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ELF1 is associated with systemic lupus erythematosus in Asian populations

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Systemic lupus erythematosus (SLE) is an autoimmune disease with a strong genetic involvement. The susceptibility genes identified so far can only explain a small proportion of disease heritability. Through a genome-wide association in a Hong Kong Chinese cohort and subsequent replication in two other Asian populations, with a total of 3164 patients and 4482 matched controls, we identified association of *ELF1* (E74-like factor 1) with SLE (rs7329174, OR = 1.26, joint $P = 1.47 \times 10^{-8}$). *ELF1* belongs to the ETS family of transcription factors and is known to be involved in T cell development and function. Database analysis revealed transcripts making use of three alternative exon1s for this gene. Near equivalent expression levels of distinct transcripts initiated from alternative exon1s were detected in peripheral blood mononuclear cells from both SLE patients and healthy controls. Although a direct association of rs7329174 with the three forms of transcripts for this gene was not detected, these findings support an important role of *ELF1* in SLE susceptibility and suggest a potentially tight regulation for the expression of this gene.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a prototype autoimmune disease characterized by autoantibody production and multiorgan damage. It mainly affects women of childbearing age and has population differences in both disease prevalence and severity (1,2). Genetic factors are known to

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play key roles in the disease, with ~30 times increased risk for siblings of those affected, and further increased risk for monozygotic twins (3–5).

Through genome-wide association studies (GWAS) and other family- or population-based studies, a number of SLE susceptibility genes have been established through replications across populations. For example, after the initial report on the association of *IRF5* with the disease (6), its association was then confirmed in other European populations (7–9) and in Asian populations (10–12). The association of *STAT4* with SLE initially identified in Caucasians (13) was quickly confirmed in other populations such as Asians and Colombians (14–17). Association of *ITGAM* to SLE was confirmed in Chinese, although the risk alleles have very low frequency (18). Interestingly, two simultaneous Asian GWAS on SLE, including our own (19,20), identified association of *ETS1* and *WDFY4* with the disease, which had not been detected in earlier studies based on the populations of European ancestry, raising a possibility of population differences for disease association for these loci. *PXK* was found to be associated with SLE in Caucasians (21,22) but not in Asians (14,23).

ELF1 (E74-like factor 1) belongs to the ETS family of transcription factors that regulate the expression of a wide range of genes and play important roles in hematopoiesis, immune cell development and function, and angiogenesis (24,25). It acts as both an enhancer and a repressor to regulate the transcription of a variety of genes (26,27). Previously, we identified *ETS1*, a prototype ETS family transcription factor, as associated with SLE in Asian populations (19). Here, we report association of *ELF1* with SLE in Chinese populations living in Hong Kong and Mainland, China, respectively, and a Thai population living in Bangkok. Database analysis and subsequent RT–PCR experiment identified interesting features of this gene, suggestive of tight regulation of its expression.

RESULTS

Table 1 shows SNPs in and around the *ELF1* gene on chromosome 13q10 with significant disease association ($P < 0.005$) from the GWAS stage, which includes 612 SLE patients of Hong Kong Chinese and 1160 matched controls. This GWAS partially overlaps with our previously reported GWAS finding ETS1 and WDFY4 associated with SLE (19), but reflecting a doubling of SLE samples genotyped by the same platform. We observe a genomic inflation factor of 1.045 and a good match between cases and controls of the Hong Kong samples, analyzed by principal component introduced in EIGEN-STRAT (28), similar to what reported before (19). SNP rs7329174 showed the most significant association with the disease, and conditional logistic regression did not present convincing evidence for any independent contributions from other SNPs in this region, although this should be viewed with caution since tests on independence are usually underpowered. Figure 1 shows linkage disequilibrium (LD) among these SNPs, ranging from low to moderate LD ($r^2 = 0.06–0.44$).

We have chosen rs7329174 for further replication in the remaining samples from Hong Kong and samples from another Chinese population in Anhui, central China, as well as samples from Bangkok, Thailand. Association analysis was done by comparing cases with controls matched geographically. Although we observed a smaller effect size and marginal significance in the Anhui samples and the samples from Bangkok, Thailand, nevertheless the same trend was observed in all three populations (Table 2). A marginal *P*-value was observed in a heterogeneity test of different ORs in the three cohorts (by the Breslow–Day test, $P = 0.09$), suggesting only minor differences, if any.

A marginal significant association with lupus nephritis was also observed for rs7329174 in a patient-only test, but the effect is only significant for the Hong Kong samples (OR = 1.25, $P = 0.02$). In addition to renal nephritis, we also saw a significant association of this SNP in a patient-only analysis to dsDNA antibodies production (OR = 1.27, $P = 0.02$). Analysis of other subphenotypes did not find significant differences through the patient-only analysis. A note of caution is that none of the subphenotype associations would survive correction by the number of subphenotypes examined and more studies would be needed to establish connections between subphenotypes for this disease and this locus.

In an effort to delineate the potential functional role of rs7329174, we performed bioinformatics analysis of databases from NCBI for transcripts of *ELF1*, especially focusing on the region where rs7329174 is located. According to Entrez_gene database, there are two alternative variants for the *ELF1* gene (http://www.ncbi.nlm.nih.gov/gene/1997). Transcript NM_172373.3 contains an exon1 (shown as E1B in Fig. 1) that is 37 kb upstream of a constitutive exon2. NM_001145353.1 lacks exon1 completely, with otherwise the same sequence as NM_172373.3. We searched the expressed sequence tags (ESTs) and refseq_rna databases from NCBI for transcripts for this gene and did not find any supporting evidence for the transcription initiation reflected by NM_001145353.1. Instead, we identified transcripts containing two other alternative first exons in addition to finding support for the exon1 represented by NM_172373.3. One of these alternative first exons, shown as E1C in Figure 1, locates 570 bp upstream of the constitutive exon2 and ~1 kb downstream of SNP rs7329174. Another alternative first exon, E1A, is ~80 kb upstream of the constitutive exon2 and is close to *WBP4* gene, which is on the other strand of the chromosome. All three alternative first exons are supported by multiple EST sequences derived from different tissues and cDNA libraries. Examples of these transcripts include, for example, BX640798 and CN312731 for E1A and CD522023 and DC296627 for E1C. According to the EST entries, the three alternative first exons seem to express with a similar prevalence.

Using peripheral blood mononuclear cell (PBMC) from SLE patients and healthy blood donors, we confirmed the prevalent expression of the three alternative exon1s (Fig. 1B). However, we did not find correlation between these alternative transcription initiations represented by these distinct exon1s and the rs7329174 genotypes. For the samples we have examined (~30 each in both cases and controls), transcripts containing the three alternative exon1s seem to have a similar expression level (Fig. 1B), although the sample size is considered small given the fact that expression experiments are usually affected by both experimental and individual variations.

Table 1. SNPs in and around ELF1 in GWAS (612 cases and 1160 controls) that showed significant association with SLE

SNP	Position on chromosome 13 (NCBI Build 36)	A1	F_A	F_U	A2	OR (95% CI)	*P*-values	Logistic regression *P*-values*	EIGAN-STRAT-corrected *P*-values**
rs7329174	40 456 110	G	0.283	0.219	A	1.41 (1.22–1.63)	7.18×10^{-6}	N/A	2.25×10^{-5}
rs10507489	40 462 422	A	0.118	0.194	G	0.78 (0.66–0.92)	3.79×10^{-3}	0.093 (1.21×10^{-5})	0.00226
rs4942016	40 492 060	T	0.391	0.441	C	0.81 (0.71–0.92)	1.49×10^{-3}	0.29 (8.10×10^{-5})	0.00046
rs3794329	40 532 954	A	0.243	0.190	G	1.37 (1.18–1.59)	4.77×10^{-5}	0.34 (0.002)	5.16×10^{-5}
rs2772179	40 560 028	C	0.424	0.471	T	0.83 (0.73–0.94)	3.37×10^{-3}	0.10 (1.75×10^{-5})	0.00077

A1, minor allele; A2, major allele; F_A, minor allele frequency in the affected; F_U, minor allele frequency in the unaffected.
*P-values by conditional logistic regression when controlling for the effect of rs7329174 (*P*-values for rs7329174 when controlling for the effect of this SNP in the same conditional logistic regression test).
**Association *P*-values on allelic test after EIGEN-STRAT correction on population substructure using the principal component method.

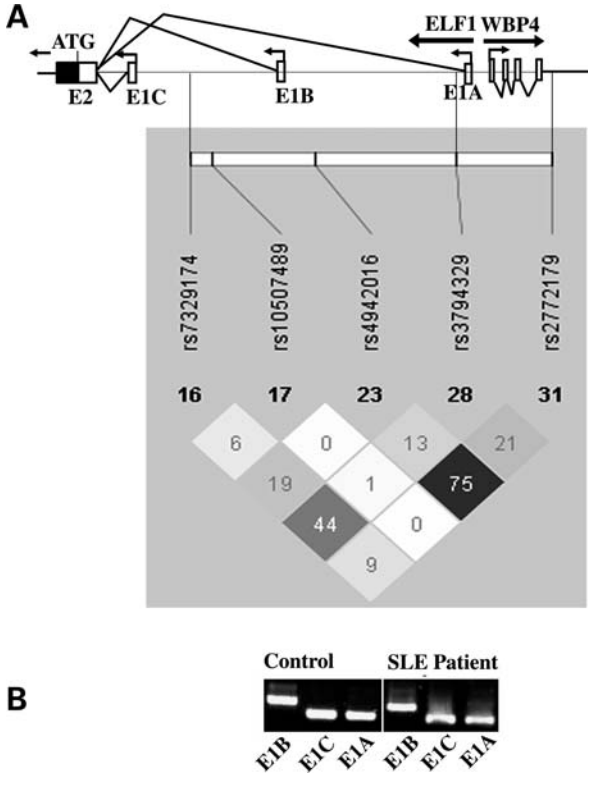

Figure 1. LD among SNPs in *ELF1* associated with SLE and transcription of the gene from three alternative exon1s. (**A**) SNPs showed significant association in the GWAS stage, their LD to each other and their relative position to *ELF1*. Shown are also the three alternative exon1s designated as E1A, E1B and E1C for this gene. SNP rs7329174 is about 1 kb upstream of E1C. LD among the SNPs was calculated by Haploview and shown are the r^2 value between the SNPs. (**B**) Confirmation of the expression of all three alternative exon1s of this gene by RT–PCR from PBMC of both patients and controls. Shown are representative results from a control and case, respectively.

DISCUSSION

SNP rs7329174 has the highest G allele frequency in Asians, 23.3 and 30.7% in Chinese and Japanese, respectively, according to HapMap. It also has high minor allele frequency in Gujarati Indians in Houston, a HapMap phase III population (16.5% on G allele). However, it has low allele frequencies in other populations and is near monomorphic A in Caucasians (for HapMap CEPH data, there are 164 AA genotypes and 1 AG genotype). Neighboring SNPs that are in LD with rs7329174 ($r^2 > 0.5$), such as rs9532692, rs7335629, rs4264266, rs17593261 and rs3794329, are either monomorphic or have extremely low minor allele frequencies (<1%) in HapMap CEPH data. The allele frequency difference raises a possibility of differences in disease associations for this locus between populations. The major allele in humans, the 'A' allele, was observed in the same position in both Rhesus Macaque and Chimpanzee (Fig. 2), suggesting a possibility that the risk allele 'G' may be gained in humans.

Regulation of *ELF1* gene expression seems a plausible mechanism, should SNP rs7329174 be the functional variant itself. Analysis of a database containing both gene expression and SNP genotyping data for the HapMap individuals did not show any correlation between rs7329174 genotype or its surrogates and the *ELF1* expression level (29,30) (data not shown). The finding of the three equally used exon1s, however, does indicate that the expression of this gene is probably under the complex, multilayer regulation.

In mouse, *ELF1* is expressed at high levels throughout thymocyte development and in all subsets of maturing thymocytes and T cells. In addition, high-level *ELF1* expression was also observed in B lymphocytes and macrophages (31). Elf-1 binds and activates or represses a list of genes important to T cell development and function, such as CD4, which plays a central role in the selection, differentiation, survival and activation of Th cells (32,33). ELf-1 is also found to increase the promoter activity of *CD3-Delta* (34), *TdT* (35), *IL3* (36) and *GM-CSF* (37). In B lymphocytes, Elf-1 binds to a B cell-specific regulatory element in the *IgH* enhancer and activates murine *IgH* expression (38). It is also thought to be the major regulator of the Ig alpha gene promoter (39) as well as the gene for Fc receptor for IgA (*FCAR*) (40).

IL-2 abnormality plays a central role in the activity and function of T cells in SLE. SLE patients were found to have decreased levels of IL-2 (41,42), as found in lupus mouse models (43–45). Elf-1 was known to bind to an *IL2* enhancer and regulate its expression (26). Essential positive regulatory element was also identified in the promoter region of *IL2Rα,* which is regulated by Elf-1. Thus, Elf-1 may play a role in IL-2 production as well as its responses (46–48).

Table 2. Replication of ELF1 association with SLE in additional samples and in other Asian populations

rs7329174	#_case	#_control	F_A	F_U	*P*-value*	OR (95% CI)
GWAS (HK)	612	1160	0.2826	0.2186	7.18×10^{6}	1.41 (1.21–1.64)
Replication						
HK	710	684	0.2611	0.201	0.0001	1.40 (1.17–1.68)
AH	1380	1297	0.2301	0.2047	0.0123	1.16 (1.02–1.32)
Thai	462	951	0.2738	0.2440	0.044	1.17 (0.98–1.40)
Joint Analysis	3164	4482			1.47×10^{8}	1.26 (1.16–1.36)

HK, Hong Kong samples; AH, Anhui samples; Thai, samples from Bangkok, Thailand.
*One-sided test was applied to the replication stage but not the joint analysis.

Figure 2. Sequence comparison among Human, Chimpanzee and Rhesus Macaque. The position of SNP rs7329174 is pointed by an arrow.

Elf-1 may also play a role in T cell receptor signaling. T cells from patients with SLE are characterized by decreased expression of CD247 (CD3 zeta chain) and increased expression of FcR γ-chain (49). Elf-1 has been shown to enhance the expression of CD247 (50–52) and suppress the expression of FcR γ-chain (53). *LAT* gene, which encodes a protein that is downstream of the signal transduction pathway following activation of the T cell receptor, is also a target gene of Elf-1 in transcription regulation (54). Interestingly, we also identified *CD247* in SLE association using the same cohorts (Li *et al*., manuscript in submission), suggesting important roles of this pathway in SLE.

Our work for the first time identifies this T cell transcription factor to be associated with SLE, and the various transcript forms found for this gene suggest a tight control in its expression and function. Further studies are still needed to replicate the association of this gene in other populations to firmly establish the role of this transcription factor in SLE pathogenesis. The monomorphic nature of rs7329174 in Caucasian populations suggests a potential difference among major ethnic groups on this locus in SLE susceptibility.

MATERIALS AND METHODS

Subjects

One thousand three hundred twenty-two SLE samples were collected from four hospitals in Hong Kong: Queen Mary Hospital, Tuen Mun Hospital, Queen Elizabeth Hospital and Pamela Youde Nethersole Eastern Hospital. The patients were all of self-reported Chinese ethnicity living in Hong Kong. Six hundred twenty of these patients were used in the GWAS stage (612 passed quality control, see below), and the rest was used in the replication of the findings. One thousand three hundred eighty SLE patients collected in Anhui were all self-reported Chinese ethnicity living in Anhui province, central China. They were recruited from the Department of Rheumatology at both Anhui Provincial Hospital and the First Affiliated Hospital of Anhui Medical University, located in Hefei, Anhui Province, central China. Four hundred sixty-two Thai patients with SLE attending the King Chulalongkorn Memorial Hospital, a tertiary referral center in Bangkok, were recruited through the Lupus Research Unit in the Department of Microbiology, Chulalongkorn University, Thailand. All subjects met the revised criteria of the American College of Rheumatology for SLE diagnoses (55). For the diagnosis, renal nephritis was defined as proteinuria of >0.5 g/day or biopsy-proven lupus nephritis.

Controls used in the GWAS stage were from both healthy individuals and from other studies conducted in the University of Hong Kong, genotyped with the same platform. For the replication stage, Hong Kong controls were healthy blood donors kindly contributed by the Hong Kong Red Cross and were all of self-reported Chinese ethnicity living in Hong Kong. Controls from Anhui were selected from a pool of healthy blood donors recruited from Hefei City, with an effort to match for the age and sex of the corresponding SLE patients. Thai controls were recruited from unrelated voluntary healthy donors from the same ethnic background and geographic area as the Thai SLE patients.

The Hong Kong study was approved by the Institutional Review Board of the University of Hong Kong and Hospital Authority Hong Kong West Cluster, New Territory West Cluster and Hong Kong East Cluster. The study on Anhui and Thai samples was approved by the Institutional Review Board of Research Ethics Committee of Anhui Medical University and the Ethics Committee of the Faculty of Medicine, Chulalongkorn University, respectively. All patients gave informed consent for this study.

Genotyping

Six hundred twenty-one SLE patients were genotyped by Illumina 610-Quad Human Beadchip. Quality control on both individual samples and SNPs was conducted as described

before (19). A total of 612 SLE patients and 1160 controls passed quality control and were analyzed on 513 108 SNPs. In the replication stage, SNP rs7329174 was genotyped by the TaqMan SNP genotyping method using assay-on-demand probes and primers (Applied Biosystems, Foster City, CA, USA). Around 100 samples were genotyped by both Illumina Beadchip and TaqMan and the results from the two platforms had 100% concordance for this SNP.

Analysis of *ELF1* transcripts, RT–PCR and PCR

EST sequences from NCBI dbEST corresponding to this locus and cDNA sequences from refseq_rna were extracted by BLAST search using the RefSeq cDNA sequence for ELF1 (GenBank: NM_172373.3) as a template, and their corresponding genomic structure was analyzed by aligning these sequences to reference human genome sequence.

PBMCs extracted from EDTA-treated blood (patients) or Buffy coat (controls) were used for the extraction of total RNA. Total RNA from patients and controls (30 individuals each) was isolated using TRIzol reagent (Invitrogen Corporation, Carlsbad, CA, USA) according to the manufacturer's instruction. First-strand cDNA synthesis was performed using high-capacity cDNA reverse transcription kit (Applied Biosystems) according to the manufacturer's instructions. Briefly, 2 μg of total RNA (10 μl) from each sample was added to a mixture of 2.0 μl of 10× RT buffer, 0.8 μl of 25× dNTP mix (100 mM), 1.0 μl of MultiScribe™ reverse transcriptase, 2 μl of 10× RT random primers, 1.0 μl of RNase inhibitor and 3.2 μl of nuclease-free water. The final reaction mix was kept at 25°C for 10 min, 37°C for 120 min and heated at 85°C for 5 s. The reaction was stopped by cooling down to 4°C until further experiment.

Transcripts containing alternative exon1s identified by the analysis of EST from NCBI were examined by RT–PCR using the following primers. Forward primers for the three alternative exon1 variants are: 5′-AAGAAGCCACTGAAGACAGG-3′ (E1A in Fig. 1); 5′-CAGACACCACTTCCCCAATC-3′ (E1B) and 5′-AAGAAGCCACTGAAGACAGG-3′ (E1C). A common reverse primer from the constitutive exon2 was used for all three reactions: 5′-GATTCACGTATCAGGCAGC-3′. PCRs were conducted using Qiagen Hotstart Taq master-mix by the following conditions: 94°C for 30 s, 55°C for 30 s, 72°C for 30 s for 40 cycles (42 cycles for SLE patients), and an extension cycle for 11 min at 72°C.

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Conflict of Interest statement. None declared.

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