

# Disease Phenotypes and Gender Association of FCRL3 Single-Nucleotide Polymorphism –169T/C in Taiwanese Patients with Systemic Lupus Erythematosus and Rheumatoid Arthritis

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**ABSTRACT. Objective.** To investigate the association of the functional *FCRL3* single-nucleotide polymorphism (SNP) –169T/C with disease phenotypes and susceptibility to systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) in Taiwanese.

**Methods.** *FCRL3* SNP –169T/C was genotyped in 573 patients with SLE, 670 patients with RA, and 758 controls. Genotype distributions and allele frequencies were compared among the 3 groups as aggregates or as stratified by clinical characteristics, autoantibody profile, and sex within patient groups.

**Results.** Overall, *FCRL3* SNP –169T/C was not associated with susceptibility to either SLE or RA. However, –169CC genotype was significantly reduced in leukopenia-positive SLE patients as compared to the leukopenia-negative SLE patients (CC vs CT+TT,  $p = 6 \times 10^{-4}$ , OR 0.444, 95% CI 0.279–0.708) and controls ( $p = 6.1 \times 10^{-3}$ , OR 0.583, 95% CI 0.396–0.857). On the other hand, –169TT genotypes were significantly more numerous in RA patients with non-destructive disease as compared with patients with destructive disease (CC+CT vs TT:  $p = 0.007$ , OR 1.672, 95% CI 1.149–2.432). The –169T allele frequency was also significantly increased in non-destructive RA compared with patients with destructive disease (C vs T:  $p = 0.010$ , OR 1.423, 95% CI 1.089–1.859). *FCRL3* SNP –169TT homozygous donors were significantly more numerous among female cyclic citrullinated peptide (CCP)-negative RA patients versus female CCP-positive RA patients (CC+CT vs TT:  $p = 0.019$ , OR 1.64, 95% CI 1.085–2.479).

**Conclusion.** The functional *FCRL3* SNP –169T/C appears to play important roles in the development of certain phenotypes such as SLE leukopenia and RA disease severity in Taiwanese patients with SLE and RA. (First Release Nov 15 2010; *J Rheumatol* 2011;38:264–70; doi:10.3899/jrheum.100437)

## Key Indexing Terms:

*FCRL3* SINGLE-NUCLEOTIDE POLYMORPHISM GENDER  
SYSTEMIC LUPUS ERYTHEMATOSUS RHEUMATOID ARTHRITIS

Immunoglobulin Fc receptors (FcR) serve as a critical link between cellular and humoral immune responses. FcR on various hematopoietic cells mediate activating or inhibitory signals to modulate the human immune system<sup>1</sup>. Activating FcR facilitate the clearance of pathogens whereas inhibitory FcR downregulate immune responses<sup>2,3</sup>. The balance between activating and inhibitory FcR signals is critical to

determine immune unresponsiveness or hyperreactivity to foreign as well as self antigens. Therefore, FcR play important roles in the pathogenesis of autoimmune disorders<sup>1,2,3,4,5</sup>.

One of the most important genetic regions associated with susceptibility to multiple human autoimmune diseases is located in chromosome 1q21-23<sup>6,7,8</sup>. Recently, a cluster

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of newly identified genes, Fc receptor-like genes (*FCRL*, also known as FcRH, IRTA, or SPAP), were located in 1q21<sup>9,10,11,12</sup>. The novel human *FCRL* family consists of at least 8 genes (*FCRL1*, *FCRL2*, *FCRL3*, *FCRL4*, *FCRL5*, *FCRL6*, *FCRLA*, and *FCRLB*) that share a common ancestry with the classic FcγR genes<sup>12,13</sup>. Subsequent studies demonstrated that *FCRL1-5* genes are preferentially expressed in B cells and that *FCRL6* gene is mainly expressed in T cells and natural killer cells<sup>14,15,16</sup>. B cells also express *FCRLA* and *FCRLB*<sup>17</sup>. Because B cells are critical effectors in the generation of autoantibodies, *FCRL* gene products are believed to have an important role in autoimmune diseases<sup>12,15</sup>.

*FCRL3*, which has structural homology with the classical FcγR genes, is expressed in B cells, natural killer cells, and T cells<sup>12</sup>. The functional promoter polymorphism of *FCRL3* [single-nucleotide polymorphism (SNP) -169C/T, rs7528684] was found to alter the binding affinity of nuclear factor-κB (NF-κB) and to affect gene expression<sup>18,19</sup>. The *FCRL3* SNP -169T/C is significantly associated with systemic lupus erythematosus (SLE) in Japanese<sup>18</sup>. However, subsequent studies failed to establish the association of *FCRL3* SNP -169T/C with SLE in Caucasian and African Americans, Spanish Caucasians, Koreans, and Chinese, suggesting that *FCRL3* SNP -169T/C may not play a major role in SLE susceptibility in other populations<sup>19,20,21,22</sup>. Additionally, the *FCRL3* SNP -169T/C was found to be significantly associated with rheumatoid arthritis (RA) in Japanese and the -169C allele is a risk factor for RA<sup>18</sup>. Subsequent studies in RA revealed conflicting results in different ethnic populations<sup>23</sup>. Of note, the opposite allele (-169T) was found to be associated with multiple sclerosis and Addison's disease<sup>24,25,26</sup>. To understand the influence of the SNP -169T/C on different autoimmune diseases, additional studies are needed<sup>22</sup>.

Our aim was to investigate whether clinical manifestations are associated with *FCRL3* SNP -169T/C genotypes or alleles in Taiwanese patients with SLE and RA.

## MATERIALS AND METHODS

**Study subjects.** Anticoagulated peripheral blood was obtained from subjects selected for study. A total of 758 healthy blood donors (628 women, 130 men) were recruited following a questionnaire survey to exclude any donors with autoimmune diseases (including RA, SLE, ankylosing spondylitis, and autoimmune thyroiditis), diabetes mellitus, and cardiovascular diseases. The age of healthy control donors ranged from 16 to 64 years (mean 40.0 ± 11.5 yrs). The study recruited 573 patients with SLE (529 women, 44 men) who fulfilled the 1982 and/or 1997 revised American College of Rheumatology criteria for SLE<sup>27</sup> and 670 Taiwanese patients with RA (577 women, 93 men) who fulfilled the 1987 American Rheumatism Association criteria for RA<sup>28</sup>. The mean age at SLE disease onset was 30.5 ± 11.8 years (range 8–77 yrs). The clinical characteristics and autoantibody status of SLE patients are shown in Table 1.

The patients with RA have been followed for at least 2 years in the rheumatology clinics of Chang Gung Memorial Hospital to evaluate disease courses. Serial radiographic damage of both hands was graded according to the Steinbrocker staging system<sup>29</sup>: soft tissue swelling and periarticular

Table 1. Clinical characteristics and autoantibody production in 573 patients with systemic lupus erythematosus (SLE) who underwent genotype analysis.

Characteristics	SLE Patients (N = 573), n (%)
Oral ulcer	168 (29.3)
Arthritis	360 (62.8)
Malar rash	351 (61.3)
Discoid rash	121 (21.1)
Photosensitivity	139 (24.3)
Pleural effusion	105 (18.3)
Pericardial effusion	75 (13.1)
Ascites	32 (5.6)
Nephritis	332 (57.9)
Neuropsychiatric manifestations	104 (18.2)
Leukopenia (WBC count < 3500/μl)	353 (64.6)
Anemia (Hb < 9 g/dl)	191 (33.3)
Thrombocytopenia (platelets < 10 <sup>5</sup> /μl)	156 (27.2)
Complement depressed	486 (84.8)
Anti-dsDNA	435/562 (77.4)
Anti-Sm	171/460 (37.2)
Anti-RNP	197/460 (42.8)
Anti-SSA	240/369 (65.0)
Anti-SSB	103/369 (27.9)
Anticardiolipin IgG	129/457 (28.2)
Anticardiolipin IgM	32/437 (7.3)

WBC: white blood cell; Hb: hemoglobin.

ular osteoporosis (stage I), bony erosion (stage II), joint subluxation (stage III), and joint ankylosis (stage IV). Two rheumatologists independently verified the hand radiography and scored the Steinbrocker stage of each patient. The rare inconsistent cases of radiography scoring were resolved through consultations among physicians and assessment of clinical function. Patients' RA severity varied during the disease course. Definite radiographic destructive changes of bilateral hands and cervical spine were evaluated among RA patients with disease duration > 5 years. Thus, patients who had erosive bony lesions within 5 years (radiographic stage II) and/or destruction of joint subluxation and/or ankylosis (stage > III) at any time in the disease course were categorized as having destructive RA. Non-destructive RA was defined when RA disease activity remained either at stage I for > 5 years or at stage II for > 10 years.

A total of 670 Taiwanese patients with RA (577 women, 93 men) were included in the study. RA patients had a mean age of 46.3 ± 13.6 years (range 6–80 yrs) at disease onset and had a mean disease duration of 13.6 ± 8.1 years. Patients had been followed for at least 2 years for assessment of disease course. A total of 531 of the 670 patients (79.7%) were positive for rheumatoid factor (RF); 487 of 624 (78%) patients were determined to be positive for anti-cyclic citrullinated peptide antibody (anti-CCP). Out of 600 RA patients assayed for antinuclear antibodies (ANA), 322 (53.7%) were positive (titers > 1:80). Based on radiography data of hand and wrist joints, 536 patients displayed bony erosion (stage > II), 423 were found to have destruction of joint subluxation and/or ankylosis (stage > III), and 212 were documented to have cervical spine involvement. Among patients with disease duration > 5 years, 158 were evaluated as having non-destructive RA and 213 were classified as negative for cervical spine involvement.

In terms of treatment, most patients followed by a rheumatology specialist received 3 major disease-modifying antirheumatic drugs (DMARD; methotrexate, hydroxychloroquine, or sulfasalazine) and < 10 mg steroid treatment. Anti-tumor necrosis factor therapy was used in 15 cases of severe destructive RA, less than 3% of the RA patients screened.

The local ethics committee of Chang Gung Memorial Hospital approved the study and all donors provided written consent.

**Nucleic acid isolation.** EDTA anticoagulated peripheral blood was obtained from healthy control donors and patients with SLE and RA. Genomic DNA was isolated using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN, USA) as described<sup>30</sup>.

**Genotyping of FCRL3 SNP -169T/C.** Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry was used for genotyping FCRL3 SNP -169T/C (rs7528684) according to the protocols recommended by the manufacturer (Sequenom, San Diego, CA, USA). Polymerase chain reaction (PCR) was carried out in 96-well plates using 10 ng genomic DNA. PCR products (253 bp) were automatically purified on the MALDI-TOF Map I I/8 robotic platform using a Bruker Genopure DS Magnetic Bead DNA purification kit (Bruker Daltonics, Bremen, Germany). DNA was extended by PCR with SNP detection primer 5'-GGT GAG ATT ACG GGA AGT CC -3' and termination mix (dCTP, ddGTP, ddATP, and ddTTP). PCR included a denaturing step at 94°C for 5 min followed by 50 cycles of denaturing at 94°C for 8 s, annealing at 52°C for 8 s, and extension at 72°C for 8 s. PCR was ended with a final extension at 72°C for 2 min. Allele-specific products were purified with the Bruker Genopure Oligo Magnetic Bead DNA purification kit and samples were automatically spotted on the 384-well Anchor Chip plate and analyzed by autoflex mass spectrometry. Genotype and allele frequencies of FCRL3 SNP -169T/C were in Hardy-Weinberg equilibrium in controls and SLE and RA patients ( $p > 0.05$ ).

**Autoantibody assay.** Autoantibody titers were determined by ELISA. ANA were considered positive if serum titers were  $> 1:80$  by Hep-G2 cell assay. Titers of anti-CCP antibodies were determined by a third-generation ELISA kit (Inova Diagnostics, San Diego, CA, USA). A cutoff value of 40 IU/ml was set to distinguish anti-CCP-positive ( $> 40$  IU/ml) and negative ( $< 40$  IU/ml) patients with RA. Anti-extractable nuclear antigen (Ro/SSA, La/SSB, Sm, and RNP) and anticardiolipin antibodies were assessed by commercial ELISA (Pharmacia Diagnostics, Uppsala, Sweden). RF was regarded as positive when the titer was  $> 20$  IU/ml in a nephelometric assay.

**Statistical analysis.** We carried out single-locus analyses to examine associations between FCRL3 SNP -169T/C polymorphism and patients with SLE or RA. Three chi-square tests (genotype test, allele test, Cochran-Armitage trend test) were performed using SAS/Genetics software 8.2 (SAS Institute, Cary, NC, USA). For SLE patients, we stratified the clinical phenotypes by leukopenia (positive or negative) and serositis (positive or negative) according to SLE diagnosis criteria and compared SNP -169T/C genotypic and allelic differences using chi-square tests. Associations of SNP -169T/C genotypes and alleles with onset ages, disease severity, and presence of anti-CCP antibody or RF were also analyzed in RA patients. We applied stepwise logistic regression models and compared stratified case groups using covariates including age, sex, and SLE clinical manifestations (malar rash, discoid rash, photosensitivity, pleural effusion, pericardial effusion, ascites, oral ulcers, arthritis, thrombocytopenia, anemia, nephritis, and neuropsychiatric manifestations) and RA severity phenotypes (RA destruction, anti-CCP antibody and RF production) to investigate the association of disease phenotype with the SNP variant together with the clinical characteristics. The statistical power of analyses was calculated using the PBAT software (<http://www.biostat.harvard.edu/~clange/default.htm>). The population prevalence (0.051% for SLE and 1% for RA were used), minor allele frequencies, odds ratios, and significance level of 0.05 were considered simultaneously in the power calculation after phenotypic stratification.

## RESULTS

**FCRL3 SNP -169T/C is associated with leukopenia and serositis in SLE.** The FCRL3 SNP -169T/C was genotyped in 573 SLE patients, 670 RA patients, and 758 healthy controls. No significant differences of genotype distributions and allele frequencies were found between SLE patients and

healthy controls (Table 2). SLE is characterized as a heterogeneous disease producing various autoantibodies and diverse clinical manifestations; thus we stratified patients according to clinical characteristics. We found there were significant differences in the genotype distribution between leukopenia-positive and negative SLE patients (trend test  $p = 0.005$  with 100,000 permutations). In addition, there was a significant reduction of -169CC genotype in leukopenia-positive SLE patients versus those without leukopenia (CC vs CT+TT, chi-square = 11.654,  $p = 6 \times 10^{-4}$ , OR 0.444, 95% CI 0.279–0.708) or compared to normal controls (CC vs CT+TT, chi-square = 7.644,  $p = 6.1 \times 10^{-3}$ , OR 0.583, 95% CI 0.396–0.857; Table 2). The -169C allele frequency was also significantly decreased in leukopenia-positive SLE patients versus those without leukopenia (chi-square = 7.552,  $p = 0.006$ , OR 0.712, 95% CI 0.559–0.907). Additionally, a minor reduction of -169C allele frequency was observed in leukopenia-positive SLE patients compared to controls ( $p = 0.054$ , OR 0.835, 95% CI 0.695–1.003). In contrast, the -169C allele frequency was significantly enriched in serositis-positive SLE patients (i.e., pleural effusion plus pericardial effusion) versus those without serositis (C vs T; chi-square = 3.942,  $p = 0.047$ , OR 1.316, 95% CI 1.004–1.725), although the increase of -169 CC genotype did not reach significance ( $p = 0.181$ ; Table 2). Moreover, stepwise logistic regression analysis indicated that the SNP -169T/C variant, along with anemia, was significantly associated with leukopenia. Our data suggest that the FCRL3 SNP -169T/C is not associated with SLE in general, but the SNP appears to be associated with leukopenia and serositis in patients with SLE.

**FCRL3 SNP -169T/C is associated with disease severity in RA.** As shown in Table 3, no significant association was found between FCRL3 SNP -169T/C and RA in Taiwanese patients. Since RA patients displayed different clinical characteristics, we stratified them using criteria as described and further examined the association between phenotypes and SNP -169T/C polymorphisms. We found that SNP -169T/C genotype distributions were significantly different between destructive RA and non-destructive RA (trend test  $p = 0.011$ ; 100,000 permutations). Donors with -169TT genotypes were significantly more numerous among RA patients with non-destructive disease compared with those with destructive RA (CC+CT vs TT: chi-square = 7.221,  $p = 0.007$ , OR 1.672, 95% CI 1.149–2.432; statistical power  $> 80\%$ ). The -169T allele frequency was also significantly increased in RA patients with non-destructive as compared with destructive disease (C vs T: chi-square = 6.689,  $p = 0.010$ , OR 1.423, 95% CI 1.089–1.859).

We also examined whether the SNP -169T/C was associated with autoantibodies in RA patients. We found no association between FCRL3 SNP -169T/C and any autoantibody production in RA patients. However, -169TT homozygotes tended to increase in RA patients who were anti-CCP-nega-

Table 2. Distribution of *FCRL3* SNP-169T/C genotype and allele frequencies in SLE patients with leukopenia and serositis (pleural effusion and pericardial effusion).

Characteristic (n)	Genotype			Minor Allele Frequency	p for Trend	Chi-square	Genotype Analysis (CC vs CT + TT)			Allele Analysis (C vs T)		
	CC	CT	TT				OR (95% CI)	p	Chi-square	OR (95% CI)	p	
SLE (573)	85	293	195	0.416	0.543	1.291	0.841 (0.625–1.131)	0.256	0.360	0.953 (0.817–1.114)	0.549	
Controls (758)	130	370	258	0.404								
Leukopenia-positive (353)	38	187	128	0.373	0.049	7.644	0.583 (0.396–0.857)	$6.1 \times 10^{-3}$	3.709	0.835 (0.695–1.003)	0.054	
Controls (758)	130	370	258	0.404								
Leukopenia-positive (353)	38	187	128	0.373	0.005*	11.654	0.444 (0.279–0.708)	$6 \times 10^{-4}$	7.552	0.712 (0.559–0.907)	0.006	
Leukopenia-negative (220)	47	106	67	0.455								
Serositis-positive (142)	26	77	39	0.454	0.044*	1.794	1.413 (0.852–2.345)	0.181	3.942	1.316 (1.004–1.725)	0.047	
Serositis-negative (431)	59	216	156	0.388								
Female SLE (529)	81	267	181	0.406	0.590	0.628	0.880 (0.642–1.206)	0.428	0.283	0.956 (0.809–1.129)	0.595	
Female controls (628)	107	309	212	0.416								
Female leukopenia-positive (322)	37	168	117	0.454	0.013*	9.021	0.481 (0.298–0.775)	$2.7 \times 10^{-3}$	6.010	0.731 (0.569–0.939)	0.014	
Female leukopenia-negative (207)	44	99	64	0.388								
Female serositis-positive (129)	26	68	35	0.465	0.025*	3.053	1.584 (0.946–2.653)	0.081	5.014	1.382 (1.041–1.834)	0.025	
Female serositis-negative (400)	55	199	146	0.386								

\* 100,000 permutations.

Table 3. Distribution of *FCRL3* SNP-169T/C genotype and allele frequencies in healthy Taiwanese controls, RA patients, and RA patients with different clinical manifestations.

Characteristic (n)	Genotype			Minor Allele Frequency	p for Trend	Chi-square	Genotype Analysis (CC vs CT + TT)			Allele Analysis (C vs T)		
	CC	CT	TT				OR (95% CI)	p	Chi-square	OR (95% CI)	p	
RA (670)	115	322	233	0.412	0.844	0.086	0.968 (0.778–1.204)	0.769	0.039	1.015 (0.874–1.178)	0.84	
Controls (758)	130	370	258	0.416								
Destructive RA (423)	79	211	133	0.436	0.011*	7.221	1.672 (1.149–2.432)	0.007	6.689	1.423 (1.089–1.859)	0.010	
Non-destructive RA (159)	22	68	69	0.352								
CCP-positive RA (487)	82	244	161	0.419	0.165	3.417	1.443 (0.978–2.128)	0.065	1.921	1.216 (0.922–1.602)	0.166	
CCP-negative RA (137)	22	58	57	0.372								
RF-positive RA (531)	90	259	182	0.413	0.836	0.283	1.111 (0.753–1.639)	0.595	0.043	1.029 (0.789–1.346)	0.836	
RF-negative RA (139)	25	63	51	0.407								
Female RA (577)	91	287	199	0.406	0.615	0.072	0.968 (0.763–1.229)	0.789	0.248	0.960 (0.816–1.129)	0.619	
Female controls (628)	107	309	212	0.416								
Female destructive RA (367)	67	137	113	0.437	0.002*	8.358	1.823 (1.214–2.736)	$3.8 \times 10^{-3}$	9.568	1.590 (1.185–2.132)	0.002	
Female non-destructive RA (134)	14	60	60	0.328								
Female CCP-positive RA (417)	66	218	133	0.420	0.049*	5.517	1.640 (1.085–2.479)	0.019	3.971	1.353 (1.005–1.821)	0.046	
Female CCP-negative RA (122)	16	53	53	0.348								
Female RF-positive RA (458)	73	232	153	0.416	0.389	1.149	1.256 (0.828–1.906)	0.284	0.719	1.135 (0.847–1.521)	0.397	
Female RF-negative RA (119)	16	53	53	0.382								

\* 100,000 permutations. CPP: cyclic citrullinated peptide; RF: rheumatoid factor.

tive ( $p = 0.065$ ). Stepwise logistic regression analysis revealed no significant associations among RA destruction and anti-CCP antibody and RF production. Our data suggest that the *FCRL3* SNP-169T/C is associated with RA disease severity and possibly with anti-CCP antibody production.

*Gender affects the association of FCRL3 SNP-169T/C with phenotypes of SLE and RA.* Development of autoimmune diseases in males may require a strong genetic contribution to disease susceptibility<sup>31</sup>. After gender stratification, similar distributions of -169T/C genotypes and allele frequen-

cies were observed among all the 3 female groups (controls, SLE patients, RA patients). The significant association of *FCRL3* -169T/C genotypes with leukopenia was preserved in female SLE patients (trend test  $p = 0.013$ , 100,000 permutations). The frequency of *FCRL3* -169CC homozygous donors was significantly decreased in leukopenia-positive female SLE patients versus leukopenia-negative patients (CC vs CT+TT: chi-square = 9.021,  $p = 2.7 \times 10^{-3}$ , OR 0.481, 95% CI 0.298–0.775; Table 2). The -169C allele frequency was also significantly reduced in leukopenia-positive female SLE patients compared to those without leukopenia (chi-square = 6.010,  $p = 0.014$ , OR 0.731, 95% CI 0.569–0.939). *FCRL3* SNP -169T/C showed a trend to be associated with serositis in female SLE patients, although the association was not significant ( $p = 0.081$ , Table 2). Of note, we observed that -169T/C genotypes were significantly associated with female CCP-positive RA compared with female CCP-negative RA (trend test  $p = 0.049$ ; 100,000 permutations; Table 3). *FCRL3* -169TT homozygous donors were significantly enriched in female CCP-negative RA patients compared with female CCP-negative RA patients (CC+CT vs TT: chi-square = 5.517,  $p = 0.019$ , OR 1.640, 95% CI 1.085–2.479). The -169T allele frequency was also significantly increased in female CCP-negative RA patients compared with female CCP-positive patients (C vs T: chi-square = 3.971,  $p = 0.046$ , OR 1.353, 95% CI 1.005–1.821). Our data indicate that gender appears to affect the association of *FCRL3* SNP -169T/C with leukopenia and serositis in SLE and destructive RA and anti-CCP antibody production.

## DISCUSSION

The precise function of *FCRL3* is undefined, but it is predicted that *FCRL3* is a membrane protein that transduces signals into cells through an immunoreceptor-tyrosine activation motif (ITAM) and an immunoreceptor-tyrosine inhibitory motif (ITIM) in the receptor cytoplasmic domain<sup>12</sup>. The existing data suggest that *FCRL3* may affect B cell development and augment the emergence of self-reactive B cells in the germinal center<sup>12,13,14,15,16,17,18</sup>. The *FCRL3* SNP -169T/C allele is located at a transcription factor NF- $\kappa$ B element and is crucial for regulation of *FCRL3* expression. The -169C allele has a much higher binding affinity for NF- $\kappa$ B than the -169T allele. Donors carrying -169C allele have higher expression of *FCRL3* than donors with only the -169T allele<sup>18</sup>. The -169C allele was found to be associated with susceptibility to several autoimmune diseases including RA and SLE in a Japanese population<sup>18</sup>. The association between the functional *FCRL3* promoter SNP -169T/C has been confirmed in another Japanese case-control study<sup>32</sup>. However, studies in other ethnic populations including other Asians have yielded conflicting results for the role of the SNP -169T/C in autoimmune diseases<sup>21,22,24,33,34,35,36,37,38</sup>. Because *FCRL3* is considered an

excellent candidate gene for autoimmune diseases, we undertook the current study to test whether *FCRL3* SNP -169T/C plays a role in the pathogenesis of SLE and RA in Taiwanese. Our genetic analysis indicated that the *FCRL3* SNP -169T/C is not an overall risk factor for SLE and RA in Taiwanese. However, SNP -169T/C was associated with clinical manifestations of SLE and RA in Taiwanese. Our findings support the theory that the SNP -169T/C has a role in development of subsets of SLE and RA in Taiwanese.

The -169C allele appears to be a risk factor for serositis in SLE patients and for destructive RA in RA patients. *FCRL3* protein contains both ITAM and ITIM and is expressed mainly in B cells, natural killer cells, and T cells<sup>12</sup>. *FCRL3* expression is regulated in B cells in the secondary lymphoid organ and is detected in lymphocytes of disease-specific tissues; *FCRL3* very probably is a functional molecule in immunity and potentially pathogenic in autoimmune disorders<sup>18</sup>. The possible explanation for the association of the highly expressed -169C allele with destructive RA and serositis in SLE is that *FCRL3* may augment the emergence of autoreactive B cells and T cells. Interestingly, the -169C allele seems to have a protective role against leukopenia, supporting suggestions that the highly functioning allele -169C may provide surviving signals to leukocytes including autoreactive lymphocytes. *FCRL3* is also expressed in synovial tissues, which may explain the pathological connection between SNP -169T/C and destructive RA.

It was previously found that the -169C allele was associated with RF and anti-CCP antibody production in a group of Japanese RA patients<sup>18</sup>. However, we failed to detect the association of SNP -169T/C with SLE autoantibody production (SSA/Ro, SSB/La, Sm, RNP, ACA IgM, and dsDNA), except for a marginal increase of -169 CC homozygous donors in anticardiolipin antibody-positive SLE patients in the  $3 \times 2$  contingency table analysis (data not shown). Although -169T/C showed a modest association with anti-CCP antibody production in female RA patients, no significant association was found between SNP -169T/C and production of other autoantibodies in SLE and RA patients. Our data indicate that the SNP -169T/C may not be a strong factor affecting production of other autoantibodies in Taiwanese patients with SLE and RA.

Ample evidence suggests strong genetic effects on male SLE and RA disease susceptibility<sup>31,39,40,41</sup>. We found a significant association of *FCRL3* SNP -169T/C with leukopenia and serositis in female patients with SLE. Similarly, a significant association of *FCRL3* SNP -169T/C with anti-CCP antibody production was observed in female patients with RA. The genetic contribution of *FCRL3* SNP -169T/C to the development of SLE and RA seems to be influenced by gender in Taiwanese. Of note, the opposite -169T allele of the SNP -169T/C is associated with susceptibility to multiple sclerosis and Addison's disease<sup>24,25,26</sup> as

compared with the -169C allele, which was associated with SLE and RA in Japanese patients in the original report<sup>18</sup>, supporting that the SNP -169T/C may have opposite roles in different autoimmune diseases. RA and SLE patients display functional immune differences (such as the Th1/Th2 paradigm axis differentiation of T effector cells) during disease development<sup>42,43</sup>. We previously demonstrated different functional effects of FcγRIIb 187-Ile/Thr alleles and genotypes on disease susceptibility in RA and SLE<sup>30,44</sup>. Our current data suggest that the SNP -169T/C may differentially associate with autoimmune disease phenotypes in SLE and RA.

Subset analysis of disease cohorts may reduce sample sizes and may increase the chances of type I errors in statistical analyses. In our study, the size of subsets of the patients with SLE leukopenia and destructive RA remained relatively large. From our power analysis, we observed that after phenotype stratification, the power for the analyses in which the trend test p values were highly significant (< 0.01) reached up to 90%. When the trend test p values were between 0.01 and 0.05, the power of the analysis hit only 80%. For analyses in which the p values of the trend test were on the borderline of significance (that is, only slightly less than 0.05), the power dropped to around 50% and was expectedly unsatisfactory. Therefore, the functional *FCRL3* SNP -169T/C appeared to play important roles in subsets of SLE and RA in Taiwanese. On the other hand, *FCRL3* SNP -169T/C was only modestly associated with SLE serositis and anti-CCP antibody production in female RA patients.

The limitations of our study were the various causes of leukopenia in SLE, although the drug-induced origin was excluded, and the heterogeneity of DMARD treatments may have affected the progression of destruction in RA. We should interpret these results cautiously. Independent studies with large control and patient populations are needed to confirm our findings.

The evidence for association of *FCRL3* SNP -169T/C SNP with SLE and RA in Caucasians and Asians is contradictory, although a metaanalysis showed the association between the SNP -169T/C and RA in East Asian populations<sup>6,21,22,23,24,32,33,34,35,36,37,45</sup>. Nevertheless, a large study in Korean subjects failed to demonstrate an association of SNP -169T/C with RA<sup>22</sup>, suggesting the association of the SNP -169T/C with RA may be specific for Japanese. Notably, the normal control distribution of *FCRL3* SNP -169T/C revealed a significant difference between Taiwanese and Japanese (CC vs CT+TT: chi-square = 8.758, p = 0.003, OR 1.386, 95% CI 1.116–1.722)<sup>32</sup>. These data indicate the possible effects of population heterogeneity in the differing results from Asian studies<sup>21,22,32</sup>. Our study generally confirmed the negative association of SNP -169T/C with SLE and RA in Taiwanese. However, our data indicate that SNP -169T/C may have a role in the phenotypic manifestations of SLE and RA patients.

In summary, the functional *FCRL3* SNP -169T/C may affect the disease phenotypes of SLE and RA in Taiwanese. *FCRL3* -169CC genotype and high expression -169C allele have protective roles in development of leukopenia in SLE, while -169C allele may be a risk factor for serositis in SLE. In contrast, -169TT genotype and -169T allele may have a protective role against destructive RA. Gender effects may influence the association of *FCRL3* SNP -169T/C with phenotypes of SLE and RA in Taiwanese. Future studies with large populations are required to validate our findings.

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