Higher genetic susceptibility to inXammation in mild disease activity of systemic lupus erythematosus

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Abstract In order to test the hypothesis that stratiWcation of Mexican ModiWcation of the Systemic Lupus Erythematosus Disease Activity Index (MEX-SLEDAI) simpliWes the genetic study of SLE, we evaluated the genetic susceptibility to inXammation and defects in clearance of immune complexes among SLE patients in Taiwan. SLE phenotypes were stratiWed according to the MEX-SLEDAI scores into two subgroups (≦10 and >10), and then accord- ing to renal disorder and neurological disorder, aiming to minimize any loss of power associated with disease heter- ogeneity. Upon stratiWcation, *IL1-β* polymorphism and LTA were signiWcantly associated with SLE within the MEX-SLEDAI ≦10 subgroup. When SLE patients were classiWed into two subgroups with or without renal disor- der to stratify the genetic study, we could Wnd that the stratiWcation with renal disorder could partially conWrm the hypothesis that stratiWcation of MEX-SLEDAI score simpliWes the genetic study of complex diseases such as SLE. So we concluded that in the mild disease state of SLE, stratiWcation of disease phenotypes, especially *IL1-β* and *LTA*, according to MEX-SLEDAI scores could reveal new associations between candidate genes and disease activity index of SLE.

Keywords Systemic lupus erythematosus · Disease activity index · Genetic susceptibility · InXammation

Introduction

Systemic lupus erythematosus (SLE) is a chronic inXam- matory autoimmune disease that can aVect many organ systems including the skin, joints, and any internal organ. In such an autoimmune disease, patients' immu- nological dysfunction may result in damage to their own tissues, resulting in chronic inXammation. Although the etiology is unknown, it is thought to result from a com- bination of hereditary, environmental, and hormonal factors [1-6].

Candidate gene association studies, which are valuable in clarifying the underlying cause of some diseases, are based on the assumption that the major genes inXuencing susceptibility to the disease of interest act in known biologi- cal pathways. For SLE, gene identification for the syndrome is clearly relevant to the eVorts to understand the pathogen- esis of the associated complex trait [7]. In association stud- ies of human SLE, the contributions of the MHC loci, Fc *y* receptors (FCGR), various cytokines and its receptors, com- ponents of the complement cascade, and proteins involved in apoptosis have been explored [8, 9]. DiVerent genotype results were seen in diVerent populations. For example, genetic variants of *FCGRIIA*, *IIIA*, and *IIIB*, and their asso- ciation with SLE have been extensively studied in various populations; results were inconsistent [10–12]. Our study strategy was to use the case–control candidate gene associa- tion study approach. To understand inflammation suscepti- bility and defects in clearance of immune complexes, we selected six candidate genes encoding major cytokines of inflammation (*TNF-c*, *IL1-β*, *LTA*, *ILJRN*) and related to auto-antibody concentration (*PDCDJ*, *TNF-c*, *IL1-β*) [13–15] and clean-up functions (*C4*) [16]. The majority of can- didate genes only has the minor eVect and through gene–gene interaction to involve the pathogenesis of SLE [17]. So the stratification with subtype of patient population is feasi- ble to find candidate gene's eVect.

There are many instruments that can measure SLE clini- cal disease activity; British Isles Lupus Assessment Group (BILAG) [18], European Consensus Lupus Activity Mea- sure (ECLAM) [19], Lupus Activity Criteria Count [20], Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [21], Mexican Modification of the SLEDAI (MEX-SLEDAI) [22], and Systemic Lupus Activity Mea- sure (SLAM) [23], and so on. Many studies have been con- ducted to determinate whether SLE disease activity index correlates with serum levels of cytokine and its receptor, complement, anti-dsDNA antibody, and other factors. Serum IL-6, TNF*c* levels (especially soluble receptor IL-2*c*, p55 srTNF*c*, and p75 srTNF*c*), C3, C4, and anti- dsDNA antibody do indeed correlate strongly with SLEDAI and ECLAM disease activity scores [24–27]. However, few studies have determined the correlation between SLE disease activity index and genotypes of cyto- kine, cytokine receptor, complement, or FCGR. SLEDAI comparison of patients with *C4A * Q0* to patients with other *C4A* allotypes has failed to discern a significant corre- lation between severe and mild disease [28]. Recently, a significant association between the *PD-J.3A* allele and renal manifestations was reported, using the damage assess- ments of the SLICC/ACR Damage Index and SLEDAI score [29]. Therefore, presently we tested the hypothesis that the stratification of disease activity indices (e.g., MEX- SLEDAI scores) simplifies the genetic study of complex diseases such as SLE. We evaluated genetic susceptibility to inflammation and defects in clearance of immune com- plexes among SLE patients in Taiwan.

Patients and methods

Collection of SLE patients and normal controls

Over the course of 1 year, 95 un-related SLE patients from either the Taipei or Taichung Veterans General Hospitals were collected. The patients fulfilled four or more of the ACR criteria. Additionally, 95 normal, non-aboriginal Taiwanese individuals were recruited as controls by the super-control project managed by the Institute of Biomedi- cal Sciences (IBMS), Academia Sinica. The super-control project was a collaborative study sponsored by Glaxo- SmithKline and IBMS to establish a cell bank and a genetic database on non-aboriginal Taiwanese healthy men and women, aged 20 and above, residing in Taiwan. The objec- tive of this project was to establish a representative super- control pool for non-aboriginal Taiwanese individuals to serve as an ethnicity panel and multiple controls for a range of diseases specified in the agreement. Our controls were not sex and age-matched with the patients with SLE. The project objective was explained to each SLE patient and normal subject, who then signed an informed consent form.

Each patient completed a flowchart for clinical and labo- ratory data collected by physician over a half-year time frame. The record of clinical and serologic features included clinical manifestations, nephrotic status, blood pressure, hematogram, urinalysis, anti-double-stranded- DNA antibody, complement titer, CH_{50} hemolytic titer, serum creatinine concentration (Cr), urine creatinine clear- ance rate (CCr), and 24-h urine protein [30].

Classification of SLE patients by disease activity index scale

Disease activity was assessed by our medical flowchart review using a modified version of the MEX-SLEDAI, in which the maximum score was 32 [22]. MEX-SLEDAI is valuable and not expensive experience. Abnormalities that were not mentioned in the medical record were considered as not present and were scored as zero.

SLE patients were divided into two subgroups: those having a MEX-SLEDAI score -10 or a score >10. Depend on their distribution, there was a half of patient population belong to each of two subgroups (Fig. 1). The SLEDAI score is a weighted activity index, giving the highest scores to patients with kidney and central nervous system involve- ment [21]. MEX-SLEDAI score -10 indicates a mild dis- ease state such as mucocutaneous disorder and arthritis, whereas >10 indicates a severe disease state such as renal and neurological disorder. To assess inflammation suscepti- bility and defects in clearance of immune complexes, and to investigate whether the chosen disease activity index could simplify the genetic study of SLE, we selected six candidate genes encoding major cytokines of inflammation (*TNF-o*, *ILJ-* , *LTA*, *ILJRN*) and related to auto-antibody concentration (*PDCDJ*, *TNF-o*, *ILJ-*) [13–15] and clean- up functions (*C4*) [16].

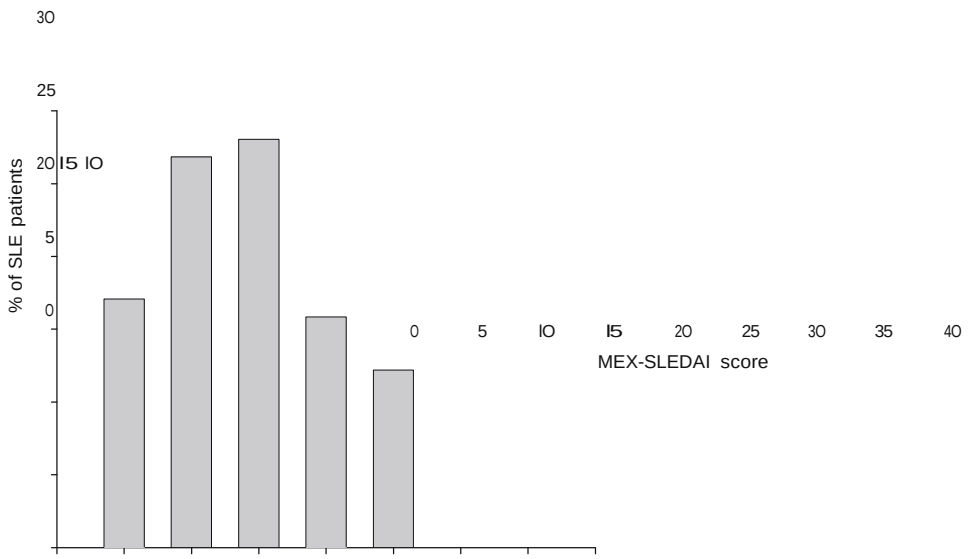

Fig. 1 Classification of SLE patients according to MEX-SLEDAI score. Scores calculated based on the available data collection sheets were used to calculate the distribution of percentage. SLEDAI scores were combined, with scores of 0–5 represented as 5, and scores of 6–10 represented as 10

Genotyping

All of single nucleotide polymorphisms (SNPs) were col- lected from the dbSNP database of the National Center for Biotechnology Information (NCBI; Hyattsville, MD, USA). The selection of SNPs focused on the exon and pro- moter regions [31], in which we preferred to select either amino acid changed or transcription factor binding SNPs. To validate SNPs from the candidate genes, minor allele frequencies that exceeded 5% were accepted for study.

Genomic DNA was extracted from blood cells using the PureGene DNA Isolation Kit (Gentra Systems, Minneapo- lis, MN, USA). For these SNP assays, we used the Seque- nom Mass-Array (Sequenom, San Diego, CA, USA) platform at Academia Sinica of Taiwan to do matrix- assisted laser desorptionIionization-time of flight (MALDI- TOF) mass spectrometry. In brief, this automated SNP genotyping platform includes primer design software, auto- mated arraying robotics, and MALDI-TOF mass spectro- metric analysis.

Restriction fragment length polymorphism genotyping of *IL1-β* and *ILJRN*

The promoter region of *IL1-β* contains an SNP at position -511 (C → T). The effect of *IL1-β* C-511T may be mediated by linkage disequilibrium with the TATA box polymor- phism *ILJ* C-31T. Linkage disequilibrium between *ILJ* C-31T and *ILJ* C-511T was with 99.5% of the inferred haplotype (*ILJ* C-31TI*ILJ* C-511T either C–C or T—T). Findings from electrophoretic mobility-shift assay have demonstrated that the transcription factor complex cannot bind to the C allele of *ILJ* C-31T [32]. The primers flank- ing this SNP used to amplify the polymorphic region by PCR were: 5'GGCATTGATCTGGTTCATC (sense) and 5'GTTTAGGAATCTTCCCACTT (antisense) [33]. The 306 bp PCR

product contained a cleavage site for *DdeI* (BioLabs Inc., New England), resulting in two fragments of 146 and 160 bp. The C allele contained an additional cleav- age site resulting in three fragments of 160, 114, and 32 bp [34]. PCR conditions were as follows: 20 cycles at 94°C for 30 s, 65°C touch down to 55°C for 30 s, and 72°C for 20 s, 15 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 20 s.

ILJRN (rs315952) is a CIT SNP. This SNP is a synony- mous change at coden position 3 of interleukin 1 receptor antagonist isoform 1 precursor (NP 776214) serine^{112} resi- due. The following primers flanking this SNP were used to amplify the polymorphic region by PCR: 5'GGTATGGCA TTTCCCCTGTA (sense) and 5'CCTTCGTCAGGCATAT TGGT (antisense). The 303 bp PCR product contained a cleavage site on C SNP for *Msp AJI* (BioLabs Inc., New England), resulting in two fragments of 104 and 199 bp. The T SNP did not contain this cleavage site, and therefore resulted in one 303 bp fragment. PCR conditions were as follows: 35 cycles at 95°C for 60 s, 60°C for 45 s, and 72°C for 45 s.

All PCR products were checked by electrophoresis on 2% agarose gel stained with ethidium bromide for visuali- zation with ultraviolet light. The fragments of PCR product cutting by restriction enzyme were determined by electro- phoresis on a 3% agarose gel.

Genotype confirmation

With the exception of the genotyping of *IL-J* and *ILJRN* (which was done using restriction enzymes), all other geno- types were confirmed by sequencing. Primers (picked from Primer3 software, http:IIfrodo.wi.mit.eduIcgi-binIprimer3I primer3_www.cgi) were used to amplify the polymorphic region by PCR. The primers were: 5'GGCTCTAGGGCTC AAGGTTT (sense) and 5'GCATCTTGTCCCCTTCTCTG (antisense) for LTA (rs2857713), 5'ACCACCCTACACCT CCTCCT (sense) and 5'AGTGGGCTCCCTCTGTTTTT (antisense) for *LTA* (rs1041981), 5'AACACAGCTTTTCC CTCCAA (sense) and 5'GATTTGGAAAGTTGGGGA CA (antisense) for *TNF-o*-308AIG, 5'GTGCCTGTGTTCT CTGTGGA (sense) and 5'ACCCTGCCTGCTTCTCCT (antisense) for *PDCDJ* (rs2227981), 5'TCAACACAAAG GCTGTGA GC (sense) and 5'CAGGAGGCTACAGAGG GAAA (antisense) for *C4* (rs22582i8), 5'CTGGGTGTTT CCTGGTTTTG (sense) and 5'GAGGTGAGGTGGGAG ACTCA (antisense) for *C4*(rs3926i0), and, finally,
PCR conditions consisted of an initial denaturation of
5 min at 95°C followed by three different annealing and elongation conditions: *TNF-o*-308AIG, *LTA* (rsi04i98i) and *C4* (rs3926i0) followed by 20 cycles of touchdown PCR in 30 s at 94°C, 30 s at 65°C (decrease 0.5°C per cycle), 40 s at 72°C, and a final 20 cycles in 30 s at 94°C, and then were sequenced using the BigDye Terminator
Cycle Sequencing Kit vi.iI3.i (Applied Biosystems, Foster City, CA, USA). Sequencing products were separated on an ABI PRISM 3700 DNA Analyzer (Applied Biosystems). Raw sequencing data were analyzed by the DNA Sequenc- ing Analysis Software v3.7 (Applied Biosystems).

Statistical analyses

Statistical significance for genotyping was determined by X^2-test or Fisher's exact test (if the number was <5), with significance defined as $P \leqq 0.05$. Other results such as age, disease duration, and MEX-SLEDAI score, represent the mean $\pm$ standard error. Clinical severity results represent the mean group clinical score. Statistical differences were calculated by the Student's *t*-test and significance was defi- ned as $P \leqq 0.05$.

Results

Clinical characteristics and MEX-SLEDAI scores of SLE patients

Of the 95 SLE patients, MEX-SLEDAI scores were deter- mined for 82. The remaining other i3 patients were excluded due to incomplete data. Among the 82 SLE patients, the distribution of MEX-SLEDAI scores is pre- sented as the percentage of the SLE population (Fig. i). In brief, there were 43.9% (n = 36) SLE patients whose MEX- SLEDAI scores were -i0 and 56.i% (n = 46) SLE patients whose MEX-SLEDAI scores were >i0. The patients were divided into two subgroups based on these MEX-SLEDAI scores. As summarized in Table i, the common symptoms of subgroup i (MEX-SLEDAI $\leqq$ i0) were mucocutaneous

SLE patients were classified using the MEX-SLEDAI score. Patients were separated them into two subgroups, with MEX-SLEDAI - i0 comprising subgroup i and MEX-SLEDAI > i0 comprising subgroup 2

Result displayed significant difference in comparison with subgroup i

disorder and arthritis. In subgroup 2 (MEX-SLEDAI > i0), the prevalent symptoms were renal and neurological disor- ders. The mean disease duration in both subgroups (8.i ± i.3 years in subgroup i and i0.2 ± i.0 years in sub- group 2) was not significantly different (Table i). No sig- nificant differences were evident between the subgroups in mean age, onset age, and sex ratio.

Different frequency of genotype and allele among SLE patients, subpopulations, and normal controls

The frequencies of all SNP genotypes and alleles per candi- date gene are presented in Table 2. All SNP genotypes in the normal control population fit the Hardy—Weinberg equi- librium model, except for *C4* SNPs (rs22582i8, rs3926i0, rs4947334). *C4* copy number was different in different indi- viduals; therefore, it is reasonable that *C4* SNPs could not fit the Hardy–Weinberg equilibrium model. Significantly different frequencies of genotype and allele were evident between SLE patients and normal control subjects when compared with the frequencies of genotype and allele in SLE patients and normal control subjects. Allele frequency determinations revealed that only *IL1-β* C-5iiT (*P*-value = 0.0i6) was significantly different (using 2×2 X^2-test or Fisher's exact test). Although the genotype of *IL1-β* C-5iiT in SLE patients did not fit the Hardy–Weinberg equilibrium model, there was no significant difference in SLE patients and normal control subjects.

Further examination of the two MEX-SLEDAI SLE patient subgroups revealed new frequencies of genotypes and alleles (Table 2). When compared to normal controls, the IL1-*J* C-511T allele frequency in the MEX-SLEDAI - 10 subgroup reached significance.

MEX-SLEDAI score for all SNP genotypes of the candidate genes

In the SLE patient MEX-SLEDAI subgroups, each SNP genotype displayed a different MEX-SLEDAI score (Table 3). In the MEX-SLEDAI - 10 subgroup, two SNPs (*IL1-β* C-511T and *LTA* rs1041981) displayed MEX-SLE- DAI scores that were significantly different from each other's genotype for the same SNP. In the same subgroup, *IL]-J* C-511T displayed significant differences CT hetero- zygote and TT homozygote scores were compared with CC homozygote scores.

Two exon region SNPs are present in the *LTA* gene. *LTA* (rs2857713) affects amino acid 13 with an Arg → Cys change, whereas LTA (rs1041981) exhibited a Thr → Asn

Table 2 Frequency of SNP genotypes and alleles in all candidate genes in the two SLE patient subgroups, and normal controls

SNP		SLE cases			Control	*P* value[a]	*P* value[b]	*P* value[c]
		Total (*N* = 95)	Subgroup 1 (*N* = 36)	Subgroup 2 (*N* = 46)	Normal (*N* = 95)			
IL1RN (rs315952)	CC	30 (31.58%)	10 (27.78%)	14 (30.43%)	36 (37.89%)			
Genotype	TT	23 (24.21%)	11 (30.56%)	10 (21.74%)	18 (18.95%)			
	CT	42 (44.21%)	15 (41.67%)	22 (47.83%)	41 (43.16%)	n.s.	n.s.	n.s.
Allele	C	0.54	0.49	0.54	0.59			
	T	0.46	0.51	0.46	0.41	n.s.	n.s.	n.s.
IL1-*J* C-511T	CC	21 (22.1%)	6 (16.67%)	12 (26.09%)	30 (31.58%)			
Genotype	TT	38 (40.0%)	15 (41.67%)	18 (39.13%)	24 (25.26%)			
	CT	36 (37.89%)	15 (41.67%)	16 (34.78%)	41 (43.15%)	n.s.	n.s.	n.s.
Allele	C	0.41	0.38	0.44	0.53			
	T	0.59	0.63	0.57	0.47	0.018	0.024	n.s.
LTA (rs1041981)	AA	24 (25.26%)	10 (27.78%)	12 (26.09%)	23 (24.21%)			
Genotype	CC	28 (29.47%)	9 (25%)	13 (28.26%)	23 (24.21%)			
	AC	43 (45.26%)	17 (47.22%)	21 (45.65%)	49 (51.58%)	n.s.	n.s.	n.s.
Allele	A	0.48	0.51	0.49	0.5			
	C	0.52	0.49	0.51	0.5	n.s.	n.s.	n.s.
LTA (rs2857713)	AA	55 (57.89%)	18 (50%)	29 (63.04%)	59 (62.11%)			
Genotype	GG	6 (6.31%)	2 (5.56%)	2 (4.35%)	2 (2.11%)			
	AG	34 (35.79%)	16 (44.44%)	15 (32.61%)	34 (35.79%)	n.s.	n.s.	n.s.
Allele	A	0.76	0.72	0.79	0.8			
	G	0.24	0.28	0.21	0.2	n.s.	n.s.	n.s.
TNF-*o* A-308G	AA	3 (3.15%)	2 (5.56%)	1 (2.17%)	2 (2.11%)			
Genotype	GG	75 (78.95%)	28 (77.78%)	35 (76.09%)	76 (80%)			
	AG	17 (17.89%)	6 (16.67%)	10 (21.74%)	17 (17.89%)	n.s.	n.s.	n.s.
Allele	A	0.12	0.14	0.13	0.11			
	G	0.88	0.86	0.87	0.89	n.s.	n.s.	n.s.
PDCD1 (rs2227981)	CC	57 (60%)	24 (66.67%)	28 (60.87%)	49 (51.58%)			
Genotype	TT	4 (4.21%)	1 (2.78%)	1 (2.17%)	7 (7.37%)			
	CT	34 (35.79%)	11 (30.56%)	17 (36.96%)	39 (41.05%)	n.s.	n.s.	n.s.
Allele	C	0.78	0.82	0.79	0.72			
	T	0.22	0.18	0.21	0.28	n.s.	n.s.	n.s.
C4 (rs2258218)	CC	2 (2.11%)	1 (2.78%)	0	2 (2.117%)			
Genotype	TT	29 (30.53%)	9 (25%)	16 (34.78%)	27 (28.42%)			
	CT	64 (67.37%)	26 (72.22%)	30 (65.22%)	66 (69.47%)	n.s.	n.s.	n.s.
Allele	C	0.36	0.39	0.33	0.37			
	T	0.64	0.61	0.67	0.63	n.s.	n.s.	n.s.
C4 (rs392610)	GG	4 (4.21%)	2 (5.56%)	1 (2.17%)	5 (5.26%)			
Genotype	TT	16 (16.84%)	8 (22.22%)	7 (15.22%)	14 (14.74%)			
	GT	75 (78.95%)	26 (72.22%)	38 (82.61%)	76 (80%)	n.s.	n.s.	n.s.
Allele	G	0.44	0.42	0.44	0.45			
	T	0.56	0.58	0.57	0.55	n.s.	n.s.	n.s.
C4 (rs4947334)	AA	55 (57.89%)	23 (63.89%)	23 (50%)	57 (60%)			
Genotype	CC	0	0	0	0			
	AC	40 (42.11%)	13 (36.11%)	23 (50%)	38 (40%)	n.s.	n.s.	n.s.
Allele	A	0.79	0.82	0.75	0.803			
	C	0.21	0.18	0.25	0.196	n.s.	n.s.	n.s.

N individual number, *n.s.* not significant

[a] Value upon comparison of total SLE and normal control

[b] Value upon comparison of MEX-SLEDAI - 10 subgroup 1 and normal control

[c] Value upon comparison of MEX-SLEDAI > 10 subgroup 2 and normal control

change in amino acid 60. Specific analysis of the differ- ences in genotype frequencies between SLE patients and normal controls failed to reveal any significance, even fol- lowing the stratification of the two MEX-SLEDAI sub- groups. However, a significant difference was observed between each genotype's MEX-SLEDAI score for the MEX-SLEDAI $\leq$ 10 subgroup of *LTA* (rs1041981) (Table 3). The MEX-SLEDAI $\leq$ 10 scores of *LTA* (rs1041981) were significantly different when the AA homozygote score was compared with the AC heterozygote score.

The confirmation of stratification with renal disorder and neurological disorder

In MEX-SLEDAI > 10 subgroup, the prevalent symptoms were renal and neurological disorders. When SLE patients were classified into two subgroups with or without renal dis- order, the new frequencies of genotypes and alleles are list

Table 3 MEX-SLEDAI scores of the SNP genotypes of the candidate genes

SNP		MEX-SLEDAI score		
		MEX-SLEDAI - 10 (*N* = 36)	MEX-SLEDAI > 10 (*N* = 46)	Total (*N* = 82)
IL1RN (rs315952)	CC	5.80 ± 0.83	15.43 ± 1.10	11.42 ± 1.22
Genotype	TT	5.91 ± 0.77	16.20 ± 1.21	10.81 ± 1.34
	CT	7.27 ± 0.49	16.45 ± 0.88	12.73 ± 0.93
IL1-*J* C-511T	CC	7.83 ± 0.17	16.08 ± 1.24	13.33 ± 1.25
Genotype	TT	6.13 ± 0.66*	15.39 ± 0.99	11.18 ± 1.02
	CT	6.2 ± 0.65*	16.88 ± 0.91	11.71 ± 1.12
LTA (rs1041981)	AA	4.8 ± 0.63**	14.33 ± 0.86	10.00 ± 1.17
Genotype	CC	6.33 ± 0.83	16.69 ± 1.31	12.45 ± 1.39
	AC	7.47 ± 0.49	16.71 ± 0.85	12.58 ± 0.91
LTA (rs2857713)	AA	6.06 ± 0.54	15.48 ± 0.68	11.87 ± 0.82
Genotype	GG	9.00 ± 1.00	21.00 ± 3.00	15.00 ± 3.70
	AG	6.56 ± 0.61	16.60 ± 1.11	11.42 ± 1.10
TNF*o* A-308G	AA	4.50 ± 1.50	13.00 ± 0.00	7.33 ± 2.96
Genotype	GG	6.39 ± 0.45	16.66 ± 0.68	12.10 ± 0.77
	AG	7.33 ± 0.88	14.40 ± 1.17	11.75 ± 1.18
PDCD1 (rs2227981)	CC	6.50 ± 0.45	15.57 ± 0.80	11.38± 0.79
Genotype	TT	3.00 ± 0.00	13.00 ± 0.00	8.00 ±5.00
	CT	6.64 ± 0.79	17.12 ± 0.87	13.00 ± 1.15
C4 (rs2258218)	CC	4.00 ± 0.00	0	4.00 ± 0.00
Genotype	TT	7.56 ± 0.67	16.00 ± 0.84	12.96 ± 1.01
	CT	6.15 ± 0.47	16.13 ± 0.79	11.50 ± 0.82
C4 (rs392610)	GG	4.50 ± 1.50	11.00 ± 0.00	6.67 ± 2.33
Genotype	TT	6.13 ± 0.79	17.43 ± 1.72	11.40 ± 1.74
	GT	6.69 ± 0.47	15.97 ± 0.63	12.20 ± 0.71
C4 (rs4947334)	AA	6.17 ± 0.45	16.13 ± 0.85	11.15 ± 0.88
Genotype	CC	0	0	0
	AC	6.92 ± 0.76	16.04 ± 0.84	12.75 ± 0.95

N individual number. MEX-SLEDAI score of every genotype was calculated and statistical significance was assessed using the Student's *t*-test in every subgroup. Significance is defined as * $P \leq 0.05$ or ** $P \leq 0.001$

in Table 4. When compared to normal controls, the *IL1-β* C-511T genotype and allele frequency in the renal disorder (−) subgroup reached significance. When compared to renal dis- order (−) subgroup, the *LTA* (rs1041981) allele frequency, and *C4* (rs4947334) genotype and allele frequency in the renal disorder (+) subgroup reached significance.

When SLE patients were classified into two subgroups with or without neurological disorders, the new frequencies of genotypes and alleles are list in Table 5. When compared to normal controls, only the *IL1-β* C-511T allele frequency in the neurological disorder (+) subgroup reached signifi- cance. Although the significant result disappeared after multiple corrections, the stratification with renal disorder could partially confirm the hypothesis that stratification of MEX-SLEDAI score simplifies the genetic study of com- plex diseases such as SLE after these analyses.

Discussion

SLE is a chronic inflammatory autoimmune disease that damages various tissues and organs. Several genes involved in the control of autoimmunity and inflammation appear to be important in the pathogenesis of this disease. Among them, genes for the control of antibody generation and clear-up function are most important. Appropriately, this study focused on six candidate genes encoding several major inflammatory cytokines of inflammation (*TNF-o, IL1-β, LTA, IL]RN*), functions related to auto-antibody concentration (*PDCD]*, *TNF-o, IL]-J*) [13–15], and clean- up functions (*C4*) [16]. Our results demonstrate that the gene for *IL1-β* is influential on SLE pathogenesis.

As a group, SLE patients do not display significant sea- sonal variation in disease manifestations and activity, except for photosensitivity [35–38]. Presently, 82 SLE patients were divided into two subgroups, based on their MEX-SLEDAI scores. A score ≦10 indicates a mild dis- ease state such as mucocutaneous disorder and arthritis, whereas a score >10 is indicative of a severe disease state such as renal and neurological disorder. When MEX-SLE- DAI scores were used to stratify candidate gene effects, we found that the gene encoding *IL1-β* C-511T was influential only on the mild disease state in SLE pathogenesis.

Assessment of MEX-SLEDAI scores of SLE patients in the same subgroup revealed that significance of the *IL1-β* C-511T in the MEX-SLEDAI ≦10 subgroup upon comparison of the CC homozygote to the TT homozygote and CT hetero- zygote. Similar results were observed using MEX-SLEDAI scores to classify SLE patients to stratify the genetic associa- tion results. Although both the genotype and allele of *LTA* were not associated with SLE, the MEX-SLEDAI scores of *LTA* were significantly different between the two alleles ($P < 0.005$). We conclude that there is a higher genetic susceptibility to inflammation in mild SLE disease activity, and it was more straightforward to stratify patients with or with- out renal disorder. But it was less straightforward to stratify patients with or without neurological disorder.

IL1-β is a member of the interleukin 1 cytokine family. The promoter region contains an SNP at position -511 (C ⟶ T) that is associated with inflammatory disease, and is in linkage disequilibrium with *IL]RN* polymorphism [32]. In a previous study, it was demonstrated that carriage of the *IL1-β*-511T allele is associated with a higher risk of SLE than carriage of the -511CIC genotype in African Americans [39]. It must be mentioned that one study failed to demonstrate an association of this SNP with SLE in Chi- nese patients [40]. However, the importance of the latter finding is debatable, since the number of patients with mild SLE disease state was much smaller than the number of those with severe disease state.

IL1RN is a natural antagonist of *IL1-β*, and can modu- late a variety of IL1-related immune and inflammatory responses. The serum concentration of IL1RN is correlated with *IL1-β* in SLE patients [41], and studies have found significant correlation between IL1RN and the BILAG musculoskeletal score [27] and SLEDAI and ECLAM dis- ease activity scores. After a dense SNP map focusing on *IL]RN* was constructed across a 360-kb region containing the *IL1-β* gene cluster, researchers found that *IL]RN* (rs315952) was associated with SLE [42]. Although our study could not replicate this result, we could find that the MEX-SLEDAI score of CT genotype was very different with CC and TT genotype in the mild disease state. Our other study had provided evidence for an association of the rs315952 C allele with SLEISLE subgroups [43].

TNFo is a member of the TNF superfamily. Its serum lev- els are strongly correlated with SLEDAI and ECLAM disease activity scores [24]. Presently, we failed to find any significant association between the A-308G SNP and disease manifesta- tions like one previous study [44]. One meta-analysis demon- strates that the *TNF-o* promoter -308 AIG polymorphism may confer susceptibility to SLE, especially in European-derived population, but not in Asia-derived population [45].

LTA is a member of the *TNF* superfamily produced by lymphocytes. We found two SNPs (rs1041981 and rs2857713) in the exon region of *LTA* gene in our Taiwan- ese population. Although neither the genotype nor the allele frequency of *LTA* was associated with SLE, we demon- strate that the polymorphism (rs1041981) at Thr^{26}IAsn of *LTA* is significantly correlated with different MEX-SLE- DAI subgroups. This result also was confirmed by using renal disorder to stratify the

genotype and allele frequency. Thr^{26}IAsn SNP of *LTA* linked with the first intron NcoI polymorphism exhibits reduced LTA production [46], and is linked with *HLA* genotypes in SLE [47], consistent with a role of the gene product in the disease progress. Thus, our

Table 4 Frequency of SNP genotypes and alleles in all candidate genes in the two SLE patient subgroups with or without renal disorder, and normal controls

SNP		SLE cases		Control	*P* value[a]	*P* value[b]	*P* value[c]
		Renal disorder (+) (*N* = 36)	Renal disorder ( ) (*N* = 58)	Normal (*N* = 95)			
IL1RN (rs315952)	CC	15 (41.67%)	15 (25.86%)	36 (37.89%)			
Genotype	TT	6 (16.67%)	17 (29.31%)	18 (18.95%)			
	CT	15 (41.67%)	26 (44.83%)	41 (43.16%)	n.s.	n.s.	n.s.
Allele	C	0.63	0.48	0.59			
	T	0.37	0.52	0.41	n.s.	n.s.	n.s.
IL1-*J* C-511T	CC	8 (22.22%)	12 (20.69%)	30 (31.58%)			
Genotype	TT	12 (33.33%)	26 (44.83%)	24 (25.26%)			
	CT	16 (44.44%)	20 (34.48%)	41 (43.15%)	n.s.	n.s.	0.0397
Allele	C	0.44	0.38	0.53			
	T	0.56	0.62	0.47	n.s.	n.s.	0.0097
LTA (rs1041981)	AA	5 (13.89%)	19 (32.76%)	23 (24.21%)			
Genotype	CC	14 (38.89%)	14 (24.14%)	23 (24.21%)			
	AC	17 (47.22%)	25 (43.10%)	49 (51.58%)	n.s.	n.s.	n.s.
Allele	A	0.38	0.54	0.5			
	C	0.62	0.46	0.5	0.0249	n.s.	n.s.
LTA (rs2857713)	AA	19 (52.78%)	36 (62.07%)	59 (62.11%)			
Genotype	GG	4 (11.11%)	2 (3.45%)	2 (2.11%)			
	AG	13 (36.11%)	20 (34.48%)	34 (35.79%)	n.s.	n.s.	n.s.
Allele	A	0.71	0.79	0.8			
	G	0.29	0.21	0.2	n.s.	n.s.	n.s.
TNF-*o* A-308G	AA	1 (2.78%)	2 (3.45%)	2 (2.11%)			
Genotype	GG	28 (77.78%)	46 (79.31%)	76 (80%)			
	AG	7 (19.44%)	10 (17.24%)	17 (17.89%)	n.s.	n.s.	n.s.
Allele	A	0.13	0.12	0.11			
	G	0.87	0.88	0.89	n.s.	n.s.	n.s.
PDCD1 (rs2227981)	CC	20 (55.56%)	36 (62.07%)	49 (51.58%)			
Genotype	TT	3 (8.33%)	1 (1.72%)	7 (7.37%)			
	CT	13 (36.11%)	21 (36.21%)	39 (41.05%)	n.s.	n.s.	n.s.
Allele	C	0.74	0.80	0.72			
	T	0.26	0.20	0.28	n.s.	n.s.	n.s.
C4 (rs2258218)	CC	0	2 (3.45%)	2 (2.117%)			
Genotype	TT	14 (38.89%)	14 (24.14%)	27 (28.42%)			
	CT	22 (61.11%)	42 (72.41%)	66 (69.47%)	n.s.	n.s.	n.s.
Allele	C	0.31	0.40	0.37			
	T	0.69	0.60	0.63	n.s.	n.s.	n.s.
C4 (rs392610)	GG	0	4 (6.90%)	5 (5.26%)			
Genotype	TT	4 (11.11%)	12 (20.70%)	14 (14.74%)			
	GT	32 (88.89%)	42 (72.41%)	76 (80%)	n.s.	n.s.	n.s.
Allele	G	0.44	0.43	0.45			
	T	0.56	0.57	0.55	n.s.	n.s.	n.s.
C4 (rs4947334)	AA	15 (41.67%)	40 (68.97%)	57 (60%)			
Genotype	CC	0	0	0			
	AC	21 (58.33%)	18 (31.03%)	38 (40%)	0.0090	n.s.	n.s.
Allele	A	0.71	0.84	0.803			
	C	0.29	0.16	0.196	0.0249	n.s.	n.s.

N individual number, *n.s.* not significant

[a] Value upon comparison of renal disorder (+) and renal disorder ( )

[b] Value upon comparison of renal disorder (+) and normal control

[c] Value upon comparison of renal disorder ( ) and normal control

Table 5 Frequency of SNP genotypes and alleles in all candidate genes in the two SLE patient subgroups with or without neurologic disorder, and normal controls

SNP		SLE cases		Control	*P* value[a]	*P* value[b]	*P* value[c]
		Neurologic disorder (+) (*N* = 35)	Neurologic disorder ( ) (*N* = 52)	Normal (*N* = 95)			
IL1RN (rs315952)	CC	11 (31.43%)	15 (28.85%)	36 (37.89%)			
Genotype	TT	7 (20%)	15 (28.85%)	18 (18.95%)			
	CT	17 (48.57%)	22 (42.31%)	41 (43.16%)	n.s.	n.s.	n.s.
Allele	C	0.56	0.50	0.59			
	T	0.44	0.50	0.41	n.s.	n.s.	n.s.
IL1-*J* C-511T	CC	7 (20%)	12 (23.08%)	30 (31.58%)			
Genotype	TT	15 (42.86%)	21 (40.38%)	24 (25.26%)			
	CT	13 (37.14%)	19 (36.54%)	41 (43.15%)	n.s.	n.s.	n.s.
Allele	C	0.39	0.41	0.53			
	T	0.61	0.59	0.47	n.s.	0.037	n.s.
LTA (rs1041981)	AA	9 (25.71%)	13 (25%)	23 (24.21%)			
Genotype	CC	9 (25.71%)	15 (28.85%)	23 (24.21%)			
	AC	17 (48.57%)	24 (46.15%)	49 (51.58%)	n.s.	n.s.	n.s.
Allele	A	0.50	0.48	0.50			
	C	0.50	0.52	0.50	n.s.	n.s.	n.s.
LTA (rs2857713)	AA	23 (65.71%)	27 (51.92%)	59 (62.11%)			
Genotype	GG	2 (5.71%)	3 (5.77%)	2 (2.11%)			
	AG	10 (28.57%)	22 (42.31%)	34 (35.79%)	n.s.	n.s.	n.s.
Allele	A	0.80	0.73	0.80			
	G	0.20	0.27	0.20	n.s.	n.s.	n.s.
TNF-*o* A-308G	AA	0	3 (5.77%)	2 (2.11%)			
Genotype	GG	29 (82.86%)	38 (73.08%)	76 (80%)			
	AG	6 (17.14%)	11 (21.15%)	17 (17.89%)	n.s.	n.s.	n.s.
Allele	A	0.09	0.16	0.11			
	G	0.91	0.84	0.89	n.s.	n.s.	n.s.
PDCD1 (rs2227981)	CC	20 (57.14%)	35 (67.31%)	49 (51.58%)			
Genotype	TT	0	2 (3.85%)	7 (7.37%)			
	CT	15 (42.86%)	15 (28.85%)	39 (41.05%)	n.s.	n.s.	n.s.
Allele	C	0.79	0.82	0.72			
	T	0.21	0.18	0.28	n.s.	n.s.	n.s.
C4 (rs2258218)	CC	0	2 (3.85%)	2 (2.117%)			
Genotype	TT	12 (34.29%)	12 (23.08%)	27 (28.42%)			
	CT	23 (65.71%)	38 (73.08%)	66 (69.47%)	n.s.	n.s.	n.s.
Allele	C	0.33	0.40	0.37			
	T	0.67	0.60	0.63	n.s.	n.s.	n.s.
C4 (rs392610)	GG	1 (2.86%)	3 (5.77%)	5 (5.26%)			
Genotype	TT	7 (20%)	8 (15.38%)	14 (14.74%)			
	GT	27 (77.14%)	41 (78.85%)	76 (80%)	n.s.	n.s.	n.s.
Allele	G	0.41	0.45	0.45			
	T	0.59	0.55	0.55	n.s.	n.s.	n.s.
C4 (rs4947334)	AA	20 (57.14%)	31 (59.62%)	57 (60%)			
Genotype	CC	0	0	0			
	AC	15 (42.86%)	21 (40.38%)	38 (40%)	n.s.	n.s.	n.s.
Allele	A	0.79	0.80	0.803			
	C	0.21	0.20	0.196	n.s.	n.s.	n.s.

N individual number, *n.s.* not significant

[a] Value upon comparison of neurologic disorder (+) and neurologic disorder ( )

[b] Value upon comparison of neurologic disorder (+) and normal control

[c] Value upon comparison of neurologic disorder ( ) and normal control

experimental approach (stratification according to MEX- SLEDAI scores) might be a good method to study the gene effect for disease pathogenesis.

Programmed cell death 1 (*PDCD1*) encodes a cell surface membrane protein of the immunoglobulin superfamily. This protein is expressed in pro-B-cells and is thought to play a role in their differentiation. This protein may also be impor- tant in T cell function and contributes to the prevention of autoimmune diseases (http:IIwww.ncbi.nlm.nih.govlentrezI dispomim.cgi?id=600244). We could not see the *PD-1.3* AIG polymorphism (RUX1 binding site) [48] in our population, although there were other SNPs present. These other SNPs and their genotypes and allele frequencies were not associ- ated with SLE, even when we used MEX-SLEDAI scores to stratify their frequencies. To resolve this controversial differ- ence from results obtained by the Japanese in their studies of the *PDCD1* gene, it might be possible to use another disease index (such as SLE damage index) to double test the gene function, or maybe increase our patient numbers.

C4 is a part of the classical complement activation path- way. Varying haplotypes of this gene cluster exist; individ- uals may have 1, 2, or 3 copies of this gene [49]. We found that three SNPs exist in the exon region of the *C4* gene: rs2258218, rs392610, and rs4947334. No genotype fit the Hardy—Weinberg equilibrium model, and they were not sig- nificantly different between SLE patients and normal con- trol subjects, even when MEX-SLEDAI scores were used to stratify the frequencies. But they were significantly different between SLE patients with or without renal disor- der. Therefore, hereditary complement deficiency states are associated with increased risk of SLE, but contribute only marginally to the incidence of SLE in the population [50].

After completing this study, it became known to us that the SLE disease activity index is correlated with the geno- types of mediators in SLE pathogenesis. MEX-SLEDAI scores might stratify the genetic approach and permit the evaluation of the allelic risk in genetic studies of SLE. Unfortunately, our sample size was so small and after mul- tiple corrections, the significant result disappeared. So the following study should enlarge the patient number to fit the criteria of population-based genetic study.

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