

## EXTENDED REPORT

# Variation in the *ICAM1–ICAM4–ICAM5* locus is associated with systemic lupus erythematosus susceptibility in multiple ancestries

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## ABSTRACT

**Objective** Systemic lupus erythematosus (SLE; OMIM 152700) is a chronic autoimmune disease for which the aetiology includes genetic and environmental factors. *ITGAM*, integrin  $\alpha_M$  (complement component 3 receptor 3 subunit) encoding a ligand for intracellular adhesion molecule (ICAM) proteins, is an established SLE susceptibility locus. This study aimed to evaluate the independent and joint effects of genetic variations in the genes that encode *ITGAM* and *ICAM*.

**Methods** The authors examined several markers in the *ICAM1–ICAM4–ICAM5* locus on chromosome 19p13 and the single *ITGAM* polymorphism (rs1143679) using a large-scale case-control study of 17 481 unrelated participants from four ancestry populations. The single-marker association and gene-gene interaction were analysed for each ancestry, and a meta-analysis across the four ancestries was performed.

**Results** The A-allele of *ICAM1–ICAM4–ICAM5* rs3093030, associated with elevated plasma levels of soluble ICAM1, and the A-allele of *ITGAM* rs1143679 showed the strongest association with increased SLE susceptibility in each of the ancestry populations and the trans-ancestry meta-analysis (OR<sub>meta</sub> = 1.16, 95% CI 1.11 to 1.22;  $p = 4.88 \times 10^{-10}$  and OR<sub>meta</sub> = 1.67, 95% CI 1.55 to 1.79;  $p = 3.32 \times 10^{-46}$ , respectively). The effect of the *ICAM* single-nucleotide polymorphisms (SNPs) was independent of the effect of the *ITGAM* SNP rs1143679, and carriers of both *ICAM* rs3093030-AA and *ITGAM* rs1143679-AA had an OR of 4.08 compared with those with no risk allele in either SNP (95% CI 2.09 to 7.98;  $p = 3.91 \times 10^{-5}$ ).

**Conclusion** These findings are the first to suggest that an ICAM integrin-mediated pathway contributes to susceptibility to SLE.

Systemic lupus erythematosus (SLE; OMIM 152700) is a chronic autoimmune disease for which the aetiology includes diverse genetic and environmental factors. The formation and deposition of immune complexes, which consist of nuclear autoantigens and autoantibodies, in vital organs result in chronic inflammation, organ failure and severe morbidity and mortality in patients with SLE.

Previous genome-wide and candidate-gene association studies reported an association of SLE with a non-synonymous variant in *ITGAM*, encoding integrin  $\alpha_M$  (complement component 3 receptor 3 subunit), among European and African descents.<sup>1</sup> *ITGAM* is a ligand for intercellular adhesion molecule (ICAM) proteins, suggesting the involvement of an integrin-ICAM-mediated adhesion pathway for SLE susceptibility.

In this candidate gene-association study, we examined the independent and combined effects of common variation in *ITGAM* and the *ICAM1–ICAM4–ICAM5* locus, encoding other components of the integrin-adhesion pathway, with SLE susceptibility using a large multi-ancestry population.

## METHODS

### Study population

As part of the first phase of this study, we included a total of 14 719 patients with SLE and controls from diverse ancestry backgrounds, recruited from multiple institutions worldwide as part of the Lupus Association Study (LLAS)-2, with approval from the appropriate institutional review boards (online supplementary table S1). The affected had a minimum of four of eleven 1997 American College of Rheumatology revised criteria for the classification of SLE.<sup>3</sup> All participants in this study were filtered

by principal component analysis to identify population outliers as previously reported.<sup>4</sup> The sample exceeding five SDs along any statistically significant principal component were defined as outliers and removed from the study.

For the second phase to demonstrate consistency of results for rs3093030 in this larger Korean population, we constituted an independent cohort consisting of 2762 SLE cases and controls of Korean ancestry. Hospital-based SLE cases and controls were recruited from six hospitals in Korea with approval from the respective institutional review boards (online supplementary table S1).

### Genotyping

We genotyped the 12 *ICAM* variants in phase I (rs5030340, rs5030390, rs5030391, rs5030351, rs5491, rs1799969, rs5498, rs5030400, rs3093032, rs281437, rs3093030, rs2228615), which tagged most of the common single-nucleotide polymorphisms (SNPs) in the *ICAM1-ICAM4-ICAM5* locus, in addition to an *ITGAM* variant (rs1143679), using customised arrays based on the Illumina iSelect platform at the Lupus Genetics Studies Unit of the Oklahoma Medical Research Foundation. For the SLE-associated SNP in the *ICAM1-ICAM4-ICAM5* locus with the strongest effect (rs3093030), we genotyped this marker for the phase II Korean cohort using the Sequenom iPlex platform at the Korea Advanced Institute of Science and Technology.

### SNP association with SLE susceptibility

The statistical assessment for SNP association with SLE susceptibility was performed by the  $\chi^2$  test with allelic genetic model for each ancestry and meta-analysis across the four ancestries using the PLINK 1.07 (<http://pngu.mgh.harvard.edu/~purcell/plink/>).<sup>5</sup> In the meta-analysis, homogeneity of effect sizes was evaluated using the  $I^2$  heterogeneity index and Cochran's Q statistic.<sup>6</sup> For  $p > 0.01$  in heterogeneity Q statistic, fixed-effects models were implemented; otherwise random-effects models were used. For meta-analysis, GWAMA 1.4 software (<http://www.well.ox.ac.uk/gwama/>) was also used to calculate OR and 95% CI.<sup>7</sup> Hardy-Weinberg equilibrium (HWE) was examined by comparison between genotype distributions expected and observed in controls using the  $\chi^2$  test. Pair-wise  $D'$  and  $r^2$  values between SNPs were calculated by the Haploview 4.2 program. Statistical power was estimated using the CaTS-Power calculator (<http://www.sph.umich.edu/csg/abecasis/CaTS/index.html>).

### Imputation

Using the genotype data for the 12 *ICAM* SNPs, 58 HapMap SNPs located in a chromosome 19 region from position 10 200 kb to 10 320 kb (build 36.3) were imputed using the MACH V.1.0 program (<http://www.sph.umich.edu/csg/abecasis/MACH/index.html>) with the ancestry-matched template data provided by the International HapMap Project (<http://www.hapmap.org>) Phase 3 (Genome Browser release 2). Europeans were imputed using the CEU (Utah residents with Northern and Western European ancestry) data, Africans using the ASW (African ancestry in Southwest USA) data, Hispanics using the MEX (Mexican ancestry in Los Angeles, California) data, and Koreans using the CHB (Han Chinese in Beijing, China) data. Among the 58 imputed SNPs, 69.0% in Europeans, 77.6% in Africans, 58.6% in Hispanics and 63.8% in Koreans showed high ( $\geq 0.70$ ) average posterior probability for the most likely genotype, although some SNPs were not confidently imputed because of their weak correlations with the 12 genotyped SNPs.

### Gene-gene interaction

Epistatic interactions between the seven SLE-associated SNPs in the *ICAM1-ICAM4-ICAM5* locus and the non-synonymous SNP rs1143679 in *ITGAM* were examined using multiplicative logistic regression with the affected status as a dependent factor, two SNPs as main factors and the two-SNP interaction term. The analysis was performed using the PLINK '--epistasis' option.<sup>5,8</sup> In addition, case-only analysis using the PLINK '--fast-epistasis' and '--case-only' options was used to examine the dependency or the correlation between the tested SNPs in the groups of SLE cases.<sup>5,8</sup> To access combined effects of the risk alleles in rs3093030 and rs1143679, fixed-effects OR and 95% CI were estimated in stratification by the number of risk alleles (0 to 4) as compared with the group having no risk allele, separately among the European, African and Hispanic populations using the Case-control And Tdt Meta-Analysis Package (<http://cran.r-project.org/web/packages/catmap/index.html>). Two-copy carriers homozygous for *ICAM* or *ITGAM* risk allele were removed from the two-risk-allele carrier group in order to see the combined effect.

### SNP association with gene expression

Using the Genevar 2.0.1 database, which includes SNP genotype and mRNA profiling data for fibroblasts, lymphoblastoid cell lines, and T cells from the 75 GenCord individuals,<sup>9,10</sup> the 19 SNPs located in a chromosome 19 region from position 10 200 kb to 10 320 kb were assessed for their associations with mRNA levels of *ICAM1*, *ICAM4* and *ICAM5* using a  $t$  statistic for Spearman's rank correlation coefficient ( $\rho$ ). To minimise the type I error caused by multiple testing, the significance level of the  $p$  value was set to  $\alpha = 0.05/(19 \text{ SNPs})/(5 \text{ probes})/(3 \text{ cell lines}) = 1.75 \times 10^{-4}$ .

## RESULTS

### *ICAM-SLE* association study

In phase I, we evaluated 12 common variants directly typed in the *ICAM1-ICAM4-ICAM5* locus spanning ~22 kb in a total of 14 719 unrelated SLE cases and controls of European ( $n=7427$ ; SLE/controls=3936/3491), African ( $n=3613$ ; 1679/1934), Hispanic ( $n=2299$ ; 1492/807) and Korean ( $n=1380$ ; 640/740) ancestries (online supplementary table S1). Proportions of genetic ancestry for the study subjects were filtered using principal component analysis as described in a previous study (online supplementary figure S1).<sup>4</sup> All variants were typed with call rates of over 97.9%, and genotype proportions were consistent with expectations of the HWE ( $p \geq 0.001$ ) among controls from each of the four ancestries (online supplementary table S2).

Of the 12 *ICAM* SNPs typed, five were significantly associated with SLE susceptibility under a statistical threshold of  $p \leq 4.17 \times 10^{-3}$  in the trans-ancestry meta-analysis after Bonferroni correction for multiple comparisons (rs5030390, rs5498, rs281437, rs3093030 and rs2228615;  $1.05 \times 10^{-8} \leq p_{\text{meta}} \leq 2.80 \times 10^{-4}$ ) (online supplementary table S2). Moreover, the directions of the effect sizes of each SNP were same among the ancestries except for rs5030390, the association of which was considered unreliable (online supplementary table S2).

The strongest association with SLE was found with rs3093030 located in the 0.4 kb intergenic region between *ICAM1* and *ICAM4* ( $p=2.49 \times 10^{-8}$ ; OR=1.16, 95% CI 1.10 to 1.22). The effect size was similar in each of the four ancestries (online supplementary table S2), with OR=1.12 (95% CI 1.05 to 1.20) in European, OR=1.18 (95% CI 1.01 to 1.37) in African, OR=1.28 (95% CI 1.13 to 1.45) in Hispanic and OR=1.15 (95% CI 0.98

**Table 1** Association of directly typed and imputed markers in the *ICAM* locus and *ITGAM* with susceptibility to systemic lupus erythematosus

Gene	SNP*	European (n=7427)		African (n=3613)		Hispanic (n=2299)		Korean (n=1380 or 4142) <sup>†</sup>		Meta-analysis (n≤17 481) <sup>‡</sup>	
		MAF (SLE/controls)	OR	MAF (SLE/controls)	OR	MAF (SLE/controls)	OR	MAF (SLE/controls)	OR	OR	p Value
<i>ICAM1-ICAM4-ICAM5</i>											
	rs3093030 (G>A)	(0.471/0.442)	1.12	(0.107/0.092)	1.18	(0.580/0.518)	1.28	(0.353/0.318)	1.17	1.16	4.88×10 <sup>-10</sup>
	rs2569693 (C>T)	(0.427/0.403)	1.11	(0.097/0.085)	1.16 <sup>§</sup>	(0.550/0.492)	1.26	(0.363/0.332)	1.14 <sup>§</sup>	1.14	4.50×10 <sup>-7</sup>
	rs5498 (A>G)	(0.470/0.445)	1.11	(0.188/0.173)	1.11 <sup>§</sup>	(0.582/0.524)	1.26	(0.414/0.384)	1.13 <sup>§</sup>	1.13	6.08×10 <sup>-7</sup>
	rs2228615 (G>A)	(0.427/0.403)	1.11	(0.098/0.087)	1.14 <sup>§</sup>	(0.552/0.496)	1.25	(0.346/0.319)	1.13 <sup>§</sup>	1.14	1.05×10 <sup>-6</sup>
	rs2569702 (T>C)	(0.431/0.408)	1.10	(0.097/0.085)	1.16 <sup>§</sup>	(0.552/0.494)	1.26	(0.345/0.318)	1.13 <sup>§</sup>	1.14	1.27×10 <sup>-6</sup>
	rs892188 (C>T)	(0.431/0.408)	1.10	(0.097/0.085)	1.16 <sup>§</sup>	(0.552/0.494)	1.26	(0.345/0.318)	1.13 <sup>§</sup>	1.14	1.27×10 <sup>-6</sup>
	rs281437 (G>A)	(0.276/0.288)	0.94 <sup>§</sup>	(0.339/0.379)	0.84 <sup>§</sup>	(0.175/0.220)	0.75	(0.088/0.092)	0.95 <sup>§</sup>	0.89	8.28×10 <sup>-6</sup>
<i>ITGAM</i>											
	rs1143679 (G>A)	(0.194/0.126)	1.67	(0.157/0.104)	1.82	(0.168/0.100)	1.59	(0.002/0.000)	NC	1.67	3.32×10 <sup>-46</sup>

\*Four SNPs (rs3093030, rs5498, rs2228615 and rs281437) were genotyped and the other three SNPs (rs2569693, rs2569702 and rs892188) were imputed. The major allele is more common than the minor allele in the whole control population but not necessarily in each subpopulation.

<sup>†</sup>A total of 4142 Korean participants from phase I and phase II were genotyped for rs3093030, and the separate analyses are shown in online supplementary table S2.

<sup>‡</sup>Fixed-effects p value and OR were calculated from the meta-analysis because the effect size of each SNP was not significantly different among the ancestry populations (p>0.01 in Cochran's Q statistic). For *ITGAM* rs1143679, the meta-analysis was performed with European, African and Hispanic results.

<sup>§</sup>Allelic associations with SLE were not statistically significant at a threshold of  $\alpha=0.05$ . The detailed information is shown in online supplementary table S2.

MAF, minor allele frequency; NC, not calculated; SLE, systemic lupus erythematosus; SNP, single-nucleotide polymorphism.

to 1.35) in Korean ancestries (p=0.307 in Q statistic,  $I^2=17.0$ ). In a conditional logistic regression analysis followed by a meta-analysis, we found that the SLE association of rs5498, rs281437 and rs2228615 accounted for that of rs3093030, and they presented a consensus signal of SLE susceptibility locus, possibly because they are in high linkage disequilibrium with rs3093030 ( $D' \geq 0.84$ ; online supplementary table S3).

Although the effect magnitude of rs3093030 among Koreans was similar to those of the other ancestry populations, it did not achieve statistical significance, in contrast with the others (p=0.0841 in Koreans versus p=5.95×10<sup>-4</sup> in Europeans, p=4.02×10<sup>-2</sup> in Africans and p=7.28×10<sup>-5</sup> in Hispanics), perhaps because of the small sample size of the Korean population relative to the other ancestry populations. With the minor allele frequency of 0.324 in the Korean controls, the statistical power to detect the effect size found in the meta-analysis (fixed-effects OR=1.16) was only 42% in the Korean population.

To increase the statistical power for Korean detection, in phase II we recruited an additional independent population of 2762 unrelated Korean participants (online supplementary table S1). Genotyping call rate was 96.9%, and the control subject genotypes were distributed under HWE (p=0.691). Indeed, a similar effect size was detected in the validation population (OR=1.18), and by combining the phase I and phase II populations of Koreans (n=4142), statistical significance (p=1.07×10<sup>-3</sup>; OR=1.17, 95% CI 1.07 to 1.29) was achieved (online supplementary table S2). Therefore, with the additional Korean validation population, each of the four ancestry populations independently showed a statistically significant association between the *ICAM* locus SNP rs3093030 and SLE susceptibility, and the meta-analysis across the four ancestral populations revealed a more significant overall relationship (p=4.88×10<sup>-10</sup>; OR=1.16, 95% CI 1.11 to 1.22; online supplementary table S1).

To further characterise this genomic region as an SLE susceptibility locus, we imputed HapMap SNPs around the *ICAM* gene cluster that included *EDG5*, *MRPL4*, *ICAM1*, *ICAM4*, *ICAM5*, *RAVER1* and *ICAM3*. Using the genotype data for the 12 *ICAM* SNPs, we imputed 58 HapMap SNPs located in a chromosome 19 region from position 10 200 kb to 10 320 kb (Genome build 36.3) using the MACHV.1.0 program, with the ancestry-matched haplotype templates provided by the HapMap3 Genome Browser release 2 of the International HapMap Project.

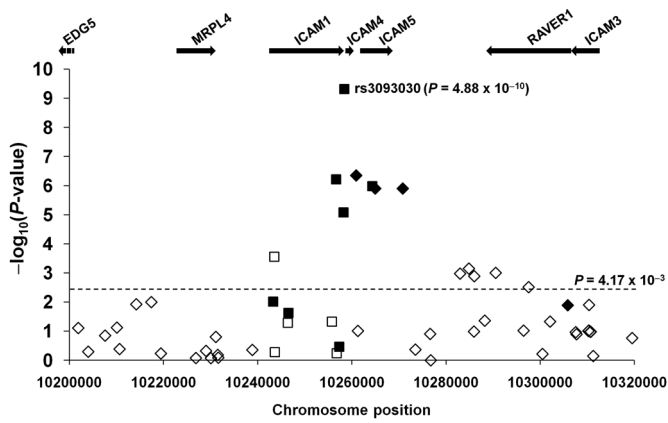
Three imputed SNPs (rs2569693, rs2569702 and rs892188) with high imputation quality (average posterior probability for the most likely genotype  $\geq 0.97$ ) were significantly associated with SLE susceptibility (table 1) while demonstrating the same direction of SLE-risk effects in each of the SNPs. In addition, all SLE-associated SNPs showed larger effects in Hispanics than in the other ethnic groups. These imputed SLE-associated SNPs were localised to the *ICAM1-ICAM4-ICAM5* region and correlated highly with the genotyped SLE-associated SNP rs3093030 ( $0.81 \leq r^2 \leq 0.96$ ). Trans-ancestry comparisons for the seven SLE-associated SNPs (ie, four genotyped and three imputed) supported that the *ICAM1-ICAM4-ICAM5* locus alone associated sufficiently with SLE susceptibility (p $\leq 8.28 \times 10^{-6}$ ; figure 1).

#### Pathway-based *ICAM* and *ITGAM* interaction study

Because *ICAM1* and *ICAM4* are binding partners of an  $\alpha_M\beta_2$  integrin, in which the  $\alpha$  subunit is encoded by an SLE-susceptibility gene, *ITGAM*, we examined the statistical interaction between SLE-risk alleles of the *ICAM1-ICAM4-ICAM5* region and the previously reported SLE-risk alleles of the *ITGAM* SNP rs1143679 to characterise the pathway-based effect on SLE susceptibility.<sup>2</sup> For this analysis, all participants of the phase I population were also genotyped for the non-synonymous SNP rs1143679 located in exon 3 of *ITGAM*. Call rates were  $\geq 99.6\%$  within each ancestry population, and genotype distributions were consistent with expectations of HWE in each of the control groups (online supplementary table S2).

The overall fixed effect of the SLE-risk-associated A allele in the *ITGAM* SNP was OR=1.67 (95% CI 1.55 to 1.79, p=3.32×10<sup>-46</sup>), showing similar effect sizes among European, African and Hispanic ancestries (p=0.541 in Q-statistic,  $I^2=0.00\%$ ; online supplementary table S2), although the risk-associated allele was too rare (0.07%) to evaluate the interaction among Koreans. The infrequency in Koreans was consistent with the previous report.<sup>11</sup> Statistical interaction between *ITGAM* rs1143679-A and each of the seven SLE-associated SNPs in the *ICAM1-ICAM4-ICAM5* locus (p $\leq 4.17 \times 10^{-3}$ ; table 1) were examined among cases and controls using the PLINK '--epistasis' analysis and among only the cases using the PLINK '--fast-epistasis' analysis.

The *ITGAM* and *ICAM* SNPs independently affected SLE susceptibility, showing no notable *ICAM-ITGAM* epistatic interactions (p>0.05; online supplementary table S4). Using five



**Figure 1** A systemic lupus erythematosus association map for the genotyped (squares) and imputed single-nucleotide polymorphisms (SNPs) (diamonds) within and around the *ICAM1-ICAM4-ICAM5* locus. Negative logarithms (base 10) of the p values calculated from the meta-analysis (y axis) are plotted against SNP positions on human chromosome 19 (x axis). The direction of effect size was the same in all four ancestries for some SNPs (filled symbols) but not for others (empty symbols). The arrows indicate transcription directions and gene sizes.

subgroups according to the number of risk-associated alleles (0 to 4) in the *ICAM* SNP, rs3093030, and the *ITGAM* SNP, rs1143679, we assessed the combined effects of the SLE risk-associated alleles in the *ICAM-integrin* pathway. Fixed-effects OR and 95% CI for SLE susceptibility in each subgroup were estimated with reference to the subgroup with no risk allele. The natural logarithm of OR correlated linearly ( $r^2=0.9803$ ) with the number of risk alleles (0 to 4), and four-copy carriers had OR of 4.08 for risk of SLE compared with carriers with 0 copies (figure 2).

### Gene expression

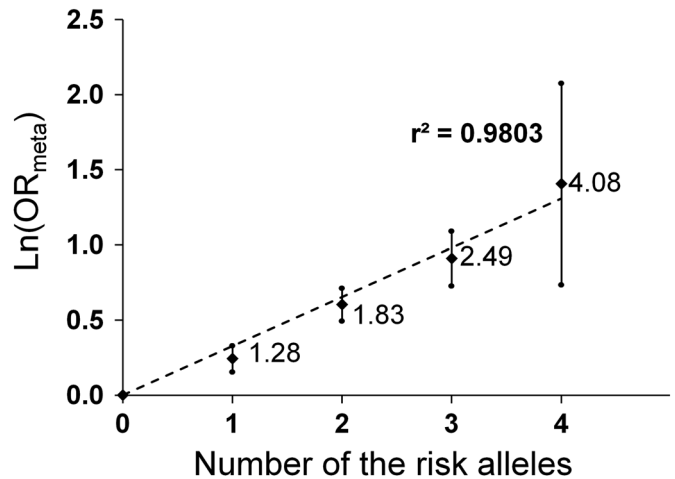
To determine whether the genetic variation has a role in gene expression, we evaluated expression quantitative trait loci (eQTL) associations within the *ICAM* locus using the Genevar (GENe Expression VARIation) database (<http://www.sanger.ac.uk/resources/software/genevar/>), which publicly provides data for 75 GenCord individuals on both SNP genotypes and expression profiles from primary fibroblasts, lymphoblastoid cell lines and T cells.<sup>9 10</sup> The 19 Genevar SNPs included rs2569693, rs5498, rs2228615 and rs281437 among the SLE-associated SNPs. However, none of the evaluated markers were associated with aberrant mRNA levels of *ICAM1*, *ICAM4* or *ICAM5* in any cell type (online supplementary figure S2).

### DISCUSSION

To our knowledge, this is the first report of a comprehensive approach used to evaluate the effect of the *ICAM1-ICAM4-ICAM5* locus on SLE risk. We observed that several SNPs within this locus are associated with susceptibility to SLE in multiple ancestry populations.

*ICAM1* is mainly expressed in the vascular endothelium, macrophages and lymphocytes, and plays a role in immunological events including extravasation and T-cell-mediated responses.<sup>12</sup> However, a plausible role for *ICAM4* and *ICAM5* in SLE pathogenesis is less clear because they are preferentially expressed in red blood cells and brain, respectively.<sup>13 14</sup>

The *ICAM* polymorphisms were not associated with altered mRNA levels of *ICAM1*, *ICAM4* or *ICAM5* in the Genevar database, although the sample size ( $n=75$ ) was too small to make this null association conclusive. However, soluble *ICAM1* levels



**Figure 2** Combined effects of *ICAM1-ICAM4-ICAM5* rs3093030-A and *ITGAM* rs1143679-A alleles on systemic lupus erythematosus (SLE) susceptibility. Natural logarithms of the relative odds of SLE (y axis) are plotted against the number of at-risk alleles (x axis) in the two putative SLE susceptibility single-nucleotide polymorphisms (SNPs), rs3093030 in *ICAM1-ICAM4-ICAM5* and rs1143679 in *ITGAM*. OR values are provided next to the data points, and error bars represent corresponding 95% CI. A linear trend line (dashed) and  $r^2$  are shown.

in plasma have been associated with two SNPs (rs3093030 and rs5498) in the *ICAM1-ICAM4-ICAM5* locus by protein QTL analysis in two independent studies involving 6578 European women ( $p=5.9 \times 10^{-23}$  and  $p=4.8 \times 10^{-25}$ ) and 9813 European individuals ( $p=3.5 \times 10^{-23}$  and  $p=2.5 \times 10^{-21}$ ).<sup>15 16</sup> The alleles that were associated with increased soluble *ICAM1* in those studies (A in rs3093030 and G in rs5498) were also associated with increased susceptibility to SLE in this study, providing a plausible mechanism for their effect. In addition, the genotype-phenotype relationships are consistent with several previously reported observations of raised plasma levels of soluble *ICAM1* in patients with SLE,<sup>17-21</sup> suggesting an underlying aetiological role.

Soluble *ICAM1* is a cleaved form consisting of the extracellular domain of the membrane-bound full-length *ICAM1* that is capable of binding to ligands. Thus soluble and membrane-bound *ICAM1* can compete with each other for binding to ligands,<sup>12</sup> but the immunological role of either soluble or membrane-bound *ICAM1* in SLE pathogenesis has not been characterised. The level of soluble *ICAM1* probably correlates with that of membrane-bound *ICAM1*,<sup>12 19</sup> but it is not known whether the increase of soluble *ICAM1* in SLE accompanies with the concurrent increase of the membrane form due to increased gene expression or with the reciprocal decrease of the membrane form due to increased proteolytic processing, as the membrane-bound *ICAM1* levels have not been measured in patients with SLE. None of the Genevar SNPs in the *ICAM1-ICAM4-ICAM5* locus was associated with *ICAM1* mRNA expression. Instead, the non-synonymous *ICAM1* SNP rs5498 (encoding Lys469Glu) was associated with both increased levels of the soluble form of *ICAM1* protein and increased SLE susceptibility. Of note is the fact that it is located at an Ig superfamily domain, which may affect the proteolytic conversion to the soluble *ICAM1*. However, the level of statistical significance for the SLE association of rs5498 was weaker than that of rs3093030 in all populations, and statistical significance was not reached in the African or Korean ancestry populations. Thus it is possible that rs5498 affects SLE susceptibility in some ancestry populations but not others.

In this study, we also showed the association of *ITGAM* rs1143679 in European, African and Hispanic descendants, which was also found in previous association studies for systemic sclerosis.<sup>22</sup> This common variant was monomorphic in Koreans, which is consistent with the literature of other Asian populations,<sup>11</sup> perhaps reflecting selective pressures in this population. *ITGAM* (also known as CD11b, Mac-1 and complement receptor type 3) is a well-characterised molecule in the integrin  $\alpha$  chain family that is expressed in a number of myeloid cells including macrophages, monocytes and neutrophils.<sup>23–25</sup> *ITGAM* encodes the  $\alpha$  chain of  $\alpha_M\beta_2$  integrin, which regulates neutrophil and monocyte adhesion and migration from the bloodstream via interactions with a wide range of structurally unrelated ligands, including but not limited to ICAM1 and ICAM2.<sup>26 27</sup>

The previously identified SLE-susceptibility *ITGAM* SNP, rs1143679, encodes a change in amino acid from arginine at position 77 to histidine (R77H). This conversion of amino acids induces an alteration in the tertiary and quaternary structures of the ligand-binding  $\alpha_M\beta_2$  domain, thereby modifying its overall binding affinity.<sup>28</sup> Nath *et al* suggest that the R77H conversion may ultimately influence the conformation of the  $\alpha$ L domain (specific to the ligand-binding domain for ICAM1), with subsequent consequences on  $\alpha_M\beta_2$  ligand binding.<sup>2</sup>

Indeed, evidence suggests that  $\alpha_M\beta_2$  levels are increased on neutrophils in SLE patients with active disease and may contribute to endothelial injury, consistent with the cumulative organ damage associated with SLE.<sup>29</sup>  $\alpha_M\beta_2$  is also involved in immune complex clearance, a process that is impaired in patients with SLE,<sup>30</sup> suggesting that the function of  $\alpha_M\beta_2$  may be altered in these people, either directly by structural modification or indirectly by alterations in receptor–ligand binding, possibly resulting from structural modification.<sup>2</sup>

In addition, this conversion is a target for alloantibodies present in mothers of neonates affected with neonatal autoimmune neutropenia.<sup>28</sup> Notably, alloantibodies reactive against the polymorphic  $\alpha_M\beta_2$  molecule are capable of blocking the  $\alpha_M\beta_2$ -dependent adhesion of neutrophils and monocytic U937 cells to a number of molecules including ICAM1.<sup>28</sup>

The functional association of *ICAM* and *ITGAM* prompted us to examine the associated SNPs in these genes for epistatic effects. Although a joint role for both *ICAM1* and *ITGAM* in SLE aetiology appears to be plausible, they independently affected SLE susceptibility in this study. This may indicate that the functional role of the *ICAM* variants is involved in a non-integrin-mediated pathway or that some factors are affected by *ICAM*–*ITGAM* binding through different pathways of *ICAM* and *ITGAM* without a common pathway.

In summary, we identified the association of several SNPs within the *ICAM1*–*ICAM4*–*ICAM5* locus with SLE susceptibility. Although the effect size was modest, the association was consistent across all four ancestries. Our findings suggest that common variations in genes involved in *ICAM*-mediated or *ICAM*/integrin-mediated adhesion play a causal role in SLE susceptibility.

**Contributors** KK, EEB, JAK, JOO, SKN, BR, AHS, KLM, PMG, JBH, CK, RPK and S-CB designed the study. EEB, C-BC, MEA-R, H-SL, SAB, LAC, GSA, JCE, AMS, COJ, GSG, DLK, BPT, J-MA, JMG, YMK, SCS, C-HS, S-KL, C-SK, JTM, MP, RRG, LMV, TBN, JM, BAP-E, TJV, BIF, KLM, PMG, JDR, JAJ, RHS, JBH, RPK and S-CB recruited and characterised the SLE cases and controls. KK, AA and KMK performed genotyping. SBG, AW, MC and CDL performed quality control analyses. KK and EEB performed statistical analyses. KK, EEB, C-BC, CK, RPK and S-CB prepared the manuscript. All authors approved the final draft.

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