Single-nucleotide Polymorphism and Haplotypes of TNIP1 Associated with Systemic Lupus Erythematosus in a Chinese Han Population

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ABSTRACT. ***Objective.*** To determine the association of systemic lupus erythematosus (SLE) with single-nucleotide polymorphisms (SNP) in the TNIP1 gene and compare the expression of this gene in cases and controls from a Chinese Han population in this replication study.

Methods. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry was used to genotype 19 SNP in TNIP1 in Chinese Han patients with SLE (n = 341) and controls (n = 356). Genotypes were analyzed by codominant, dominant, and recessive models. Analysis of allele frequencies and linkage disequilibrium was also performed. Western blotting and qRT-PCR were used to measure the expression of these genes in peripheral blood mononuclear cells of SLE cases and controls.

Results. Seven SNP loci were significantly associated with SLE in our population ($p < 0.05$ for all comparisons). Two TNIP1 gene haplotypes (ATTGCGC and GTCCTAT) were associated with SLE ($p = 0.0246$ and $p = 0.0024$, respectively). Western blotting and qRT-PCR results provide evidence that patients with SLE had significantly reduced expression of TNIP1/ABIN-1 relative to controls.

Conclusion. Analysis of SNP in the TNIP1 gene and expression of this gene in peripheral blood lymphocytes indicated these SNP were associated with the occurrence of SLE in Han Chinese patients. Future studies should examine the roles of these SNP in the pathogenesis of SLE. (First Release July 15 2013; J Rheumatol 2013;40:1535–44; doi:10.3899/jrheum.121391)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS TNIP1 SINGLE-NUCLEOTIDE POLYMORPHISMS

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the formation of autoantibodies and immune complex and symptoms that include skin lesions and disorders of multiple organs including the joints, kidneys, heart, liver, and nervous system[1,2]. The pathogenesis of SLE is not entirely clear, but genetic and environmental factors play important roles[3]. Genetic analysis indicates non-Mendelian inheritance, but there are multiple SLE susceptibility genes, interactions between these genes, and interactions between these genes and environmental factors[4]. Several recent genome-wide association studies (GWAS) have identified multiple SLE susceptibility genes[5,6]. Two genes appear to play particularly important roles in the regulation of the autoimmune inflammatory response in SLE: (1) TNFAIP3 (tumor necrosis factor α-l induced protein 3), which codes for the protein A20, and (2) TNIP1, a gene that codes for TNFAIP3-interacting protein-1 (ABIN-1), which binds to A20.

ABIN-1 binds with A20[7] and is expressed in various tissues, including lymphocytes, spleen, and skeletal muscle, all of which play roles in cell differentiation, apoptosis, and regulation of inflammatory responses[8]. A20/TNFAIP3 is involved in cell activation, regulation of cytokine signals, and apoptosis[9]. SNP rs2230926 of this gene is associated with SLE in Han Chinese patients[10] and downregulation of this gene occurs in peripheral blood mononuclear cells of patients with SLE[11]. Gateva, *et al* identified ABIN-1/TNIP1 as one of 5 loci associated with risk for SLE in European patients[6]. The rs7708392 locus of this gene is associated with SLE in European, Japanese, and Chinese Han populations[12,13,14,15]. Thus, ABIN-1/TNIP1 appears to be important in the regulation of autoimmune responses in diverse populations of patients with SLE[5,12,13].

Several recent animal studies have also examined the

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Supported by the National Natural Science Foundation of China (grant numbers 81071293 and 81201232).

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Accepted for publication May 13, 2013.

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roles of these genes in autoimmune responses. For example, Höevelmeyer, *et al*[16] developed a new mouse strain with tissue-specific deletion of A20. Their experiments indicated that B cell-specific deletion of A20 reduced the number of marginal zone B cells and that A20-deficient B cells had enhanced proliferation upon activation. These new mice also had higher levels of serum immunoglobulins and upregulation of autoantibodies. These results suggest a B cell-specific role of A20 in the development of autoimmunity due to a dysfunctional interaction of B cells and T cells. In addition, Chu, *et al*[17] reported that selective loss of A20 in the B cells of aged mice caused inflammation and autoimmune responses, with elevation of interleukin 6 (IL-6), plasma cell expansion, and formation of autoantibodies. These results indicate that downregulation of A20 increased B cell hyperreactivity and suggest that this mechanism may underlie the association of heritable human mutations or polymorphisms in A20 with certain autoimmune diseases.

Our studies of TNFAIP3 are continuing. In this replication study, we report our analysis of the significance of TNIP1 in SLE by studying a Han Chinese population in southwestern China and compare patients who had definite clinical diagnoses of SLE with age and sex-matched controls. In particular, we studied polymorphisms of TNIP1 in 341 patients with SLE and 356 controls and compared the expression of this gene in the peripheral blood lymphocytes of patients and controls.

MATERIALS AND METHODS

Patients and controls. In this replication study, patients with SLE who were of Han ethnicity were enrolled. They lived in the Chongqing, Sichuan, Yunnan, and Guizhou areas of southwestern China and were admitted to the Dermatology Department of The Third Military Medical University Southwestern Hospital (Chongqing, China) from January 2008 to April 2011. The diagnosis of all patients was based on the 1997 criteria of the American Rheumatism Association[18]. Patients with other systematic diseases or autoimmune diseases were excluded. A 5 ml sample of peripheral venous blood was collected, and relevant epidemiological and clinical data were recorded.

The healthy control group consisted of age- and sex-matched individuals from the same geographic area. Blood samples were collected as described. All controls were healthy based on the results of physical examinations and from routine testing of blood, urine, liver function, renal function, and autoantibodies.

The Ethics Committee of the First Affiliated Hospital of the Third Military Medical University approved our study and all patients provided signed informed consent.

Genotyping assays. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used for genotyping of 19 SNP loci of the TNIP1 gene. SNP sequence-specific extension primers were added into the PCR amplification products. One base was extended at the SNP locus and the primers are given in Table 1. Then sample analytes were co-crystallized with the chip substrate, and the crystal was placed in the vacuum tube of the mass spectrometer and measured using transient (10^{-9} ns) strong laser excitation. Finally, the results were analyzed using Typer 4.0 Genotyping software (Sequenom). The genotyping success rate was > 95%.

SLE severity classification and Western blotting. SLE severity was classified by the Systemic Lupus Erythematosus Disease Activity Index-2000 (SLEDAI-2K), in which a score of 0–4 indicates inactive disease, 5–9 indicates mild disease, 10–14 indicates moderate disease, and a score ≥ 15 indicates severe disease[19]. Peripheral blood lymphocytes were isolated, and proteins were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred to PVDF membranes, as described in the technical document provided by Abcam (Website: www.abcam.com/ps/pdf/protocols/Western_blot_diagram.pdf). The primary antibody was TNIP1 (14990382; Eastern Biotech) and chemiluminescence was used for detection (Supersignal West Femto Luminol Enhancer Solution; cat. 1856192, Thermo Fisher).

Western blot and qRT-PCR. The peripheral blood lymphocytes were isolated following the instructions from the manufacturer (Haoyang Inc.). Total RNA was extracted using Trizol (Invitrogen). First-strand cDNA was synthesized using 1μg extracted total RNA as template and random hexanucleotide as primer for extension by Rever Tra Ace qRT-PCR kit (Toyobo). The primers used in qRT-PCR were TNIP1 forward primer 5'-CAG AAT GAG TTG CTG AAA CA-3', TNIP1 reverse primer 5'-TCT CCT CAT CTT TGA ATG CT-3'; β-actin forward primer 5'-CAA CCA ACT GGG ACG ACA T-3', β-actin reverse primer 5'-GCA CAG CCT GGA TAG CAA C-3'. The first cDNA synthesized was used in the qRT-PCR reaction under the following conditions: SYBR premix ExTaq (2×) 10 μl, forward and reverse primer (10 μM) 1 μl each, first-strand cDNA 1 μl, 7 μl of milli-Q water. The mixture was denatured in 95°C for 1 min, followed by 40 cycles of 95°C for 10 s, and 60°C for 30 s. qRT-PCR was analyzed using the PCR 7500 system (Applied Biosystems, Invitrogen). The relative quantification was obtained using the ΔΔCT method relative to a reference gene (β-actin).

Statistical analysis. Demographic variables of cases and controls were compared using Student's t test for continuous variables and Pearson's chi-square test for categorical variables. A goodness-of-fit chi-square test was used to test for deviation of genotype frequencies of each SNP in the controls from those expected under the Hardy-Weinberg equilibrium. The association between the distributions of SNP genotypes and SLE was assessed by a chi-square test or Fisher's exact test. The risk of SLE associated with each genotype was expressed as an OR and 95% CI based on logistic regression.

Each subject has 2 alleles for each SNP, so the observations might not be truly independent. Thus, generalized estimating equations (GEE), which are similar to general regression models but account for the dependence among alleles in an individual, were used to identify the risk of SLE associated with each allele. The SAS procedure Proc Genmod (Version 9.1.3, SAS Institute Inc.) was used to fit GEE models. OR and 95% CI were also calculated for each risk factor.

Linkage disequilibrium (LD) blocks of all SNP were analyzed by Haploview analysis. The degree of LD between individual SNP was assessed by Lewontin's coefficient D'. The 95% confidence bounds on D' were generated and each comparison was classified as "strong LD," "inconclusive," or "strong recombination." A block was created if it was at least 95% informative (i.e., not inconclusive). For each LD block, each individual has 2 haplotypes, so the data may not be independent. Thus, we also used GEE to assess the risk of SLE associated with different haplotypes. All statistical tests were performed using SAS version 9.1.3 on 2-sided probabilities. The Benjamini and Hochberg method was used to control the false discovery rate due to multiple testing.

RESULTS

Characteristics of patients with SLE and controls. During the study period (January 2008 to April 2011), we enrolled 341 consecutive patients with SLE (332 women and 9 men, mean age 32.65 yrs) and 356 controls (345 women and 11 men, mean age 30.28 yrs). There were no significant differ-

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Table 1. Primers used for genotyping.

SNP	Forward Primer	Reverse Primer Extension	Direction of Primer	Single-strand Extension
rs6889239	ACGTTGGATGTTACGCACCTGTGCATCAAG	ACGTTGGATGCTCTCCCAAGCATCATTTCC	R	CTCCTGTGCCCAGTGCC
rs2233311	ACGTTGGATGTTGTGTCATTTGGCTCCACC	ACGTTGGATGCGTGCAAGTGGCTTCTAGTT	F	AGAGCCAGCTGATCTCA
rs3792789	ACGTTGGATGGAAACAGTTTTTGTCTTGGG	ACGTTGGATGGGGACCTCAGAAGGGTTTG	F	CCTTGGGAGGAAGGTGC
rs960709	ACGTTGGATGTCAGGCCCCCAGAGTTCCGT	ACGTTGGATGTATGGGTCTTTTCAGCTCGG	R	TTAAGAAGAGTCCTGGAG
rs12658895	ACGTTGGATGGAAAGACTGGACTAGACTGG	ACGTTGGATGTGCAGTCCATTCCGCCTCTT	R	CTCTTCCCATGGATCTCAC
rs871269	ACGTTGGATGAATTAATTCCTGAGCTCCCG	ACGTTGGATGCTGGGGATCTGGCAGTGAA	F	tGCTGGGACCCCTAACCAT
rs8177834	ACGTTGGATGAGTGGAGAGAGGCCTCATC	ACGTTGGATGCACTCTTAGGATTGCTGCTC	R	CTCCTGGAGGCTTCTGCAA
rs888989	ACGTTGGATGGGAATTGTGGCCACTCACAG	ACGTTGGATGGAGAGGCAGGGACTTGATTG	F	GAGCAAGGAGGCCCAAGTTC
rs13168551	ACGTTGGATGTGTGTGCTTGGTCCACTGTA	ACGTTGGATGCAGTGGTGTTTTCTGCAAGG	F	TCCACTGTACCAAGTTGTCTT
rs7708392	ACGTTGGATGGCCAACTGGTCAATTCTCCC	ACGTTGGATGGGGTCTCTTCTGGAACTTAG	F	aAGAGGCTGATTCCAGTTATT
rs10463312	ACGTTGGATGTGGCTCCATTTCTGCACAAC	ACGTTGGATGACAGGGAGGCCAAGTCCATC	R	ccCCAAGTCCATCGCAACATGA
rs12655899	ACGTTGGATGACAAGGGCAAGTCGCTCCCA	ACGTTGGATGTCTTCCTTGAATGCCCCGAG	R	aCCGAGCCAGATGCTCTTCCTGC
rs13153275	ACGTTGGATGAAAAGAGGATCTCCATGGGC	ACGTTGGATGGCCAGGGAAACATGCTGATA	R	cTGCTGATAAATGGTGAAAGTCT
rs4958437	ACGTTGGATGAGGTAAACACCGGCTGCCTG	ACGTTGGATGAAGTCGCTCCTAGAGGGGAA	F	GCGGTGTCTCTCGTCCC
rs918498	ACGTTGGATGCATCCATCCTGCACATGCAC	ACGTTGGATGCTCCAGAGTTCCTACTATAC	F	GCACATGCACACAAATG
rs2277940	ACGTTGGATGAAGGAGGGACCCAGGAAAG	ACGTTGGATGGAAGATCAACATTCTGACTC	F	GGTTGACAGGGGAACAT
rs4958436	ACGTTGGATGATATGCCAGCTCCAAGTGAC	ACGTTGGATGATTTAATGAGCAGCTACTGTG	R	ACTGTGTGCCAGGCATCGC
rs10036748	ACGTTGGATGCTTTCATAGCATGATACACG	ACGTTGGATGGCAAAGCAGCCCCTTTTTTC	R	CTTTTTTCACTTTCTGTCAC
rs6862024	ACGTTGGATGGTGCTGGGACTAGAGGATAG	ACGTTGGATGAGGGTGTTGGCTAGTGTCTG	R	GTGATGCTGCAGGGCTGGCC

ences between these 2 groups in age or sex. All genotype frequencies in the control population were in agreement with the predictions of Hardy-Weinberg equilibrium ($p > 0.05$).

The clinical data were recorded from 219 of the 341 patients (Table 2). The mean age at SLE onset was 28.4 years, 25.6% of patients experienced onset when they were younger than 20 years, and 63% of patients experienced onset when they were 20 to 40 years old. The most common signs/symptoms were immunological disorder (85.8%), hematologic disorder (80.4%), malar rash (79.0%), renal disorder (78.1%), and arthritis (74.9%). Analysis of immunological indices indicated that 96.8% of patients were positive for antinuclear antibodies, 83.6% of patients had low complement, and 64.7% patients were positive for anti-Sjögren syndrome A (Table 2). There were no significant associations between individual SNP and clinicopathological variables or immunohistochemical indices (data not shown).

Associations of genotypes with SLE. Table 3 shows the results of our codominant analysis of all identified SNP. All p values in this and subsequent multiple comparisons were adjusted by the Benjamini and Hochberg method. The results indicate that SNP rs10036748 from TNIP1 (adjusted $p = 0.015$) was significantly associated with SLE. Five other SNP had adjusted p values of 0.05 to 0.10.

Table 4 shows the results of our analysis of genotype frequencies of all SNP based on dominant and recessive models, with the p values and adjusted p values indicated for the model that yielded the most significant risk. The results

Table 2. Characteristics of enrolled patients with SLE (n = 219).

Characteristic	Mean ± SD or n (%)
Age, yrs	32.6 ± 11.8
Female	211 (96.3)
Male	8 (3.7)
Onset age, yrs	28.4 ± 10.9
< 20	56 (25.6)
20–40	138 (63.0)
> 40	25 (11.4)
Symptoms	
Immunologic disorder	187 (85.8)
Neurologic disorder	9 (4.1)
Vasculitis	27 (12.3)
Hematologic disorder	176 (80.4)
Renal disorder	171 (78.1)
Arthritis	164 (74.9)
Serositis	38 (17.4)
Oral ulcers	54 (24.8)
Discoid	51 (23.3)
Photosensitive	114 (52.1)
Malar rash	173 (79.0)
Immunological indices	
Low complement (C3/C4)	183 (83.6)
Anti-U1RNP/Sm	104 (47.7)
RO-52	120 (55.0)
Anti-SSA	141 (64.7)
Anti-SSB	55 (25.2)
Anti-Sm	114 (52.3)
Anti-dsDNA	147 (67.1)
Antinuclear antibody	211 (96.8)
Hypertension	35 (16.0)
Hyperlipidemia or high blood lipids	23 (10.5)

SLE: systemic lupus erythematosus; anti-SSA: anti-Sjögren syndrome A.

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Table 3. Codominant analysis of single-nucleotide polymorphisms (SNP) in TNIP1.

SNP	Genotype	Cases (frequency)	Controls (frequency)	p	Adjusted p†
rs2277940	CC	67 (0.197)	72 (0.202)	0.4121	0.4579
	TT	107 (0.315)	127 (0.357)		
	CT	166 (0.488)	157 (0.441)		
rs8177834	AA	3 (0.009)	9 (0.025)	0.1029	0.2144
	AG	84 (0.246)	102 (0.286)		
	GG	254 (0.745)	246 (0.689)		
rs2233311	TT	3 (0.009)	10 (0.028)	0.1072	0.2144
	GG	250 (0.733)	246 (0.687)		
	GT	88 (0.258)	102 (0.285)		
rs10463312	CC	53 (0.156)	57 (0.159)	0.1746	0.2843
	TT	130 (0.383)	160 (0.447)		
	CT	156 (0.460)	141 (0.394)		
rs12658895	CC	326 (0.956)	334 (0.933)	0.1844	0.2843
	CT	15 (0.044)	24 (0.067)		
rs12655899	AG	172 (0.506)	160 (0.447)	0.287	0.3587
	AA	65 (0.191)	74 (0.207)		
	GG	103 (0.303)	124 (0.346)		
rs13153275	CC	301 (0.888)	311 (0.869)	0.5397	0.5681
	GG	2 (0.006)	5 (0.014)		
	CG	36 (0.106)	42 (0.117)		
rs6862024	AA	35 (0.103)	30 (0.084)	0.199	0.2843
	AG	143 (0.422)	134 (0.374)		
	GG	161 (0.475)	194 (0.542)		
rs888989	CC	3 (0.009)	12 (0.034)	0.0338	0.0966
	TT	249 (0.730)	240 (0.670)		
	CT	89 (0.261)	106 (0.296)		
rs871269	CC	52 (0.152)	71 (0.198)	0.1973	0.2843
	TT	135 (0.396)	124 (0.346)		
	CT	154 (0.452)	163 (0.455)		
rs4958436	CC	116 (0.341)	108 (0.306)	0.4079	0.4579
	TT	61 (0.179)	76 (0.215)		
	CT	163 (0.479)	169 (0.479)		
rs3792789	CC	20 (0.059)	20 (0.056)	0.9824	0.9824
	TT	195 (0.577)	207 (0.578)		
	CT	123 (0.364)	131 (0.366)		
rs7708392	CC	223 (0.660)	206 (0.575)	0.0180	0.060
	GG	9 (0.027)	22 (0.061)		
	CG	106 (0.314)	130 (0.363)		
rs6889239	CC	223 (0.654)	206 (0.575)	0.0138	0.060
	TT	8 (0.023)	22 (0.061)		
	CT	110 (0.323)	130 (0.363)		
rs10036748	CC	15 (0.046)	35 (0.112)	0.0015	0.015*
	TT	206 (0.638)	165 (0.527)		
	CT	102 (0.316)	113 (0.361)		
rs918498	CC	45 (0.142)	60 (0.188)	0.2303	0.3071
	TT	130 (0.410)	116 (0.364)		
	CT	142 (0.448)	143 (0.448)		
rs4958437	CC	113 (0.331)	95 (0.268)	0.0557	0.1392
	TT	64 (0.188)	90 (0.254)		
	CT	164 (0.481)	170 (0.479)		
rs960709	AA	8 (0.024)	21 (0.059)	0.0153	0.06
	AG	105 (0.311)	128 (0.359)		
	GG	225 (0.666)	208 (0.583)		
rs13168551	CC	225 (0.664)	208 (0.586)	0.0076	0.0507
	TT	9 (0.027)	26 (0.073)		
	CT	105 (0.310)	121 (0.341)		

† Adjusted p value was calculated by the Benjamini and Hochberg method for multiple comparisons.
* Significant association ($p < 0.05$).

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Table 4. Genotype frequencies of all single-nucleotide polymorphisms (SNP) in TNIP1 in dominant or recessive models. None of the SNP were in protein-coding regions.

SNP	Genotype	Cases (frequency)	Controls (frequency)	p	Adjusted p†	OR (95% CI)
rs2277940	TT	107 (0.315)	127 (0.357)	0.2406	0.2673	0.828 (0.604–1.135)
	CC+CT	233 (0.685)	229 (0.643)			Reference
rs8177834	AG+GG	338 (0.991)	348 (0.975)	0.0954	0.159	2.914 (0.782–10.855)
	AA	3 (0.009)	9 (0.025)			Reference
rs2233311	TT	3 (0.009)	10 (0.028)	0.0612	0.136	0.309 (0.084–1.132)
	GG+GT	338 (0.991)	348 (0.972)			Reference
rs10463312	TT	130 (0.383)	160 (0.447)	0.0894	0.159	0.770 (0.569–1.041)
	CC+CT	209 (0.617)	198 (0.553)			Reference
rs12658895	CC	326 (0.956)	334 (0.933)	0.1844	0.2459	1.562 (0.805–3.030)
	CT	15 (0.044)	24 (0.067)			Reference
rs12655899	AA+AG	237 (0.697)	234 (0.654)	0.2209	0.2673	1.219 (0.887–1.675)
	GG	103 (0.303)	124 (0.346)			Reference
rs13153275	CC+CG	337 (0.994)	353 (0.986)	0.2857	0.3007	2.387 (0.460–12.386)
	GG	2 (0.006)	5 (0.014)			Reference
rs6862024	AG+AA	178 (0.525)	164 (0.458)	0.0771	0.1542	1.308 (0.971–1.761)
	GG	161 (0.475)	194 (0.542)			Reference
rs888989	CC	3 (0.009)	12 (0.034)	0.0242	0.0691	0.256 (0.072–0.915)
	TT+CT	338 (0.991)	346 (0.966)			Reference
rs871269	CC	52 (0.152)	71 (0.198)	0.1117	0.1674	0.727 (0.491–1.078)
	TT+CT	289 (0.848)	287 (0.802)			Reference
rs4958436	TT	61 (0.179)	76 (0.215)	0.2357	0.2673	0.797 (0.547–1.160)
	CC+CT	279 (0.821)	277 (0.785)			Reference
rs3792789	CC	20 (0.059)	20 (0.056)	0.8514	0.8514	1.063 (0.561–2.013)
	TT+CT	318 (0.941)	338 (0.944)			Reference
rs7708392	CG+GG	115 (0.340)	152 (0.425)	0.0222	0.0691	0.699 (0.514–0.951)
	CC	223 (0.660)	206 (0.575)			Reference
rs6889239	TT	8 (0.023)	22 (0.061)	0.0132	0.066	0.367 (0.161–0.836)
	CC+CT	333 (0.977)	336 (0.939)			Reference
rs10036748	CC	15 (0.046)	35 (0.112)	0.0022	0.022*	0.387 (0.207–0.724)
	TT+CT	308 (0.954)	278 (0.888)			Reference
rs918498	CC	45 (0.142)	60 (0.188)	0.1172	0.1674	0.714 (0.468–1.089)
	TT+CT	227 (0.858)	259 (0.812)			Reference
rs4958437	TT	64 (0.188)	90 (0.254)	0.0365	0.0913	0.680 (0.474–0.977)
	CC+CT	277 (0.812)	265 (0.746)			Reference
rs960709	AG+GG	330 (0.976)	336 (0.941)	0.0205	0.0691	2.578 (1.126–5.903)
	AA	8 (0.024)	21 (0.059)			Reference
rs13168551	TT	9 (0.027)	26 (0.073)	0.005	0.0333*	0.345 (0.159–0.748)
	CC+CT	330 (0.973)	329 (0.927)			Reference

† Adjusted p value was calculated by the Benjamini and Hochberg method for multiple comparisons.
* Significant association ($p < 0.05$).

indicate 2 significant associations (adjusted $p < 0.05$) for decreased risk of SLE from recessive models: (1) individuals with 2 variant C alleles (CC) of rs10036748 in TNIP1 had decreased risk compared with others (TT+CT) (OR 0.387, 95% CI 0.207–0.724); and (2) individuals with 2 variant T alleles (TT) of rs13168551 in TNIP1 had decreased risk compared with others (CC+CT; OR 0.345, 95% CI 0.159–0.748). Five other SNP had adjusted p values of 0.05 to 0.10.

Associations of the alleles with SLE. Table 5 shows the associations of different alleles with SLE. The results indicate that 6 of 19 SNP from TNIP1 were significantly associated with SLE. In particular, rs7708392 C (OR 1.429, 95% CI 1.106–1.847), rs6889239 C (OR 1.417, 95% CI 1.099–1.825), rs4958437 C (OR 1.299, 95% CI 1.048–1.609), and rs13168551 C (OR 1.454, 95% CI 1.120–1.887) were associated with an increased risk of SLE, whereas rs10036748 C (OR 0.622, 95% CI 0.476–0.812) and rs960709 A (OR 0.698, 95% CI 0.539–0.903) were associated with a decreased risk of SLE.

Linkage disequilibrium analysis. Figure 1 shows the results of linkage disequilibrium analysis of the 19 SNP and their physical locations based on Haploview analysis. These results indicate the presence of 2 haplotype blocks. Block 1 is 21 kb and has the following 7 SNP: rs8177834, rs2233311, rs10463312, rs12655899, rs13153275,

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Table 5. Association of different TNIP1 alleles with SLE

SNP	Allele	SLE	Control	GEE OR (95% CI)	p	Adjusted p†
rs2277940	C	300 (44.12%)	301	1.078 (0.867–1.340)	0.4989	0.5543
	T	380 (55.88%)	411	Reference		
rs8177834	A	90	120	0.753 (0.564–1.004)	0.0533	0.1184
	G	592	594	Reference		
rs2233311	G	588	594	1.285 (0.967–1.708)	0.0844	0.1407
	T	94	122	Reference		
rs10463312	C	262	255	1.139 (0.908–1.428)	0.2614	0.3268
	T	416	461	Reference		
rs12658895	C	667	692	1.542 (0.809–2.939)	0.1879	0.2505
	T	15 (2.20%)	24	Reference		
rs12655899	A	302	308	1.058 (0.854–1.312)	0.6049	0.6367
	G	378	408	Reference		
rs13153275	C	638	664	1.249 (0.802–1.946)	0.3253	0.3827
	G	40 (5.90%)	52	Reference		
rs6862024	A	213	194	1.233 (0.974–1.560)	0.0818	0.1407
	G	465	522	Reference		
rs888989	C	95	130	0.730 (0.551–0.966)	0.0279	0.0698
	T	587	586	Reference		
rs871269	C	258	305	0.820 (0.658–1.022)	0.0769	0.1407
	T	424	411	Reference		
rs4958436	C	395	385	1.156 (0.932–1.433)	0.1876	0.2505
	T	285	321	Reference		
rs3792789	C	163	171	1.013 (0.792–1.295)	0.9201	0.9201
	T	513	545	Reference		
rs7708392	C	552	542	1.429 (1.106–1.847)	0.0063	0.0237*
	G	124	174	Reference		
rs6889239	C	556	542	1.417 (1.099–1.825)	0.0071	0.0237*
	T	126	174	Reference		
rs10036748	C	132	183	0.622 (0.476–0.812)	0.0005	0.005*
	T	514	443	Reference		
rs918498	C	232	263	0.823 (0.653–1.038)	0.0993	0.1528
	T	402	375	Reference		
rs4958437	C	390	360	1.299 (1.048–1.609)	0.0169	0.0483*
	T	292 (42.82%)	350	Reference		
rs960709	A	121	170	0.698 (0.539–0.903)	0.0062	0.0237*
	G	555 (82.10%)	544	Reference		
rs13168551	C	555	537	1.454 (1.120–1.887)	0.005	0.0237*
	T	123	173	Reference		

† Adjusted p value was calculated by the Benjamini and Hochberg method for multiple comparisons.
* Significant association ($p < 0.05$). SLE: systemic lupus erythematosus; GEE: generalized estimating equations.

rs6862024, and rs888989. Block 2 is 5 kb and has the following 7 SNP: rs7708392, rs6889239, rs10036748, rs918498, rs4958437, rs960709, and rs13168551.

Next, we examined the combined effect of these 19 SNP by analysis of the frequency of different haplotypes in block 1 (Table 6) and block 2 (Table 7). There were 18 of the possible haplotypes in each block. We combined all haplotypes that had frequencies of 9 or less to simplify the statistical comparisons. Then we used the most common haplotype as the reference group for analysis of the association of specific haplotypes with SLE and estimated haplotype-specific OR by GEE. This method of using a common haplotype as a reference is preferable to using a pool of different haplotypes, because each haplotype is compared with the same reference group. For block 1, the GGTACGT haplotype was most common in cases and controls (41.94% and 39.94%, respectively) and the ATTGCGC haplotype (OR 0.695, 95% CI 0.506–0.954; p = 0.0246) was associated with SLE (Table 6). For block 2, the CCTTCGC haplotype was the most common in cases and controls (55.72% and 49.86%, respectively) and the GTCCTAT haplotype (OR 0.649, 95% CI 0.491–0.858; p = 0.0024) was associated with SLE (Table 7).

Expression of TNIP1/ABIN-1 in patients and controls. Next, we determined whether any of the identified SNP were associated with changes in gene expression. The mRNA and

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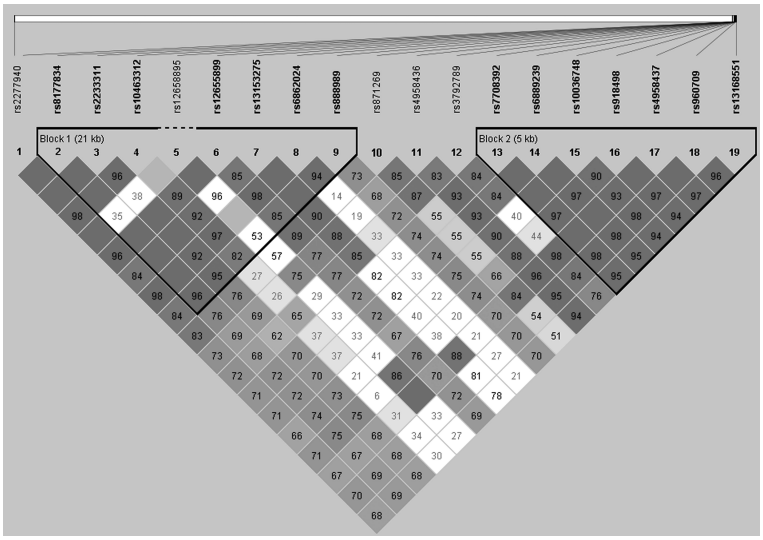

Figure 1. Linkage disequilibrium (LD) analysis of TNIP1 in 341 Han Chinese patients with systemic lupus erythematosus. The location of each single-nucleotide polymorphism (SNP) is given on top and each box gives the coefficient of determination (r^2). SNP positions represent the LD between the adjacent SNP.

Table 6. Association of block 1 haplotypes of TNIP1 with SLE.

	Haplotype	Total (%)	SLE (%)	Control (%)	GEE OR (95% CI)	p
Block 1	GGTACGT	572 (40.92)	286 (41.94)	286 (39.94)	Reference	
	GGCGCAT	391 (27.97)	205 (30.06)	186 (25.98)	1.102 (0.844–1.438)	0.4741
	ATTGCGC	200 (14.31)	82 (12.02)	118 (16.48)	0.695 (0.506–0.954)	0.0246*
	GGCGGGT	90 (6.44)	40 (5.87)	50 (6.98)	0.800 (0.501–1.277)	0.3496
	GGTGCGT	66 (4.72)	25 (3.67)	41 (5.73)	0.610 (0.367–1.014)	0.0564
	GGCACGT	22 (1.57)	11 (1.61)	11 (1.54)	1.000 (0.429–2.332)	> 0.9999
	GGTACGC	12 (0.86)	6 (0.88)	6 (0.84)	1.000 (0.322–3.110)	> 0.9999
	GGTGCAT	12 (0.86)	7 (1.03)	5 (0.70)	1.400 (0.442–4.437)	0.5675
Other	GGCGCGC	8 (0.57)	3 (0.44)	5 (0.70)	1.538 (0.758–3.122)	0.2328
	ATTGCGT	8 (0.57)	7 (1.03)	1 (0.14)		
	GGTAGGT	3 (0.21)	1 (0.15)	2 (0.28)		
	GGTGCGC	3 (0.21)	3 (0.44)	0 (0.00)		
	GTCGCGT	3 (0.21)	3 (0.44)	0 (0.00)		
	GGCGCAC	2 (0.14)	1 (0.15)	1 (0.14)		
	GTCGCAT	2 (0.14)	1 (0.15)	1 (0.14)		
	ATTACGT	2 (0.14)	0 (0.00)	2 (0.28)		
	GGCACAT	1 (0.07)	0 (0.00)	1 (0.14)		
	ATTGCAT	1 (0.07)	1 (0.15)	0 (0.00)		

* Significant association ($p < 0.05$). GEE: generalized estimating equations; SLE: systemic lupus erythematosus.

protein levels of TNIP1/ABIN-1 were determined by quantitative RT-PCR and Western blotting, respectively. Expression of TNIP1/ABIN-1 mRNA and protein were lower in patients with SLE than in controls ($p < 0.001$; Figure 2). Further analysis indicated controls had significantly higher levels of TNIP1/ABIN-1 mRNA and protein than those with inactive, mild, moderate, or severe SLE ($p < 0.001$; Figure 3). We also determined the association of TNIP1 SNP with expression of the corresponding proteins, but none of the results were significant (data not shown).

DISCUSSION

We analyzed SNP in the TNIP1 gene and expression of this gene in peripheral blood lymphocytes of Han Chinese patients with SLE and controls in this replication study. Although the role of this gene in SLE has been investigated in an American population[30], a Chinese Han population[31], and a Japanese population[14], our replication study identified several new SNP associated with SLE in a Chinese Han population. Our findings suggested that 7 SNP loci were significantly associated with SLE and that 2 TNIP1 haplo-

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Table 7. Association of block 2 haplotypes of TNIP1 with SLE.

	Haplotype	Total (%)	SLE (%)	Control (%)	GEE OR (95% CI)	p
Block 2	CCTTCGC	737 (52.72)	380 (55.72)	357 (49.86)	Reference	
	GTCCTAT	279 (19.96)	114 (16.72)	165 (23.04)	0.649 (0.491–0.858)	0.0024*
	CCTCTGC	236 (16.88)	122 (17.89)	114 (15.92)	1.005 (0.743–1.361)	0.9722
	CCTTTGC	81 (5.79)	38 (5.57)	43 (6.01)	0.830 (0.518–1.331)	0.4396
	CCCCTGC	20 (1.43)	7 (1.03)	13 (1.82)	0.506 (0.191–1.341)	0.1705
Other	CCCTCGC	9 (0.64)	4 (0.59)	5 (0.70)	0.822 (0.441–1.531)	0.5367
	GTCCTAC	8 (0.57)	5 (0.73)	3 (0.42)		
	CCTCTGT	6 (0.43)	2 (0.29)	4 (0.56)		
	CCTTCGT	4 (0.29)	2 (0.29)	2 (0.28)		
	GTCCTGC	4 (0.29)	1 (0.15)	3 (0.42)		
	GTCCCAT	3 (0.21)	3 (0.44)	0 (0.00)		
	GTCTTAT	3 (0.21)	1 (0.15)	2 (0.28)		
	CCCTTGC	2 (0.14)	0 (0.00)	2 (0.28)		
	GTCCTGT	2 (0.14)	1 (0.15)	1 (0.14)		
	CCTCTAC	1 (0.07)	0 (0.00)	1 (0.14)		
	CCTTTAT	1 (0.07)	0 (0.00)	1 (0.14)		
	CTTTCGT	1 (0.07)	1 (0.15)	0 (0.00)		
	GCCCTGC	1 (0.07)	1 (0.15)	0 (0.00)		

* Significant association ($p < 0.05$). GEE: generalized estimating equations; SLE: systemic lupus erythematosus.

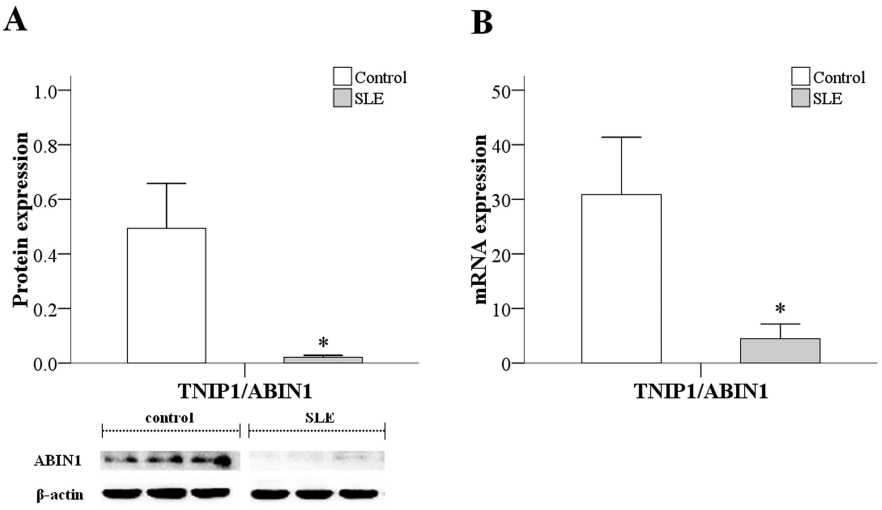

Figure 2. Protein and mRNA expression of TNIP1/ABIN-1 in peripheral blood lymphocytes of patients with systemic lupus erythematosus (SLE). (A) Expression of protein based on Western blotting of TNIP1/ABIN-1 in patients with SLE (n = 18) and controls (n = 10). (B) Expression of mRNA based on qRT-PCR of TNIP1/ABIN-1 in patients with SLE (n = 18) and controls (n = 10). *Significant difference ($p < 0.05$) relative to the control.

types were associated with SLE. Moreover, patients with SLE had significantly lower TNIP1 expression in their peripheral blood lymphocytes.

ABIN-1 (coded by TNIP1) binds to A20 (coded by TNFAIP3), a protein that contains multiple zinc finger domains at the carboxyl terminus that have E3 ubiquitin ligase activity, and an ovarian tumor domain at the N-terminus that has deubiquitinating activity[20]. Thus, these proteins are involved in inhibition of the nuclear factor-κB (NF-κB) pathway, inhibition of TNF-mediated apoptosis[21,22], and blocking of inflammatory signals[20,21,23]. Previous research indicated that a severe inflammatory response occurs in A20-deficient mice and that these mice were more sensitive to lipopolysaccharide and TNF[24]. A20-deficient cells cannot terminate TNF-induced NF-κB activation, so they are more prone to TNF-mediated apoptosis. Therefore, signaling from TNIP1 and A20 is involved in regulation of the inflammatory response, and

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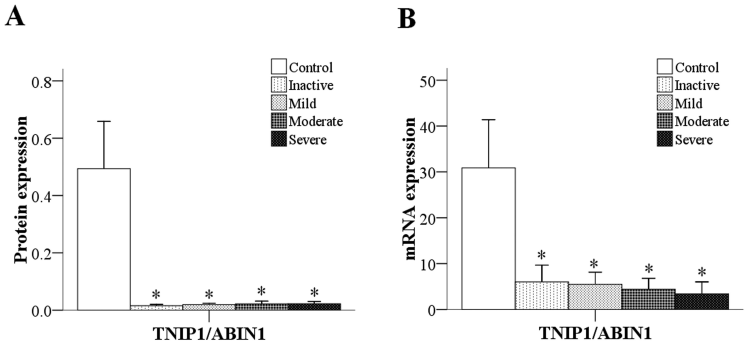

Figure 3. Expression of TNIP1/ABIN-1 protein (A) and mRNA (B) in controls and patients with different systemic lupus erythematosus severity. Control group, n = 10; inactive group, n = 3; mild group, n = 3; moderate group, n = 5; severe group, n = 7. *Significant difference relative to control.

may also have a role in the induction of autoimmune inflammatory reactions[25].

ABIN-1/TNIP1 contains 2 major functional domains, AHD and UBAN (ubiquitin binding in ABIN-1 and NEMO)[25]. Expression of TNIP1 mRNA occurs in multiple tissues and is especially high in lymphocytes, the spleen, and skeletal muscle, regions associated with cell differentiation, apoptosis, and inflammatory responses[26]. AHD1 mediates the binding of ABIN-1 and A20, and AHD2 is located in the UBAN motif and is closely related to the inhibition of NF-κB signaling. ABIN-1 connects A20 with the ubiquitinated NEMO, leading to A20-mediated NEMO deubiquitination and NF-κB inhibition[25]. Most functions of ABIN-1 appear to be A20-dependent, but some of its functions do not require A20[25,27]. Other research indicates that expression of TNIP1 mRNA is low in resting T lymphocytes and elevated in activated CD4+/CD8+ T cells, indicating that upregulation of TNIP1 is associated with T cell activation[28,29]. A recent study by Adrianto, *et al*[30] examined the association of 2 independent functional risk haplotypes in TNIP1 with SLE in cases and controls of European ancestry, and of African American, Hispanic, East Asian (76% of whom were Korean), and African American Gullah populations. In agreement with our results, these researchers reported an association of SLE with TNIP1 variants in several populations and reported that reduced ABIN-1 expression was associated with SLE. Taken together with our results, this previous research indicates that TNIP1 affects the TNF-mediated inflammatory response and appears to have an important role in the regulation of immune function.

Previous research[5] reported that the CC genotype of rs10036748 in TNIP1 was protective against SLE in European populations, in agreement with our results. A previous report indicated no association of the rs13168551 locus with SLE in Chinese subjects from the Yunnan region[31]. However, our study showed that the allele frequency distribution of that locus was significantly different in cases and controls, and that the TT genotype was associated with significantly reduced risk of SLE. We speculate that this difference may be due to the different racial constitution of subjects in these 2 studies. We studied a Chinese Han population, whereas Yunnan is a highly diverse multiethnic province. Again, further studies with larger sample sizes are needed for confirmation of this hypothesis.

Our study had several limitations that should be noted. The number of cases was relatively small and all subjects were Han Chinese from southwestern China, so our results should not be generalized to other populations. Second, we have not yet performed functional studies of the identified SNP. These 2 limitations can be easily overcome by future studies.

Our replication study confirmed that the rs7708392 C/G and rs10036748 T/C loci of TNIP1 are associated with SLE in the Chinese Han population. We also identified 4 SLE susceptibility loci (rs6889239 C/T, rs4958437 C/T, rs960709 G/A, and rs13168551 C/T) and 2 SLE-associated haplotypes (ATTGCGC from block 1 and GTCCTAT from block 2). However, our results need to be validated by more comprehensive analysis of different genetic models. Future research should analyze the biological functions of the mutated loci and seek to clarify the influence of SNP in TNIP1 on SLE in the Chinese Han population.

ACKNOWLEDGMENT

The authors are grateful to all participants involved in the study and thank all colleagues for coordination and sample collection.

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