Supporting Information

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SI Materials and Methods

Western Blot Analysis. Proteins were extracted from PBMCs of Chinese male controls carrying rs3853839 G and C allele ($n = 6, 2$ respectively). Following SDS/PAGE, the proteins were transferred onto a PVDF membrane (Millipore). After blocking with 5% nonfat milk, the membrane was successively incubated with the mouse anti-TLR7 primary antibody (Santa Cruz Biotechnology) and HRP-conjugated secondary antibody (Cell Signaling). The peroxidase activity was detected using SuperSignal West Pico kit (Pierce). β-Actin was used as internal control.

CNV Experiments. ***SYBR Green quantitative real-time PCR.*** We tested CNVs of *TLR7* and *TLR8* gene in 223 SLE patients and 139 normal control subjects, using SYBR Green quantitative real-time PCR. Demographic data of study cohorts are shown in Table S3. *CD36* was used as one copy control. Primer sequences were as follows: *TLR7* forward primer, 5′-aggtggaagcagacaggaga-3′; *TLR7* reverse primer, 5′- tctcccagacacacttgcag-3′; *TLR8* forward primer, 5′-tccttcagtcgtcaatgctg-3′; *TLR8* reverse primer, 5′- cgtttggggaacttcctgta-3′; *CD36* forward primer, 5′-taagttcaggttcctggaatgc-3′; and *CD36* reverse primer, 5′-caaattatggtatggactgtgc -3′. Each sample was analyzed in duplicates or triplicates and real-time PCR reactions were performed using ABI 7900HT system (Applied Biosystems). The relative standard curve method was used to calculate the CN of the target gene. In brief, we serially diluted CEPH 1347–02 commercial DNA (as a two-copy control; Applied Biosystems) by 3 logs of DNA concentrations that cover a Ct range of approximately 23 to 33. After the PCR was completed, a standard curve was generated with the log of DNA for *TLR7*, *TLR8*, and *CD36* gene versus their corresponding CT for each dilution (Fig. S3 *C–E*). Based on these standard curves, the initial CNs of *TLR7* or *TLR8* and *CD36* gene in the test samples were calculated. The ratio of the CN of *TLR7* or *TLR8* to the CN of *CD36* gene was therefore a measure for *TLR7* or *TLR8* CN in each sample. Using CEPH 1347–02 DNA as a two-copy control, the calculated CNs of test samples were corrected and rounded to the closest integers. To verify initial result, two additional sets of primers for each of *TLR7* and *TLR8* gene were used to detect the CNVs in some of the samples (Fig. S3 *A* and *B*). Consistent results were achieved using different sets of primers.

Southern blot. A total of 250 human subjects were studied for the potential CNVs of *TLR7–TLR8* locus using PmeI-PFGE or TaqI genomic Southern blot analyses, among them 203 female subjects and 47 male subjects with a total of 453 X chromosomes. Categories of study cohorts are shown in Table S4. In addition, 49 patients with SLE who were tested for CNVs by real-time PCR assay were also included in PmeI-PFGE analysis. cDNA probes for human *TLR7* was generated from MGC clone 45096/IMAGE:5582912) by PmeI-HindIII restriction digests, which yielded a 0.8-kb HindIII-fragment corresponding to the 5′ region of *TLR7*, and a 2.3-kb PmeI-HindIII (vector) fragment corresponding to the 3′ region of *TLR7*. gDNA probes that include sequences surrounding exon 1 of *TLR7* (765 bp) and exon 1 of *TLR8* (755 bp) were generated by single specific primer PCR and purified by agarose gel electrophoresis.

PmeI-PFGE. A long-range sequence analysis for rare-cutter restriction enzyme PmeI sites of the genomic region 200 kb upstream of *TLR7* and 200 kb downstream of *TLR8* was performed. Physical map of the *TLR7-TLR8* locus is shown in Fig. S4*A*. There is one PmeI site located inside the *TLR7* gene, which is 19 kb from the 5′ end of the gene. Application of *TLR7* 0.8-kb HindIII 5′ cDNA probe and 2.3-kb 3′ cDNA probe for Southern blot analysis of gDNA digested with PmeI reveals 66.5-kb and 185-kb PmeI fragments, respectively. Ninety-three patients with SLE, 74 first-degree family members of patients with SLE, and 19 healthy controls were subjected to PmeI-PFGE by 5′ probe or 3′ probe. Peripheral blood leukocytes of these samples were isolated via Ficoll gradient centrifugation and then made into DNA plugs in low gelling temperature agarose to avoid shearing and mechanical breakage. The gDNA trapped in agarose plugs was then digested with PmeI and resolved by PFGE and then underwent Southern blot analysis.

TaqI genomic Southern blot analyses. gDNA digested with TaqI, processed and transferred to nylon membrane according to standard Southern blot procedure, were hybridized to 2.3-kb *TLR7* 3′ probe labeled by ^{32}P-dCTP that yielded 7.25-kb and 2.23-kb restriction fragments, to 0.8-kb HindIII *TLR7* 5′ probe that yielded 2.25-kb restriction fragment, to 0.755-kb *TLR8* probe that yielded a 1.1-kb restriction fragment, or to 0.765-kb *TLR7* exon 1 probe that yielded a 10.8-kb restriction fragment. A total of 113 gDNA samples were processed by this methodology.

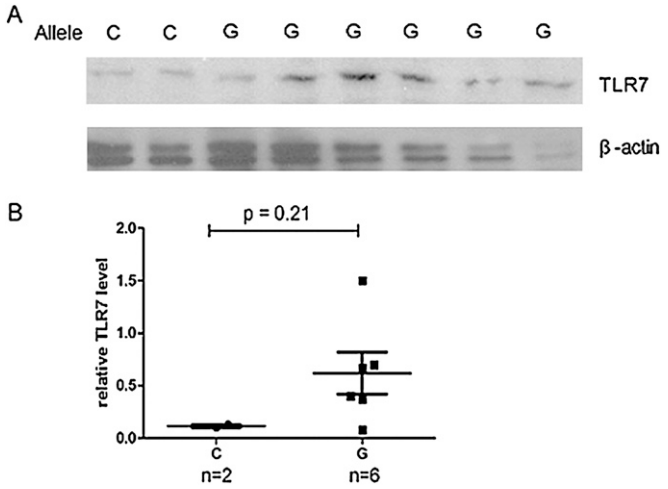

Fig. S1. Allelic-specific expression levels of TLR7 protein in Chinese male controls. (*A*) TLR7 proteins extracted from PBMCs of Chinese male controls carrying allelic genotype of rs3853839 (n = 2 and n = 6 for C males and G males, respectively) were determined by Western blot. (*B*) The normalized values are shown relative to the β-actin control. Each symbol represents an individual, horizontal lines indicate mean values, and the error bars indicate ± SD.

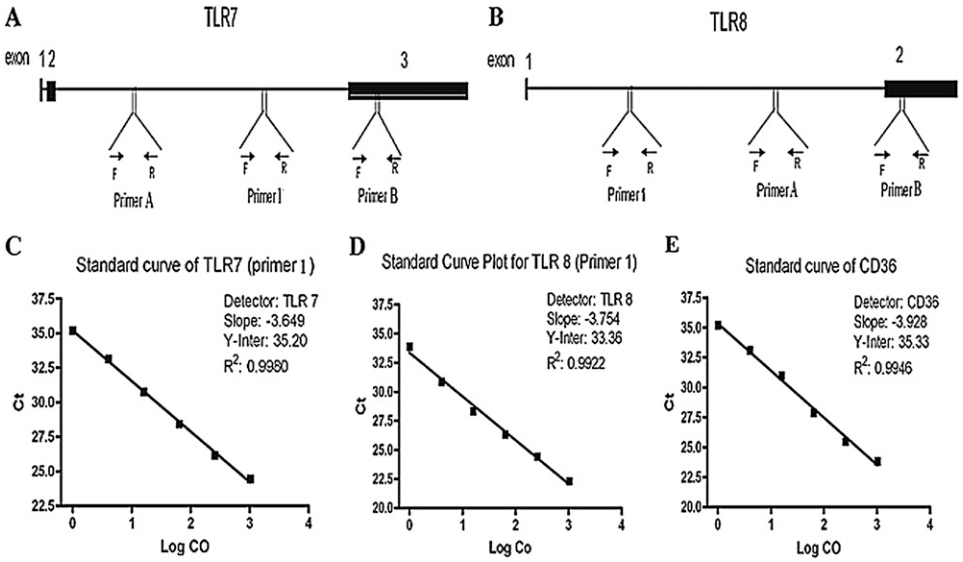

Fig. S2. Detection of CNV at *TLR7–TLR8* locus using SYBR Green quantitative real-time PCR. (*A*) Locations of primers across *TLR7*. (*B*) Locations of primers across *TLR8*. Primer 1 was used for CNV detection in each gene. Then, two additional primers (primer A and B, respectively) designed in other parts of each gene were used in some samples to verify the initial results. (*C–E*) Standard curves of primers for *TLR7*, *TLR8*, and *CD36* to calculate the CN of the target gene.

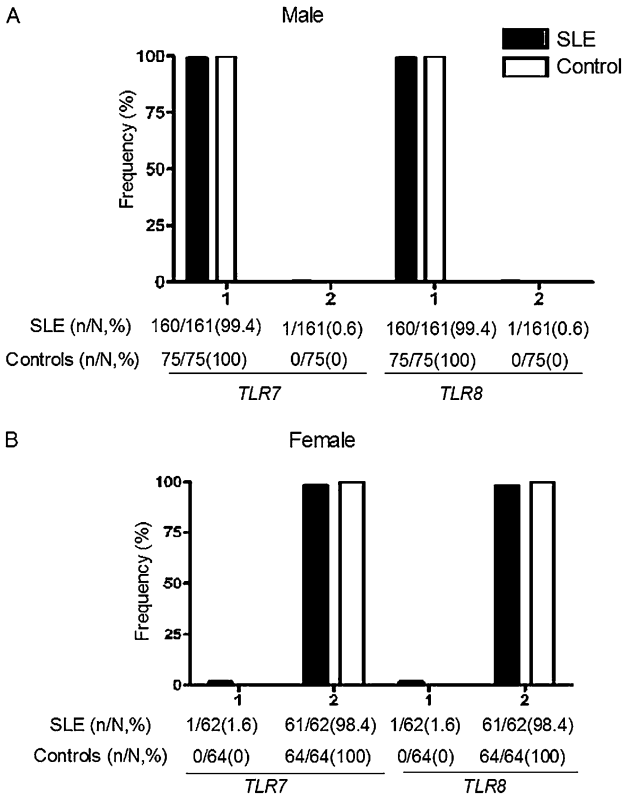

Fig. S3. Frequency of copy number (CN) at *TLR7-TLR8* locus among 223 individuals. No CNV was identified in male and female controls. (*A*) Distributions of *TLR7-TLR8* CNVs in male samples showed one male patient having a two-copy variant of both *TLR7* and *TLR8*. (*B*) Distributions of *TLR7–TLR8* CNVs in female samples showed one female patient having a one-copy variant of *TLR7* and another having a one-copy variant of *TLR8*.

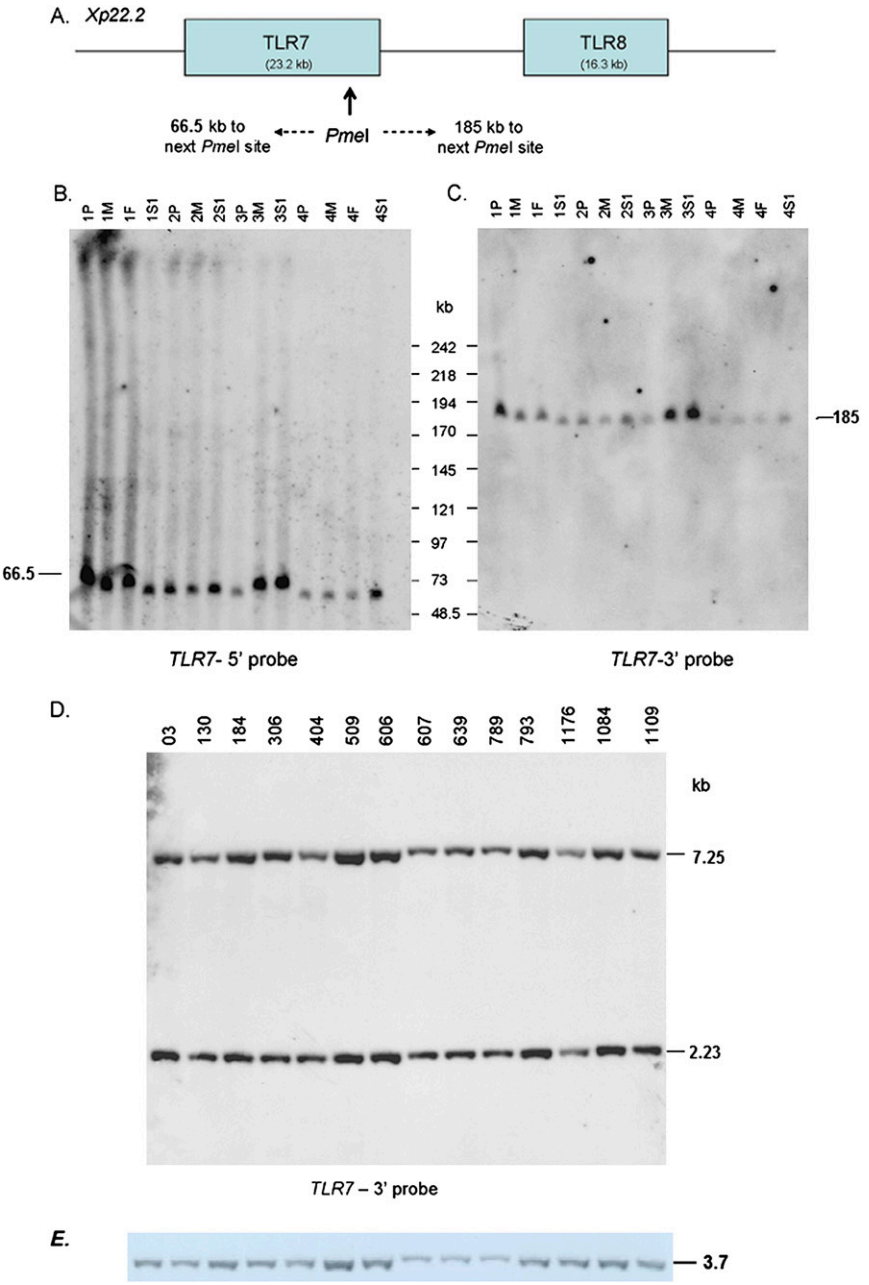

Fig. S4. Southern blot analyses of *TLR7–TLR8* locus. (*A*) Physical map of the *TLR7–TLR8* locus. A PmeI restriction site is present 19 kb from the 5′ end of the *TLR7* gene. Locations of the upstream and downstream PmeI sites are stated. (*B* and *C*) Southern blot analysis of PmeI-digested gDNA from four SLE families resolved by pulsed field gel electrophoresis and hybridized to 0.8-kb *TLR7* 5′ probe (*B*) and to 2.3-kb *TLR7* 3′ probe (*C*), respectively. (*D*) TaqI Southern blot analyses of 14 SLE patients using 2.3-kb *TLR7* 3′ probe, which yielded 7.25-kb and 2.23-kb restriction fragments. (*E*) TaqI Southern blot hybridized to CYP21 to show the 3.7-kb CYP21B with two copies of autosomal genes of the same Southern blot (internal control).

Table S1. Association of SNPs investigated in the discovery panel

SNP	Functional region	Major allele	Major allele frequency		*P* value	OR (95% CI)	Bonferroni-corrected *P* value
			Case (*n* = 1,434)	Control (*n* = 1,591)			
TLR7							
rs2897827	Promoter	G	0.908	0.899	0.289	1.10 (0.92–1.31)	
rs5935436	Promoter	G	0.966	0.949	0.0018*	1.52 (1.17–1.97)	0.0414*
rs2302267	Intron 1	A	0.905	0.899	0.392	1.08 (0.91–1.29)	
rs179019	Intron 2	C	0.760	0.755	0.617	1.03 (0.91–1.17)	
rs5743740	Intron 2	A	0.848	0.847	0.905	1.01 (0.87–1.17)	
rs179016	Intron 2	C	0.840	0.842	0.81	0.98 (0.85–1.14)	
rs1638596	Intron 2	A	0.941	0.949	0.168	0.85 (0.67–1.08)	
rs179012	Intron 2	G	0.914	0.908	0.427	1.08 (0.90–1.29)	
rs179010	Intron 2	G	0.678	0.663	0.231	1.07 (0.96–1.20)	
rs179009	Intron 2	A	0.825	0.831	0.526	0.95 (0.83–1.10)	
rs179008	Exon (L11Q)	T	0.997	0.998	NA	NA	
rs3853839	3′UTR	G	0.795	0.757	0.0007*	1.24 (1.10–1.41)	0.0161*
rs3764880	Exon 1(V1M) or 5′UTR	G	0.844	0.823	0.033*	1.16 (1.01–1.34)	0.759
rs17256081	Intron 1	A	0.861	0.848	0.153	1.11 (0.96–1.29)	
rs5020841	Intron 1	G	1.000	1.000	NA	NA	
rs2109134	Intron 1	T	1.000	0.999	NA	NA	
rs4830805	Intron 1	A	0.827	0.804	0.026*	1.16 (1.02–1.33)	0.598
rs3827469	Intron 2	G	0.781	0.780	0.956	1.00 (0.89–1.14)	
rs4830806	Intron 2	A	0.805	0.808	0.750	0.98 (0.85–1.11)	
TLR8							
rs4830808	Intron 2	A	0.779	0.780	0.989	1.00 (0.88–1.14)	
rs1013151	Intron 2	A	0.805	0.809	0.749	0.98 (0.85–1.11)	
rs5741886	Intron 2	T	0.196	0.188	0.502	0.95 (0.83–1.10)	
rs2159377	Exon 3 D136D	A	0.776	0.775	0.931	1.01 (0.89–1.14)	
rs5744080	Exon 3 H233H	A	0.803	0.807	0.755	0.98 (0.86–1.11)	
rs2407992	Exon 3 L669L	C	0.196	0.190	0.553	1.04 (0.91–1.19)	
rs5744082	Exon 3 Q715R	G	1.000	1.000	NA	NA	
rs3747414	Exon 3 I769I	A	0.784	0.819	0.432	0.81 (0.47–1.39)	

*Significant at $P < 0.05$.

Table S2. Primers used in resequencing three exons and 5′promoter region (up to 2 kb upstream) of *TLR7* gene

Region	Forward primer	Reverse primer
Exon1–2	1F 5′-cccgacctgatctttgtag-3′	1R5′-gagagcagagcatgagagg-3′
	2F 5′-tggttttctggaatatgtctc-3′	2R 5′-ccccaattcaaacatctg-3′
Exon3	1F 5′-aaatgctgcttctaccctct-3	1R 5′-gcggtatctctagtagctggt-3′
	2F 5′-gaggtattcccacgaacac-3′	2R 5′-ttaattctgtcagcgcatc-3′
	3F 5′-tccctactgttttgccatct-3′	3R 5′-tgcacgatagacctgaagttc-3′
	4F 5′-cagtaactctcttcagcatgtg-3′	4R 5′-gacagattcaggcatttgag-3′
	5F 5′-agtatgggcagaccttggat-3′	5R 5′-ggtccaaagtttccaggttc-3′
	6F 5′-tggagagaaggtgataacagat-3′	6R 5′-tcccagaaatagaggtgactt-3′
	7F 5′-cccagaaaatgtcctcaac-3′	7R 5′-tctttgcatacttgtctgtcat-3′
	8F 5′-gttgctatgatgcttttattgt-3′	8R 5′-gcctctccttggtaaactag-3′
	9F 5′-ctggcagtgtctaaagaacg-3′	9R 5′-attacaagcatgagccacc-3′
	10F 5′-aaacatggggctctgattc-3′	10R 5′-aggaagcactgaagcagc-3′
	11F 5′-accccgtctgtactaaaaat-3′	11R 5′-ctccttttagggtttaccatc-3′
	12F 5′-accaattgcttccgtgtcat-3′	12R 5′-gtttcttctcccatcctccag-3′
	13F 5′-cctttgataatttacctgctt-3′	13R 5′-agaacggaaactatgaaacac-3′
Up to 2 kb upstream	1F 5′-agccagttgcccaataatc-3′	1R 5′-ctcactttaacatgccgtctg-3′
	2F 5′-gcaccccagtttagaatcagt-3′	2R 5′-gctcttgtttggtgatgctc-3′
	3F 5′-atatctttgtgtctgggcag-3′	3R 5′-ggcagtaaaaaccaaatgtc-3′
	4F 5′-gtaaagcattatagtccccatc-3′	4R 5′-gttggttcactcactttcct-3′
	5F 5′-aaaaccctcaataaatgtcact-3′	5R 5′-cccttgctctatacccact-3′
	6F 5′-agaggaaagtgagtgaaccaac-3′	6R 5′-ccaactacaaagatcaggtcg-3′
	7F 5′-gtgattgtcataactggaagg-3′	7R 5′-atatgatttcggttccctct-3′

Table S3. Study cohorts for CNV of *TLR7* and *TLR8* gene using real-time PCR assay

	SLE			Normal controls		
Ethnicity	All	Female	Male	All	Female	Male
White	89	46	43	88	48	40
Asian	126	14	112	32	12	20
Mexican American	5	1	4	16	4	12
Black	3	1	2	3	0	3
Total	223	62	161	139	64	75

Table S4. Study cohorts for CNV of *TLR7* and *TLR8* gene using Southern blot analyses

Category*	SLE	SLE-R	T1D	T1D-R	Other dis	Controls	Total	X-chromosomes
Females	97	49	10	10	18	19	203	406
Males	5	25	4	2	3	8	47	47
All	102	74	14	12	21	27	250	453
PmeI-PFGE all[†]	44	74	—	—	—	19	137	—
TaqI all	58	—	14	12	21	8	113	—

*SLE-R, relatives of SLE patients; T1D, type 1 diabetes mellitus; T1D-R, relatives of T1D patients; Other Dis, patients and relatives with other autoimmune diseases including juvenile rheumatoid arthritis, immune thrombocytopenia purpura and multiple sclerosis; Controls, healthy subjects.

[†]In addition to 137 subjects listed in this table, 49 SLE patients who were previously tested for CNVs by real-time PCR were also included in PmeI-PFGE analysis.