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Genome-wide association study identifies three novel susceptibility loci for systemic lupus erythematosus in Han Chinese

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DEAR EDITOR, Systemic lupus erythematosus (SLE) is a common prototypic autoimmune disease with substantial genetic predispositions. It is more prevalent in Asians than in Caucasians. Genome wide association studies (GWAS) have discovered more than 80 genetic loci for the risk of SLE[1], which improve the understanding of SLE etiology and provide potential therapeutic targets. However, each GWAS finding only confers a relatively small effect, and they in total cannot

fully explain SLE heritability, suggesting more genetic variants are yet to be discovered.

To detect novel susceptibility loci for SLE, we conducted a two-stage GWAS in a Han Chinese population. All patients met the revised American College of Rheumatology SLE classification criteria[2]. The age- matched controls were recruited without SLE, family history of SLE or any other autoimmune diseases. Each participant provided written informed consent. The study was approved by the Institutional Review Board of Anhui Medical University, China, and was conducted according to the Declaration of Helsinki.

In the discovery stage, we performed strict quality control on variants and samples in the SLE GWAS data set, which have been described before[2]. After quality control, the genotype data of 493,955 autosomal SNPs in 1,047 SLE cases and 1,205 controls were imputed using IMPUTE 2 together with the 1000 Genomes Project reference data (phase 1 integrated set, March 2012, build 37). The SNPs with imputation INFO scores of >0.9 were included in further analyses. A logistic regression model (additive model) was used for single-variant association analysis with gender as a covariate.

In the replication stage, we genotyped 82 top SNPs in an independent cohort of 3,509 cases and 8,246 controls using the Sequenom MassARRAY system. The SNPs were chosen if they satisfied several criteria: (1) beyond 500kb from any SLE locus; (2) not within the major histocompatibility complex region; (3) with $P < 5.00\times10^{-4}$ in the discovery stage; (4) Hardy-Weinberg Equilibrium $P \geqslant 10^{-4}$ in both controls and cases; (5) Minor allele frequency > 1%; (6) within known susceptibility genes/loci for autoimmune disorders. We tested their association for the risk of SLE in Plink 1.07 using logistic regression (additive model) with gender as a covariate.

We aggregated the association evidence through a meta-analysis using METAL and evaluated the heterogeneity between the two stages via the I^2 and Q statistics. There were 17 SNPs that met the threshold of genome-wide significance but 14 of them failed a Hardy-Weinberg Equilibrium test at $P < 10^{-4}$ or with $I^2 > 30\%$. We identified three novel loci associated with disease: *KIT* (rs2855772_C, Odds ratio (OR) = 1.40, $P_{meta} = 1.21\times10^{-15}$), *GPR78* (rs13116227_T, OR = 1.34, $P_{meta} = 3.05\times10^{-11}$),

and *TRAPPC11* (rs10018951_T, OR = 1.31, $P_{meta} = 1.18\times10^{-14}$) (Table 1). Rs2855772 is resided in an intron of *KIT* which is a tyrosine kinase receptor and plays a key role in cell differentiation and survival of immune cells. The *KIT* ligand-binding can promote the activation of STAT family members in JAK/STAT signaling which is critical in SLE[3]. Moreover, the soluble *KIT* (s*KIT*) level is significantly lower in SLE patients and is correlated with titer of anti-DNA antibody and the SLE activity index (SLEDAI) score. It is also negatively affected by high doses of corticosteroid for SLE therapy[4]. Rs13116227 locates ~2.2 kb 5'-upstream of *GPR78* ,which is a member of G Protein–Coupled Receptor and coupled to stimulatory (Gs) protein resulting in increased cyclic AMP (cAMP) which then inhibits NF-κB activation[5]. Rs10018951 locates in an intron of the *TRAPPC11* gene and encodes the enhancer regulatory function for *TRAPPC11* in CD14+ monocytes and skin[6]. *TRAPPC11* encodes a component of the transport protein particle (TRAPP) complex. Depletion of TRAPPC11 will cause a stressed unfolded protein response (UPR). Persistent UPR results in endoplasmic reticulum stress, which in turn is associated with cell dysfunction and apoptosis[7]. Apoptosis-related genes have been found to have more than twofold higher expression levels in SLE patients with active disease compared to those with inactive disease[8].

We conducted Real-Time PCR (RT-PCR) and expression quantitative trait loci（eQTL）to determine gene expression differences and regulatory effect of SNPs, respectively. We also analyzed the correlation between genes' mRNA expression levels and clinical parameters of SLE. Unfortunately, the results were statistically insignificant after correcting for multiple testing.

In conclusion, we identified three novel susceptibility regions at *KIT, GPR78* and *TRAPPC11* for SLE. These discoveries provide new insights into the genetic and biological basis of SLE.

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Table 1. Three novel genome-wide significant SLE SNPs that was identified in this study.

SNP	Chr	Positon (hg19)	Gene*	Allele**	GWAS				Replication				Meta		
					1,047 cases, 1,205 controls				3,509 cases, 8,246 control				4,556cases, 9451controls		
					MAF		OR	P***	MAF		OR	P***	P_{hew}	OR	P^{****}_{meta}	I^2
					Case	Control	(95%CI)		Case	Control	(95%CI)					
rs2855772	4	55548475	KIT	C/T	0.1433	0.1041	1.44(1.20-1.72)	6.44E-05	0.1312	0.09772	1.39(1.27-1.53)	2.95E-12	0.04074	1.40	1.21E-15	0
rs13116227	4	8558266	2.2kb 5' of GPR78	T/C	0.1299	0.09253	1.46(1.21-1.77)	6.37E-05	0.1211	0.09561	1.30(1.18-1.44)	5.57E-08	0.3896	1.34	3.05E-11	13.71%
rs10018951	4	184609373	TRAPPC11	T/C	0.2101	0.1622	1.37(1.81-1.60)	3.60E-05	0.193	0.1555	1.30(1.20-1.41)	4.86E-11	0.5372	1.31	1.18E-14	0

*Annotated by Haploreg v4.1 (http://archive.broadinstitute.org/mammals/haploreg/haploreg.php）

**Minor allele/Major allele.

***Association statistic adjusted for gender.

****Association statistic adjusted for gender and study.

The details of other SNPs selected for the replication study can be obtained by application to the corresponding author.