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Association of *FCGR3A* and *FCGR3B* Copy Number Variations With Systemic Lupus Erythematosus and Rheumatoid Arthritis in Taiwanese Patients

Ji-Yih Chen,[1] Chin-Man Wang,[1] Su-Wei Chang,[1] Ching-Hui Cheng,[2] Yeong-Jian Jan Wu,[1] Jing-Chi Lin,[1] Bing Yang,[3] Huei-Huang Ho,[1] and Jianming Wu[4]

Objective. **To determine whether copy number variations (CNVs) in *FCGR3A* and *FCGR3B* are associated with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) in Taiwanese individuals.**

Methods. ***FCGR3A*** **and** ***FCGR3B*** **CNV genotypes were determined in 846 patients with SLE, 948 patients with RA, and 1,420 healthy control subjects, using custom TaqMan CNV assays. The *FCGR3A* and *FCGR3B* CNV genotypes were compared between healthy control subjects and patients and among patients stratified according to clinical characteristics.**

Results. **A low (<2) *FCGR3A* copy number was significantly associated with SLE (for <2 copies versus 2 copies, $P = 5.06 \times 10^{-4}$, false discovery rate–corrected P [P_{FDR}] = 0.001, odds ratio [OR] 3.26, 95% confidence interval [95% CI] 1.68−6.35) and RA (for <2 copies versus 2 copies, $P = 5.83 \times 10^{-4}$, $P_{FDR} = 0.0012$, OR 2.82, 95% CI 1.56−5.1). A low *FCGR3B* copy number was also significantly associated with SLE (for <2 copies versus 2 copies, $P = 0.0032$, $P_{FDR} = 0.0032$, OR 1.59, 95% CI 1.17−2.18). Notably, a high (>2) *FCGR3A* copy number was also associated with SLE (for >2 copies versus 2 copies, $P = 0.003$, $P_{FDR} = 0.0061$, OR 1.6, 95% CI 1.17−2.18). Additionally, the *FCGR3A* low copy number genotype was significantly enriched in subsets of patients with SLE (those with ulcer, arthritis, rash, discoid rash, photosensitivity, nephritis, leukopenia, thrombocytopenia, depressed complement levels, and autoantibody positivity) and patients with RA (those positive for rheumatoid factor) compared with healthy control subjects. The *FCGR3B* low copy number genotype was also significantly enriched in SLE patients with ulcer, rash, discoid rash, photosensitivity, ascites, nephritis, complement level depression, and anti–double-stranded DNA antibody positivity compared with control subjects. However, *FCGR3B* CNVs were not associated with RA susceptibility (for <2 copy numbers versus 2 copy numbers, $P = 0.3584$, OR 1.15, 95% CI 0.85–1.55) and clinical characteristics.**

Conclusion. **In Taiwanese individuals, a low *FCGR3A* copy number is a common risk factor for SLE and RA, while a low *FCGR3B* copy number confers a risk of SLE but not RA.**

IgG Fcγ receptors (FcγRs) mediate a variety of immune functions that are critical in immune responses. In humans, 5 classic low-affinity FcγRs (FcγRIIA, FcγRIIB, FcγRIIC, FcγRIIIA, and FcγRIIIB) are coded by 5 genes (*FCGR2A, FCGR2B, FCGR2C, FCGR3A,* and *FCGR3B*, respectively) in the *FCGR* cluster on chromosome 1. Activating FcγRs (FcγRIIA,

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[1]Ji-Yih Chen, MD, Chin-Man Wang, MD, Su-Wei Chang, PhD, Yeong-Jian Jan Wu, MD, Jing-Chi Lin, MD, Huei-Huang Ho, MD: Chang Gung University College of Medicine, Taiwan, Republic of China; [2]Ching-Hui Cheng, BS, MT: Chang Gung Memorial Hospital, Taiwan, Republic of China; [3]Bing Yang, PhD: Xin-Jhu Blood Center, Taiwan Blood Services Foundation, Taiwan, Republic of China; [4]Jianming Wu, DVM, PhD: University of Minnesota, St. Paul.

Address correspondence to Ji-Yih Chen, MD, Department of Medicine, Division of Allergy, Immunology and Rheumatology, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, No. 5, Fu-Shin St. Kuei-Shan, Tao-Yuan, Taiwan, Republic of China (e-mail: jychen31@adm.cgmh.org.tw); or to Jianming Wu, DVM, PhD, Department of Veterinary and Biomedical Sciences, University of Minnesota, 235B AnSc/VetMed Building, 1988 Fitch Avenue, St. Paul, MN 55108 (e-mail: jmwu@umn.edu).

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FcγRIIC, FcγRIIIA, and FcγRIIIB) mediate immune cell activation promoting inflammation, while the classic inhibitory FcγRIIB dampens immune responses and restricts inflammation (1,2). Activating FcγRs mediate functions including immune complex clearance, phagocytosis, antigen presentation, antibody-dependent cellular cytotoxicity, and cytokine production (3). In contrast, the inhibitory FcγRIIB abrogates immune cell activation. FcγRIIB also plays a role in the maintenance of peripheral B cell tolerance and the prevention of autoimmunity (2).

The variations in FcγR expression significantly affect IgG immune complex–mediated signal thresholds (3,4). Notably, proinflammatory and antiinflammatory cytokines could modulate the expression levels of activating and inhibitory FcγRs (5) that affect the threshold immune cell response to IgG immune complexes (6). FcγR-knockout mouse models indicate that both activating and inhibitory FcγRs influence the development of autoimmune diseases (7–9). The contributions of FcγR genes to autoimmune diseases have attracted substantial attention, and functional FcγR polymorphisms have been reported to play important roles in the pathogenesis of autoimmune diseases (4,10,11).

Gene copy number variation (CNV) is a rich source of genetic heterogeneity (12,13). The *FCGR* cluster on chromosome 1q23 shows a complex pattern of CNVs. Among 5 FcγR genes in the cluster, 3 genes (*FCGR3A*, *FCGR2C,* and *FCGR3B*) have CNVs, while *FCGR2A* and *FCGR2B* do not have CNVs (14,15). CNVs play important roles in human disease pathogenesis (16). *FCGR3B* copy number deficiency is associated with autoimmune disease, including systemic lupus erythematosus (SLE) (17–19), Sjögren's syndrome (20), and systemic sclerosis (21). Although *FCGR3B* CNVs were reported to associate with rheumatoid arthritis (RA) (22–24), no association between RA and *FCGR3B* CNVs was observed in other studies (25,26). Moreover, no association between *FCGR3A* CNVs and RA was observed (24,27). In the current study, we investigated whether *FCGR3A* and *FCGR3B* CNVs are associated with susceptibility to SLE and RA in Taiwanese individuals. The results provide new insights into the role of FcγRIII family members in the pathogenesis of SLE and RA in an Asian population.

PATIENTS AND METHODS

Study subjects. Taiwanese healthy control donors (512 men and 908 women) were recruited locally. The mean ± SD age of the healthy control donors was 40.2 ± 11.6 years (range 18–64 years). Taiwanese patients with SLE (72 men and 774 women) who fulfilled the 1982 and/or 1997 revised American College of Rheumatology criteria for SLE (28,29) and patients with RA (141 men and 807 women) who fulfilled the 1987 American College of Rheumatology criteria for the classification of RA (30) were recruited at Chang Gung Memorial Hospital, Tao-Yuan, Taiwan. Stratification of the clinical characteristics of the patients with RA was previously described (31). The ethics committee of Chang Gung Memorial Hospital approved the human study, and all donors provided written consent.

Nucleic acid isolation. Anticoagulated peripheral blood was obtained from healthy control donors, patients with SLE, and patients with RA. All genomic DNA samples were isolated from anticoagulated peripheral blood using a Puregene DNA isolation kit (Gentra Systems) in the same laboratory as previously described (32).

Determination of *FCGR3* CNVs. *FCGR3A* and *FCGR3B* CNVs were genotyped using custom TaqMan CNV real-time quantitative polymerase chain reaction (PCR) assays with FAM/minor groove binder dual-labeled probes that were produced by Applied Biosystems (see Supplementary Table 1, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38813/abstract). A TaqMan Copy Number Reference Assay (RNase P) with a VIC dye–labeled TAMRA probe (catalog no. 4403328; Applied Biosystems) was used as the internal control for copy number targets.

Duplex quantitative real-time PCRs were carried out on an Applied Biosystems ViiA 7 Real-Time PCR System (Life Technologies), according to the manufacturer's instructions. All samples were tested in duplicate, and fluorescence signals were normalized to ROX. The quantitative PCR amplification curves were analyzed using ViiA 7 software (Applied Biosystems) on a plate-by-plate basis, and the copy number was assigned from the raw quantification cycle (Cq) values using CopyCaller software version 2.0 (Applied Biosystems). This software uses a clustering algorithm and assigns a copy number value of 2 to the cluster with the most samples. CopyCaller software also provides extensive diagnostics for the validity of the results, which were set to accept the copy number assignment only when confidence was >95%, the SD of the sample replicate ΔCq estimates was <0.20, and a reference gene Cq was <32. More than 85% of samples had >99% confidence level in the copy number assignment. In addition, a repeat copy number assay was carried out on all samples with a copy number of <2, 10% of healthy control samples with a copy number of 2, and 20% of all samples with a copy number of >2, to confirm the copy number calls. Copy number assignment with >95% confidence levels completely matched (100% reproducibility) for all samples in repeat copy number assays. Overall, our methodology resulted in clear assignment of *FCGR3* copy numbers for 99% of the samples (histograms of *FCGR3A* and *FCGR3B* CNV analyses are shown in Supplementary Figure 1, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38813/abstract). The accuracy of the assays was further confirmed by determining CD16 expression on natural killer (NK) cells and neutrophils.

Evaluation of FcγRIII (CD16) expression levels. To determine the expression of CD16 on NK cells and neutro-

phils, 100-μl samples of fresh whole blood were stained with fluorescein isothiocyanate (FITC)–conjugated anti-human CD16 monoclonal antibodies (clone 16B; eBiosciences) and phycoerythrin (PE)–conjugated anti-human CD56 (Beckman Coulter). Whole blood samples stained with FITC-conjugated murine IgG1 and PE-conjugated anti-human CD56 (in separate tubes) were used as isotype controls. After incubation at room temperature for 30 minutes, blood samples were treated with 1× BD FACS Lysing Solution (BD Biosciences) to lyse red blood cells, followed by analysis on a Beckman Coulter FC500 flow cytometer. NK cells within the lymphocyte population were identified as CD56+ cells. Characteristic light scatter properties were used to identify neutrophils in flow cytometric analyses. The expression of FcγRIII (CD16) was analyzed using FlowJo software (Tree Star).

Autoantibody assays. Autoantibody titers were determined by enzyme-linked immunosorbent assay (ELISA). The autoantibody status of the patients was assessed at the time of diagnosis of SLE or RA. Antinuclear antibody (ANA) positivity was defined as a serum titer of ≥1:80 in a Hep-G2 cell assay. Anti–extractable nuclear antigen antibodies (Ro/SSA, La/SSB, Sm, and RNP) and anticardiolipin antibodies were assessed by commercial ELISA according to the manufacturer's instructions (Pharmacia Diagnostics).

Statistical analysis. The distribution of *FCGR3* copy numbers between patients with SLE or patients with RA and healthy control subjects was compared using chi-square and Fisher's exact tests. *P* values, odds ratios (ORs), and 95% confidence intervals (95% CIs) were calculated based on the identified risk copy number. To investigate the association of copy number with clinical manifestations of SLE and RA, we assigned those SLE and RA patients with a clinical phenotype as "1" cases and those without it as "0" cases. The clinical phenotypes of SLE patients were stratified according to the SLE diagnostic criteria. The phenotypes of RA patients were stratified based on the presence or absence of rheumatoid factor (RF), anti–cyclic citrullinated peptide (anti-CCP) antibodies, and radiographic erosions. We compared the copy number distributions between "1" cases and "0" cases and between "1" cases and healthy controls using chi-square and Fisher's exact tests. In addition, logistic regression models adjusted for sex and age were used to investigate the relationship between each clinical manifestation and copy number among patients. The logistic regression models adjusted for sex and age were also used to calculate the ORs of high copy number (>2) and low copy number (<2) in disease susceptibility.

To correct for multiple comparisons, an SAS MULTTEST procedure was performed, in which false discovery rate (FDR)–adjusted *P* values (P_{FDR}) (33) were defined in a step-up manner but with less conservative multipliers. Mann-Whitney U tests were used to evaluate the effect of *FCGR3* copy numbers on FcγRIII (CD16) expression levels. *P* values less than 0.05 were considered significant.

RESULTS

Characteristics of the SLE patients and the RA patients. The SLE cohort comprised 846 patients ranging in age from 8 years to 77 years (mean ± SD age 30.88 ± 11.87 years). Seventy-two (8.51%) of the patients were male, and 774 (91.49%) were female. The mean ± SD age of the male patients was 31.46 ± 12.52 years, and that of the female patients was 30.64 ± 11.56 years. The clinical characteristics of the patients with SLE are shown in Table 1. The RA cohort comprised 948 patients (141 males and 807 females). The mean ± SD age of these patients was 46.49 ± 13.9 years; 76.4% (670 of 887) were anti-CCP antibody positive, 78.81% (744 of 944) were RF positive, 50.9% (424 of 833) were ANA positive, and 71.04% (601 of 846) had destructive disease.

Association of both *FCGR3A* and *FCGR3B* CNVs with SLE susceptibility. We examined the single-locus associations between *FCGR3* CNVs and susceptibility to SLE. As shown in Table 2, a low (<2) *FCGR3A* copy number was significantly associated with SLE disease susceptibility (for <2 copies versus 2 copies, $P = 5.06 \times 10^{-4}$, $P_{FDR} = 0.001$, OR 3.26, 95% CI 1.68–6.35). Notably, the high (>2) *FCGR3A* copy number was also a risk factor for SLE susceptibility (for >2 copies versus 2 copies, $P = 0.003$, $P_{FDR} = 0.0061$, OR 1.6, 95% CI 1.17–2.18), suggesting that an *FCGR3A* abnormality has a role in the development of SLE. Similarly, a low *FCGR3B* copy number was significantly associated with SLE disease susceptibility (for <2 copies versus 2 copies, $P = 0.0032$, $P_{FDR} = 0.0032$, OR 1.59, 95% CI 1.17–2.18).

Table 1. Clinical characteristics of the patients with SLE (n = 846)*

Oral ulcer	224/846 (26.48)
Arthritis	532/846 (62.88)
Malar rash	466/846 (55.08)
Discoid rash	161/846 (19.03)
Photosensitivity	189/846 (22.34)
Pleural effusion	162/846 (19.15)
Pericardial effusion	102/846 (12.06)
Ascites	44/846 (5.2)
Nephritis	471/846 (55.67)
Neuropsychiatric manifestations	135/846 (15.96)
Leukopenia†	473/846 (55.91)
Anemia‡	257/846 (30.38)
Thrombocytopenia§	220/846 (26)
Anti-dsDNA	628/828 (75.85)
Complement level depression	643/833 (77.19)
Anti-RNP	295/685 (43.07)
Anti-Sm	260/686 (37.9)
Anti-SSA	365/568 (64.26)
Anti-SSB	150/568 (26.41)
Anticardiolipin IgG	187/664 (28.16)
Anticardiolipin IgM	55/607 (9.06)

* Values are the no. of positive cases/no. of total cases (%). SLE = systemic lupus erythematosus; anti-dsDNA = anti–double-stranded DNA.
† White blood cell count <3,500 units/liter.
‡ Hemoglobin concentration <9 gm/dl.
§ Platelet count $<10^5/\mu l$.

Table 2. Association of *FCGR3A* and *FCGR3B* CNVs with SLE susceptibility*

	FCGR3A		*FCGR3B*	
	SLE	Controls	SLE	Controls
Unadjusted				
CN <2	31 (3.68)	19 (1.34)	121 (14.34)	126 (8.97)
CN = 2	700 (83.04)	1,280 (90.14)	605 (71.68)	1,026 (73.02)
CN >2	112 (13.29)	121 (8.52)	118 (13.98)	253 (18.01)
χ^2	27.8166		19.1486	
P	9.11×10^{-7}		6.95×10^{-5}	
Fisher's exact *P*	1.23×10^{-6}		7.95×10^{-5}	
Adjusted for age and sex				
CN >2 vs. CN = 2				
P	0.0030		0.0574	
P_{FDR}	0.0061		0.0574	
OR (95% CI)	1.6 (1.17–2.18)		0.77 (0.59–1.01)	
CN <2 vs. CN = 2				
P	5.06×10^{-4}		0.0032	
P_{FDR}	0.001		0.0032	
OR (95% CI)	3.26 (1.68–6.35)		1.59 (1.17–2.18)	

* Except where indicated otherwise, values are the number (%). CNVs = copy number variations; SLE = systemic lupus erythematosus; P_{FDR} = false discovery rate–adjusted *P* value; OR = odds ratio; 95% CI = 95% confidence interval.

Moreover, a high *FCGR3B* copy number tended to have a protective role against SLE disease development (adjusted *P* [P_{adj}] = 0.0574, P_{FDR} = 0.0574, OR 0.77, 95% CI 0.59–1.01). Our data suggested that the FcγRIIIB deficiency is also a risk factor for SLE in Taiwanese individuals.

Association of RA susceptibility with *FCGR3A* CNVs but not with *FCGR3B* CNVs. As shown in Table 3, a low (<2) *FCGR3A* copy number was significantly associated with RA disease susceptibility (for <2 copies versus 2 copies, $P = 5.83 \times 10^{-4}$, P_{FDR} = 0.0012, OR 2.82, 95% CI 1.56–5.10). In contrast to the findings in SLE patients, the high (>2) *FCGR3A* copy number had no effect on RA susceptibility (*P* = 0.3335). In addition, neither low *FCGR3B* copy number nor high *FCGR3B* copy number was associated with RA susceptibility. Our data indicated that *FCGR3A* deficiency is a susceptibility factor for RA, while *FCGR3B* CNVs appear not

Table 3. Association of *FCGR3A* and *FCGR3B* CNVs with RA susceptibility*

	FCGR3A		*FCGR3B*	
	RA	Controls	RA	Controls
Unadjusted				
CN <2	36 (3.81)	19 (1.34)	98 (10.35)	126 (8.97)
CN = 2	836 (88.47)	1,280 (90.14)	685 (72.33)	1,026 (73.02)
CN >2	73 (7.72)	121 (8.52)	164 (17.32)	253 (18.01)
χ^2	15.5197		1.3208	
P	4.27×10^{-4}		0.5167	
Fisher's exact *P*	5.00×10^{-4}		0.5160	
Adjusted for age and sex				
CN >2 vs. CN = 2				
P	0.3335		0.4320	
P_{FDR}	0.432		0.432	
OR (95% CI)	0.86 (0.62–1.17)		0.91 (0.72–1.15)	
CN <2 vs. CN = 2				
P	5.83×10^{-4}		0.3584	
P_{FDR}	0.0012		0.3584	
OR (95% CI)	2.82 (1.56–5.1)		1.15 (0.85–1.55)	

* Except where indicated otherwise, values are the number (%). CNVs = copy number variations; RA = rheumatoid arthritis; P_{FDR} = false discovery rate–adjusted *P* value; OR = odds ratio; 95% CI = 95% confidence interval.

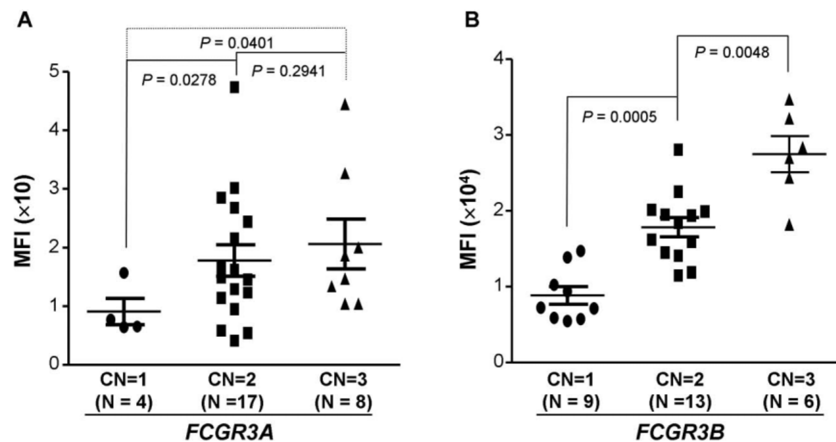

Figure 1. *FCGR3A* and *FCGR3B* copy numbers (CNs) affect Fcγ receptor expression. **A,** Effect of *FCGR3A* copy number on CD16A expression on natural killer (NK) cells. NK cells carrying 1 copy of *FCGR3A* expressed significantly less CD16A than those carrying 2 copies ($P = 0.0278$). NK cells carrying 3 copies of *FCGR3A* expressed significantly more CD16A than those carrying 1 copy ($P = 0.0401$). **B,** Effect of *FCGR3B* copy number on CD16B expression on neutrophils. Neutrophils from donors carrying 1 copy of *FCGR3B* expressed significantly less CD16B than those from donors carrying 2 copies ($P = 0.0005$). Neutrophils from donors carrying 3 copies of *FCGR3B* expressed significantly more CD16B than those from donors carrying 2 copies ($P = 0.0048$). Each symbol represents an individual subject; bars show the mean ± SD. MFI = mean fluorescence intensity.

to have a role in the development of RA in Taiwanese individuals.

Effects of *FCGR3A* and *FCGR3B* CNVs on SLE phenotypes and autoantibody production. Patients with SLE exhibit heterogeneous disease manifestations and variations in the severity, nature, and spectrum of clinical involvement. We subsequently examined the effects of *FCGR3A* and *FCGR3B* CNVs on SLE clinical phenotypes. Copy number frequencies were compared between the SLE patients with each disease characteristic and the healthy control subjects (positive versus healthy controls) and among SLE patients stratified by each clinical characteristic (positive versus negative).

The *FCGR3A* low copy number genotype was significantly enriched in SLE patients positive for ulcer, arthritis, rash, discoid rash, photosensitivity, nephritis, leukopenia, thrombocytopenia, complement level depression, anti–double-stranded DNA (anti-dsDNA), anti-RNP, anti-Sm, anti-SSA, anti-SSB, and anticardiolipin IgG compared with healthy control subjects (see Supplementary Table 2, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38813/abstract). However, a low (<2) *FCGR3A* copy number was only marginally associated with nephritis among subsets of SLE patients stratified according to clinical manifestations (for patients with nephritis versus patients without nephritis, $P_{adj} = 0.0457$, OR 2.32, 95% CI 1.02–5.28) (see Supplementary Table 3, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38813/abstract). Our data suggested that *FCGR3A* deficiency may play a role in the development of lupus nephritis.

FCGR3B low copy number genotypes were significantly enriched in SLE patients with ulcer, rash, discoid rash, photosensitivity, ascites, nephritis, complement level depression, and anti-dsDNA antibody positivity compared with healthy controls (see Supplementary Table 4, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38813/abstract). Nevertheless, only the phenotypes of oral ulcer and nephritis were associated with a low (<2) *FCGR3B* copy number ($P_{FDR} < 0.05$) among SLE patients stratified according to clinical manifestations (see Supplementary Table 5, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38813/abstract).

***FCGR3* CNVs and clinical characteristics of patients with RA.** We also examined whether *FCGR3A* CNVs are associated with RA disease characteristics. The *FCGR3A* low copy number genotype was significantly increased among RF-positive patients with RA and patients with destructive RA compared with healthy control subjects (see Supplementary Table 6, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38813/abstract), but the increased enrichment was not significant when the association was compared among the subsets of patients with RA (see Supplementary Table 7, available on the *Arthritis & Rheumatology* web site at http://

onlinelibrary.wiley.com/doi/10.1002/art.38813/abstract). In contrast, a low (<2) *FCGR3A* copy number was protective against anti-CCP antibody production (P_{FDR} = 0.008, OR 0.35, 95% CI 0.17–0.72) in RA patients stratified according to anti-CCP positivity (see Supplementary Table 7). These data suggested that the functions of *FCGR3A* play a role in RA. However, *FCGR3B* was not associated with any clinical characteristics of RA (see Supplementary Tables 8 and 9, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38813/abstract).

Correlation between *FCGR3A* CNVs and CD16A expression on NK cells. To evaluate whether the *FCGR3A* CNVs affect FcγRIIIA (CD16A) expression on NK cells, peripheral blood samples from individuals carrying 1 (n = 4), 2 (n = 17), and 3 (n = 8) copies of *FCGR3A* were used in flow cytometric assays. As shown in Figure 1A, NK cells carrying 1 copy of *FCGR3A* expressed significantly less CD16A than those carrying 2 copies of *FCGR3A* (*P* = 0.028), and the NK cells carrying 3 copies of *FCGR3A* also expressed significantly more CD16A than those carrying 1 copy of *FCGR3A* (*P* = 0.040), suggesting that *FCGR3A* CNVs may affect NK cell functions.

Significant effect of *FCGR3B* CNVs and CD16B expression on neutrophils. To assess the effect of *FCGR3B* copy number on neutrophil FcγRIIIB (CD16B) expression, we determined the expression of CD16B in individuals carrying 1 (n = 9), 2 (n = 13), or 3 (n = 6) copies of *FCGR3B*. As shown in Figure 1B, neutrophils from individuals carrying 1 copy of *FCGR3B* expressed significantly less CD16B compared with neutrophils from individuals carrying 2 copies of *FCGR3B* (*P* = 0.0005). Neutrophils from individuals carrying 3 copies of *FCGR3B* expressed significantly more CD16 compared with neutrophils from individuals carrying 2 copies of *FCGR3B* (*P* = 0.0048). These data confirmed the previous observation that FcγRIIIB gene doses affect the expression of CD16B on neutrophils (19).

DISCUSSION

Although *FCGR3A* CNVs were previously detected (14,34,35), the role of *FCGR3A* CNVs in SLE is unknown. Moreover, no association between *FCGR3A* CNVs and RA was observed in European Caucasians (24,27). In this study, we observed that a low (<2) *FCGR3A* copy number was significantly associated with both SLE and RA in Taiwanese individuals, suggesting that FcγRIIIA deficiency may be a common risk factor for SLE and RA in Taiwanese. Surprisingly, a high (>2) *FCGR3A* copy number was also associated with susceptibility to SLE (Table 2), suggesting that the unbalanced functions of *FCGR3A* (both a deficiency and an overload) play a role in the pathogenesis of SLE.

FCGR3A copy number frequencies are similar between healthy European individuals (24,27) and healthy Taiwanese individuals. However, *FCGR3A* deficiency is a risk factor for RA in Taiwanese, while no association between *FCGR3A* CNVs and RA susceptibility was observed in European Caucasians (24,27). There are 2 explanations for this discrepancy.

First, because the frequency of *FCGR3A* deficiency (copy number <2) is very low in all populations (<2%), large numbers of patients and control subjects are required to detect an effect of *FCGR3A* CNVs. Compared with previous studies (24,27), the current study included more healthy control subjects and patients with RA. To detect associations of *FCGR3A* CNVs with SLE and RA by setting the alpha level at 0.05, the current study had >95.8% power to detect an OR of 1.55 in 846 patients with SLE and 1,420 healthy control subjects based on a 13.29% frequency of a high (>2) copy number. The study had >99.5% power to detect an OR of 2.86 in 945 patients with RA and 1,420 healthy control subjects based on 3.81% frequency of a low (<2) copy number. Therefore, the current study had much better power to detect associations of *FCGR3A* CNVs with SLE and RA.

Second, epistatic interactions between *FCGR3A* CNVs and other genes may be required for development of the RA phenotype. In European and African American populations, the composition of epistatic modifiers may differ from that in the Taiwanese population. Therefore, the effect of *FCGR3A* CNVs on RA may not be the same in different races. Similarly, the functional FcγRIIB single-nucleotide polymorphism (SNP) is associated with SLE in Asians (32,36–38), while the association between the FcγRIIB SNP and SLE in Caucasians requires studies with a large sample size because of epistatic interactions (39). Notably, a low *FCGR3A* copy number showed a negative association with anti-CCP antibody positivity, which highlighted a complicated role that *FCGR3A* CNVs play in autoimmune disease phenotypes and suggested that the genetic backgrounds may be different between anti-CCP antibody–positive and anti-CCP antibody–negative patients with RA. Most importantly, *FCGR3A* deficiency is associated with 2 distinct autoimmune diseases (SLE and RA), suggesting that defective FcγRIIIA functions may represent a common risk factor for various autoimmune diseases.

The functional *FCGR3A* SNP (rs396991) has been associated with lupus nephritis in several different ethnic groups in multiethnic meta-analyses (40,41). In the current study, we observed that a low *FCGR3A* copy number is a strong risk factor for nephritis in patients with SLE compared with healthy control subjects (OR 4.51, $P < 0.0001$) (Supplementary Table 2, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38813/abstract). However, the association with nephritis was marginal among patients with SLE (OR 2.32, $P = 0.0457$) stratified according to the presence of nephritis (see Supplementary Table 3, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38813/abstract). Because a low *FCGR3A* copy number is also a predisposing factor for SLE, the overall enrichment of the *FCGR3A* low copy number genotype in the entire SLE patient population may somehow decrease the significance of the association between lupus nephritis and a low *FCGR3A* copy number among patients with SLE. The association of a low *FCGR3A* copy number with nephritis in Taiwanese individuals reaffirms the notion that less-efficient immune complex handling by FcγRIIIA is an important contributor to lupus nephritis pathogenesis (11).

Surprisingly, we observed that a high *FCGR3A* copy number is also a risk factor for SLE. Higher frequencies of cytokine-producing FcγRIIIA-positive dendritic cells (DCs) were observed in SLE patients, particularly in those with active disease, suggesting that FcγRIIIA-mediated inflammatory cytokine production in DCs might contribute to disease pathogenesis (42). The higher density of activating FcγRIIIA on the surface of immune cells (NK cells, monocytes, DCs, macrophages, and subsets of T cells) could tip the delicate balance of immune responses toward intense inflammation, which may result in the development of SLE.

Our in vitro data demonstrated a correlation between a low *FCGR3A* copy number and low CD16A expression on NK cells, suggesting that *FCGR3A* copy number has physiologic implications in NK cell functions. The disease associations suggest that modulation of FcγRIIIA function may be an important therapeutic target for lupus nephritis. Because the sample size of subjects with a low (<2) *FCGR3A* copy number in the current study was not very large, further study using a much larger sample size is required to confirm our findings.

Consistent with previous studies in SLE (17–19,25,43), we observed that an *FCGR3B* deficiency was significantly associated with SLE susceptibility in Taiwanese individuals, with an OR of 1.59 (99.4% statistical power based on 14.34% frequency of a low [<2] copy number and an alpha level of 0.05), which is within the range of a meta-analysis (OR 1.32–1.92 for SLE) (44). Moreover, a high (>2) *FCGR3B* copy number seems to have a protective role in SLE. Our findings highlight the growing evidence that FcγRIIIB functions play important roles in the pathogenesis of SLE across multiple ethnicities. Additionally, our data demonstrate a clear correlation between *FCGR3B* copy number and CD16B cell surface expression, suggesting that *FCGR3B* copy number has physiologic implications in neutrophil functions. FcγRIIIB plays a critical role in the adherence of neutrophils to immune complexes and their subsequent clearance (19,45). The high density of FcγRIIIB on neutrophils with weak signaling capacity may suit FcγRIIIB for efficient capture and internalization of immune complexes with minimal neutrophil activation (45,46). Thus, FcγRIIIB expression on neutrophils may have an important role in autoimmunity, and the expression levels of FcγRIIIB might have a significant impact on the pathogenesis of autoimmune diseases. Insufficient FcγRIIIB-mediated immune complex clearance may be the underlying mechanism of a low *FCGR3B* copy number predisposing to SLE and a high *FCGR3B* copy number having a protective role in SLE.

Our study failed to show an association between *FCGR3B* CNVs and RA susceptibility in Taiwanese individuals, suggesting that *FCGR3B* CNVs may not have a significant role in the development of RA, which is consistent with the results of a previous meta-analysis (44). The association between *FCGR3B* CNVs and RA observed in different studies was not consistent (22–26). If the lowest estimate (OR 1.36) of an association in the meta-analysis (44) is used, our current study would only have 55.8% power to detect a significant difference between 945 RA patients and 1,420 healthy control subjects based on the low (<2) copy number frequency of 10.35%, which may be the reason that we failed to detect a significant association of *FCGR3B* CNVs with RA in Taiwanese individuals. Nevertheless, the lack of an association between a low *FCGR3B* copy number and RA in Taiwanese individuals suggests that CD16B function may not play a critical role in the development of RA in an Asian population.

Two recent studies demonstrated that FcγR gene CNVs in chromosomal region 1q23 are the result of independent and recurrent non-allelic homologous recombination (NAHR) events between the 2 segments that carry *FCGR3A* or *FCGR3B* (15,47). Deletions of

either *FCGR3A* or *FCGR3B* differ only in the position of the NAHR breakpoint. The *FCGR* CNV mutation rate per generation is estimated to be $\sim 1.008 \times 10^{-3}$ (15). No linkage disequilibrium exists between *FCGR3A* CNVs and *FCGR3B* CNVs (15,47) or between *FCGR3* CNVs and functional SNPs in FcγRIII genes (34). Therefore, the association of individual *FCGR3* CNVs with SLE and RA most likely reveals independent FcγRIII gene effects in autoimmune diseases.

In conclusion, the present study demonstrates a role of *FCGR3* CNVs in autoimmunity. The deficiency of *FCGR3* family members is one of mechanisms involved in the development of SLE and RA in Taiwanese individuals.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Drs. Chen and Wu had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Chen, Wang, J. Wu.

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