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Lupus 2012 21: 75 originally published online 17 October 2011
DOI: 10.1177/0961203311422724

Lupus (2012) 21, 75–83
http://lup.sagepub.com

PAPER

Association of CD247 with systemic lupus erythematosus in Asian populations

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Objective: Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease with complex genetic inheritance. CD247 (CD3Z, TCRZ) plays a vital role in antigen recognition and signal transduction in antigen-specific immune responses, and is known to be involved in SLE pathogenesis. Weak disease association was reported for genetic variants in this gene in Caucasian studies for SLE, Crohn's disease and systemic sclerosis, but its role as a genetic risk factor was never firmly established. **Methods:** In this study, using a collection of 612 SLE patients and 2193 controls of Chinese ethnicity living in Hong Kong in a genome-wide study, single nucleotide polymorphisms (SNPs) in and around CD247 were identified as being associated with SLE. The two most significant SNPs in this locus were selected for further replication using TaqMan genotyping assay in 3339 Asian patients from Hong Kong, Mainland China, and Thailand, as well as 4737 ethnically and geographically matched controls. **Results:** The association of CD247 with SLE in Asian populations was confirmed (rs704853: odds ratio [OR] = 0. 81, $p = 2.47 \times 10^{-7}$; rs858543: OR = 1.10, $p = 0.0048$). Patient-only analysis suggested that rs704853 is also linked to oral ulcers, hematologic disorders and anti-double-stranded DNA (dsDNA) antibody production. **Conclusion:** A significant association between variants in CD247 and SLE was demonstrated in Asian populations. Understanding the involvement of CD247 in SLE may shed new light on disease mechanisms and development of new treatment paradigms. *Lupus* (2012) **21,** 75–83.

Key words: CD247; GWAS; Asian; association; systemic lupus erythematosus

Introduction

Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by production of auto-antibodies directed against a host of nuclear components.[1] Genetic factors are known to play an important role in the pathogenesis of SLE,

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Received 4 June 2011; Accepted 11 August 2011

10.1177/0961203311422724

which is supported by a higher monozygotic twin concordance rate compared with dizygotic twins. The risk for siblings of those affected is also 30 times higher than that for the general population.[2–4] There are also population differences in terms of both disease prevalence and manifestations.[5] The disease is more common in African Americans, Hispanics, and Asians than in Caucasians, and Asians are also known to have more lupus nephritis than patients of European ancestry.[6–7]

Genome-wide association studies (GWAS) enable genome-wide search for variants associated with this disease without selecting candidates beforehand. In the last few years, more than a dozen genes have been established as conferring disease susceptibility to SLE in studies of different racial backgrounds.[8–13] Recently, several novel susceptibility genes have been uncovered for SLE, including *IL21R*,[14] *IRAK*1,[15] *ETS1*, *WDFY*4,[16] and a number of other genes.[17,18]

CD247 is an amplification module in the T-cell receptor (TCR) signaling cascade and is important for assembly and surface expression of the TCR/CD3 complex. Reduced expression level of CD247 has been found in various conditions, including chronic infection and inflammatory diseases, as well as neoplastic conditions. Various studies have shown that, of all the TCR subunits, only expression of CD247 is specifically downregulated, and these T cells have impaired TCR-mediated function (reviewed in Baniyash[19]). It was reported that the T cells of SLE patients have a reduced level of CD247, and FCER1G (Fc receptor common gamma chain) may replace the zeta chain in TCR signaling.[20,21] Recently, two studies reported weak association of genetic variants in the 3′UTR region of this gene with SLE disease risk,[22,23] but the role of this gene is far from soundly established, due to the small sample sizes examined in these studies.

In this study, we first genotyped 612 cases of SLE collected in Hong Kong by Illumina 610-Quad Beadchip and analyzed the data against 2193 control individuals genotyped on the same platform. Association of two SNPs with the disease was confirmed in four independent sample collections from Hong Kong, Mainland China (Anhui and Shanghai), and Thailand (Bangkok). Joint analysis of the entire dataset showed consistent association of these SNPs with SLE and suggested connection of the genetic variants with sub-phenotype manifestations.

Materials and methods

Subjects

The Hong Kong cohort included 1224 SLE patients recruited from four hospitals in Hong Kong: Queen Mary Hospital, Queen Elizabeth Hospital, Tuen Mun Hospital, and Pamela Youde Nethersole Eastern Hospital. The patients were all of self-reported Chinese ethnicity, living in Hong Kong, and they were studied in the GWAS stage and the subsequent replication in this study. The 1483 SLE patients collected in the Anhui province of China were attending the Department of Rheumatology at Anhui Provincial Hospital and the First Affiliated Hospital of Anhui Medical University. The Shanghai cohort contained 771 SLE patients recruited from Shanghai Renji Hospital. SLE patients collected in Thailand were all of self-reported Thai ethnicity and were recruited from King Chulalongkorn Memorial Hospital, a tertiary referral center in Bangkok. All patients were diagnosed according to the criteria of the American College of Rheumatology.[24]

The Hong Kong study was approved by the Institutional Review Board of the University of Hong Kong and Hospital Authority, Hong Kong West Cluster, New Territory West Cluster, and Hong Kong East Cluster. The study of Anhui, Shanghai, and Thai samples was approved by the Institutional Review Board of Research Ethics Committee of Anhui Medical University, Shanghai Jiao Tong University School of Medicine, and the Ethics Committee of the Faculty of Medicine, Chulalongkorn University, respectively. All patients provided informed consent for the collection of samples and the subsequent research.

For the GWAS stage, controls were from both healthy individuals and individuals from other studies conducted in the same institution, genotyped by the same platform. For the replication stage, Hong Kong controls were blood donors recruited from the Hong Kong Red Cross and were all of self-reported Chinese ethnicity, living in Hong Kong. Controls in the Anhui and Shanghai cohorts were healthy blood donors recruited from Hefei and Shanghai, respectively. Thai controls were healthy blood donors recruited from the same geographic areas as the Thai patients.

Genome-wide association study

Six hundred and twelve SLE patients and 2193 controls were genotyped by Illumina 610-Quad Human Beadchip. Initial quality control of the chip data

included exclusion of SNPs and individuals of low genotype call rate (<90%), low minor allele frequency (<1%), and violation of Hardy–Weinberg equilibrium in controls ($p < 0.0001$), as well as individuals of close relationship detected by PLINK,[25] as reported in detail before.[16] Population substructure was analyzed by principal component introduced in EIGEN-STRAT.[26]

Genotyping in the replication stage

Two of the CD247 SNPs were genotyped by TaqMan SNP assay using Assay-on-Demand probes and primers (Applied Biosystems, Foster City, CA, USA; catalogue nos. C_8918129_10 for rs858543, C_8918140_10 for rs704853). Genotyping accuracy was confirmed by direct sequencing of polymerase chain reaction (PCR) products for some randomly chosen samples, which showed 100% concordance. Genotyping concordance between Illumina Human 610-Quad Beadchip and TaqMan SNP genotyping methods was also checked on selected samples and the two methods showed complete concordance.

Statistical analysis

The SNPs were analyzed for association with the disease by χ^2 test using PLINK.[25] Linkage disequilibrium (LD) patterns were analyzed by Haploview.[27] Testing of the independent contribution of a SNP in disease association was performed by conditional logistic regression controlling for the effect of other SNPs or haplotype-based test. Sub-phenotype stratification analysis was performed by a case-only method, in which basic allelic association was performed by comparing minor allele frequency of patients with a specific sub-phenotype with those without the sub-phenotype.

Overall odds ratios (ORs) and p values jointly analyzed from the Hong Kong, Anhui, Shanghai, and Thai populations were obtained by Cochran–Mantel–Haenszel test of disease association conditional on SNP differences between populations. Potential heterogeneity for OR between populations was tested both by Breslow–Day test and by partitioning the χ^2 statistic among these populations.

Results

Genome-wide association study

Population substructure was analyzed for the samples in the GWAS stage by principal component method proposed by Price et al.[26] A good match between the cases and controls in the Hong Kong samples used in GWAS was observed, similar to that previously reported for a smaller set of GWAS on the same cohort.[16] A genome-wide inflation factor of 1.045 was observed, which is consistent with the result from principal component analysis. A combination of strategies was used to choose a certain locus for further replication, based on the initial p values, annotated function of the nearby gene, and previous reports of association with other autoimmune diseases. We examined GWAS data on the CD247 gene locus in a 400 kb span, including both upstream and downstream regions of the gene. One hundred and thirty-seven SNPs were interrogated in this region, from rs6677499 to rs13374598. Fourteen SNPs showed a suggestive association with nominal $p < 0.05$, and five of them with $p < 0.02$ are shown in Table 1 (see also Figure 1 for their positions relative to *CD247*). Association p values after correction for population substructure are also presented in Table 1, and it seems that population substructure is unlikely to be a factor causing inflation in association seen in this region. Independence test by conditional logistic regression showed that rs704853 is the major contributor to disease association in this locus and its p value changed significantly only when controlling for the effect of rs858543. Therefore, these two SNPs, rs704853 and rs858543, were chosen for further replication in a much larger collection of samples.

Replication experiment

Making use of the remaining samples from Hong Kong not included in the GWAS stage, and sample collections from Anhui and Shanghai, China, and Bangkok, Thailand, we went on to replicate these two SNPs using TaqMan assay. As shown in Table 2, both rs704853 and rs858543 showed significant association with the disease by joint analysis of data from all these cohorts. The same trend was observed for all the cohorts for the two SNPs, although they did not reach nominal significance in some cohorts, possibly due to both sample and effect sizes. Testing between-population OR heterogeneity by the Breslow–Day method on these populations, however, did not show evidence of significant difference ($p = 0.52$).

Independence test

For data from all the Hong Kong samples (both GWAS and replication), conditional logistic regression revealed that the SNP rs704853 was

Table 1 SNPs showing significant disease association in GWAS in and around *CD247*

SNP	*Position (1q24. 2. NCBI Build 36,UCSC hg18)*	*Allele*	*MAF–SLE*	*MAF–Control*	*OR (95% CI)*	p *values (allelic test)*	p *values after EIGEN-STRAT correction*	*conditional* p *values*[a]	*conditional* p *values*[b]
rs2949659	165706162	G < A	0.369	0.332	1.17 (1.03–1.34)	0.018	0.011	0.016	6.50×10^{-5}
rs858543	165733922	C < T	0.520	0.465	1.24 (1.09–1.41)	0.001	0.001	0.0917	0.006
rs704853	165735488	A < C	0.166	0.218	0.73 (0.62–0.86)	8.70×10^{-5}	6.94×10^{-5}	n/a	n/a
rs17534481	165737441	T < C	0.179	0.144	1.29 (1.09–1.52)	0.004	0.008	0.039	6.20×10^{-4}
rs3820393	165779182	G < T	0.336	0.305	1.15 (1.01–1.31)	0.020	0.017	0.062	1.22×10^{-4}

CI: confidence interval, GWAS: genome-wide association studies, MAF: minor allele frequency, OR: odds ratio, SLE: systemic lupus erythematosus, SNP: single nucleotide polymorphism.
[a]*p* values for each of the four SNPs when controlling for the effect of rs704853 in each of the four conditional logistic regression tests, respectively.
[b]*p* values of rs704853 when controlling for each of the other four SNPs in the same logistic regression tests, respectively.

significantly associated with the disease ($p = 0.0037$) when controlling for the effect of rs858543. Independent contribution from rs858543 is questionable, with a p value of 0.0936 when controlling for the effect of rs704853, although this could be caused by the usually low power in detecting independent variants in association studies. There is weak LD between the two SNPs ($r^2 = 0.23$ in Hong Kong samples, $r^2 = 0.15$ and 0.16 in Anhui and Thai samples, respectively). This is consistent with the result of logistic regression using data from GWAS. There is also no evidence showing interaction between these two SNPs by logistic regression test.

Haplotype analysis on the Hong Kong samples indicates that the CC haplotype formed by rs858543 and rs704853 is the major risk haplotype ($p = 8.11 \times 10^{-4}$ for the Hong Kong cohort), whilst the TA haplotype is the major protective haplotype ($p = 2.03 \times 10^{-5}$). For the Anhui samples, only marginal significance in disease association was observed for the TA haplotype formed by rs858543 and rs704853 ($p = 0.045$).

Sub-phenotype analysis

A case-only analysis was performed to determine potential genetic association with specific sub-phenotypes (for example, cases with arthritis versus cases without arthritis). It showed that rs704853 has suggested association with oral ulcers (OR = 0.78, $p = 0.048$), hematologic disorders (OR = 0.78, $p = 0.033$), and anti-dsDNA antibody production (OR = 0.76, $p = 0.028$), although the p values do not withstand multiple testing correction and need further confirmation from additional cohorts (Table 3).

Discussion

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. SNP rs704853 is located in this intron 1, at about 19 kb downstream of exon 1. Most other associated SNPs found in this study also locate in this region (see Figure 1), suggestive of roles in regulation of expression of this gene. Through imputation based on Phase II HapMap data on CHB (Chinese Han in Beijing), rs858536, a SNP about 300 bp upstream of rs704853 in intron 1, also showed significant association (OR = 0.74, $p = 0.000156$). In addition, two sets of Caucasian GWAS data on rs704853 (dbGaP accession

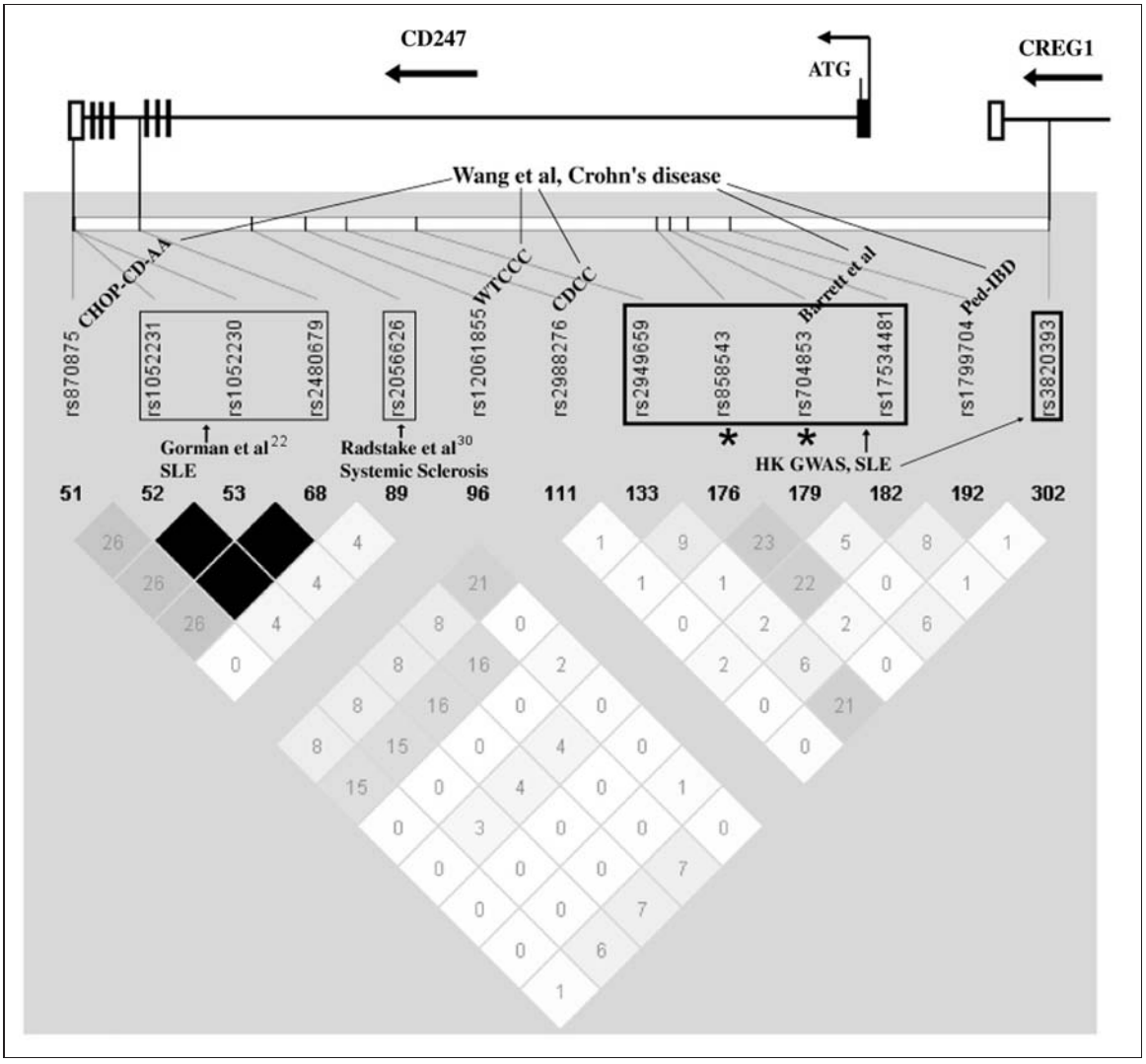

Figure 1 LD among SNPs detected in our GWAS and those reported in previous studies. Shown are LD values for CHB individuals in HapMap (r^2). CHOP-CD-AA, WTCCC. CDCC, Barrett et al., and Ped-IBD are various association studies on Crohn's disease summarized in Wang et al.[31] Also see Table 4 for our GWAS results on these SNPs or their high LD surrogates. Stars point to the two SNPs replicated in this study.
LD: linkage disequilibrium, GWAS: genome-wide association studies, SNP: single-nucleotide polymorphism.

number phs000122.v1.p1: 1311 cases and 3340 controls, $MAF_{case} = 0.096$, $MAF_{control} = 0.103$; $p = 0.076$, $OR = 0.93$; SLEGEN: 767 cases and 383 controls, $MAF_{case} = 0.092$, $MAF_{control} = 0.114$; $p = 0.113$, $OR = 0.79$) showed a similar trend, suggesting a cross-population association on this locus, although further work is needed to establish the role of this locus in other populations.

Although our data support a significant role for rs704853, rs858543 and a few other SNPs in this region may also contribute to disease association, as reflected by the marginally significant p values from these SNPs when controlling for the effect of rs704853 in the GWAS data (Table 1). Having multiple independent signals from a single locus is another layer of information supportive of true positive association, even if no individual SNP reaches genome-wide significance. This notion is significant as GWAS enters a stage of attempting to identify variants of smaller effect size. It is also realized that multiple contributing alleles from a single locus may be a common phenomenon. Examined by both a set-based association test installed in PLINK[25] and an in-house program designed to detect regions with multiple independent SNPs contributing to disease association,

Table 2 Replication of SLE association in additional cohorts

SNP	*Cohort (no. of controls/no. of cases)*	*Control MAF*	*SLE MAF*	*OR*	p *value*	*Joint analysis*	
						OR (95% CI)	p *value*
rs704853	HK_GWAS (2193/612)	0.217	0.166	0.72	8.70×10^{-5}	0.81 (0.75–0.88)	2.47×10^{-7}
	HK_TaqMan (1152/612)	0.204	0.184	0.88	0.183		
	HK_all (3345/1224)	0.213	0.175	0.78	8.56×10^{-5}		
	AH_TaqMan (1463/1483)	0.184	0.163	0.87	0.0455		
	Thai_TaqMan (970/473)	0.162	0.146	0.88	0.277		
	SH_TaqMan(1152/771)	0.187	0.148	0.76	3.56×10^{-3}		
rs858543	HK_GWAS (2193/612)	0.467	0.52	1.24	8.37×10^{-4}	1.10 (1.03–1.18)	0.0048
	HK_TaqMan (2193/612)	0.467	0.501	1.15	0.0233		
	HK_all (2193/1224)	0.467	0.508	1.18	9.80×10^{-4}		
	AH_TaqMan (1463/1483)	0.436	0.444	1.03	0.58		
	Thai_TaqMan (552/324)	0.505	0.52	1.06	0.556		

AH: Anhui populations in mainland China, CI: confidence interval, HK: Hong Kong Chinese, MAF: minor allele frequency, OR: odds ratio, SH: Shanghai populations in mainland China, SLE: systemic lupus erythematosus, SNP: single nucleotide polymorphism, Thai: Thai population in Bangkok, Thailand.

Table 3 Association of rs704853 with SLE analyzed by sub-phenotype stratification in Hong Kong samples

Sub-phenotype (number of patients)*		*MAF–SLE (+)*	*MAF–SLE (−)*	p	*OR*
ANA	(+) 1123 (−) 108	0.168	0.163	0.859	1.04
Renal disorder	(+) 433 (−) 798	0.165	0.170	0.762	0.964
Age at onset	(<27) 367 (≥27) 423	0.160	0.178	0.355	0.880
Malar rash	(+) 680 (−) 551	0.177	0.158	0.251	1.15
Discoid rash	(+) 132 (−) 1099	0.176	0.167	0.735	1.07
Photosensitivity	(+) 347 (−) 884	0.149	0.175	0.168	0.825
Oral ulcers	(+) 288 (−) 943	0.146	0.180	0.0476	0.779
Arthritis	(+) 616 (−) 615	0.153	0.181	0.0958	0.819
Serositis	(+) 118 (−) 1113	0.168	0.168	0.978	1.01
Neurologic disorder	(+) 88 (−) 1143	0.137	0.170	0.347	0.778
Hematologic disorder	(+) 696 (−) 535	0.152	0.187	0.0334	0.776
Immunologic disorder	(+) 778 (−) 453	0.163	0.174	0.522	0.925
Anti-dsDNA Ab	(+) 869 (−) 362	0.155	0.195	0.0276	0.759
Anti-Sm Ab	(+) 140 (−) 1091	0.168	0.168	0.979	1.01
Anti-Ro Ab	(+) 598 (−) 633	0.165	0.170	0.787	0.968
Anti-La Ab	(+) 159 (−) 1072	0.177	0.166	0.668	1.08
Anti-RNP Ab	(+) 282 (−) 949	0.174	0.166	0.684	1.06

*Number of patients: (+) patients positive for the manifestation and (−) patients negative for the manifestation.
MAF–SLE (+): minor allele frequency for patients positive for a certain sub-phenotype, MAF–SLE (−): minor allele frequency for patients negative for the sub-phenotype.
ANA: antinuclear antibodies, dsDNA: double-stranded DNA, MAF: minor allele frequency, OR: odds ratio, RNP: ribonucleoprotein, SLE: systemic lupus erythematosus.

Table 4 GWAS results on SNPs reported in other studies

Associated disease	*References*	*SNP*	*Position (NCBI Build 36, UCSC hg18)*	*Allele*	*MAF case*	*MAF control*	*OR (95% CI)*	p *(allelic test)*	*Conditional* p[a]	*Conditional* p[b]
Systemic sclerosis	Radstake et al.[30]	rs2056626	165687049	G < T	0.0891	0.1005	0.88 (0.70–1.09)	0.229	0.149	5.70×10^{-5}
SLE	Gorman et al.[22]	rs2480679[c]	165674141	A < G	0.228	0.221	1.04 (0.90–1.21)	0.577	0.422	7.00×10^{-5}
Crohn's disease	Barrett et al.[31]	rs704853[d]	165735488	A < C	0.168	0. 218	0.73 (0.62–0.86)	1.30×10^{-4}	N/A	N/A
	WTCCC[31]	rs12061855[e]			N/A	N/A	N/A	N/A	N/A	N/A
	Ped-IBD[31]	rs1799704	165742495	C < A	0.0556	0.0693	0.79 (0.60–1.03)	0.085	0.031	6.50×10^{-5}
	CDCC[31]	rs2988276	165698027	G < A	0.359	0.378	0.92 (0.81–1.05)	0.21	0.111	6.50×10^{-5}
	CHOP-CD-AA[31]	rs870875	165666358	C < A	0.444	0.464	0.92 (0.81–1.05)	0.274	0.187	7.30×10^{-5}

[a] p values for this SNP in conditional logistic regression test when controlling for the effect of rs704853; [b] p values for rs704853 when controlling for the effect of the analyzed SNP in the conditional logistic regression test.
[c] In high LD with rs1052231 and rs1052230 ($r^2 = 1$ in CHB, and $r^2 = 0.47$ and 0.60, respectively, in CEU in HapMap), the two SNPs reported in a previous study.[22]
[d] replicated in this study (see Table 2).
[e] monomorphic in CHB, HapMap.
CI: confidence interval, GWAS: genome-wide association study, MAF: minor allele frequency, OR: odds ratio, SNP: single-nucleotide polymorphism.

this gene showed highly significant association after permutation on the number of SNPs tested in this locus ($p = 0.021$ and 0.003, respectively).

In Hong Kong samples, the SNP rs704853 may be involved more closely in patients with oral ulcers (OR = 0.78, $p = 0.048$), hematologic disorders (OR = 0.78, $p = 0.033$), and anti-dsDNA Ab production (OR = 0. 76, $p = 0.028$), although these p values do not withstand multiple testing correction for the number of sub-phenotypes examined and replication from other cohorts is needed to confirm these findings. Using the same cohorts, we recently also uncovered *ELF1* (E74-like factor 1) as a susceptibility gene for SLE.[28] Interestingly, it has been shown that the encoded protein, Elf-1, an Ets family transcription factor, is known to regulate CD247 expression, and itself is known to be reduced in SLE patients.[29] Association of both genes with SLE in Asian populations suggests a potential connection in the underlying mechanisms.

Recently, GWAS on systemic sclerosis, an autoimmune disease with certain similarities to SLE, identified *CD247* as a major susceptibility gene[30] (rs2056626, OR = 0.86, $p = 3.39 \times 10^{-9}$). The same SNP was genotyped in our GWAS stage and showed an OR of 0.88, with a p value of 0.23 in allelic analysis (Table 4). Although it is possible that rs2056626 may also be associated with SLE (it has no LD with the SNPs shown in Table 1; see Figure 1), based on conditional logistic regression on our GWAS data, the effect from rs7043853 apparently is the major signal in SLE association. Interestingly, some other susceptibility loci for systemic sclerosis also overlapped with those for SLE, including the MHC locus, *IRF5*, and *STAT4*. For *IRF5* and *STAT4*, the associated SNPs as well as the risk alleles were the same for these two diseases, suggesting overlaps in the underlying mechanisms.

Due to its important function and known involvement in SLE, the expression of CD247 in SLE patients was studied recently and found to be significantly lower than that in healthy controls.[22] In the same study, two SNPs in high LD with each other, rs1052230 and rs1052231 in 3′UTR of the gene, were found to be associated with CD247 expression level 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.[22] We do not have coverage on these SNPs, described in the study of Gorman et al.[22] A SNP in absolute LD with rs1052231 and rs1052230 based on CHB data in HapMap was interrogated in our GWAS stage (rs2480679, Table 4), but did not show significant disease association ($p = 0.5033$, OR = 1.053).

Imputation result on rs1052231 and rs1052230 also showed an insignificant result (OR = 1.06, $p = 1.479$).

Several GWAS also showed suggested association of *CD247* with Crohn's disease, summarized by Wang et al.[31] (the relevant SNPs are depicted in Figure 1 and Table 4). These studies examined five different SNPs in the gene and each of them showed marginal p values for disease association (0.024 to 0.0018). It can be seen from Figure 1 that none of these SNPs is in high LD with any other. Of these SNPs, rs704853 was found to be associated with Crohn's disease with the same risk allele as found in this study. Although potential association with SLE for these SNPs found in the previous studies needs further investigation, it does raise a possibility that multiple alleles in this gene may affect its function and disease association.

CD247 is undoubtedly a very important gene in T cell function, and both our study and previous association studies on this gene, both for SLE and for other autoimmune diseases, raise interesting questions on the role of genetic variants in this gene in disease susceptibility. The SNPs in this study did not reach genome-wide significance, and it seems that there could be population differences among the different Asian populations. Population substructure can cause false positive results, but can also obscure true association signals. Many of the questions on this gene warrant further replication from other cohorts, both for SLE and for other autoimmune disorders.

Acknowledgements

We thank PK and AC for their contributions to Thai paediatric sample collections. The CEU data used for the meta-analyses described in this paper were obtained from dbGaP at http://www.ncbi.nlm.nih.gov/gap (dbGaP accession number phs000122.v1.p1) provided by Timothy W. Behrens, Peter K. Gregersen, Lindsey Criswell, and Susan Manzi. The Caucasian association result of SLEGEN data was kindly provided by the International Consortium on the Genetics of Systemic Lupus Erythematosus.

Funding

This study was partially supported by a generous donation from Shun Tak District Min Yuen Tong of Hong Kong (to YLL). WY is thankful for support from Research Grant Council of Hong Kong (GRF HKU781709M). DQY is thankful for grant support from the key program of National Natural Science Foundation of China (No. 30830089). NH and YA are thankful for support from the National Research Council of Thailand. Genotyping of samples serving as controls in this study was supported by Research Grants Council of Hong Kong GRF grants HKU 7623/08M, HKU 7747/07M and HKU775608M. JZ and YZ are supported by Edward the Sai Kim Hotung Paediatric Education and Research Fund and University Postgraduate Studentship.

Conflicts of interest

The authors confirm that there are no conflicts of interest.

References

1 Danchenko N, Satia JA, Anthony MS. Epidemiology of systemic lupus erythematosus: a comparison of worldwide disease burden. *Lupus* 2006; 15: 308–318.
2 Arnett FC, Shulman LE. Studies in familial systemic lupus erythematosus. *Medicine (Baltimore)* 1976; 55: 313–322.
3 Ramos-Niembro F, Alarcon-Segovia D. Familial aspects of mixed connective tissue disease (MCTD). I. Occurrence of systemic lupus erythematosus in another member in two families and aggregation of MCTD in another family. *J Rheumatol* 1978; 5: 433–440.
4 Sestak AL, Shaver TS, Moser KL, Neas BR, Harley JB. Familial aggregation of lupus and autoimmunity in an unusual multiplex pedigree. *J Rheumatol* 1999; 26: 1495–1499.
5 Pons-Estel GJ, Alarcon GS, Scofield L, Reinlib L, Cooper GS. Understanding the epidemiology and progression of systemic lupus erythematosus. *Semin Arthritis Rheum* 2010; 39: 257–268.
6 Mok CC, Lau CS. Lupus in Hong Kong Chinese. *Lupus* 2003; 12: 717–722.
7 Wong SN, Tse KC, Lee TL, *et al*. Lupus nephritis in Chinese children–a territory-wide cohort study in Hong Kong. *Pediatr Nephrol* 2006; 21: 1104–1112.
8 Hom G, Graham RR, Modrek B, *et al*. Association of systemic lupus erythematosus with C8orf13-BLK and ITGAM-ITGAX. *N Engl J Med* 2008; 358: 900–909.
9 Remmers EF, Plenge RM, Lee AT, *et al*. STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus. *N Engl J Med* 2007; 357: 977–986.
10 Graham RR, Cotsapas C, Davies L, *et al*. Genetic variants near TNFAIP3 on 6q23 are associated with systemic lupus erythematosus. *Nat Genet* 2008; 40: 1059–1061.
11 Harley JB, Alarcon-Riquelme ME, Criswell LA, *et al*. Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in ITGAM, PXK, KIAA1542 and other loci. *Nat Genet* 2008; 40: 204–210.
12 Yang W, Ng P, Zhao M, *et al*. Population differences in SLE susceptibility genes: STAT4 and BLK, but not PXK, are associated with systemic lupus erythematosus in Hong Kong Chinese. *Genes Immun* 2009; 10: 219–226.
13 Yang W, Zhao M, Hirankarn N, *et al*. ITGAM is associated with disease susceptibility and renal nephritis of systemic lupus erythematosus in Hong Kong Chinese and Thai. *Hum Mol Genet* 2009; 18: 2063–2070.

14 Webb R, Merrill JT, Kelly JA, *et al*. A polymorphism within IL21R confers risk for systemic lupus erythematosus. *Arthritis Rheum* 2009; 60: 2402–2407.
15 Jacob CO, Zhu J, Armstrong DL, *et al*. Identification of IRAK1 as a risk gene with critical role in the pathogenesis of systemic lupus erythematosus. *Proc Natl Acad Sci U S A* 2009; 106: 6256–6261.
16 Wu ZM, Zhang SZ, Liu XJ, Lan L, Yang WY, Han DY. [Analysis of clinical features of concomitant vertigo in idiopathic sudden deafness]. *Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi* 2010; 45: 916–918.
17 Gateva V, Sandling JK, Hom G, *et al*. A large-scale replication study identifies TNIP1, PRDM1, JAZF1, UHRF1BP1 and IL10 as risk loci for systemic lupus erythematosus. *Nat Genet* 2009; 41: 1228–1233.
18 O'Malley S, Su H, Zhang T, Ng C, Ge H, Tang CK. TOB suppresses breast cancer tumorigenesis. *Int J Cancer* 2009; 125: 1805–1813.
19 Baniyash M. TCR zeta-chain downregulation: curtailing an excessive inflammatory immune response. *Nat Rev Immunol* 2004; 4: 675–687.
20 Liossis SN, Ding XZ, Dennis GJ, Tsokos GC. Altered pattern of TCR/CD3-mediated protein-tyrosyl phosphorylation in T cells from patients with systemic lupus erythematosus. Deficient expression of the T cell receptor zeta chain. *J Clin Invest* 1998; 101: 1448–1457.
21 Tsokos GC, Wong HK, Enyedy EJ, Nambiar MP. Immune cell signaling in lupus. *Curr Opin Rheumatol* 2000; 12: 355–363.
22 Gorman CL, Russell AI, Zhang Z, Cunninghame Graham D, Cope AP, Vyse TJ. Polymorphisms in the CD3Z gene influence TCRzeta expression in systemic lupus erythematosus patients and healthy controls. *J Immunol* 2008; 180: 1060–1070.
23 Warchol T, Piotrowski P, Lianeri M, *et al*. The CD3Z 844 T>A polymorphism within the 3'-UTR of CD3Z confers increased risk of incidence of systemic lupus erythematosus. *Tissue Antigens* 2009; 74: 68–72.
24 Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997; 40: 1725.
25 Purcell S, Neale B, Todd-Brown K, *et al*. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007; 81: 559–575.
26 Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* 2006; 38: 904–909.
27 Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; 21: 263–265.
28 Yang J, Yang W, Hirankarn N, *et al*. ELF1 is associated with systemic lupus erythematosus in Asian populations. *Hum Mol Genet* 2011; 20: 601–607.
29 Tsokos GC, Nambiar MP, Juang YT. Activation of the Ets transcription factor Elf-1 requires phosphorylation and glycosylation: defective expression of activated Elf-1 is involved in the decreased TCR zeta chain gene expression in patients with systemic lupus erythematosus. *Ann N Y Acad Sci* 2003; 987: 240–245.
30 Radstake TR, Gorlova O, Rueda B, *et al*. Genome-wide association study of systemic sclerosis identifies CD247 as a new susceptibility locus. *Nat Genet* 2010; 42: 426–429.
31 Wang K, Zhang H, Kugathasan S, *et al*. Diverse genome-wide association studies associate the IL12/IL23 pathway with Crohn Disease. *Am J Hum Genet* 2009; 84: 399–405.