

# Dense mapping of *IL18* shows no association in SLE

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**Systemic lupus erythematosus (SLE) is an autoimmune disease which behaves as a complex genetic trait. At least 20 SLE risk susceptibility loci have been mapped using both candidate gene and genome-wide association strategies. The gene encoding the pro-inflammatory cytokine, *IL18*, has been reported as a candidate gene showing an association with SLE. This pleiotropic cytokine is expressed in a range of immune cells and has been shown to induce interferon- $\gamma$  and tumour necrosis factor- $\alpha$ . Serum interleukin-18 has been reported to be elevated in patients with SLE. Here we aimed to densely map single nucleotide polymorphisms (SNPs) across *IL18* to investigate the association across this locus. We genotyped 36 across *IL18* by Illumina bead express in 372 UK SLE trios. We also genotyped these SNPs in a further 508 non-trio UK cases and were able to accurately impute a dense marker set across *IL18* in WTCCC2 controls with a total of 258 SNPs. To improve the study's power, we also imputed a total of 158 SNPs across the *IL18* locus using data from an SLE genome-wide association study and performed association testing. In total, we analysed 1818 cases and 10 770 controls in this study. Our large well-powered study (98% to detect odds ratio = 1.5, with respect to rs360719) showed that no individual SNP or haplotype was associated with SLE in any of the cohorts studied. We conclude that we were unable to replicate the SLE association with rs360719 located upstream of *IL18*. No evidence for association with any other common variant at *IL18* with SLE was found.**

## INTRODUCTION

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease with complex symptoms affecting the skin, blood cells, kidneys and joints (1). SLE is caused by multiple environmental and genetic factors, with an incidence of 9:1 females to males, and a peak incidence age of 20–30 years in females (2). A number of SLE genome-wide association studies (GWASs) have been performed, confirming at least 20 risk susceptibility loci (3–6). However, there are still a number of published candidate gene studies which need further investigation. One published candidate gene is *IL18* (7), which reported to have a strong association between a single nucleotide polymorphism (SNP) (rs360719) at the *IL18* locus,  $P = 3.8 \times 10^{-7}$ , and SLE in three European populations (Spanish, Italian and Argentinean).

*IL18* encodes a pro-inflammatory cytokine [interleukin-18 (IL-18)] located within a linkage region of SLE on chromosome 11 at 11q22.2–q22 (8–10), affecting both the innate and acquired immunity (11). This cytokine is pleiotropic which is influential to the development of T-helper 1 responses (12). *IL18* is expressed by a spectrum of immune cells such as natural killer (NK) cells, dendritic cells and macrophages (13), which induces interferon- $\gamma$ , tumour necrosis factor- $\alpha$  and granulocyte–macrophage colony-stimulating factor production. Consequently, causing an increase in the cytotoxicity of NK and T cells (14), a characteristic found in SLE patients.

Elevated IL-18 serum levels in SLE patients were previously reported (11), showing an increase as disease severity intensifies [361–1081 pg/ml in SLE patients compared with 100–126 pg/ml in controls (15)]. *IL18* has functional relevance to *OX40L* (16), a known SLE risk locus, where it

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has been shown to induce *OX40L* on stimulated T cells and dendritic cells (16). *OX40L*–*OX40* interactions were reported in maintaining T cell memory and survival of activated T cells (17). Polymorphisms in this gene could result in the imbalance of the immune response, which is a characteristic found in SLE. Therefore, *IL18* has many characteristics which suggest it to be an SLE risk locus and so it is a good candidate gene to investigate further.

Thus, genetic analysis of the *IL18* locus in SLE is warranted on the basis of it being a good functional candidate and prior genetic data. Here we used a dense marker set across *IL18* (Fig. 1) in a European SLE population, and we imputed and analysed genetic data obtained from a GWAS in SLE.

## RESULTS

### Individual SNP association analysis in UK SLE trios

Three hundred and eighty UK trios were genotyped using a customized Illumina BeadXpress chip, and the genotype data were subjected to quality control (QC) checks, as described in the Materials and Methods. From this, eight families were removed due to Mendelian errors (families with >5%), together with SNP 24 (rs4988359) which displayed deviation from Hardy–Weinberg equilibrium (HWE) ( $P < 0.001$ ). Only SNPs with genotyping >90% were analysed. SNP 20 (rs360717) failed to genotype (genotyping <90%) on the Illumina platform and, therefore, was not included in further analysis. All other SNPs were within HWE ( $P > 0.001$ ) and showed genotyping frequency of >90%.

The transmission disequilibrium test (TDT) analysis on the 372 trios was performed using PLINK-TDT. The results (Table 1) of this analysis demonstrated that no individual SNP association was found ( $P < 0.05$ ). In addition, a haplotype-TDT analysis using Haploview across the locus showed no haplotypic associations.

### Individual SNP association analysis in UK case–control cohort (UK|CC)

Owing to the lack of association found in the UK SLE trios cohort, an independent UK case–control cohort was analysed to increase the power of the study. This case–control cohort consisted of 508 UK non-trio cases, which were genotyped on an Illumina bead express custom chip, and 2910 Wellcome Trust Case Control Consortium controls (WTCCC2) [previously genotyped (18)]. However, since three SNPs genotyped in the UK cases were not present in the WTCCC2 controls, these SNPs were imputed in the WTCCC2 controls. In a second phase of imputation, 508 cases and 2910 controls were subjected to an imputation analysis to impute SNPs between positions 111 464 762 and 111 653 000 bp (dbSNP 126) on chromosome 11. In total, 258 SNPs were imputed in this region. Samples were imputed using the 1000 genomes CEU data as the phased reference (HapMap data were not used as rs360719 is not a HapMap SNP) and trio parents were used as the un-phased reference panel. Therefore, the final UK case–control association analysis cohort (UK|CC) included both genotyped and imputed data from 258 SNPs. All imputed data were subjected to QC checks. Two samples were removed for poor certainty (<90%).

SNP 9 (rs7938116) and SNP 24 (rs4988359) were excluded for poor HWE ( $P < 0.001$ ). All other SNPs showed good HWE and genotyping greater than 90%. Five hundred and eight cases and 2908 controls were then subjected to a case–control analysis using SNPTEST.

First, we analysed the imputed UK|CC cohort for the 36 SNPs initially genotyped in the UK SLE trio cohort. As seen in Table 1, rs12797880, rs5744256, rs543810 and rs9919624 showed weak associations ( $P = 0.016$ ,  $P = 0.0027$ ,  $P = 0.015$  and  $P = 0.036$ , respectively); however, after permutations to adjust for multiple testing (10000 permutations) no individual SNP associations were found ( $P < 0.05$ ). To extend this analysis, we analysed the remaining 222 imputed SNPs for individual SNP associations. No other strong individual SNP associations were found (data not presented) ( $P < 0.05$ ) in the remaining 222 SNPs.

### Imputation certainty

To ascertain whether the 1000 genomes CEU data were a good source to use as the phased reference panel, we imputed the UK|CC cohort using only the trio parents ( $n = 744$ ) as the unphased reference and no phased reference was used. We therefore imputed all 36 SNPs in both the cases and controls (508 and 2908, respectively).

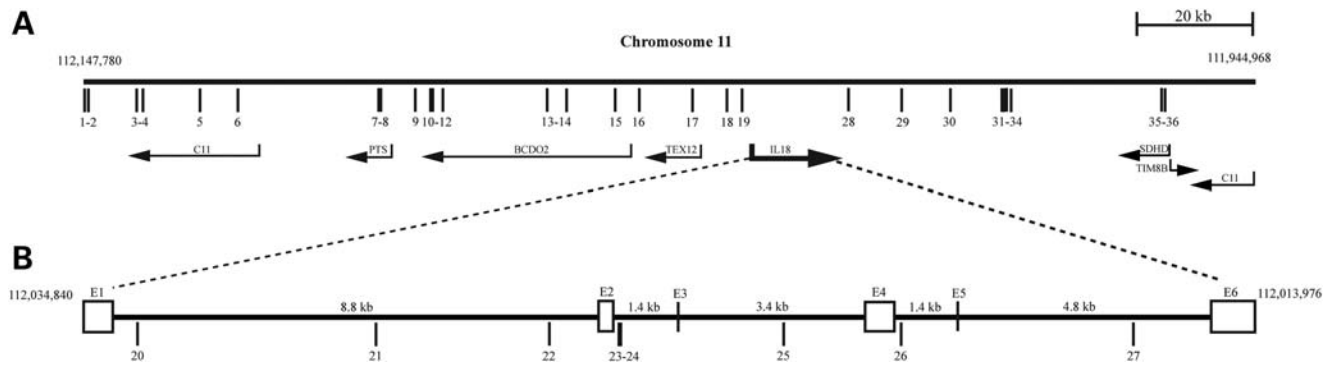
After imputation, the probabilistic genotypes were analysed using SNPTEST; here an additive Frequentist model was applied. The analysis showed weak individual SNP associations in SNPs rs12797880, rs4937113, rs5744256, rs543810 and rs9919624; however, after adjusting for multiple testing no single significant SNP association was found. Therefore, when comparing this with the imputation performed in the UK|CC cohort, using the 1000 genomes and unphased reference (Table 1), it can be seen that using only the unphased reference does not alter individual SNP associations. Weak associations are seen in the same SNPs when comparing both imputations. Therefore, we can conclude that the 1000 genomes reference data were a good reference set to use for this imputation, as using only the unphased reference panel for the imputation gave results similar to when using the 1000 genomes and unphased reference simultaneously.

### Haplotypic association analysis

The haplotypic architecture across the locus was constructed using Haploview, with the solid spine algorithm and a  $D'$  cut-off >0.8. None of the haplotypes showed association ( $P < 0.05$ ) in either the UK trios or UK|CC cohort. No risk haplotype (haplotype more prevalent in the cases) or protective haplotype (haplotype more prevalent in the controls) was observed (Fig. 2).

### Previous association with rs360719 analysis

As previously reported by Sanchez *et al.* (7), an association was found for rs360719 ( $P_{\text{combined}} = 3.8 \times 10^{-7}$ ) (our SNP 17) in three southern European populations. Consequently, we aimed to investigate this potential association further. SNP rs360719 was not genotyped by Illumina (as described in the Materials and Methods) because this SNP was <100 bp away from other genotyped SNPs and therefore



**Figure 1.** (A) Gene diagram across chromosome 11 showing SNPs genotyped in this study. (B) *IL18* gene showing the six exons along with the SNPs typed in 380 UK SLE trios. SNP 1 (rs7120010), SNP 2 (rs2564866), SNP 3 (rs2853127), SNP 4 (rs891360), SNP 5 (rs2518360), SNP 6 (rs2564871), SNP 7 (rs2518345), SNP 8 (rs1367038), SNP 9 (rs7938116), SNP 10 (rs11214139), SNP 11 (rs2250417), SNP 12 (rs2518356), SNP 13 (rs11214127), SNP 14 (rs10891337), SNP 15 (rs12797880), SNP 16 (rs11214108), SNP 17 (rs360719), SNP 18 (rs1946519), SNP 19 (rs187238), SNP 20 (rs360717), SNP 21 (rs4937113), SNP 22 (rs360722), SNP 23 (rs360721), SNP 24 (rs4988359), SNP 25 (rs5744256), SNP 26 (rs549908), SNP 27 (rs5744280), SNP 28 (rs543810), SNP 29 (rs243908), SNP 30 (rs578784), SNP 31 (rs4937075), SNP 32 (rs10891323), SNP 33 (rs11214093), SNP 34 (rs360726), SNP 35 (rs10789859), SNP 36 (rs9919624).

was genotyped in the UK SLE trios using a Taqman assay and then imputed in the UK|CC cohort. The Taqman genotyping for rs360719 in UK trios showed good clustering of genotypes, indicating an accurate allele call rate. From these results, we were unable to replicate the association previously found in this SNP. The UK trio data showed that the A allele was over-transmitted in this cohort and therefore not replicating the previous finding (7) where the G allele was found to be the risk over-transmitted allele. However, in the UK|CC cohort, the results show that the G allele was over-represented in the cases; however, the *P*-value of 0.6 showed no significant association.

### GWAS imputation and association analysis

As the results in the UK samples show that we were unable to replicate the previous association (SNP 17 rs360719) (7), we then used an SLE GWAS (19) data set and we imputed missing SNPs at *IL18* using the 1000 genomes project as reference data and the trios parents as the unphased reference (as previously described). The European American GWAS genotype data comprised 60 SNPs across this region, where we again imputed between positions 111 464 762 and 111 653 000 bp on chromosome 11. A total of 158 SNPs were imputed (including rs360719). The imputation was performed using IMPUTE V2 in 9170 individuals [1310 cases and 7860 controls (European American), using the 1000 genomes (CEU) data as a phased reference panel].

Following imputation, the SLE GWAS was analysed using SNPTEST where no individual SNP association was found. SNP 17 (rs360719) demonstrated no evidence of an association ( $P = 0.784$ ); however, the risk allele G, previously shown to be associated, was found to be equal in both the cases and controls and therefore we were unable to replicate the finding by Sanchez *et al.* (7) in SLE. The marginal evidence of association shown in the UKICC analysis, for SNPs rs12797880, rs5744256, rs543810 and rs9919624, was not replicated here. We also did not find any haplotypic association in this cohort.

### Power calculation for rs360719

To ensure the two-study cohort had power to ascertain association, a genetic power calculation was performed using the sample size in the SLE GWAS European American case-control cohort and UK|CC data. The result showed that we have 99, 98 and 75% power to detect effect sizes of 1.81, 1.53 and 1.26, respectively, which is the range of odds ratios (ORs) declared by Sanchez *et al.* (7), using an additive model with one degree of freedom. This demonstrates that the combined data set has sufficient power to detect an association in an SNP (rs360719) if present.

### Bayes factors

Rather than formulate our results based on the null hypothesis of no association (which we fail to reject for rs360719), we can formally ask the question as to whether the data support no association (null; OR = 1) or an association as declared by Sanchez *et al.* (7). This can be achieved by using the estimated OR and standard error declared by Sanchez *et al.* (7) to formulate a prior distribution, and then calculating the Bayes factor (BF) for the null model against the associated model. We performed this analysis using SNPTEST.

For the UK case-control data, the BF in favour of the null was 5.9 which is positive support (20), while for the European data the BF was 50.5 which is strong. Combining the two BFs gives a BF of 298 in favour of the null, which is very strong. If we have equal prior belief in the models then this translates to a probability for the null of 0.997 [probability of the null given the data = 0.997; probability that the OR is 1.53 (1.29, 1.81) = 0.003]. The prior belief in models is subjective; however, the BF is objective and coherent in that it averages over a plausible range of ORs using the estimate and standard error declared by Sanchez *et al.* (7).

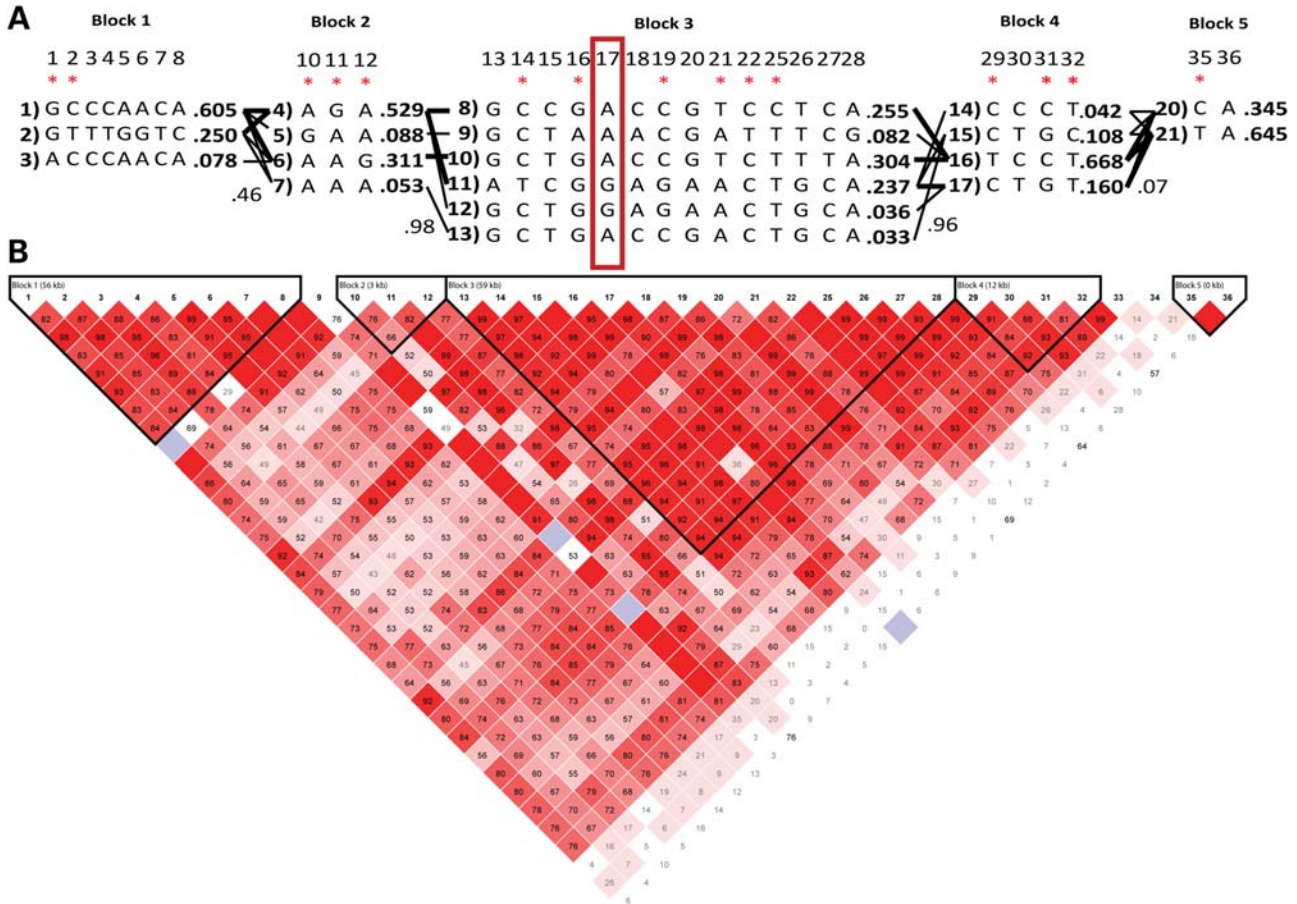
### Meta-analysis

A meta-analysis was performed on the joint UK SLE cohorts using METAL (21) in the 36 genotyped SNPs. This analysis

**Table 1.** Allelic association in the UK SLE trio cohort and independent UK|CC cohort

SNP number	SNP	Trios 372 families							Case control, $n = 3416$ (508 cases, 2908 controls)						$i^2P_{META}$
		HWE	Percentage genotyping	MAF	Alleles (Maj:MA)	T:U	Chi-square	$P$ -value	HWE	Percentage genotyping	Associated allele	Frequency case, control	Chi-square	$P$ -value	
1	rs7120010	0.025	99.4	0.072	G:A	59:44	2.18	0.120	0.198	99.8	A	0.089, 0.080	0.918	0.338	0.263
2	rs2564866	0.694	99.3	0.272	T:C	146:136	0.355	0.516	0.444	99.8	T	0.268, 0.259	0.361	0.548	0.330
3	rs2853127	0.768	99.2	0.302	C:T	150:148	0.013	1.000	0.412	99.4	T	0.303, 0.289	0.869	0.351	0.336
4	rs891360	0.909	98.8	0.283	T:C	148:139	0.282	0.558	0.431	99.9	T	0.283, 0.259	2.747	0.097	0.060
5	rs2518360	0.887	99.2	0.283	G:A	149:144	0.085	0.728	0.527	99.7	G	0.287, 0.266	1.86	0.173	0.092
6	rs2564871	0.684	99.1	0.270	G:A	142:138	0.057	0.767	0.634	99.9	G	0.266, 0.259	0.197	0.657	0.413
7	rs2518345	0.755	99.2	0.296	T:C	156:146	0.331	0.530	0.853	99.9	T	0.281, 0.271	0.410	0.522	0.329
8	rs1367038	0.644	99.1	0.305	C:A	157:147	0.329	0.569	0.850	99.8	C	0.294, 0.279	0.984	0.321	0.184
9	rs7938116	1.000	100	0.009	T:C	7:4	0.818	0.405	–	–	–	–	–	–	0.405
10	rs11214139	0.422	99.1	0.112	A:G	74:72	0.027	0.935	0.039	100	G	0.112, 0.106	0.378	0.539	0.457
11	rs2250417	0.443	97.6	0.473	G:A	168:167	0.003	0.952	0.128	97.5	A	0.479, 0.453	2.24	0.134	0.177
12	rs2518356	0.468	98.1	0.341	A:G	152:151	0.003	0.823	0.717	99.9	G	0.341, 0.311	3.46	0.063	0.113
13	rs11214127	0.576	99.2	0.266	G:A	142:130	0.529	0.549	0.633	99.8	A	0.255, 0.243	0.717	0.397	0.235
14	rs10891337	0.696	99.4	0.267	C:T	145:132	0.610	0.513	0.513	99.4	T	0.259, 0.246	0.789	0.374	0.245
15	rs12797880	0.568	99.2	0.235	T:C	129:128	0.004	0.951	0.573	99.4	T	0.771, 0.734	5.81	0.016	0.021
16	rs11214108	0.334	98.8	0.115	G:A	80:69	0.812	0.522	0.344	99.9	A	0.110, 0.101	0.846	0.358	0.269
17	rs360719	0.651	92.0	0.298	A:G	92:72	2.43	0.118	0.930	100	G	0.280, 0.272	0.262	0.608	0.276
18	rs1946519	0.502	98.1	0.416	C:A	171:154	0.889	0.386	0.199	99.6	A	0.409, 0.386	1.99	0.158	0.058
19	rs187238	0.088	99.2	0.291	C:G	143:132	0.440	0.476	0.951	99.9	G	0.282, 0.273	4.07	0.523	0.336
20	rs360717	–	–	–	–	–	–	–	0.951	99.9	A	0.281, 0.273	0.306	0.580	0.465
21	rs4937113	0.181	98.5	0.427	T:A	164:162	0.012	0.828	0.859	99.7	A	0.425, 0.406	1.34	0.247	0.166
22	rs360722	0.800	98.5	0.096	C:T	68:59	0.638	0.486	0.047	99.9	T	0.105, 0.096	0.896	0.344	0.257
25	rs5744256	0.353	99.6	0.229	T:C	126:126	0.000	0.900	0.483	99.9	T	0.780, 0.736	8.98	0.0027	0.004
26	rs549908	0.443	98.0	0.332	T:G	149:145	0.054	0.818	0.899	99.7	G	0.321, 0.312	0.344	0.557	0.540
27	rs5744280	0.454	98.1	0.347	C:T	155:147	0.212	0.612	0.799	99.7	T	0.346, 0.329	1.05	0.305	0.370
28	rs543810	0.197	96.8	0.110	A:G	83:61	3.37	0.103	0.049	99.8	G	0.121, 0.096	5.97	0.015	0.009
29	rs243908	0.442	98.5	0.330	T:C	151:143	0.218	0.605	0.780	99.8	C	0.320, 0.312	0.258	0.612	0.529
30	rs578784	0.417	99.2	0.297	C:T	140:140	0.000	0.906	0.713	99.9	T	0.287, 0.284	0.048	0.827	0.824
31	rs4937075	0.061	98.8	0.304	C:G	143:140	0.032	0.814	0.500	99.8	G	0.296, 0.286	0.419	0.518	0.532
32	rs10891323	0.219	99.5	0.125	C:T	82:78	0.100	0.752	1.0	99.8	T	0.887, 0.883	0.131	0.717	0.526
33	rs11214093	0.315	97.6	0.430	T:C	168:157	0.372	0.786	0.763	99.8	T	0.424, 0.418	0.132	0.716	0.878
34	rs360726	0.352	99.2	0.228	C:T	136:115	1.76	0.351	0.598	99.8	T	0.225, 0.198	3.85	0.050	0.020
35	rs10789859	0.451	98.0	0.344	C:T	174:162	0.429	0.830	0.087	99.4	T	0.670, 0.652	1.24	0.265	0.307
36	rs9919624	0.026	99.9	0.013	G:A	11:9	0.200	0.513	0.487	100	G	0.015, 0.008	4.39	0.036	0.045

Table showing the allelic association comparison between the UK SLE trio cohort and the UK|CC cohort. SNP numbers correspond to those in Figure 1, all HWE and MAF are stated.  $i^2P_{META}$  represents the meta-analysis unpermuted  $P$ -value.



**Figure 2.** (A) UK|CC data showing SNPs which were genotyped and imputed. Haplotype constructed from 508 cases and 2608 controls. Tagger SNPs are indicated by an asterisk. Numbers across the top of the haplotypes correspond to the SNP numbers in Figure 1. The haplotype number is in bold on the left of each haplotype. Haplotype frequencies are shown after each block in bold. (B) LD plot from Haploview which shows the pattern of LD across the UK|CC population with the brightest red showing regions of high pairwise  $D'$ .

combines the  $P$ -values from both cohorts to ascertain association between these SNPs and SLE. From the analysis, four SNPs were found to have marginal association, rs12797880 (SNP15)  $P = 0.021$ , rs5744256 (SNP25)  $P = 0.004$ , rs360726 (SNP34)  $P = 0.02$  and rs9919624 (SNP36)  $P = 0.045$ . However, after permutation to adjust for multiple testing (10 000 permutations), these SNPs showed no evidence of association.

**Subphenotype analysis**

We performed a subphenotype analysis on anti-Ro, anti-La, age of onset (juvenile lupus versus adult lupus and mean age interquartile range) and renal disease in 508 non-trio cases using PLINK. No individual SNP association was found in any of the subphenotype groups.

**DISCUSSION**

The work presented in this manuscript describes a detailed fine-mapping of variants across the *IL18* locus, in a large UK SLE data set followed by a meta-analysis and analysis of a SLE GWAS cohort. The results of the association studies in the UK SLE trios and UK|CC showed no individual

SNP or haplotypic association observed across *IL18*. This suggests that common genetic polymorphisms at *IL18* do not exert a significant genetic effect in the development of SLE. In this study, we analysed three cohorts to investigate the possible association between *IL18* and SLE; however, even in the largest cohort (GWAS data), no individual SNP association was found. A power calculation for the imputed case-control SLE GWAS cohort showed 98% power [effect size 1.53 (7)] to detect a previously reported association. To confirm our results, we performed a meta-analysis between the UK SLE trios cohort and the imputed UK|CC. This analysis showed marginal associations in four SNPs; however, after permutations these SNPs did not demonstrate significant statistical associations.

In this study, we failed to replicate the association found by Sanchez *et al.* (7) in rs360719 (upstream of *IL18*) in our initial UK cohorts. We therefore imputed European American SLE GWAS data using the 1000 genomes data set as a phased reference panel, to try and replicate this finding. After imputation of the SLE GWAS cohort, no association was found with rs360719, therefore we were unable to replicate the previous finding (7). SNPs in linkage disequilibrium (LD) with SNP 17 (rs360719) were also analysed to discover whether another SNP in LD with rs360719 was

associated in the European populations; however, no individual SNP associations were found.

A previous association found at SNP rs360719 (Sanchez *et al.* (7)) was shown to be strongly associated in a Spanish cohort ( $P$ -value of  $7.8 \times 10^{-7}$ ). As we were unable to replicate this find in our European cohort, we further explored whether there are differences in the minor allele frequency (MAF) at this SNP between southern and northern European populations. Here we compared the MAF found by Sanchez *et al.* (7) control data with our UK|CC control data and dbSNP. The MAF in Sanchez *et al.* (7) control data was 25% compared with 27% in our UK|CC data and 26% in dbSNP CEU control data. This suggests that the MAF does not differ between the southern and northern European populations and therefore cannot account for the association found in the southern European population. We also hypothesized that the previous association found was due to long-range LD with rs360719 and other SNPs; however, after long-range LD analysis of 500 kb up- and downstream of SNP rs360719, we can conclude that no other SNP in LD with this SNP was found to be associated and therefore cannot account for the previous association.

Our study covers a wider range across the *IL18* locus, genotyping 36 SNPs (Fig. 1A and B) and imputing 158 SNPs in the SLE GWAS cohort and 258 SNPs in the UK|CC cohort. We therefore had a dense analysis across *IL18* to ascertain association between these SNPs and SLE. Our cohort sizes are also large, allowing us to have good power to detect the previously reported association.

*IL18* polymorphisms have previously been reported to be associated in other autoimmune diseases such as rheumatoid arthritis (RA) and type 1 diabetes (T1D). SNPs rs1946518 and rs187238 were found to contribute to RA pathogenesis in German patients (12) and rs1946518 was found to be present in younger RA patients (22); this SNP may only be associated with certain RA disease parameters such as the age of onset. In our analysis, we are unable to report any associations between these polymorphisms and SLE. SNPs rs1946518 and rs187238 have also been reported to be associated with T1D susceptibility and *CTLA-4* gene–gene interactions (23), however we did not find association in either of these SNPs in our cohorts for SLE. Disruption and differences in transcription binding sites in T1D caused by *IL18* polymorphisms rs1946518 and rs187238 (24) have been reported, however we again did not find any association with these SNPs in any of our cohorts with SLE patients. These findings suggest that *IL18* polymorphisms have a greater effect in other autoimmune diseases compared with SLE.

In conclusion, we found no individual SNP or haplotypic association across *IL18* in the UK samples between the 36 SNPs genotyped or the 258 imputed SNPs and SLE. We also failed to replicate the association for rs360719 previously reported (7) after imputation analysis. Therefore, it is unlikely that common variants in *IL18* make a significant contribution to disease susceptibility in SLE.

## MATERIALS AND METHODS

### Study cohorts

*UK cohort.* The UK samples were a collection of UK lupus patients and parental samples available in the laboratory. In

total, there were 380 trios (parents and their affected offspring). SLE patients were diagnosed through written questionnaires, telephone interviews and clinical notes, and family members were contacted. Both patients and family members gave written consent for the use of their DNA for research within the laboratory. Ethical approval was granted by the MREC (Multi-Centre Research Ethics Committee). A total of 2910 Wellcome Trust case control consortium controls (WTCCC2) were also used for the analysis of data.

*SLE European American GWAS cohort.* Genotype data on a total of 1310 cases and 7860 controls (European population) were obtained from Dr Robert Graham (Genentech) (25). These controls were of mixed European populations compared with only a UK population in the WTCCC2 controls. Samples were subjected to QC tests as reported (19), together with EIGENSTRAT analysis for population substructure clustering using 10 principal components. This EIGENSTRAT analysis excluded 141 samples as described (19).

### Sample collection

*Preparation of DNA.* Whole blood was collected and lymphocytes were separated from whole blood by centrifugation; genomic DNA was isolated using the phenol-chloroform method.

*SNP selection.* Using HapMap B36 assembly dbSNP 126, 21 haplotype-tagging SNPs across *IL18* were selected (tagging parameters set at  $R^2$  cut-off 0.8, MAF cut-off 0.2) together with 14 functional SNPs from previous association studies (Fig. 1). SNPs rs360718, rs360719, rs1946518, rs12721559 and rs360721 were removed from selection, as these SNPs were less than 100 bp apart and therefore could not be pooled together for the customized Illumina chip.

### Genotyping

Samples were genotyped on an Illumina bead express custom chip at the Oklahoma Medical Research Foundation (OMRF). SNP rs360719 was genotyped using a Taqman assay from Applied Biosystems according to the manufacturer's instructions. The genotype data for the variant were quality controlled using the SDS 2.3 software (Applied Biosystems).

### Statistical analysis

All genotype and phenotype data were managed and analysis files produced within BC|SNPmax (Biocomputing platforms, Finland).

All data underwent general quality checks which consisted of Mendelian error analysis (families with  $>5\%$ ) for the trios along with HWE analysis for both trios and case–control data (cut-off 0.001).

Haplotype structures were generated for both the UK trios and the UK|CC cohorts using the solid spine algorithm in Haploview based on the LD between SNPs (<http://www.broadinstitute.org/mpg/haploview>) with confidence intervals of 0.98 and 0.70 for LD. Markers with minor allele frequencies of  $<5\%$  were excluded from the haplotype blocks.

Allelic associations for the UK SLE trios were tested using the TDT in PLINK, which compares expected and observed transmission of alleles from parents (heterozygous) to the affected offspring. Haplotypic architecture of the genotyped SNPs was constructed using Haploview. A case-control analysis in Haploview was then performed in UK|CC cohort and SLE European American GWAS.

### Bayes factors

We calculated the BFs by running a logistic model in SNPTEST using the Bayesian option with a Gaussian prior distribution on the log ORs with a mean of 0.425 and standard deviation of 0.086, which corresponds to an expectation of 1.53 for the OR with upper and lower 95% confidence bounds of 1.29 and 1.81, respectively. These figures are taken directly from Sanchez *et al.* (7).

### Imputation

The 508 UK cases and 2910 UK controls were subjected to an imputation analysis to impute SNPs between positions 111 464 762 and 111 653 000 bp (dbSNP 126). A total of 258 SNPs were imputed in this region using IMPUTE V2. Samples were imputed using the 1000 genomes CEU data as the phased reference. Imputed data were uploaded as probabilistic genotypes and individual SNP associations were analysed using SNPTEST, while haplotypic associations were analysed as previously described.

SLE European American GWAS genotype data were obtained and imputed using IMPUTE V2 (26) within BC|SNPmax. Genotype data were imputed using 1000 genomes (Caucasian) data as a phased reference panel and *IL18* Caucasian trio data as the unphased reference. Imputation regions were set to 111 464 762 to 111 653 000 bp on chromosome 11. Imputed data were uploaded as probabilistic genotypes and analysed for HWE and haplotypic associations as described previously.

### Meta-analysis

A meta-analysis using METAL (21) in the UK SLE trios and the imputed UK|CC cohort in the 36 genotyped SNPs was performed. This analysis allowed the combination of *P*-values in both cohorts to ascertain association between these SNPs and SLE.

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*Conflict of Interest statement.* None declared.

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