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Fine Mapping of Xq28: Both *MECP2* and *IRAK1* Contribute to Risk for Systemic Lupus Erythematosus in Multiple Ancestral Groups

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Abstract

Objectives—The Xq28 region containing *IRAK1* and *MECP2* has been identified as a risk locus for systemic lupus erythematosus (SLE) in previous genetic association studies. However, due to the strong linkage disequilibrium between *IRAK1* and *MECP2*, it remains unclear which gene is affected by the underlying causal variant(s) conferring risk of SLE.

Methods—We fine-mapped ≥136 SNPs in a ~227kb region on Xq28, containing *IRAK1*, *MECP2* and 7 adjacent genes (*L1CAM*, *AVPR2*, *ARHGAP4*, *NAA10*, *RENBP*, *HCFC1* and *TMEM187*), for association with SLE in 15,783 case-control subjects derived from 4 different ancestral groups.

Results—Multiple SNPs showed strong association with SLE in European Americans, Asians and Hispanics at $P<5\times10^{-8}$ with consistent association in subjects with African ancestry. Of these, 6 SNPs located in the *TMEM187-IRAK1-MECP2* region captured the underlying causal variant(s) residing in a common risk haplotype shared by all 4 ancestral groups. Among them, rs1059702 best explained the Xq28 association signals in conditional testings and exhibited the strongest *P* value in trans-ancestral meta-analysis ($P_{meta}=1.3\times10^{-27}$, OR=1.43), and thus was considered to be the most-likely causal variant. The risk allele of rs1059702 results in the amino acid substitution S196F in IRAK1 and had previously been shown to increase NF-κB activity *in vitro*. We also found that the homozygous risk genotype of rs1059702 was associated with lower mRNA levels of *MECP2,* but not *IRAK1*, in SLE patients ($P=0.0012$) and healthy controls ($P=0.0064$).

Conclusion—These data suggest contributions of both *IRAK1* and *MECP2* to SLE susceptibility.

Keywords

Systemic Lupus Erythematosus; Gene Polymorphism; Xq28; IRAK1; MECP2

INTRODUCTION

SLE (OMIM 152700), a chronic multi-organ autoimmune disease, is associated with significant morbidity and mortality. A large body of literature supports a role for genetic, environmental and epigenetic factors in the pathogenesis of SLE.[1–5]

Previously, single nucleotide polymorphisms (SNP) in *IRAK1* (interleukin-1 receptor-associated kinase 1) and its adjacent gene *MECP2* (methyl CpG binding protein 2), separated by 1.7 kb on Xq28, have been independently associated with risk of SLE, mainly in subjects with European and Asian ancestries.[6–10] Both *IRAK1* and *MECP2* are strong candidate genes for SLE susceptibility. IRAK1 associates with interleukin-1 receptor, up-regulates transcription factor NF-κB [11] and activates the innate immune system which is important in SLE pathogenesis.[12, 13] IRAK1 deficiency in mice abrogated SLE-associated phenotypes, including IgM and IgG autoantibodies, lymphocytic activation and renal disease, and reversed the dendritic cell “hyperactivity” associated with the *Sle3* lupus susceptibility interval.[7] MECP2 plays a role in two epigenetic repression mechanisms, DNA methylation and histone deacetylation, leading to a chromatin configuration inaccessible for transcription, thereby silencing gene expression.[14, 15] In both humans and mice, defects of DNA methylation have been implicated in the pathogenesis of SLE.[2, 3] The strong linkage disequilibrium (LD) between these two genes has led to the hypothesis that only one or the other of *IRAK* or *MECP2* is the SLE risk gene on Xq28,[16] a debate which has not yet been solved. Furthermore, rs2269368 in *ARHGAP4* has been associated with SLE in subjects with European ancestry,[17] suggesting that genes located upstream of the *IRAK1-MECP2* region may also contribute to SLE susceptibility.

Leveraging different LD patterns among multiple ancestral groups, the trans-ancestral fine-mapping approach has shown its power in identifying underlying causal variants at SLE-associated loci.[18–20] Here, we fine mapped 9 genes in Xq28 using 136–173 SNPs and assessed their association with SLE in subjects from 4 different ancestral groups. After localizing the candidate causal variant, we tested its association with the mRNA level of *IRAK1* and *MECP2*.

METHODS

Sample collection

DNA samples used in the collaborative Large Lupus Association Study 2 (LLAS2) were from subjects recruited by multiple participating institutions and processed at the Oklahoma Medical Research Foundation (OMRF). Each institution had Institutional Review Board (IRB) approval to recruit subjects and the overall study was approved by the IRB of OMRF. Each patient met at least four of eleven 1997 American College of Rheumatology revised criteria for the classification of SLE.[21]

Genotyping and data cleaning

We selected potential functional SNPs, previously reported SLE-associated Xq28 SNPs and tag SNPs based on HapMap datasets (r24) for genotyping. Fifty-five Xq28 SNPs and 347 admixture informative markers (AIMs) were successfully genotyped using an Illumina custom array on the iSCAN instrument (San Diego, CA, USA).

Subjects with genotype missing rate >10% (due to low quality), shared identical by descent >0.4 or showing mismatch between the reported and estimated gender were removed. The global ancestry of each subject was estimated based on genotype of AIMs, using principal components analysis [22] and ADMIXMAP,[23–25] as described in another LLAS2 study.[19] Genetic outliers were removed.

Final clean data from 15,783 subjects were divided into 4 groups according to ancestry, including EA (European Americans), AA (composed of 92.5% African Americans and 7.5% Gullahs), AS (comprised of 74.6% of Koreans, 16.1% of Chinese and subjects from Japan and Singapore) and HA (Hispanics enriched for the Amerindian-European admixture) (Table S5). Some subjects were previously analyzed in two published *MECP2/IRAK1* studies (Table S6).[6,7]

Imputation

To obtain genotypes of additional Xq28 SNPs, SNP genotypes of 381 Europeans, 246 Africans, 286 Asians and 181 Americans from the 1000 Genomes Project (June 2011 data release) were used as references in imputation for our EA, AA, AS and HA subjects, respectively. Imputation was performed using IMPUTE 2.1.2;[26] imputed SNPs with an information score >0.9 were included for further analyses.

Statistical analyses

The same quality control criteria were applied to genotyped and imputed SNPs. SNPs with minor allele frequency (MAF) <1% or Hardy Weinberg equilibrium $P<0.001$ in controls were excluded. SNPs with genotype missing rate >5% or showing significantly different missing rates between cases and controls (missing rate >2% and $P<0.05$) were also excluded.

In each ancestral group, SNPs were assessed for association with SLE under a logistic regression model adjusting for gender and the first 3 principal components estimated using AIMs. Haplotype-based conditional association testings were also performed by adjusting for gender and the first 3 principal components. The trans-ancestry meta-analysis was conducted across all 4 ancestral groups. For each SNP, if the Cochran's Q statistic showed no evidence of genetic heterogeneity ($P>0.05$), a fixed effect model was applied. Otherwise, a random effect model was used. All analyses described above were performed using PLINK v1.07.[27] Pairwised LD values shown in Figure S2 were calculated using Haploview 4.2.[28]

Real-time quantitative PCR (RT-PCR)

Total mRNA extracted from peripheral blood mononuclear cells (PBMC) using the All Prep DNA/RNA mini kit (QIAGEN, Valencia, CA, USA) were reverse-transcribed into cDNA (Invitrogen, Carlsbad, CA, USA). Using RT-PCR (Applies Biosystems, Foster City, CA, USA), the expression of *IRAK1* and *MECP2*, including all isoforms, were measured with TaqMan probe Hs01018347_m1 and Hs00172845_m1, respectively. The relative expression levels of *IRAK1* and *MECP2*, normalized to housekeeping gene *RPLP0*, were calculated using the $2^{-\Delta\Delta Ct}$ method. Log_{10} transformed *IRAK1* and *MECP2* levels were compared between individuals carrying different genotypes using Student's t test.

RESULTS

We genotyped 55 SNPs at Xq28, together with 347 AIMs, in 15,783 case-control subjects from 4 ancestral groups including EA, AA, AS and HA. In addition, we imputed genotypes for ungenotyped SNPs using reference data from the 1000 Genomes Project, resulting in 173 (EA), 157 (AA), 157 (AS) and 136 (HA) SNPs with MAF>1% that covers a ≥227kb region in Xq28 containing genes *L1CAM*, *AVPR2*, *ARHGAP4*, *NAA10*, *RENBP*, *HCFC1*, *TMEM187*, *IRAK1* and *MECP2* (Figure 1A). SNPs were assessed for the association with SLE under a logistic regression model adjusting for gender and global ancestry. The significance level was defined as Bonferroni-corrected $P<0.05/173=2.9\times10^{-4}$, using the most stringent criterion.

Xq28 SNPs were associated with SLE in four different ancestral groups

To confirm the previously reported association of Xq28 region with SLE,[6,7,17] we firstly performed association testing in the largest EA dataset (3,915 cases and 3,462 controls). Eighty-six SNPs located in the region containing *ARHGAP4*, *NAA10*, *RENBP, HCFC1*, *TMEM187*, *IRAK1* and *MECP2* were significantly associated with SLE, of which 61 SNPs had $P<5.0\times10^{-8}$ exceeding the genome-wide association study (GWAS) significance level and rs5945377 in *RENBP* exhibited the strongest association signal ($P=8.4\times10^{-11}$, OR=1.38) (Figure 1B, Table S1). These data confirmed that Xq28 is a risk locus for SLE in EA.

Association of Xq28 with SLE was also confirmed in our AS (1,262 cases and 1,256 controls) and HA (1,487 cases and 807 controls) datasets. In total, 85 and 40 SNPs were significantly associated with SLE in AS and HA, respectively, of which 48 and 10 SNPs had $P<5.0\times10^{-8}$ (Figure 1B, Table S2 and S3). Both datasets showed the strongest association signal in the *IRAK1-MECP2* region. SNPs in the upstream *ARHGAP4-NAA10-RENBP* region did not reach the GWAS significance level.

In the AA dataset (1,674 cases and 1,920 controls), 16 SNPs showed modest association with SLE ($P<0.05$) (Figure 1B, Table S4), of which SNPs in the *IRAK1-MECP2* region exhibited peak association signals but none of them reached the Bonferroni-corrected significance level.

Comparing across EA, AS and HA, 34 SNPs located in a ~187 kb region spanning from *ARHGAP4* to *MECP2* were significantly associated with SLE in all 3 datasets (Table 1). Of them, 7 SNPs (rs13397, rs4898375, rs1059702, rs2734647, rs2075596, rs1734787 and rs1616369) were consistently associated with SLE in AA at $P<0.05$ (Table 1), had no genetic heterogeneity ($P>0.05$) across 4 ancestral groups, and generated a combined $P_{meta}<5\times10^{-8}$ in trans-ancestral meta-analysis (Figure 1C, Table 1). We performed association testing in females and males, respectively, which yielded no evidence for a gender-specific association with SLE. Consistent association detected in EA, AS, HA and AA indicated that Xq28 is a risk locus of SLE in all these 4 ancestral groups.

SLE-associated SNPs shared by four different ancestral groups were localized to the *TMEM187-IRAK1-MECP2* region

Haplotypes with frequency >1% were constructed using the 34 SNPs that were significantly associated with SLE in EA, AS and HA. Only haplotype H1 showed consistent association with increased SLE risk in EA (frequency of 17.4% in cases vs. 13.3% in controls, $P=3.8\times10^{-9}$), AS (69.1% vs. 64.2%, $P=1.7\times10^{-4}$), HA (52.1% vs. 40.1%, $P=1.4\times10^{-9}$) and AA (6.3% vs. 4.3%, $P=4.9\times10^{-4}$) (Figure 2). H1 shared by these 4 ancestral groups could be perfectly tagged by the risk allele of 6 SNPs (rs13397, rs4898375, rs1059702, rs2734647, rs2075596 and rs1616369) in AA, which suggested that the underlying risk variant(s) of SLE was best captured by these 6 SNPs located in the *TMEM87-IRAK1-MECP2* region in this study.

In conditional haplotype-based association testing, after conditioning on rs13397, rs4898375, rs1059702, rs2734647, rs2075596 and rs1616369, association signals of all other SNPs were completely eliminated or reduced to baseline in EA, AS, HA and AA (Table S1–S4), which supported that association signals detected in Xq28 could be attributed to these 6 SNPs.

Of note, in EA, rs2269368, rs2071129, rs2071130, rs5945377 and rs5945378 (named as group 1) in the *ARHGAP4-NAA10-RENBP* region exhibited even stronger association with SLE than rs13397, rs4898375, rs1059702, rs2734647, rs2075596 and rs1616369 (named as group 2) in the *TMEM187-IRAK1-MECP2* region (Table 1). Genetic effects of these two regions could not be distinguished in EA using conditional testing, in which association signals detected at either group of SNPs were completely eliminated when conditioning on another group (Figure S1). In contrast to EA, a stronger association with SLE was detected at group 2 rather than group 1 SNPs in AS and HA (Table 1). In these two datasets, when conditioning on group 2 SNPs, association signals detected at group 1 SNPs were completely eliminated or reduced to baseline (Figure S1). Whereas, when conditioning on group 1 SNPs, group 2 SNPs retained strong residual association signals. Thus, in AS and HA, association signals detected at group 1 SNPs might be attributed to group 2 SNPs. In AA, SNPs in the *ARHGAP4-NAA10-RENBP* region were not associated with SLE (Table 1). LD analysis showed that these two groups of SNPs were in strong LD in EA and HA ($r^2>0.7$), modest LD in AS ($r^2<0.5$) and low LD in AA ($r^2<0.3$). Taken together, these data suggest that association signals detected in the *ARHGAP4-NAA10-RENBP* region are driven by SLE-associated SNPs in the *TMEM187-IRAK1-MECP2* region.

IRAK1 SNP rs1059702 could best explain association signals detected in the Xq28 region

To further localize underlying causal variant(s) in the *TMEM187-IRAK1-MECP2* region, we performed conditional testing among rs13397, rs4898375, rs1059702, rs2734647, rs2075596 and rs1616369. In EA and AA, genetic effects of these 6 SNPs could not be distinguished (Figure S2), probably because they were in strong LD in EA and had too low MAFs in AA. In HA, when conditioning on rs13397, rs1059702, rs2075596 or rs1616369, association signals of the other 5 SNPs were completely eliminated. In contrast, conditioning on rs4898375 or rs2734647 showed residual signals at rs13397, rs1059702, rs2075596 and rs1616369. Thus, association signals detected at rs4898375 and rs2734647 could be attributed to rs13397, rs1059702, rs2075596 and rs1616369. In AS, rs4898375 and rs1059702 explained association signals detected at the other 4 SNPs. Taken together, only rs1059702 could explain the association signals of the other 5 SNPs in EA, AA, HA and AS, suggesting that rs1059702 was the most likely causal variant among the 6 SLE-associated SNPs. In meta-analysis, rs1059702 exhibited the strongest *P* value ($P_{meta}=1.3\times10^{-27}$, OR=1.43) (Table 1). Furthermore, the risk minor allele of rs1059702 (S196F in exon 5) of

IRAK1 leads to increased NF-κB activity,[29] suggesting it may confer risk of SLE by affecting the biological function.

The risk allele of rs1059702 was associated with lower mRNA levels of *MECP2*

Using RT-PCR, we measured mRNA levels of *IRAK1* and *MECP2* in PBMCs from 70 SLE cases (56 females and 14 males) and 73 healthy controls (32 females and 41 males) and assessed their association with rs1059702 genotypes. To exclude the influence of X-inactivation, EA males or females carrying a homozygous rs1059702 genotype were used. Compared to those carrying the non-risk GG or G genotype, subjects carrying the SLE-risk AA or A genotype had decreased *MECP2*, but not *IRAK1*, levels in both cases (P=0.0012) and controls (P=0.0064) (Figure 3). These data suggested that rs1059702, or other SLE-risk variants tagged by rs1059702, may confer risk of SLE by affecting expression of *MECP2*.

DISCUSSION

We comprehensively investigated the genetic association between Xq28 and SLE susceptibility. In addition to previously reported *IRAK1*, *MECP2* and *ARHGAP4*, 6 other genes on Xq28 were assessed. We identified SLE-associated SNPs in the *TMEM187-IRAK1-MECP2* region in 4 different ancestral groups, and identified rs1059702 (S196F) in *IRAK1* as the most likely causal variant. Furthermore, we showed that the SLE-risk genotype of rs1059702 was associated with lower mRNA levels of *MECP2*. Thus, our data suggested that both *IRAK1* and *MECP2* are SLE risk genes on Xq28.

The successful localization of a causal variant in our study should be attributed to conducting fine-mapping using high-density SNP markers and performing association testing in subjects with African ancestry. Using fine-mapping, we identified multiple Xq28 SNPs that were strongly associated with SLE in EA, AS and HA. However, these SNPs spanned a ~187 kb region from *ARHGAP4* to *MECP2* and their independent effects were difficult to distinguish due to strong LD. Compared to EA, AS and HA, the weaker LD at Xq28 in AA helped us localize association signals to a narrower region. Based on the findings that 6 SNPs in the *TMEM187-IRAK1-MECP2* region were associated with SLE in EA, AS, HA and AA with similar odds ratios and they could explain association signals of other Xq28 SNPs, we concluded that these 6 SNPs captured the underlying risk variant(s) shared by 4 ancestral groups. Because the risk allele frequency of these 6 SNPs are much lower in AA (~5%) than in EA (~15%), HA (40%) and AS (~75%), the association signals in AA did not reach the Bonferroni-corrected significance level.

IRAK1 plays a pivotal role in the activation of NF-κB. We identified the minor allele of rs1059702 on *IRAK1*, resulting in a serine to phenylalanine substitution at amino acid 196, as a likely causal variant for SLE. Previous functional study has shown that 196F IRAK1 variant confers increased NF-κB activity *in vitro*,[29] which is consistent with abrogation of all SLE-associated phenotypes in a IRAK1 deficient mouse lupus model.[7] Of note, the minor allele of rs1059703 (L532S) in exon 12 of *IRAK1* also confers increased NF-κB activity *in vitro*,[29] and minor alleles of both rs1059702 and rs1059703 have been associated with worse outcomes in sepsis [30] and increased risk of systemic sclerosis [31] in European-derived subjects. In this study, rs1059703 was associated with SLE in EA, AS and HA (Table 1), but not in AA (MAF of 34.7% in cases vs. 34.6% in controls, P=0.897. This P value was not shown in Table 1 and S4, because rs1059703 had a genotype missing rate of 6.7% in AA which exceeded our threshold of 5%). LD analysis showed that rs1059703 and rs1059702 were in strong LD in EA (r^2=0.94), AS (r^2=0.92) and HA (r^2=0.86), but in low LD in AA (r^2=0.10), suggesting that the association of rs1059703 with SLE in EA, AS and HA might be attributed to rs1059702. IRAK regulates signal transduction of IL-1R and toll-like receptors (TLR), playing a pivotal role in innate

immunity and autoimmunity. Of interest, IRAK-M, which mediates suppression of TLR7 signalling, has also been shown as a genetic risk for murine lupus.[32]

It is well recognized that rare variants in *MECP2* cause neurodevelopmental disorder Rett syndrome.[33] In this study, the SLE-risk genotype of rs1059702 was associated with lower mRNA levels of *MECP2* but not *IRAK1* in both cases and controls. Consistent with our results, the risk minor allele of rs1059702 was associated with lower mRNA levels of *MECP2* in an eQTL study using peripheral blood from 1,469 unrelated European subjects, [34] in which the minor allele of rs1059702 was also associated with lower levels of *RENBP* and *TMEM187* but at less significant levels. The finding suggests that lower MECP2 levels may have consequences similar to hypomethylation at CpG islands in genes that are regulated epigenetically leading to dysregulated expression of SLE-risk genes. The SLE-risk haplotype tagged by the minor allele of rs1059702 has been associated with the up-regulation of 13 interferon signature genes in B cell lines from female SLE patients.[10] The biological mechanism by which rs1059702 may alter the expression of *MECP2* is not known at present. Due to the strong LD in the Xq28 region, it is possible that rs1059702 tags a functional SNP that affects the expression of *MECP2*, but whether that SNP predisposes to SLE awaits confirmation in subjects from non-EA ancestral groups.

SNPs in the *ARHGAP4-NAA10-RENBP* region exhibited peak association with SLE in EA, although this strong association pattern was not replicated in AS, HA and AA. Based on our data, we favor the explanation that association signals detected in the *ARHGAP4-NAA10-RENBP* region are driven by SLE-associated SNPs in the *TMEM187-IRAK1-MECP2* region. However, it is also possible that SNPs used in this study failed to capture underlying causal variant(s) in the *ARHGAP4-NAA10-RENBP* region in AS, HA and AA due to different LD patterns in these ancestral groups. *ARHGAP4* is a hematopoietic specific gene that belongs to the RhoGAP familiy. A deletion spanning *AVPR2* and *ARHGAP4* causes congenital nephrogenic diabetes insipidus and has been associated with severe immumodeficiency.[35] *NAA10* encodes the catalytic subunit of the major human N-terminal acetyltrasferase.[36] NAA10 knockdown reduced the growth rate in human cancer cell lines.[37] *NAA10* variant Ser37Pro results in an X-linked lethal disorder of infancy due to N-terminal acetyltransferase deficiency.[38] RENBP inhibits the activity of renin, [39] and the renin-angiotensinogen system has been implicated in SLE susceptibility.[40] Whether *ARHGAP4*, *NAA10* and *RENBP* are SLE susceptibility genes will need further investigation.

In conclusion, by taking advantage of the power of trans-ancestral mapping, we identified rs1059702 as the likely causal variant predisposing to SLE susceptibility in 4 different ancestral groups. This SNP leads to an amino acid change on *IRAK1* (S196F), with known function of increasing NF-κB activity, and is associated with lower levels of *MECP2*, suggesting both *IRAK1* and *MECP2* are SLE susceptibility genes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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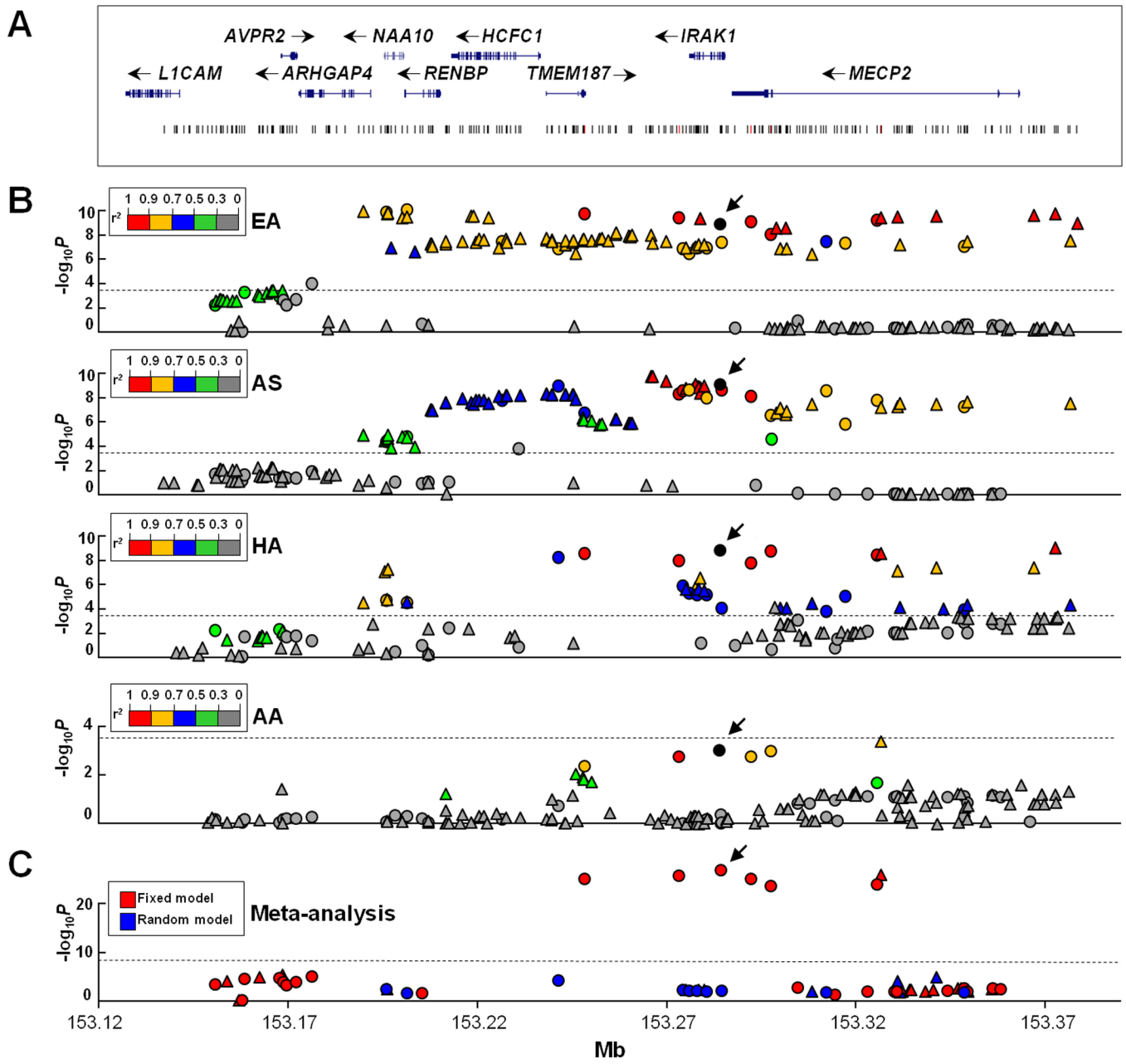
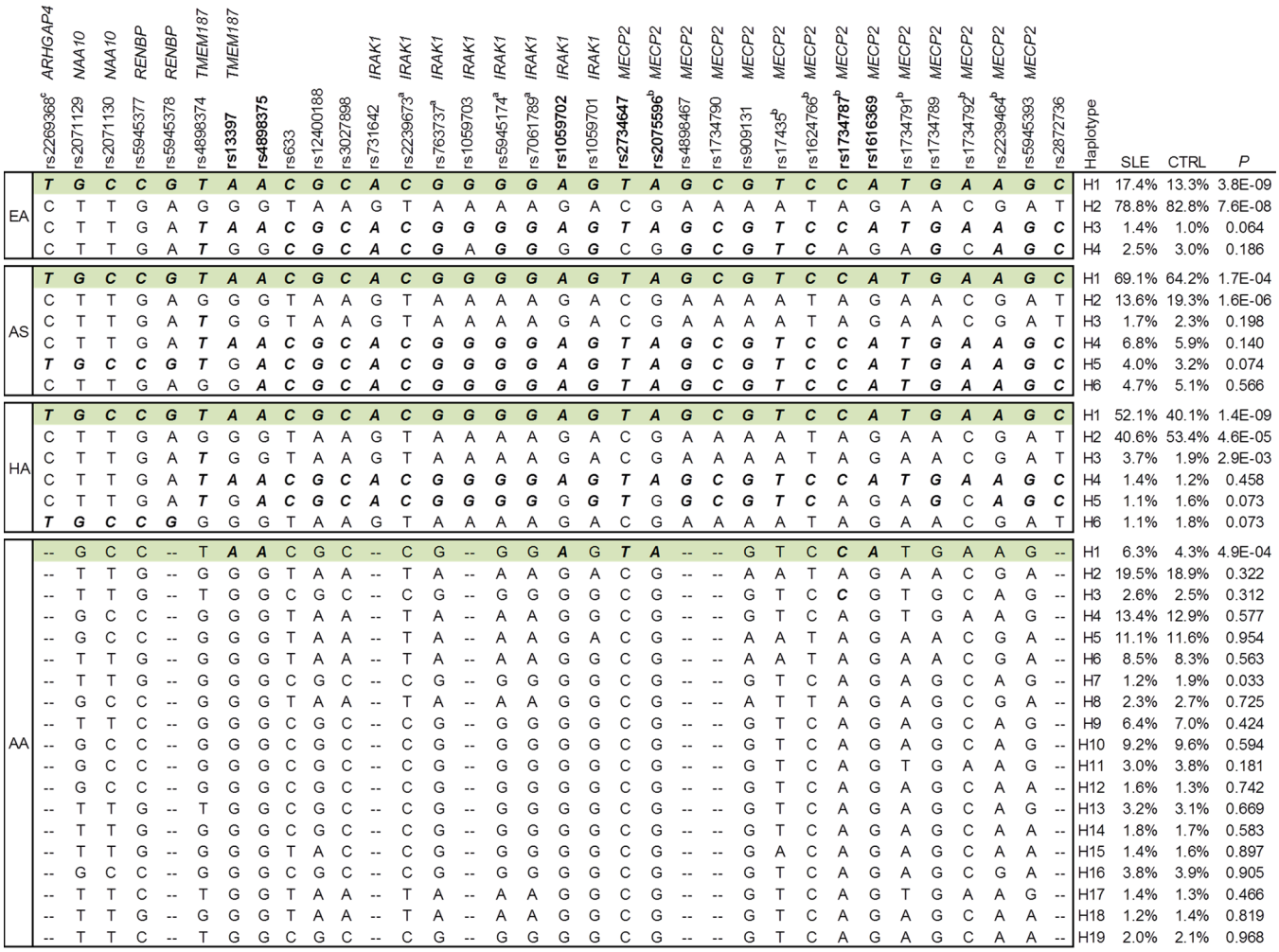


Figure 1. Association of SNPs in the Xq28 region with SLE

A) The genomic structure of the Xq28 region and the location of all SNPs are indicated. B) Association signals ($-\log_{10}P$) are plotted against the position of each SNP in EA, AS, HA and AA, respectively. Genotyped and imputed SNPs are indicated as circles and triangles, respectively. SNPs are highlighted using different colors according to their LD strength (r^2) with rs1059702 (shown as a black circle). An arrowhead is used to indicate the position of rs1059702. The dashed line represents the significance level after Bonferroni correction. C) Trans-ancestry meta-analysis *P* value generated using fixed and random model are highlighted as red and blue, respectively. The dashed line represents the significance level of 5×10^{-8}.



	ARHGAP4	NAA10	NAA10	RENBP	RENBP	TMEM187	TMEM187					IRAK1	IRAK1	IRAK1	IRAK1	IRAK1	IRAK1	IRAK1	IRAK1	IRAK1	MECP2	MECP2	MECP2	MECP2	MECP2	MECP2	MECP2	MECP2	MECP2	MECP2	MECP2	MECP2	MECP2	MECP2				
	rs2269368[c]	rs2071129	rs2071130	rs5945377	rs5945378	rs4898374	**rs13397**	**rs4899375**	rs633	rs12400188	rs3027898	rs731642	rs2239673[a]	rs763737[a]	rs1059703	rs5945174[a]	rs7061789[a]	**rs1059702**	rs1059701	**rs2734647**	**rs2075596**[b]	rs4898467	rs1734790	rs909131	rs17435[b]	rs1624766[b]	**rs1734787**[b]	**rs1616369**	rs1734791[b]	rs1734789	rs1734792[b]	rs2239464[b]	rs5945393	rs2872736	Haplotype	SLE	CTRL	*P*
EA	***T***	***G***	***C***	***C***	***G***	***T***	***A***	***A***	***C***	***G***	***C***	***A***	***C***	***G***	***G***	***G***	***G***	***A***	***G***	***T***	***A***	***G***	***C***	***G***	***T***	***C***	***C***	***A***	***T***	***G***	***A***	***A***	***G***	***C***	H1	17.4%	13.3%	3.8E-09
	C	T	T	G	A	G	G	G	T	A	A	G	T	A	A	A	A	G	A	C	G	A	A	A	A	T	A	G	A	A	C	G	A	T	H2	78.8%	82.8%	7.6E-08
	C	T	T	G	A	***T***	***A***	***A***	***C***	***G***	***C***	***A***	***C***	***G***	***G***	***G***	***G***	***A***	***G***	***T***	***A***	***G***	***C***	***G***	***T***	***C***	***C***	***A***	***T***	***G***	***A***	***A***	***G***	***C***	H3	1.4%	1.0%	0.064
	C	T	T	G	A	***T***	G	G	***C***	***G***	***C***	***A***	***C***	***G***	A	***G***	***G***	G	***G***	C	G	***G***	***C***	***G***	***T***	***C***	A	G	A	***G***	C	***A***	***G***	***C***	H4	2.5%	3.0%	0.186
AS	***T***	***G***	***C***	***C***	***G***	***T***	***A***	***A***	***C***	***G***	***C***	***A***	***C***	***G***	***G***	***G***	***G***	***A***	***G***	***T***	***A***	***G***	***C***	***G***	***T***	***C***	***C***	***A***	***T***	***G***	***A***	***A***	***G***	***C***	H1	69.1%	64.2%	1.7E-04
	C	T	T	G	A	G	G	G	T	A	A	G	T	A	A	A	A	G	A	C	G	A	A	A	A	T	A	G	A	A	C	G	A	T	H2	13.6%	19.3%	1.6E-06
	C	T	T	G	A	***T***	G	G	T	A	A	G	T	A	A	A	A	G	A	C	G	A	A	A	A	T	A	G	A	A	C	G	A	T	H3	1.7%	2.3%	0.198
	C	T	T	G	A	***T***	***A***	***A***	***C***	***G***	***C***	***A***	***C***	***G***	***G***	***G***	***G***	***A***	***G***	***T***	***A***	***G***	***C***	***G***	***T***	***C***	***C***	***A***	***T***	***G***	***A***	***A***	***G***	***C***	H4	6.8%	5.9%	0.140
	T	***G***	***C***	***C***	***G***	***T***	G	***A***	***C***	***G***	***C***	***A***	***C***	***G***	***G***	***G***	***G***	***A***	***G***	***T***	***A***	***G***	***C***	***G***	***T***	***C***	***C***	***A***	***T***	***G***	***A***	***A***	***G***	***C***	H5	4.0%	3.2%	0.074
	C	T	T	G	A	G	G	***A***	***C***	***G***	***C***	***A***	***C***	***G***	***G***	***G***	***G***	***A***	***G***	***T***	***A***	***G***	***C***	***G***	***T***	***C***	***C***	***A***	***T***	***G***	***A***	***A***	***G***	***C***	H6	4.7%	5.1%	0.566
HA	***T***	***G***	***C***	***C***	***G***	***T***	***A***	***A***	***C***	***G***	***C***	***A***	***C***	***G***	***G***	***G***	***G***	***A***	***G***	***T***	***A***	***G***	***C***	***G***	***T***	***C***	***C***	***A***	***T***	***G***	***A***	***A***	***G***	***C***	H1	52.1%	40.1%	1.4E-09
	C	T	T	G	A	G	G	G	T	A	A	G	T	A	A	A	A	G	A	C	G	A	A	A	A	T	A	G	A	A	C	G	A	T	H2	40.6%	53.4%	4.6E-05
	C	T	T	G	A	***T***	G	G	T	A	A	G	T	A	A	A	A	G	A	C	G	A	A	A	A	T	A	G	A	A	C	G	A	T	H3	3.7%	1.9%	2.9E-03
	C	T	T	G	A	***T***	***A***	***A***	***C***	***G***	***C***	***A***	***C***	***G***	***G***	***G***	***G***	***A***	***G***	***T***	***A***	***G***	***C***	***G***	***T***	***C***	***C***	***A***	***T***	***G***	***A***	***A***	***G***	***C***	H4	1.4%	1.2%	0.458
	C	T	T	G	A	***T***	G	***A***	***C***	***G***	***C***	***A***	***C***	***G***	***G***	***G***	***G***	G	***G***	***T***	G	***G***	***C***	***G***	***T***	***C***	A	G	A	***G***	C	***A***	***G***	***C***	H5	1.1%	1.6%	0.073
	T	***G***	***C***	***C***	***G***	G	G	G	T	A	A	G	T	A	A	A	A	G	A	C	G	A	A	A	A	T	A	G	A	A	C	G	A	T	H6	1.1%	1.8%	0.073
AA	--	G	C	C	--	T	***A***	***A***	C	G	C	--	C	G	--	G	G	***A***	G	***T***	***A***	--	--	G	T	C	***C***	***A***	T	G	A	A	G	--	H1	6.3%	4.3%	4.9E-04
	--	T	T	G	--	G	G	G	T	A	A	--	T	A	--	A	A	G	A	C	G	--	--	A	A	T	A	G	A	A	C	G	A	--	H2	19.5%	18.9%	0.322
	--	T	T	G	--	T	G	G	C	G	C	--	C	G	--	G	G	G	G	C	G	--	--	G	T	C	***C***	G	T	G	C	A	G	--	H3	2.6%	2.5%	0.312
	--	G	C	C	--	G	G	G	T	A	A	--	T	A	--	A	A	G	G	C	G	--	--	G	T	C	A	G	T	G	A	A	G	--	H4	13.4%	12.9%	0.577
	--	G	C	C	--	G	G	G	T	A	A	--	T	A	--	A	A	G	A	C	G	--	--	A	A	T	A	G	A	A	C	G	A	--	H5	11.1%	11.6%	0.954
	--	T	T	G	--	G	G	G	T	A	A	--	T	A	--	A	A	G	G	C	G	--	--	A	A	T	A	G	A	A	C	G	A	--	H6	8.5%	8.3%	0.563
	--	T	T	G	--	G	G	G	C	G	C	--	C	G	--	G	G	G	G	C	G	--	--	G	T	C	A	G	A	G	C	A	G	--	H7	1.2%	1.9%	0.033
	--	G	C	C	--	G	G	G	T	A	A	--	T	A	--	A	A	G	G	C	G	--	--	A	T	T	A	G	A	G	C	G	A	--	H8	2.3%	2.7%	0.725
	--	T	T	C	--	G	G	G	C	G	C	--	C	G	--	G	G	G	G	C	G	--	--	G	T	C	A	G	A	G	C	A	G	--	H9	6.4%	7.0%	0.424
	--	G	C	C	--	G	G	G	C	G	C	--	C	G	--	G	G	G	G	C	G	--	--	G	T	C	A	G	A	G	C	A	G	--	H10	9.2%	9.6%	0.594
	--	G	C	C	--	G	G	G	C	G	C	--	C	G	--	G	G	G	G	C	G	--	--	G	T	C	A	G	T	G	A	A	G	--	H11	3.0%	3.8%	0.181
	--	G	C	C	--	G	G	G	C	G	C	--	C	G	--	G	G	G	G	C	G	--	--	G	T	C	A	G	A	G	C	A	A	--	H12	1.6%	1.3%	0.742
	--	T	T	G	--	T	G	G	C	G	C	--	C	G	--	G	G	G	G	C	G	--	--	G	T	C	A	G	A	G	C	A	G	--	H13	3.2%	3.1%	0.669
	--	T	T	G	--	G	G	G	C	G	C	--	C	G	--	G	G	G	G	C	G	--	--	G	T	C	A	G	A	G	C	A	A	--	H14	1.8%	1.7%	0.583
	--	T	T	G	--	G	G	G	T	A	C	--	C	G	--	G	G	G	G	C	G	--	--	G	A	C	A	G	A	G	C	A	A	--	H15	1.4%	1.6%	0.897
	--	G	C	C	--	G	G	G	C	G	C	--	C	G	--	G	G	G	G	C	G	--	--	G	T	C	A	G	A	G	C	G	A	--	H16	3.8%	3.9%	0.905
	--	T	T	C	--	T	G	G	T	A	A	--	T	A	--	A	A	G	G	C	G	--	--	G	T	C	A	G	T	G	A	A	G	--	H17	1.4%	1.3%	0.466
	--	T	T	G	--	G	G	G	T	A	A	--	T	A	--	A	A	G	G	C	G	--	--	G	T	C	A	G	A	G	C	A	A	--	H18	1.2%	1.4%	0.819
	--	T	T	C	--	T	G	G	C	G	C	--	C	G	--	G	G	G	G	C	G	--	--	G	T	C	A	G	A	G	C	A	A	--	H19	2.0%	2.1%	0.968

Figure 2. A SLE-risk haplotype share by all four ancestral groups

Haplotypes were constructed using 34 SNPs shown in Table 1. Haplotype H1 (highlighted in green) was consistently associated with SLE in all 4 ancestral groups. Allele conferring risk of SLE is bolded and italicized.

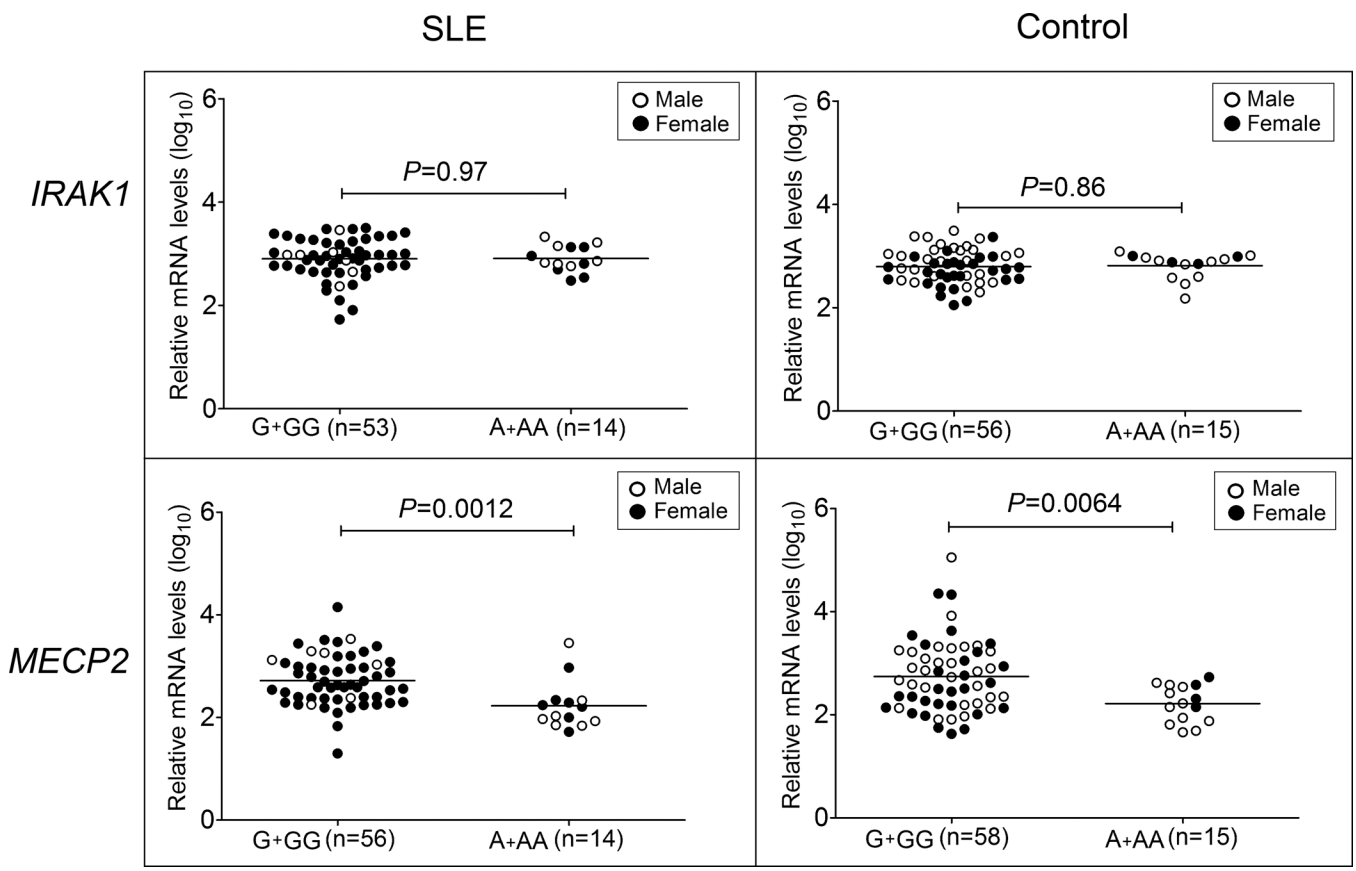


Figure 3. Association of rs1059702 genotype with *IRAK1* and *MECP2* mRNA levels

Expression levels of *IRAK1* and *MECP2* (total level of all isoforms) were measured in PBMCs of SLE patients and healthy controls with European ancestry using real-time quantitative PCR. The expression level of housekeeping gene *RPLP0* was used as an endogenous control. Log10 value of relative mRNA levels of *IRAK1* and *MECP2* were compared between different genotypes of rs1059702 (G+GG vs. A+AA) in SLE and control groups, respectively, using t test. Females are highlighted as black.

Table 1

Significant association of SNPs at Xq28 with SLE

				Tested	EA			AS			HA			AA			Meta-analysis	
Type	**SNP**	**Gene**	**Annotation**	**allele**	***P***	**OR**	***Pc***	***P***	**OR**	***Pc***	***P***	**OR**	***Pc***	***P***	**OR**	***Pc***	***P***	**OR**
I	rs2269368[c]	*ARHGAP4*	intronic	T	1.3E-10	1.38[1.25-1.52]	0.98	1.3E-05	1.32[1.17-1.49]	0.06	3.4E-05	1.32[1.16-1.50]	0.05	M	M	--	M	M
G	rs2071129	*NAA10*	intronic	G	1.3E-10	1.38[1.25-1.52]	0.87	4.1E-05	1.29[1.14-1.46]	0.02	1.9E-05	1.32[1.16-1.50]	0.19	0.818	1.01[0.92-1.12]	--	4.7E-03	1.24
I	rs2071130	*NAA10*	intronic	C	2.0E-10	1.37[1.25-1.52]	0.99	2.7E-05	1.30[1.15-1.47]	0.03	1.9E-05	1.32[1.16-1.51]	0.11	0.773	1.02[0.92-1.12]	--	3.9E-03	1.24
G	rs5945377	*RENBP*	intronic	C	8.4E-11	1.38[1.25-1.52]	0.86	1.7E-05	1.31[1.16-1.48]	0.02	3.2E-05	1.31[1.16-1.49]	0.22	0.465	0.96[0.87-1.07]	--	2.2E-02	1.23
I	rs5945378	*RENBP*	intronic	G	3.6E-10	1.37[1.24-1.51]	0.79	2.0E-05	1.31[1.16-1.48]	0.03	2.9E-05	1.32[1.16-1.50]	0.19	M	M	--	M	M
G	rs4898374	*TMEM187*	intronic	T	1.3E-07	1.28[1.17-1.40]	0.42	1.2E-09	1.53[1.34-1.76]	0.32	5.6E-09	1.48[1.30-1.69]	0.03	0.184	1.09[0.96-1.25]	--	6.2E-05	1.33
G	**rs13397**	*TMEM187*	T245T	A	1.9E-10	1.37[1.24-1.51]	*	1.8E-07	1.39[1.23-1.58]	*	3.0E-09	1.48[1.30-1.69]	*	4.2E-03	1.41[1.12-1.79]	*	8.4E-26	1.40[F]
G	**rs4898375**		Intergenic	A	3.9E-10	1.36[1.23-1.49]	*	5.2E-09	1.52[1.32-1.75]	*	1.2E-08	1.46[1.28-1.67]	*	1.7E-03	1.44[1.15-1.82]	*	1.5E-26	1.42[F]
G	rs633		Intergenic	C	1.5E-07	1.26[1.16-1.37]	0.94	2.6E-09	1.55[1.34-1.79]	0.34	1.3E-06	1.37[1.21-1.56]	0.53	0.950	1.00[0.91-1.11]	--	6.0E-03	1.27
I	rs12400188		Intergenic	G	1.2E-07	1.26[1.16-1.38]	0.82	1.7E-09	1.56[1.35-1.80]	0.30	2.3E-06	1.37[1.20-1.55]	0.42	0.998	1.00[0.99-1.11]	--	6.7E-03	1.27
G	rs3027898		Intergenic	C	3.4E-07	1.25[1.15-1.36]	0.77	2.3E-09	1.55[1.34-1.79]	0.28	5.5E-06	1.35[1.19-1.54]	0.43	0.948	1.00[0.99-1.19]	--	7.9E-03	1.26
I	rs731642	*IRAK1*	intronic	A	1.0E-07	1.27[1.16-1.39]	0.92	8.7E-10	1.58[1.36-1.82]	ND	1.9E-06	1.37[1.21-1.57]	0.34	M	M	--	M	M
G	rs2239673[a]	*IRAK1*	intronic	C	1.3E-07	1.26[1.16-1.38]	0.96	1.3E-09	1.57[1.35-1.81]	0.35	7.3E-06	1.34[1.18-1.53]	0.46	0.976	1.00[0.90-1.19]	--	7.5E-03	1.27
I	rs763737[a]	*IRAK1*	intronic	G	7.0E-08	1.27[1.16-1.39]	0.99	1.5E-09	1.56[1.35-1.81]	0.29	2.5E-06	1.36[1.20-1.55]	0.57	0.999	1.00[0.90-1.11]	--	6.7E-03	1.28
I	rs1059703	*IRAK1*	L532S/intronic	G	4.9E-10	1.35[1.23-1.48]	ND	4.3E-09	1.53[1.33-1.77]	ND	2.8E-07	1.41[1.24-1.60]	0.73	M	M	--	M	M
I	rs5945174[a]	*IRAK1*	intronic	G	6.9E-08	1.27[1.16-1.39]	0.98	1.1E-09	1.57[1.36-1.82]	0.29	3.4E-06	1.36[1.19-1.55]	0.53	0.945	1.00[0.91-1.11]	--	6.0E-03	1.28
G	rs7061789[a]	*IRAK1*	intronic	G	1.3E-07	1.26[1.16-1.38]	0.96	1.0E-08	1.53[1.32-1.77]	0.22	6.8E-06	1.34[1.18-1.53]	0.28	0.650	0.98[0.88-1.08]	--	1.3E-02	1.26
G	**rs1059702**	*IRAK1*	S196F	A	1.2E-09	1.35[1.22-1.48]	*	8.2E-10	1.56[1.35-1.79]	*	1.5E-09	1.49[1.31-1.70]	*	1.0E-03	1.48[1.17-1.87]	*	1.3E-27	1.43[F]
G	rs1059701	*IRAK1*	V562V	G	3.9E-08	1.27[1.17-1.39]	0.75	2.5E-09	1.55[1.34-1.79]	0.24	9.0E-05	1.30[1.14-1.48]	0.09	0.879	0.99[0.89-1.11]	--	7.3E-03	1.26
G	**rs2734647**	*MECP2*	3'UTR/intergenic	T	7.8E-10	1.35[1.23-1.48]	*	7.9E-09	1.51[1.32-1.74]	*	1.7E-08	1.46[1.28-1.66]	*	1.7E-03	1.42[1.14-1.78]	*	7.1E-26	1.41[F]
G	**rs2075596**[b]	*MECP2*	intronic	A	9.0E-09	1.33[1.21-1.46]	*	3.0E-07	1.44[1.25-1.66]	*	1.8E-09	1.50[1.31-1.71]	*	9.8E-04	1.45[1.16-1.80]	*	2.2E-24	1.40[F]
I	rs4898467	*MECP2*	intronic	G	1.4E-07	1.27[1.16-1.39]	0.93	8.0E-08	1.48[1.28-1.71]	ND	9.0E-05	1.30[1.14-1.48]	0.03	M	M	--	M	M
I	rs1734790	*MECP2*	intronic	C	1.4E-07	1.27[1.16-1.39]	0.93	1.5E-07	1.47[1.27-1.70]	ND	9.0E-05	1.30[1.14-1.48]	0.02	M	M	--	M	M
I	rs909131	*MECP2*	intronic	G	4.1E-07	1.25[1.15-1.37]	0.91	3.6E-08	1.50[1.30-1.73]	ND	3.8E-05	1.32[1.16-1.50]	0.14	0.775	0.98[0.89-1.09]	--	1.0E-02	1.24
G	rs17435[b]	*MECP2*	intronic	T	3.6E-08	1.28[1.17-1.39]	0.67	2.7E-09	1.53[1.33-1.76]	ND	1.8E-04	1.28[1.13-1.46]	0.02	0.522	0.97[0.87-1.07]	--	1.8E-02	1.24

				Tested	EA			AS			HA			AA			Meta-analysis	
Type	**SNP**	**Gene**	**Annotation**	**allele**	***P***	**OR**	***Pc***	***P***	**OR**	***Pc***	***P***	**OR**	***Pc***	***P***	**OR**	***Pc***	***P***	**OR**
G	rs1624766[b]	*MECP2*	intronic	C	5.0E-08	1.28[1.17-1.40]	0.77	1.5E-06	1.42[1.23-1.64]	ND	9.9E-06	1.35[1.18-1.54]	0.34	M	M	--	M	M
G	**rs1734787**[b]	*MECP2*	intronic	C	6.4E-10	1.35[1.23-1.49]	ND	1.6E-08	1.50[1.31-1.73]	ND	4.0E-09	1.48[1.30-1.69]	ND	0.021	1.22[1.03-1.45]	0.91	9.4E-25	1.39[F]
I	**rs1616369**	*MECP2*	intronic	A	4.2E-10	1.36[1.23-1.49]	*	6.8E-08	1.48[1.28-1.71]	*	2.9E-09	1.49[1.30-1.69]	*	4.0E-04	1.50[1.20-1.88]	*	1.2E-26	1.43[F]
I	rs1734791[b]	*MECP2*	intronic	T	3.4E-10	1.36[1.23-1.49]	ND	5.7E-08	1.49[1.29-1.71]	ND	7.8E-08	1.43[1.26-1.63]	0.14	0.210	1.08[0.96-1.21]	--	9.1E-05	1.32
I	rs1734789	*MECP2*	intronic	G	6.9E-08	1.27[1.16-1.38]	0.91	3.3E-08	1.51[1.30-1.74]	ND	7.6E-05	1.30[1.14-1.48]	0.08	0.492	0.96[0.87-1.07]	--	1.7E-02	1.24
I	rs1734792[b]	*MECP2*	intronic	A	3.1E-10	1.36[1.24-1.49]	ND	3.8E-08	1.50[1.30-1.73]	ND	4.4E-08	1.44[1.26-1.64]	0.14	0.134	1.10[0.97-1.24]	--	1.4E-05	1.34
G	rs2239464[b]	*MECP2*	intronic	A	8.4E-08	1.27[1.16-1.39]	0.79	5.3E-08	1.49[1.29-1.72]	ND	1.2E-04	1.29[1.13-1.46]	0.03	0.517	0.97[0.87-1.07]	--	1.8E-02	1.23
I	rs5945393	*MECP2*	intronic	G	3.5E-08	1.28[1.17-1.40]	0.84	2.3E-08	1.51[1.31-1.74]	ND	5.1E-05	1.31[1.15-1.49]	0.08	0.665	0.98[0.88-1.08]	--	1.3E-02	1.25
I	rs2872736		Intergenic	C	3.4E-08	1.28[1.17-1.39]	0.89	3.0E-08	1.51[1.31-1.75]	ND	5.3E-05	1.31[1.15-1.48]	0.09	M	M	--	M	M

Abbreviation: G, genotyped SNP; I, imputed SNP; OR, odds ratio; *Pc*, *P* value after conditioning on 6 SNPs shown as "*"; ND, non-distinguishable in conditional testing; F, fixed effect model in meta-analysis; M, missing data. Only SNPs that remained significant association with SLE after correction for multiple comparisons in EA, AS and HA are listed in this table. Position of each SNP is based on GRch37. SNPs that showed consistent association with SLE in all 4 ancestral groups are highlighted in bold. For SNPs that were not tested in conditional testing ($P>0.05$), the *Pc* value is denoted as "--". Previously reported SLE-associated SNPs located in *IRAK1*,[7] *MECP2* [6] and *ARHGAP* [17] were noted using "a", "b" and "c", respectively, all of which were confirmed to be significantly associated with SLE in EA, AS and HA.

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