	0.3792	1.06[0.93-1.20]	0.0707
9	rs7523351	165703888	Intron 1	G/C	0.1479	1.10[0.97-1.25]	G/C	0.6238	1.03[0.92-1.15]	G/C	0.2454	0.93[0.83-1.05]	G/C	0.1771	0.87[0.70-1.07]	0.8799
10	rs2949659	165706163	Intron 1	G/A	0.6102	1.03[0.92-1.16]	G/A	0.9564	1.00[0.88-1.13]	G/A	0.6354	1.03[0.92-1.16]	G/A	0.5844	0.95[0.81-1.13]	0.8530
11	rs1214611	165715729	Intron 1	A/G	7.81E-03	1.17[1.04-1.30]	A/G	0.6487	0.98[0.92-1.05]	G/A	2.95E-02	1.11[1.01-1.23]	A/G	0.2017	0.92[0.81-1.05]	0.4358
12	rs12737372	165717740	Intron 1	A/G	1.96E-02	0.88[0.78-0.98]	A/G	0.3261	0.96[0.89-1.04]	A/G	0.4384	1.08[0.90-1.29]	A/G	0.6820	1.03[0.89-1.20]	0.2823
13	rs12141731	165718065	Intron 1	A/G	1.25E-02	0.87[0.78-0.97]	A/G	0.2225	0.96[0.89-1.03]	A/G	0.2380	1.10[0.94-1.29]	A/G	0.6833	1.03[0.90-1.18]	0.2664
14	rs12144621	165720283	Intron 1	G/C	3.91E-03	1.18[1.05-1.32]	C/G	0.6756	1.02[0.94-1.10]	C/G	0.2346	1.08[0.95-1.23]	C/G	0.7843	1.02[0.88-1.18]	0.8532
15	rs858554	165721539	Intron 1	A/G	4.99E-04	1.32[1.13-1.55]	A/G	0.2795	1.04[0.97-1.11]	A/G	0.6303	1.03[0.93-1.13]	G/A	0.2774	0.93[0.82-1.06]	5.80E-03
16	rs858545	165728016	Intron 1	A/C	1.41E-02	1.17[1.03-1.32]	A/C	4.51E-02	1.07[1.00-1.15]	C/A	5.92E-03	1.15[1.04-1.27]	A/C	0.3180	1.07[0.94-1.22]	0.1583
17	rs704848*	165728498	Intron 1	G/C	1.36E-02	1.16[1.03-1.31]	C/G	1.12E-02	0.92[0.85-0.98]	C/G	0.5043	1.04[0.93-1.16]	C/G	3.39E-02	0.87[0.76-0.99]	1.40E-03
18	rs10918706	165732746	Intron 1	A/G	0.3030	1.06[0.95-1.20]	A/G	0.2756	0.96[0.89-1.03]	A/G	0.6949	1.03[0.89-1.20]	A/G	0.8676	1.01[0.86-1.20]	0.9309
19	rs858543	165733923	Intron 1	G/A	0.0797	1.10[0.99-1.23]	A/G	0.6240	1.02[0.94-1.10]	A/G	0.5157	1.04[0.93-1.16]	A/G	0.0677	1.15[0.99-1.32]	0.5225

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Abbreviations: Mb, Megabases; *P*_{meta}, meta-analysis *P*-values; *P*_{meta,corr}, Bonferroni corrected meta-analysis *P*-values; OR, odds ratio; 95% CI, 95% confidence interval.

Meta-analysis *P*-values are under the additive model and adjusted for the first three principal components and gender. Only SNPs with association results in the four study populations were tested. Significant *P*-values (<0.05) are highlighted in bold.

*r/major allele.

rs848 marker is still significant after Bonferroni correction for multiple testing (*P*_{meta}=2.66×10⁻²).

Table 3

Demographic characteristics of the four populations (after quality control)

Population Ancestry	Samples after QC	Cases	Age of onset (mean \pm SD)	Controls	Male	Female
Asian	2 488	1 246	26.4 \pm 0.3	1 242	245	2 243
European	7 248	3 842	33.6 \pm 0.3	3 406	1 452	5 796
African	3 568	1 669	34.0 \pm 0.3	1 899	713	2 855
Hispanic / Amerindians	2 247	1 457	29.5 \pm 0.4	790	199	2 048
Total	15 551	8 214		7 337	2 609	12 942

Abbreviations: QC, quality control.

Populations: African ancestry includes 274 Gullah and 3 294 other African Americans; Hispanic/Amerindian ancestry includes 1 252 Hispanics and 995 Native Americans. Information for Age of onset was available for most of the cases in each population.

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