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Contents

Original Articles

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目 錄

Original Article

Association between Hydroxychloroquine Use and the Risk of Dengue in Patients With Systemic Lupus Erythematosus: A Nationwide, Population-based Case Control Study

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Purpose: To determine the association between hydroxychloroquine (HCQ) and the risk of dengue in patients with systemic lupus erythematosus (SLE).
Methods: Using claims data from the 1997–2013 Taiwan's National Health Insurance Research Database, we identified 23,936 SLE patients. From them, we identified 14 patients who were diagnosed with dengue (International Classification of Diseases, Ninth Revision, Clinical Modification code 061). We randomly selected 140 SLE patients who did not have dengue to match dengue cases for sex, residence, date of dengue and SLE diagnosis; in a 10:1 ratio. We conducted univariable conditional logistic regression analyses to examine the associations of dengue with the baseline characteristics and SLE-related medications within 3 months before the index date, and identified those with a p-value of <0.15 as covariates in the multivariable analysis. We calculated the odds ratio (OR) with 95% confidence intervals (CI) using multivariable conditional logistic regression analyses to examine the association between HCQ use within 3 months and dengue.
Results: The risk of dengue was not significantly associated with HCQ use within 3 months before the index date (OR, 0.46; 95% CI, 0.13–1.66; p = 0.237). We did not find a significant association between dengue risk either the use of lower HCQ doses (≤ 200 mg/day) (OR, 0.52; 95% CI, 0.11–2.44; p = 0.409) or the use of higher HCQ doses (> 200 mg/day) (OR, 0.41, 95% CI, 0.09–1.93; p = 0.260).
Conclusions: This nationwide, population-based study did not demonstrate a significant association between the use of HCQ and the risk of dengue in SLE patients.

Key words: dengue, hydroxychloroquine, risk, claims data

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INTRODUCTION

Dengue, an arthropod-borne viral disease, is transmitted by the Aedes mosquito. This disease is responsible for a serious global burden. About 390 million people are infected annually worldwide. Dengue causes vascular leak, leading to systemic manifestations such as fever, hemorrhagic fever, spontaneous bleeding, organ failure, shock, and death.[1-3] Currently, there is no effective treatment for the dengue virus infection. The efficiency of vaccines is controversial.[4] Hydroxychloroquine(HCQ), an antimalarial drug, also used in autoimmune disease, is a potential medication for dengue infection. HCQ was found to induce reactive oxygen species (ROS) and mitochondrial antiviral signaling protein (MAVS) mediated innate immunity, leading to protecting against dengue virus infection.[5, 6] HCQ is widely used in systemic lupus erythematosus (SLE) patients.[7] However, whether or not HCQ can reduce the risk of dengue virus infection in patients with SLE was still unknown. In Taiwan, the National Health Insurance Database (NHIRD) has been used in longitudinal epidemiologic studies. Therefore, we aimed to investigate the association between HCQ use and the risk of dengue virus infection in SLE patients using the NHIRD.

METHODS

Study design

This was a nationwide, population-based case-control study that used claims data.

Data source

In 1995, Taiwan's government implemented a compulsory National Health Insurance (NHI) program and had included over 99% of the population in Taiwan. It included data on ambulatory care, inpatient services, dental services, traditional Chinese medical services, prescription medication, and catastrophic illness certificate. The National Health Research Institute managed the NHIRD and released comprehensive claims data for study use after anonymizing personal information. The Bureau of the NHI (BNHI) regularly audited the diagnosis by reviewing original medical charts, laboratory data, imaging, and pathology report by at least two specialists to improve the validity of the diagnosis. The BNHI also set up a catastrophic illness registry (CIR) to include patients with severe disease, such as malignancy and some autoimmune diseases including SLE, rheumatoid arthritis, systemic sclerosis, etc. Patients were issued a certificate for the CIR if their diagnoses were validated after chart reviews performed by two independent specialists.

Definition of dengue fever infection

Patients were defined as having dengue fever infection if they had at least one outpatient or inpatient visits with a diagnosis of dengue (International Classification of Diseases, Ninth Revision, Clinical Modification [ICD-9-CM] code 061).

Study samples

From the CIR using the 1997–2013 Taiwan's NHIRD, we identified a total of 23,936 patients with SLE (ICD-9-CM code 710.0). From these SLE patients, we identified 14 patients who developed dengue (ICD-9-CM code 061) after the time of SLE diagnosis as the dengue cases. We randomly selected 140 SLE patients who were never diagnosed with dengue and matched them for age, sex, year of dengue diagnosis, year of SLE diagnosis, and region of residence (represented by the postal code) in a 10:1 ratio.

Exposure of HCQ

Exposure of HCQ was defined as use of HCQ within three months before the index date. To assess the potential dose-response relationship between HCQ and the risk of dengue, we further divided HCQ users into two groups based on the median cumulative dose (i.e., > median and ≤ median) within three months before the index date.

Potential confounders

Potential confounders included urbanization level of patient's residence, insured amount according to the payroll, Charlson comorbidity index (CCI) within one year before the index date, and use of anti-rheumatic medications within 3 months before the index date. Payroll-related insured amount was used as a proxy measures of individual socioeconomic status and was divided into quantiles. We used the Deyo *et al.* revised version of CCI to represent the general comorbid condition.[8] We defined the presence of comorbidities used to calculate CCI if patients had at least three ambulatory visits or at least one inpatient visits with a corresponding ICD-9-CM code within one year before the index date. Anti-rheumatic medications included HCQ, methotrexate, cyclosporine, azathioprine, cyclophosphamide, mycophenolate/

mycophenolic acid, non-steroidal anti-inflammatory drugs (NSAIDs), and corticosteroid (average daily prednisolone equivalent). We only included use of HCQ and the potential confounders that had significantly differential distributions between dengue cases and non-dengue controls in the multivariable conditional logistic regression model.

Statistical analysis

We presented continuous variables using mean ± standard deviation and categorical variables using frequencies and proportions. We estimated the differences between groups in continuous variables using the Student's *t*-test and categorical variables using Pearson's χ^2 test. We firstly conducted univariable conditional logistic regression analyses to determine the associations between covariates and dengue. Given the small number of dengue cases in the study, only use of HCQ and other covariates with p-values of <0.15 in the univariable analyses were included in the multivariable model. Using a multivariable conditional logistic regression analysis, we calculated the odds ratio (OR) with 95% confidence intervals (CI) to determine the association of dengue with HCQ with two models based on the HCQ exposure status (i.e., model A, use of HCQ, not use of HCQ; model B, use of HCQ with more than median dose, use of HCQ with median dose or less, not use).

RESULTS

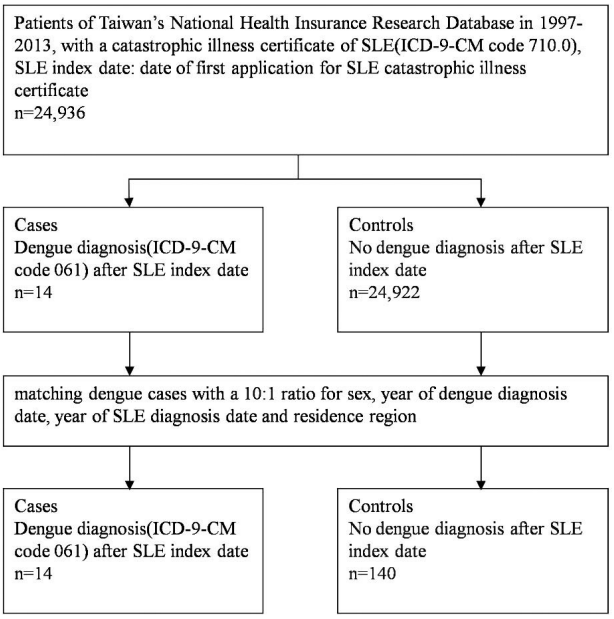
Characteristics of the study population

A total of 14 SLE patients diagnosed with dengue (ICD-9-CM code 061) were identified as the dengue cases and 140 non-dengue controls were selected after matching (10:1) the dengue cases for sex, year of dengue diagnosis, year of SLE diagnosis, and region of residence as represented by the postal code (Figure 1).

(Table 1) compares the baseline characteristics of the dengue cases with those of the non-dengue controls. The ages of the dengue and non-dengue patients ranged from 25 years old to 54 years. The proportion of NSAIDs usage was significantly higher in dengue patients than in patients without dengue.

As shown in Table 2, after adjusting for use of NSAIDs and use of cyclophosphamide, the risk of dengue was not significantly associated with HCQ use within three months before the index date (OR, 0.46; 95% CI, 0.13–1.66; p = 0.237). We also did not find a

Figure 1. Flowchart of the selection of patients from the Taiwan's National Health Insurance Research Database



Abbreviations: SLE, systemic lupus erythematosus; ICD-9-CM, International Classification of Diseases, Ninth Revision, Clinical Modification.

significant association between dengue risk and the use of either lower HCQ dose (≤ 200 mg/day) (OR, 0.52; 95% CI, 0.11–2.44; p = 0.409) or higher HCQ dose (> 200 mg/day) (OR, 0.41, 95% CI, 0.09–1.93; p = 0.260). However, the risk of dengue fever was significantly associated with use of NSAIDs and cyclophosphamide.

DISCUSSION

This is the first study to examine the association between HCQ use and the risk of dengue in lupus patients. However, this study did not demonstrate a significant protective effect of HCQ on the risk of dengue virus infection in patients with SLE although a potential protective effect of HCQ had been suggested in previous studies.[5] However, the point estimates of OR for a higher dose of HCQ and a lower dose of HCQ seemed to show a dose-response relationship and the low case number of dengue patients limited its power to demonstrate a significant protective effect of HCQ. Wang et al found that HCQ may induce ROS and MAVS mediated innate immunity, leading to protection against dengue virus infection.[5, 6] While an increase of ROS is also found to trigger the release of cytokines, result in plasma leakage, and lead to other severe symptoms. The details and etiology still need to be clarified.[9]

Table 1. Demographic data among systemic lupus erythematosus patients with and without dengue infection.

	None Dengue n=140	Dengue n=14	p Value
Dengue Age, years (mean ± SD)	39.7±14.3	39.6±13.3	0.983
Disease duration, years (mean ± SD)	5.8±4.6	5.8±4.9	0.956
Gender			1.000
Female	120 (85.7)	12 (85.7)	
Male	20 (14.3)	2 (14.3)	
CCI[a]	1.5±1.2	1.5±1.1	0.916
Urbanisation			0.947
Level 1	39 (27.9)	4 (28.6)	
Level 2	59 (42.1)	6 (42.9)	
Level 3	25 (17.9)	3 (21.4)	
Level 4	17 (12.1)	1 (7.1)	
Income (NTDs)			0.346
≤15,840	60 (42.9)	4 (28.6)	
15,841–28,800	46 (32.9)	5 (35.7)	
28,801–45,800	26 (18.6)	5 (35.7)	
≥45,801	8 (5.7)	0 (0.0)	
Hydroxychloroquine	75 (53.6)	7 (50)	0.798
Hydroxychloroquine, accumulated dose(mg) [b]			0.205
Not use	65 (46.4)	7 (50)	
≤25 percentile (11,200)	25 (17.9)	2 (14.3)	
26–50 percentile (16,800)	15 (10.7)	2 (14.3)	
51–75 percentile (33,600)	31 (22.1)	1 (7.1)	
>75 percentile	4 (2.9)	2 (14.3)	
NSAIDs	49 (35)	9 (64.3)	0.031
Other DMARDs			
Methotrexate	8 (5.7)	1 (7.1)	0.828
Cyclosporin	3 (2.1)	0 (0.0)	0.58
Azathioprine	30 (21.4)	1 (7.1)	0.204
Cyclophosphamide	5 (3.6)	2 (14.3)	0.067
Mycophenolate/ Mycophenolic	5 (3.6)	0 (0.0)	0.472
Steroid (Pd equivalent, mg/day)	1.3±1.8	1.4±2.0	0.828

Results are shown as number (%).
a One year prior to the index date. b Three months prior to the index date
Abbreviations: CCI: Charlson comorbidity index; NTDs: New Taiwan dollars; NSAIDs: non-steroidal anti-inflammatory drugs; DMARDs: disease-modifying antirheumatic drugs; Pd: prednisolone.

We incidentally found that NSAIDs use was associated with an increased risk of dengue in SLE patients. A possible explanation was a potential immunosuppressive effect of NSAIDs revealed by Bancos, et al. They reported that selective NSAIDs, especially ibuprofen are reported to blunt IgM and IgG synthesis, in human peripheral blood mononuclear cells and B lymphocyte, which lead to lower host defense.[10] However, a more likely explanation was that the use of NSAIDs actually reflected its application to treat dengue-related headache, arthralgia, or myalgia before the diagnosis of dengue was made.

Cyclophosphamide also enhances the proportion of dengue diagnosis. UC Chaturvedi et al found that in dengue virus-infected mice, following cyclophosphamide treatment, there was a decrease in the production of antibody-forming cells by the spleen. The cyclophosphamide treated mice, following dengue virus infection, produced fewer antibody-forming cells against the dengue virus. The report shows the important role of

Table 2. Crude and adjusted odds ratios (ORs) with 95% confidence intervals (CIs) for the associations between variables and the risk of dengue infection

	Univariable		Multivariable			
	OR (95% CI)	p Value	Model A		Model B	
			OR (95% CI)	p Value	OR (95% CI)	p Value
Hydroxychloroquine	0.85 (0.29–2.53)	0.773				
Hydroxychloroquine, accumulated dose(mg)			0.46 (0.13–1.66)	0.237		
Not use	Ref.				Ref.	
≤50 percentile(16,800)	0.92 (0.26–3.31)	0.897			0.52 (0.11–2.44)	0.409
>50percentile	0.78 (0.19–3.16)	0.724			0.41 (0.09–1.93)	0.260
NSAIDs	3.53 (1.04–11.97)	0.043	4.42 (1.16–16.89)	0.030	4.28 (1.11–16.44)	0.034
Cyclophosphamide	3.72 (0.64–21.55)	0.142	8.09 (1.08–60.82)	0.042	7.99 (1.05–60.58)	0.044

Abbreviations: OR: odds ratio; CI: confidence intervals, NSAIDs: non-steroidal anti-inflammatory drugs; DMARDs: disease-modifying antirheumatic drugs; Pd: prednisolone.

humoral immunity in dengue virus infection.[11]

Although the study had the strength of using a nationwide, population-based cohort, we have to mention some of its limitations. First, the small number of dengue patients led to an inadequate power of the study to test the association between HCQ and the risk of dengue. Also, the incidence of dengue may be underestimated due to the occurrence of no or mild dengue-related symptoms,[3] or attribution of dengue-related symptoms such as headache, and arthralgia to lupus-related presentations. Second, the NHIRD lacked information on the laboratory data required to confirm the diagnosis of dengue. Third, although the validity of the diagnosis using claims data is of concern, there is less concern in the diagnosis of SLE because at least two rheumatologists were selected to validate SLE diagnosis before a catastrophic illness certificate was issued. Also, the BNHI had randomly checked patients' original medical charts to minimize the bias of miscoding.[12] Fourth, given that dengue infection may be related to the development of SLE[13] and some SLE patients may have an insidious onset leading to a delay in diagnosis, we cannot prevent the possibility of reverse causality.

CONCLUSION

This nationwide population-based case-control study in Taiwan failed to show an association between HCQ use and the dengue virus infection in SLE patients. Future longitudinal studies using larger sample sizes are required to further investigate our findings.

Conflict of interest

Medical writing support was provided by Enago (www.enago.com) and was funded by personal private payment by the authors.

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紅斑性狼瘡病患使用氫氧奎寧與登革熱之風險

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目的：闡明系統性紅斑狼瘡患者中氫氧奎寧與登革熱的風險之間的關聯。

方法：1997-2013年台灣全民健康保險研究資料庫中，有23,936名SLE患者。

這之中有14名被診斷出登革熱（國際疾病分類第九版臨床修訂ICD-9-CM，代碼061）。我們根據性別、居住地、登革熱和SLE診斷日期來配對登革熱病例，以10：1的比例，隨機選擇了140名沒有感染登革熱的SLE患者，進行統計分析。

結果：在登革熱診斷日期之前的3個月內，登革熱的風險與使用HCQ無關（OR, 0.46；95%CI，0.13-1.66；p = 0.237）。

使用較低的HCQ劑量（≤200 mg /天）（OR, 0.52；95%CI，0.11-2.44；p = 0.409）。

使用較高的HCQ劑量（>200 mg /天）（OR, 0.41，95%CI，0.09–1.93；p = 0.260）。

結論：這項基於台灣全民健康保險研究資料庫的研究，並未證明HCQ的使用與登革熱的風險，在SLE患者之間存在顯著相關性。

關鍵詞：登革熱、氫氧奎寧、風險、給付資料

Original Article

Risk Factors for Progression to End-Stage Renal Disease among Systemic Lupus Erythematosus Patients: A Medical Center Retrospective Cohort Study

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Objectives: This study aimed to determine the risk factors for progression to end-stage renal disease (ESRD) in systemic lupus erythematosus (SLE) patients and to analyze the distributions of renal pathological classifications in lupus nephritis patients.

Methods: We retrospectively evaluated the medical records of 689 SLE patients who were admitted during 2005-2012. The follow-up duration was from admission to ESRD, death, loss of follow-up, or 2019. Twenty-three patients were excluded due to a diagnosis of ESRD before admission (n=22) or no initial serum creatinine data (n=1). The Cox proportional hazard model was performed to determine the risk factors for ESRD in SLE patients.

Results: A total of 666 SLE patients were included in the study, and 46 (6.9%) patients developed ESRD. The multivariate Cox proportional hazard model demonstrated a significant increase in the hazard ratio (HR) of ESRD in SLE patients with proteinuria (HR 13.54, 95% CI 1.81-101.09, p=0.011), elevated creatinine levels (for every 1 mg/dL increase, HR 1.65, 95% CI 1.31-2.07, p<0.001), seizure (HR 2.84, 95% CI 1.48-5.45, p=0.002), and hypertension (HR 3.50, 95% CI 1.71¬7.15, p=0.001). Among the 666 patients included in the study, 72 of these patients had received a renal biopsy. The biopsy results showed 51 (70.8%) of the 72 patients were class IV with regard to the classification of glomerulonephritis in SLE.

Conclusions: We reported the independent potential risk factors for progression to ESRD among SLE patients, including proteinuria, azotemia, hypertension, and seizure. Seizure has seldom been mentioned as a risk factor in previous studies.

Key words: systemic lupus erythematosus, end-stage renal disease, seizure, pathological classifications

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease involving multiple organs and systems [1, 2]. In comparison with other populations in the world, especially Caucasians, Asian populations had both a higher prevalence and a higher incidence of SLE [3-6]. Nonwhite racial groups had more end-organ damage and severe systemic involvement [7]. A population-based study of the National Health Insurance Research Database (NHIRD) in Taiwan revealed an average SLE prevalence of 97.5 per 100,000 population between 2003 and 2008 [8], which was higher than that in many countries [5].

Mortality in SLE patients has improved much in the past decades: a multisite international SLE cohort study revealed a dramatic 60% decrease in the standardized

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mortality rate (SMR), from an SMR of 4.9 during 1970-1979 to an SMR of 2.0 during 1990-2001 [9]; the twenty-year survival rate increased to about 80% [10, 11]. However, SLE patients still have higher mortality than the general population [12, 13]. The major causes of death in SLE patients included cardiovascular disease, malignancy, infection, renal disease, and central nervous system lupus [9, 12, 13]. The renal cause of death in SLE patients has a high SMR of 5.6-7.9 [9, 12, 14], and there is an extremely high SMR of 26.1 in SLE patients with end-stage renal disease (ESRD) [12].

According to previous studies, lupus nephritis and ESRD play important roles in mortality and morbidity in SLE patients [9, 15]. The development of ESRD showed a significant mortality risk in SLE patients [12]. The classifications of renal pathology in lupus nephritis also predict the prognosis of SLE patients [15, 16]. However, recently, there have been few studies in Taiwan on risk factors for ESRD in SLE patients and analysis of renal pathological classifications in lupus nephritis patients. Thus, the present study aimed to determine the risk factors for progression to ESRD in SLE patients and to analyze the distributions of renal pathological classifications in lupus nephritis patients.

Patients and Methods

Patient enrollment and study design

We retrospectively evaluated the medical records of 689 SLE patients who were first admitted to the Rheumatology Ward of Linkou Chang Gung Memorial Hospital from January 2005 to December 2012. The study was approved by the Institutional Review Board at this institution (103¬2394C). It was a retrospective review of the cohort, and thus, written informed consent was waived. The diagnosis of SLE was based on the American College of Rheumatology (ACR) revised criteria in 1997 [17]. A total of 689 SLE hospitalized patients were included in the study and followed up until ESRD, death, loss of follow-up, or March, 2019. The definition of a patient with ESRD was a patient undergoing regular dialysis. Among 689 patients, 22 patients who had been diagnosed with ESRD before admission were excluded. Under consideration of the unignorable impact of initial serum creatine on developing ESRD, we excluded one patient with a lack of initial serum creatinine data. Finally, 666 patients were included in our study.

Data collection

Collected data included gender, age at SLE onset, age at admission, initial clinical features, laboratory test results, comorbidities, and renal biopsy results.

Statistical analyses

The continuous data were presented as the means and standard deviations (SDs), and categorical data were presented as numbers and percentages. The two-tailed Student's t-test was used for group comparisons of continuous data. The Chi-squared test or Fisher's exact test was used for group comparisons of categorical data. A two-sided p-value of less than 0.05 was considered to be statistically significant. Univariate and multivariate Cox proportional hazard models were performed to determine the risk factors for ESRD in SLE patients. The hazard ratio (HR) was presented with a 95% confidence interval (CI). Moreover, the renal pathological classifications among SLE patients who had received renal biopsy were analyzed. The statistical analyses were performed using SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA).

Results

Study patients

There was a total of 666 SLE patients included in the present study. There were 592 (88.9%) females and 74 (11.1%) males. The average age at admission was 40.7 ± 16.0 years old. The mean duration from SLE onset to admission was 4.5 ± 6.2 years. The mean follow-up duration of the present study was 5.4 ± 4.1 years. In total, 46 (6.9%) patients developed ESRD during the follow-up period. The incidence rate of ESRD among SLE patients in the study was 1,284.5 (95% CI 962.1¬1714.9) per 100,000 person-years. Table 1 shows the characteristics of SLE patients with and without ESRD, including gender, admission age, SLE duration from diagnosis to admission, serum creatinine level, leukocyte count, leukopenia, hemoglobin level, autoimmune hemolytic anemia, platelet count, thrombocytopenia, serum complement 3 (C3) level, serum complement 4 (C4) level, malar rash, discoid rash, photosensitivity, oral ulcer, arthritis, pleuritis, pericarditis, proteinuria, seizure, psychosis, anti-dsDNA antibody, hypertension, and diabetes mellitus. Proteinuria was defined as total protein of urine > 0.5 g/day. There were significant differences between the ESRD and non-ESRD patients in the initial presentations of pleuritis (52.2% vs. 28.5%, p=0.001), pericarditis (28.3% vs. 13.5%, p=0.006), proteinuria (97.8% vs. 56.6%, p<0.001), seizure (37.0%

Table 1. Characteristics in systemic lupus erythematosus patients with and without end-stage renal disease.

	ESRD (n=46)	non-ESRD (n=620)	p-value
Female, N (%)	40 (87.0%)	552 (89.0%)	0.666
Admission age (years)	38.3 ± 16.4	40.9 ± 16.0	0.291
SLE duration (years)	5.8 ± 5.8	4.4 ± 6.2	0.138
Clinical presentation			
Malar rash, N (%)	22 (47.8%)	283 (45.6%)	0.775
Discoid rash, N (%)	7 (15.2%)	56 (9.0%)	0.187[a]
Photosensitivity, N (%)	7 (15.2%)	84 (13.5%)	0.750
Oral ulcer, N (%)	10 (21.7%)	159 (25.6%)	0.557
Arthritis, N (%)	22 (47.8%)	330 (53.2%)	0.479
Pleuritis, N (%)	24 (52.2%)	177 (28.5%)	0.001*
Pericarditis, N (%)	13 (28.3%)	84 (13.5%)	0.006*
Proteinuria[b], N (%)	45 (97.8%)	351 (56.6%)	<0.001*
Seizure, N (%)	17 (37.0%)	53 (8.5%)	<0.001[a]*
Psychosis, N (%)	3 (6.5%)	47 (7.6%)	1.000[a]
Serological profile			
Creatinine (mg/dL)	1.67 ± 1.55	0.89 ± 0.54	0.001*
Leukocytes (per μL)	7011 ± 3489	6245 ± 3961	0.203
Leukopenia[c], N (%)	7 (15.2%)	163 (26.3%)	0.097
Hemoglobin (g/dL)	10.2 ± 2.1	11.2 ± 2.3	0.003*
AIHA, N (%)	4 (8.7%)	41 (6.6%)	0.541[a]
Platelet (1000/μL)	211.9 ± 86.3	198.6 ± 97.9	0.369
Thrombocytopenia[d], N (%)	5 (10.9%)	109 (17.6%)	0.244
C3 (mg/dL)	63.6 ± 27.2	69.2 ± 33.4	0.268
C4 (mg/dL)	12.2 ± 8.9	13.3 ± 8.5	0.385
Anti-dsDNA positivity, N (%)	37 (80.4%)	441 (71.1%)	0.176
Comorbidity			
Hypertension, N (%)	34 (73.9%)	175 (28.2%)	<0.001*
Diabetes mellitus, N (%)	6 (13.0%)	42 (6.8%)	0.132[a]

The continuous data were presented as the mean ± SD.
[a] Fisher's exact test.
[b] total protein of urine > 0.5 g/day.
[c] Leukocyte < 4,000 /uL.
[d] platelet < 100,000 /uL.
* p-value < 0.05.
ESRD, end-stage renal disease; AIHA, autoimmune hemolytic anemia; C3, complement 3; C4, complement 4.

vs. 8.5%, p<0.001), and hypertension (73.9% vs. 28.2%, p<0.001). There were also significant differences between the ESRD and non-ESRD patients in initial creatinine level (1.67 ± 1.55 mg/dL vs. 0.89 ± 0.54 mg/dL, p=0.001) and hemoglobin level (10.2 ± 2.1 g/dL vs. 11.2 ± 2.3 g/dL, p=0.003). There was no significant difference between the ESRD and non-ESRD patients in gender, admission age, SLE duration, leukocyte count, leukopenia, autoimmune hemolytic anemia, platelet count, thrombocytopenia, C3 level, C4 level, malar rash, discoid rash, photosensitivity, oral ulcer, arthritis, psychosis, anti-dsDNA antibody, or diabetes mellitus.

Factors associated with ESRD

Gender, admission age, and several significant factors were selected for the Cox proportional hazard model to identify predictors of ESRD in SLE patients. Table 2 shows univariate and multivariate Cox proportional hazard models in the cohort of ESRD in SLE patients. Multivariate analysis demonstrated a significant increase in the HR of ESRD in SLE patients with initial presentation of proteinuria (HR 13.54, 95% CI 1.81-101.09, p=0.011), with elevated initial creatinine level (for every 1 mg/dL increase, HR 1.65, 95% CI 1.31-2.07, p<0.001), with initial presentation of seizure (HR 2.84,

Table 2. Univariate and multivariate analyses of hazard ratios of end-stage renal disease in systemic lupus erythematosus patients.

	Crude HR (95% CI)	p-value	Adjusted HR (95% CI)	p-value
Female	0.63 (0.27-1.49)	0.294	0.67 (0.27-1.68)	0.397
Admission age[a]	1.00 (0.98-1.02)	0.666	0.99 (0.97-1.01)	0.460
Pleuritis	2.73 (1.53-4.87)	0.001*	1.28 (0.64-2.55)	0.480
Pericarditis	2.70 (1.42-5.13)	0.002*	1.52 (0.67-3.43)	0.315
Proteinuria	33.30 (4.59-241.61)	0.001*	13.54 (1.81-101.09)	0.011*
Creatinine[b]	2.32 (1.91-2.81)	< 0.001*	1.65 (1.31-2.07)	< 0.001*
Hemoglobin[c]	0.82 (0.73-0.92)	0.001*	0.94 (0.82-1.09)	0.425
Seizure	5.90 (3.24-10.76)	< 0.001*	2.84 (1.48-5.45)	0.002*
Hypertension	6.80 (3.52-13.14)	< 0.001*	3.50 (1.71-7.15)	0.001*

[a] increase for every 1 year in age.
[b] increase for every 1 mg/dL.
[c] increase for every 1 g/dL.
* p-value < 0.05.
HR, hazard ratio; CI, confidence interval.

95% CI 1.48-5.45, p=0.002), and with initial presentation of hypertension (HR 3.50, 95% CI 1.71-7.15, p=0.001). In addition, under consideration of diabetes mellitus as a traditional risk factor for ESRD [18], if we included diabetes mellitus in univariate and multivariate Cox proportional hazard models, the results still were nonsignificant (univariate analysis HR 1.96, 95% CI 0.83-4.62, p=0.125; multivariate analysis HR 0.56, 95% CI 0.20-1.55, p=0.261). The comparisons of cumulative incidence of ESRD between SLE patients with seizure

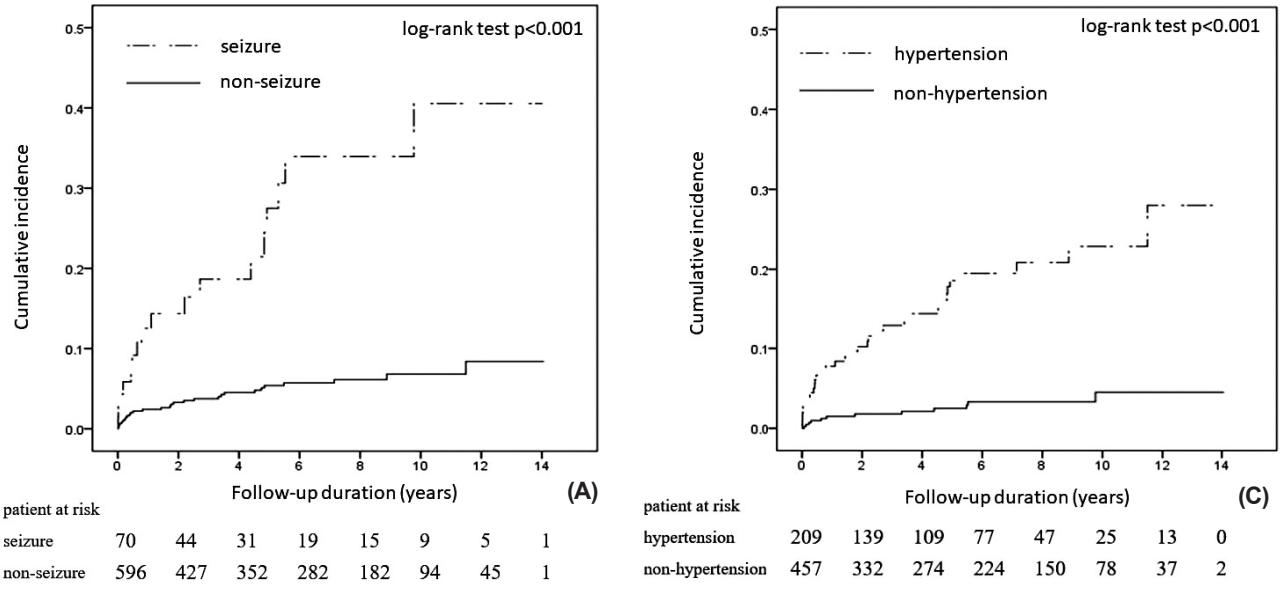

Figure 1. The cumulative incidence of end-stage renal disease between systemic lupus erythematosus patients with seizure and without seizure(A), with proteinuria and without proteinuria(B), with hypertension and without hypertension(C).

Table 3. Comparison of end-stage renal disease with different groups of pathological classifications among 72 patients who had received renal biopsy.

Class	Group	total (n=72)	ESRD (n=12)	non-ESRD (n=60)	p-value
Class I/II	Group 1	2	0 (0%)	2 (100.0%)	0.027[a]
Class V	Group 2	11	1 (9.1%)	10 (90.9%)	
Class III/IV	Group 3	55	8 (14.5%)	47 (85.5%)	
Class VI	Group 4	4	3 (75.0%)	1 (25.0%)	

[a] Cochran-Armitage trend test.

and without seizure, with proteinuria and without proteinuria, with hypertension and without hypertension were calculated by Kaplan-Meier and log-rank tests (Figure 1 A-C). There were significant increases in the cumulative incidence of ESRD among SLE patients with seizure (log¬rank test, p<0.001), with proteinuria (log-rank test, p<0.001), and with hypertension (log-rank test, p<0.001).

Classification of lupus nephritis

Among 666 SLE patients included in the present study, 72 patients had received a renal biopsy. The pathological results were based on the Renal Pathology Society/ International Society of Nephrology (RPS/ ISN) classification [19], demonstrated as follows: class I (2 patients, 2.7%), class II (0 patients, 0%), class III (4 patients, 5.6%), class IV (51 patients, 70.8%), class V (11 patients, 15.3%), and class VI (4 patients, 5.6%). We defined class I/II as group 1, class V as group 2, class III/ IV as group 3, and class VI as group 4. Table 3 shows the presence of a linear trend in proportions on ESRD across levels of group of pathological classification (proportion on ESRD of group 1: 0%, proportion on ESRD of group 2: 9.1%, proportion on ESRD of group 3: 14.5%, proportion on ESRD of group 4: 75.0%, Cochran-Armitage trend test p=0.027). Cox proportional hazard model for ESRD risk analysis among these 72 patients was also performed. Multivariate analysis demonstrated a significant increase in the HR of ESRD with elevated group level (for every 1 group level increase, HR 27.54, 95% CI 2.47-307.65, p=0.007, Table 4).

Discussion

SLE is a chronic autoimmune disease with a broad range of clinical and laboratory manifestations involving multiple organs and systems [1, 2]. Renal involvement is one of the most critical issue among SLE patients due to potential progression to ESRD and mortality, and ESRD is more common in SLE patients than in non-

Table 4. Multivariate analyses of hazard ratios of end-stage renal disease among 72 patients who had received renal biopsy.

	Adjusted HR (95% CI)	p-value
Female	0.36 (0.03-4.76)	0.439
Admission age[a]	0.96 (0.89-1.04)	0.307
Pleuritis	11.32 (1.39-92.02)	0.023*
Pericarditis	0.35 (0.04-3.10)	0.342
Proteinuria	8704.99 (<0.01 ->10000)	0.994
Creatinine[b]	2.31 (1.16-4.59)	0.017*
Hemoglobin[c]	1.13 (0.77-1.67)	0.529
Seizure	1.15 (0.25-5.41)	0.859
Hypertension	6.90 (1.48-32.22)	0.014*
Group of lupus nephritis[d]	27.54 (2.47-307.65)	0.007*

[a] increase for every 1 year in age.
[b] increase for every 1 mg/dL.
[c] increase for every 1 g/dL.
[d] increase for every 1 group level, class I/II defined as group 1, class V defined as group 2, class III/IV defined as group 3, and class VI defined as group 4.
* p-value < 0.05.
HR, hazard ratio; CI, confidence interval.

SLE patients [20, 21]. Several studies have investigated ESRD prevalence among SLE patients which ranged from 2.5%-10% [15, 21-23]. According to two NHIRD studies, ESRD prevalence among SLE patients ranged from 2.5%-4.85% [21, 22]. The present study revealed that 6.9% of SLE hospitalized patients developed ESRD during the follow-up period. The incidence rate of ESRD among SLE patients in the present study was 1,284.5 per 100,000 person-years which was higher than that in the previous NHIRD study with 612.8 per 100,000 person-years [21]. The possible explanation of both the higher prevalence and incidence of ESRD in the present study might be the more complicated disease status in the medical center compared with the general population of SLE patients.

The present study aimed to determine the risk factors for progression to ESRD in SLE patients. Multivariate analysis demonstrated initial presentation of proteinuria, elevated initial creatinine level, initial presentation of seizure, and initial presentation of hypertension were independent risk factors for developing ESRD in SLE patients (Table 2). Diabetic nephropathy and hypertensive nephrosclerosis account for nearly half of all the causes of ESRD in Asia [24]. An NHIRD study in Taiwan revealed significant risk factors for chronic kidney disease progression to dialysis including decreased age, creatinine, urea nitrogen, and diabetes mellitus [18]. Previous studies have revealed initial renal function [25, 26], ethnicity [26-28], younger age [27, 28], hypertension [25], proteinuria [27, 29], male gender [29], low C3 level [27], and cardiovascular history [25] were independent predictors of ESRD among SLE patients. A French nationwide epidemiologic study showed that significant risk factors for ESRD in SLE patients were chronic kidney disease (HR 15.9, 95% CI 11.6-21.9), lupus nephritis (HR 2.1, 95% CI 1.5-3.0), hypertension (HR 1.7, 95% CI 1.3-2.4), and cardiovascular history (HR 1.7, 95% CI 1.1-2.5) in multivariate Cox proportional hazard model [25]. A retrospective analysis comprising 186 Japanese lupus nephritis patients revealed nephrotic proteinuria (HR 3.71, 95% CI 1.15-12.0) and male gender (HR 3.33, 95% CI 1.14-9.73) were independent poor prognostic factors for renal survival [29]. Overall, the studies above were similar to the present study about the independent risk factors for developing ESRD among SLE patients including proteinuria, elevated creatinine level and hypertension.

In addition to well-known risk factors for ESRD, an interesting finding of the present study was the initial presence of seizure as an independent risk factor for ESRD which was not mentioned before. Renal disease was one of the comorbidities of epileptic patients [30]. A retrospective study of 72 children with lupus nephritis at one center from 1965 to 1999 revealed that patients with neuropsychiatric (NP) manifestations had a much higher incidence of ESRD than patients without NP manifestations (70% vs 40%, $p<0.035$). Patients with NP manifestations had a much higher progression rate to ESRD compared to patients without NP manifestations in an univariate analysis (OR 5.7, $p=0.007$), but there was no statistical significance in the multivariate analysis [31]. However, the present study demonstrated seizure as an independent risk factor for progression to ESRD among SLE patients in multivariate regression analysis. Although to our knowledge, no reports describe seizure as an independent risk factor for developing ESRD among SLE patients, the possible reasons for renal function deterioration in patients initially presenting with seizure might include increased disease activity that has resulted from NP manifestations and chronic epilepsy-related vascular damage [32-37]. However, the causal relationship and pathophysiology of seizure resulting in the development of ESRD in SLE patients may require further studies.

There was no significant difference in diabetes mellitus between SLE patients with and without ESRD in the present study (Table 1). Under consideration of diabetes mellitus as a traditional independent risk factor for chronic kidney disease progression to ESRD (HR 1.68, 95% CI 1.45¬1.88) [18], if we also selected diabetes mellitus in the multivariate Cox proportional hazard model, the result was still nonsignificant which was compatible with previous studies [25, 38]. A French nationwide epidemiologic study showed diabetes mellitus had no significant association with ESRD in SLE patients (HR 0.9, 95% CI 0.4-1.7) [25]. Using the NHIRD of Taiwan, diabetes mellitus was not an independent risk factor for ESRD in SLE patients (adjusted HR 1.64; 95% CI 0.97-2.76) [38].

The outcome of lupus nephritis is better and the proportion of the population diagnosed with lupus nephritis is lower in European patients than in nonwhite populations [39-42]. As a result, it is worthwhile to investigate SLE patients with lupus nephritis in Taiwan. Among 72 patients who had received a renal biopsy, the present study demonstrated the highest proportion of class IV (70.8%) lupus nephritis and significant increased risk of ESRD in higher level of group of pathological classifications (Table 3, Table 4). The SLICC cohort study also demonstrated the highest percentile of class IV lupus nephritis (class I: 2.4%, class II: 9.5%, class III: 26.8%, class IV: 43.2%, class V: 31.8%, and class VI:

0.8%) [15]. In addition, the Asian SLE population tends to develop lupus nephritis at a greater rate than the white population, and 10% to 30% of SLE patient with lupus nephritis progressed to ESRD, especially proliferative forms of lupus nephritis [16], which was compatible with a higher percentage of ESRD of group 3 (class III/IV) in the present study (Table 3).

There were limitations in the present study. First, confounding factors for adjustment in multivariate analysis of the Cox proportional hazard model did not include SLE disease activity assessments (e.g., Systemic Lupus Erythematosus Disease Activity Index). Instead, confounding factors for adjustment in the present study included gender, age, pleuritis, pericarditis, proteinuria, seizure, hypertension, creatinine, and hemoglobin. Second, a socioeconomic status that might be associated with ESRD onset was not included in the collected data [43]. Therefore, multivariate analysis in the present study could not adjust the socioeconomic status as one of the confounding factors. Third, the treatment regimen which played an important role in ESRD among SLE patients was not be assessed in the present study. There are lots of concerns about treatment regimen, such as patient's compliance with drugs, duration of drugs use, switch of drugs, dosage adjustment of drugs, and combination of drugs use. However, an ideal design which standardized treatment regimen would be difficult to the present retrospective study.

In conclusion, we reported the independent potential risk factors for progression to ESRD among SLE patients, including proteinuria, azotemia, hypertension, and seizure. Seizure has seldom been mentioned as a risk factor in previous studies. Further studies may be required to identify the causal relationship of seizure resulting in the development of ESRD among SLE patients.

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全身紅斑性狼瘡病患進展至末期腎病的危險因子：單一醫學中心的回溯性世代研究

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目的：探討全身紅斑性狼瘡病患進展至末期腎病的危險因子，並分析狼瘡腎炎病患的腎臟切片病理分類。

方法：回溯性調查689位2005-2012年住院的全身紅斑性狼瘡病患，自住院日開始追蹤，至發生末期腎病、死亡、失去追蹤、或2019年為止。其中23位病患被排除，包括22位住院前就已是末期腎病狀態，以及1位缺少初始血清肌酸酐值。本研究使用Cox proportional hazard model來分析全身紅斑性狼瘡病患的末期腎病危險因子。

結果：總共666位病患被納入研究，其中46（6.9%）位病患進展至末期腎病，Cox proportional hazard model的多變數分析顯示，顯著上升末期腎病風險的危險因子包括蛋白尿（HR 13.54，95% CI 1.81-101.09，p=0.011）、高肌酸酐（每增加1 mg/dL，HR 1.65，95% CI 1.31-2.07，p<0.001）、癲癇（HR 2.84，95% CI 1.48-5.45，p=0.002）、以及高血壓（HR 3.50，95% CI 1.71-7.15，p=0.001）。而在666位病患中，有72位接受腎臟切片，其中的51（70.8%）位的腎臟切片病理分類是第IV類。

結論：本研究顯示全身紅斑性狼瘡病患進展至末期腎病的獨立危險因子包括蛋白尿、氮血症、高血壓、以及癲癇，其中癲癇較少在之前的研究被提及。

關鍵詞：全身紅斑性狼瘡、末期腎病、癲癇、病理分類

Original Article

The Impact of Antiphospholipid Antibody Profile on the Clinical Outcomes Associated with Systemic Lupus Erythematosus in Taiwanese Patients

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Objectives: The aim of this study was to determine the impact of antiphospholipid (aPL) profile on thrombosis and pregnancy morbidity associated with systemic lupus erythematosus (SLE) in Taiwanese patients.
Methods: Ninety-nine out of the 689 patients with SLE who were admitted to the Chang Gung Memorial Hospital between January 2005 and December 2012 and for whom data pertaining to the aPL profile were available were included in this study. The 99 SLE patients were tested for all three aPL antibody tests including lupus anticoagulants (LA), anticardiolipin (aCL) antibodies (IgM and IgG), and anti-beta2-glycoprotein I (anti-β2-GPI) antibodies (IgM and IgG).
Results: Of the 99 patients, 46 (46.5%) patients had at least one positive aPL test (including LA, IgM/IgG aCL antibodies, IgM/IgG anti-β2-GPI antibodies), while 53 patients had negative aPL. The 46 aPL-positive SLE patients were further classified into two subgroups: multiple aPL positivity (more than one aPL test positive) (n = 15) and single aPL positivity (LA, aCL antibodies, or anti-β2-GPI antibodies) (n = 31). Multiple aPL positivity was associated with approximately 5.7 times greater risk of venous or arterial thromboembolism [adjusted odds ratio (OR) 5.72. 95% confidence interval (CI) 1.59–20.60, P = 0.008]; however multiple aPL positivity showed no significant association with pregnancy morbidity. Single aPL test positivity was not associated with significantly greater vascular thrombotic risk or pregnancy morbidity.
Conclusions: Among **Taiwanese** patients with SLE, those with more than one positive aPL test showed greater risk of vascular thrombosis.

Key words: Systemic lupus erythematosus, antiphospholipid antibody, thrombosis, pregnancy complications

Introduction

The association between antiphospholipid antibodies (aPL) and thrombosis is well established. Nonetheless, aPL are a heterogeneous group of autoantibodies and the available evidence of their pathogenic significance is inconsistent; moreover, aPL are found in 1%–5% of the general population [1, 2]. Currently, intermittent or low titers of aPL, particularly those of isolated anticardiolipin antibody (aCL), are no longer considered as risk factors for thrombosis [2-5]. To avoid misdiagnosis, the following revised Sapporo classification criteria were defined for a definitive diagnosis of antiphospholipid

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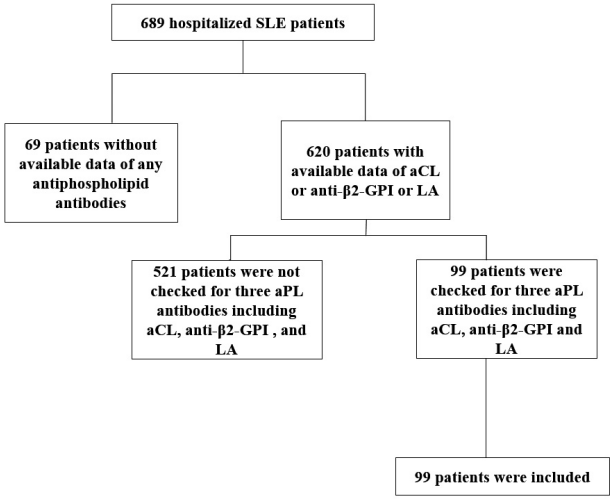
syndrome (APS): vascular thrombosis and/or pregnancy morbidity and positive results of at least one of the following aPL tests: lupus anticoagulant (LA) assay, IgM/IgG anticardiolipin (aCL), and IgM/IgG anti-β2 glycoprotein I (anti-β2-GPI) [6]. Classification of APS should be avoided if less than 12 weeks or more than 5 years have elapsed between the positive aPL test and the clinical manifestations; however, these time intervals are based on expert opinions [6]. In patients who qualified the revised Sapporo classification criteria for APS [6], aPL significantly increases the risk of thrombosis recurrence [2, 7, 8]. Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with variable clinical features ranging from mild joint and skin involvement to life-threatening renal, hematologic, and/or central nervous system manifestations [9, 10]. In previous studies, 30%–55% of patients with SLE were found positive for aPL [1, 10-15]. In several studies, SLE patients with aPL had higher prevalence of thrombosis and pregnancy morbidity compared to those without aPL [8, 10, 16-26]. Given the relatively high prevalence of aPL in SLE patients, it is conceivable that aPL-positive SLE patients may have a more severe clinical phenotype and worse prognosis than those without aPL. Recently, the term "aPL profile" has been used to define the number and type of positive aPL tests. The aPL profile distinguishes the presence of multiple (double or triple) versus single aPL type, their titers (medium-high titer vs. low titer), and the persistence of aPL positivity on repeat measurements [5, 25, 27-29]. The aPL profile is an important determinant of the risk of thrombotic and obstetric events, and consequently the intensity of treatment [8, 29, 30]. Few studies have comprehensively assessed the aPL antibodies in Taiwanese patients with SLE. Furthermore, the impact of aPL profile on the outcomes associated with SLE in Taiwanese patients has rarely been evaluated. The aim of this study was to determine the association between the aPL profile and specific clinical events, including venous or arterial thrombosis and pregnancy morbidity in Taiwanese patients with SLE.

Materials and Methods

Patients

We identified 689 patients who were admitted to the Linkou Chang Gang Memorial Hospital between 2005 and 2012 with the International Classification of Diseases, Ninth Revision (ICD-9) diagnostic code of 710.0. All patients qualified four or more criteria of the American College of Rheumatology for the classification of SLE [31]; of these, data pertaining to all three aPL tests (LA, IgM/IgG aCL antibodies, and IgM/IgG anti-β2GPI antibodies) were available for only 99 patients. The aPL profile is determined based on the aPL type, presence of multiple (double or triple) versus single aPL type, their titer, and the persistence of aPL positivity in repeated measurements. However, simultaneous tests for aPL such as LA, IgM/IgG aCL, and IgM/IgG anti-β2GPI are not performed as part of routine clinical investigations for SLE patients. The 99 SLE patients for whom data pertaining to all three aPL tests were available for aPL profile analysis were included in the study (Figure 1) and followed up until March, 2019 or until the death of the patients. We reviewed patient charts for gender, age, antiphospholipid antibodies, aPL profile, and clinical events of venous/arterial thrombosis and pregnancy morbidity. Thrombosis was confirmed by venography, arteriography, Doppler ultrasonography, or magnetic resonance angiography in each patient.

Figure 1. Schematic illustration of the patient-selection criteria



Abbreviations: aPL = antiphospholipid antibodies, LA = lupus anticoagulants, aCL = anticardiolipin antibodies (IgM/IgG), anti-β2-GPI = anti-beta2-glycoprotein I antibodies (IgM/IgG)

Methods

The following variables were included in the analysis: age at admission, gender, aPL antibodies, aPL profile, thrombotic events, pregnancy morbidity, hypertension, diabetes mellitus, smoking habit, and APS. The distribution of IgM and IgG aCL, IgM, and IgG anti-β2-GPI antibodies, and LA in the study population was analyzed. Patients were further classified into two groups according to the aPL antibody profile:

multiple aPL positivity (>1 laboratory criterion present in any combination [LA, aCL antibodies, or anti-β2-GPI antibodies]) and single aPL positivity (only a single laboratory criterion present). Those with multiple aPL positivity were further classified into two subgroups: triple positivity (presence of all three laboratory criteria) and double positivity (presence of two laboratory criteria in any combination). Patients with single aPL positivity were divided into three subgroups: LA present alone, aCL IgM or IgG present alone, and anti-β2-GPI IgM or IgG present alone. Perisistent positivity for antiphospholipid antibodies were defined as positive antiphospholipid antibodies being detected on at least two occasions at least 12 weeks apart.

Anticardiolipin (aCL) antibodies: aCL antibodies were measured with a semiquantitative ELISA assay using the commercially-available QUANTA Lite aCL IgM/IgG kit (INOVA Diagnostics, San Diego, CA, USA). The IgM and IgG isotope results were expressed in IgM phospholipid (MPL) and IgG phospholipid (GPL) units; one unit is equal to 1 mg/mL IgM or IgG. In accordance with the manufacturer's instructions, 20 MPL units/mL for IgM and 20 GPL units/mL for IgG were considered positive values for this study.

Anti-beta2-GPI (anti-β2GPI) antibodies: Anti-β2-GPI antibodies were measured with a semiquantitative ELISA assay using the commercially-available QUANTA Lite β2-GPI IgG/IgM ELISA kit (INOVA Diagnostics, San Diego, CA, USA). Polystyrene plates coated with purified β2-GPI antigen were used. The values of anti-β2-GPI I antibodies were expressed in standard IgM anti-β2-GPI units (SMU) or IgG anti-β2-GPI units (SGU). In accordance with the manufacturer's instructions, the cut-off values for the positive results were set at > 20 SMU or > 20 SGU, respectively.

Lupus anticoagulant (LA): The Dilute Russell's viper venom test system was used for detection of LA in this study. We dispensed 200 μL of test plasma, and warmed for one minute at 37°C; subsequently, 200 μL of prewarmed reagent was added to the plasma and timed from the moment of addition of reagent to a clotting end point (Gradipore, NSW, Australia) [32]. Abnormal LA clotting time is > 44s (> 2 standard deviations longer than the mean of normal plasma).

Statistical Analyses

Statistical analyses were performed using the IBM SPSS version 22 (IBM Corp, Armonk, NY, USA). Owing to the non-normal distribution of variables, data are presented as median and percentile (25 and 75) or as percentage. Nonparametric test (Mann-Whitney U test) was used to compare the quantitative data and the Chi-squared test or Fisher's exact test was used to compare proportions. Additionally, the estimated risk of thromboembolic events for each individual antiphospholipid antibody test and their combination profiles was evaluated by means of multivariate analysis with the parameters that were statistically associated with arterial/venous thrombosis by univariate analysis, with a P value of 0.05 or less, and adjusted for age and gender. P values of < 0.05 were considered indicative of statistical significance.

Results

Table 1 summarizes the demographic and clinical characteristics of the study population. Of the 99 SLE patients who were tested for the presence of LA, IgM/IgG aCL antibodies, and IgM/IgG anti-β2-GPI antibodies, at least one type of aPL positivity was detected in 46 (46.5%) patients. Fifty-three (53.5%) of the 99 patients had never tested positive for any of the three aPL antibodies; these patients were categorized as the aPL-negative group. For the 46 aPL-positive lupus patients, the median age at admission was 30.5 years, which was not significantly different from that of aPL-negative patients (34.0 years; p = 0.398). There were also no significant differences with respect to the baseline characteristics such as gender, hypertension, diabetes mellitus, smoking habit, and presence of arterial/venous thrombosis, pregnancy morbidity, or thrombocytopenia (Table 1). The mean follow-up period was 8.41 (+2.91) years. Among the 99 SLE patients (87 females and 12 males), 26 (26.3%) presented with arterial/venous thrombosis or pregnancy morbidity, whereas 73 (73.7%) did not. The frequency of pregnancy morbidity in SLE patients with and without aPL was 2.1% and 1.8%, respectively (p = 0.285) (Table 1). However, only nine out of the 46 (19.6%) aPL-positive lupus patients qualified the updated Sapporo classification criteria for APS. Table 1 shows the prevalence of LA, IgM/IgG aCL antibodies, and IgM/IgG anti-β2-GPI antibodies in lupus patients. The aCL IgG (27.3%) was the most commonly detected antibody, followed by aCL IgM (26.3%), LA (18.2%), anti-β2-GPI IgG (10.1%), and anti-β2-GPI IgM (6.1%). Overall, 15 (15.2%) patients showed multiple aPL positivity: seven (7.1%) patients were triple positive while eight (8.1%) patients were double positive. Thirty-one (31.3%) patients presented with single aPL positivity: five (5.1%) patients were

Table 1. Demographic data from patients with systemic lupus erythematosus checked for three aPL (n = 99)

	Total (n = 99)	Positive aPL (n = 46)	Negative aPL (n = 53)	*p* value
Age, years median (25–75th percentile)	32.0 (26.0–45.0)	30.5 (26.0–41.0)	34.0 (26.0–48.0)	0.398
Female, n (%)	87 (87.8%)	41 (89.1%)	46 (86.7%)	0.722
Cardiovascular risk factors			1.28 (0.64-2.55)	0.480
Hypertension, n (%)	32 (32.3%)	15 (32.6%)	17 (32.1%)	0.955
Diabetes mellitus, n (%)	10 (10.1%)	6 (13.0%)	4 (7.5%)	0.365
Smoking habit	17 (17.2%)	8 (17.4%)	9 (17.0%)	0.957
Clinical manifestation			0.94 (0.82-1.09)	0.425
Arterial/venous thrombosis, n (%)	26 (26.3%)	13 (28.3%)	13 (24.5%)	0.674
Pregnancy morbidity*, n (%)	2 (2.0%)	1 (2.1%)	1 (1.8%)	0.285
Thrombocytopenia, n (%)	50 (50.5%)	25 (54.3%)	25 (47.2%)	0.476
Antiphospholipid syndrome$^{\Delta}$	9 (9.0%)	9 (19.6%)	0	
aPL test	46 (46.5%)	46 (100.0%)	0	
LA	18 (18.2%)	18 (39.1%)	0	
aCL IgM	26 (26.3%)	26 (56.5%)	0	
aCL IgG	27 (27.3%)	27 (58.7%)	0	
anti-β2-GPI IgM	6 (6.1%)	6 (13.0%)	0	
anti-β2-GPI IgG	10 (10.1%)	10 (21.7%)	0	
Multiple aPL positivity	15 (15.2%)	15 (32.6%)	0	
Triple positive	7 (7.1%)	7 (15.2%)	0	
Double positive	8 (8.1%)	8 (17.4%)	0	
Single aPL positivity	31 (31.3%)	31 (67.4%)	0	
LA positive only	5 (5.1%)	5 (10.9%)	0	
aCL positive only	23 (23.2%)	23 (50.0%)	0	
anti-β2-GPI+ positive only	3 (3.0%)	3 (6.5%)	0	

Abbreviations: aPL = antiphospholipid antibodies, LA = lupus anticoagulants, aCL = anticardiolipin antibodies, anti-β2-GPI = anti-beta2-glycoprotein I antibodies.
* pregnancy morbidity was defined according to revised Sapporo Classification Criteria for Antiphospholipid syndrome [6].
$^{\Delta}$ APS was diagnosed according to the revised Sapporo Classification Criteria [6].

positive for only LA; 23 (23.2%) were positive for only aCL antibodies and three (3.0%) were positive only for anti-β2-GPI antibodies. Seven of the 31 (22.6%) patients with single aPL positivity were persistently positive for aPL antibodies and 13 of the 15 (86.7%) patients with multiple aPL positivity were persistently positive for aPL antibodies (not shown in Table).

Table 2 shows the relationship between individual

Table 2. Relationship between aPL testing and thrombosis/pregnancy morbidity in 99 patients with SLE checked for three aPL (n = 99)

	Arterial/venous thrombosis				Pregnancy morbidity*			
	Yes (n = 26)	No (n = 73)	OR (95% CI)	*p* value	Yes (n = 2)	No (n = 97)	OR (95% CI)	*p* value
anti-β2-GPI IgM+ (n = 6)	4	2	6.46 (1.11–37.65)	0.040	1	5	18.40 (0.99–339.21)	0.118
anti-β2-GPI IgG+ (n = 10)	5	5	3.24 (0.85–12.28)	0.122	1	9	9.78 (0.56–169.95)	0.193
aCL IgM+ (n = 26)	7	19	1.05 (0.38–2.88)	0.929	1	25	2.88 (0.17–47.48)	0.458
aCL IgG+ (n = 27)	8	19	1.26 (0.47–3.38)	0.641	1	26	2.73 (0.17–45.27)	0.473
LA+ (n = 18)	7	11	2.08 (0.71–6.10)	0.236	1	17	1.06 (0.95–1.18)	0.182

Abbreviations: OR = odds ratio; CI = confidence interval; aPL = antiphospholipid antibodies, LA = lupus anticoagulants, aCL = anticardiolipin antibodies, anti-β2-GPI = anti-beta2-glycoprotein I antibodies.

Table S1. Risk factors for arterial/venous thrombosis in 99 patients with SLE checked for three aPL (n = 99)

Characteristic	Arterial/venous thrombosis		Univariate analysis		Multivariate analysis	
	Yes (n = 26)	No (n = 73)	OR (95% CI)	*p* value	Adjusted OR$^{\Delta}$ (95% CI)	*p* value
Age (> 60 year old) (n = 9)	2	7	0.79 (0.15–4.05)	1.000	0.38 (0.06–2.57)	0.318
Male gender (n = 12)	3	9	0.93 (0.23–3.73)	1.000	0.39 (0.07–2.18)	0.285
Hypertension (n = 32)	13	19	2.84 (1.12–7.20)	0.025	3.86 (1.23–12.12)	0.020
Diabetes mellitus (n = 10)	4	6	2.03 (0.52–7.86)	0.448	-	-
Smoking habit (n = 17)	9	8	4.30 (1.44–12.82)	0.013	5.44 (1.42–20.85)	0.013
aPL positive (n = 46)	13	33	1.21 (0.50–2.97)	0.674	-	-
anti-β2-GPI IgM+ (n = 6)	4	2	6.46 (1.11–37.65)	0.040	4.33 (0.63–29.57)	0.135
anti-β2-GPI IgG+ (n = 10)	5	5	3.24 (0.85–12.28)	0.122	-	-
aCL IgM+ (n = 26)	7	19	1.05 (0.38–2.88)	0.929	-	-
aCL IgG+ (n = 27)	8	19	1.26 (0.47–3.38)	0.641	-	-
LA+ (n = 18)	7	11	2.08 (0.71–6.10)	0.236	-	-
multiple aPL positivity (n = 15)	8	7	4.19 (1.34–13.11)	0.021	5.72 (1.59–20.60)	0.008
single aPL positivity (n = 31)	5	26	0.43 (0.15–1.28)	0.122	-	-

Abbreviations: aPL = antiphospholipid antibodies, LA = lupus anticoagulants, aCL = anticardiolipin antibodies, anti-β2-GPI = anti-beta2-glycoprotein I antibodies

Δ Adjusted for age, gender, smoking habit, and hypertension

aPL positivity and thrombosis/pregnancy morbidity in the study population. Among patients who tested positive for anti-β2-GPI antibodies, anti-β2-GPI IgM was significantly associated with arterial/venous thrombosis [odds ratio (OR) 6.46, 95% confidence interval (CI) 1.11–37.65, P = 0.040] in univariate analysis; however, the association was no longer significant after adjusting for age, gender, smoking habit, and hypertension in the multivariate analysis (adjusted OR 4.33, 95% CI 0.63–29.57, P = 0.135), as shown in Table S1. Arterial/venous

Table 3. Distribution of aPL antibodies and relationship between aPL profile and thrombosis/pregnancy morbidity in 99 SLE patients checked for three aPL (n = 99)

aPL profile	Arterial/venous thrombosis				Pregnancy morbidity			
	Yes (n = 26)	No (n = 73)	OR (95% CI)	*p* value	Yes (n = 2)	No (n = 97)	OR (95% CI)	*p* value
Multiple aPL positivity (n = 15)	8	7	4.19 (1.34–13.11)	0.021	1	14	5.93 (0.35–100.37)	0.281
Triple positivity (n = 7)	4	3	4.24 (0.88–20.43)	0.075	1	6	15.17 (0.84–273.53)	0.137
Double positivity (n = 8)	4	4	3.14 (0.72–13.60)	0.201	0	8	0.98 (0.95–1.01)	1.000
(LA+, aCL+, anti-β2-GPI-)	1	3	0.93 (0.09–9.39)	1.000	0	4	0.98 (0.95–1.01)	1.000
(LA+, aCL-, anti-β2-GPI+)	1	0	0.26 (0.18–0.36)	0.263	0	1	0.98 (0.95–1.01)	1.000
(LA-, aCL+, anti-β2-GPI+) *	2	1	6.00 (0.52–69.15)	0.168	0	3	0.98 (0.95–1.01)	1.000
Single aPL positivity (n = 31)	5	26	0.43 (0.15–1.28)	0.122	0	31	0.97 (0.93–1.01)	1.000
LA+ (n = 5)	1	4	0.69 (0.07–6.47)	1.000	0	5	0.98 (0.96–1.01)	1.000
aCL+ (n = 23)	3	20	0.35 (0.09–1.28)	0.100	0	23	0.97 (0.94–1.01)	1.000
anti-β2-GPI+ (n = 3)	1	2	1.42 (0.12–16.35)	1.000	0	3	0.98 (0.95–1.01)	1.000
Negative aPL (n = 53)	13	40	0.82 (0.34–2.02)	0.674	1	52	0.87 (0.05–14.24)	1.000

Abbreviations: aPL = antiphospholipid antibodies, LA = lupus anticoagulants, aCL = anticardiolipin antibodies, anti-β2-GPI = anti-beta2-glycoprotein I antibodies

*Negative results for LA testing with double positivity of aCL and anti-β2-GPI were classified as high risk profile according to EULAR recommendations [29]. In comparison, negative results for LA test with moderate-to-high titer of aCL or anti-β2-GPI IgG or IgM were classified as moderate risk profile by another author [30].

Table S2. Outcome parameters between multiple aPL positivity and aPL-negative patients checked for three aPL (n = 68)

	Multiple aPL positivity (n = 15)	Negative aPL (n = 53)	OR (95% CI)	*p* value
Arterial/venous thrombosis	53.5% (8/7)	24.5% (13/40)	3.52 (1.07-11.58)	0.055
Pregnancy morbidity	6.7% (1/14)	1.9% (1/52)	3.71 (0.22-63.19)	0.395

Table S3. Outcome parameters between single aPL positivity and aPL negative patients checked for three aPL (n = 84)

	Single aPL positivity (n = 31)	Negative aPL (n = 53)	OR (95% CI)	*p* value
Arterial/venous thrombosis	16.1% (5/26)	24.5% (13/40)	0.59 (0.19-1.86)	0.365
Pregnancy morbidity	0.0% (0/31)	1.9% (1/52)	0.98 (0.95-1.02)	1.000

thrombosis showed no significant association with anti-β2-GPI IgG (OR 3.24, 95% CI 0.85–12.28, P = 0.122), aCL IgM (OR 1.05, 95% CI 0.38–2.88, P = 0.929), aCL IgG (OR 1.26, 95% CI 0.47–3.28, P = 0.641), or LA (OR 2.08, 95% CI 0.71–6.10, P = 0.236). Moreover, pregnancy morbidity showed no significant association with any aPL positivity including anti-β2-GPI IgM (OR 18.40, 95% CI 0.99–339.21, P = 0.118), anti-β2-GPI IgG (OR 9.78, 95% CI 0.56–169.95, P = 0.193), aCL IgM (OR 2.88, 95% CI 0.17–47.48, P = 0.458), aCL IgM (OR 2.73, 95% CI 0.17–45.27, P = 0.473), or LA (OR 1.06, 95% CI 0.95–1.18, P = 0.182).

Table 3 shows the distribution of aPL antibodies and the relationship between aPL profile and thrombosis/pregnancy morbidity in 99 SLE patients. Multiple aPL positivity showed a significant association with venous/arterial thrombosis (OR 4.19, 95% CI 1.34–13.11, P = 0.021). Triple positivity (OR 4.24, 95% CI 0.88–20.43, P = 0.075) and double positivity (OR 3.14, 95% CI 0.72–13.60, P = 0.201) show no significant association with increased vascular thrombotic risk. On multivariate analysis, as shown in Table S1, multiple aPL positivity was an independent risk factor for venous/arterial thrombosis after adjusting for age, gender, smoking habit, and hypertension (adjusted OR 5.72. 95% CI 1.59–20.60, P = 0.008). There was no significant association of single aPL positivity or aPL-negative group with arterial/venous thrombosis. Moreover, pregnancy morbidity showed no significant association with multiple aPL positivity, single aPL positivity, or aPL-negative group.

The OR of arterial/venous thrombosis or pregnancy morbidities between patients with different aPL profile and aPL-negative patients with SLE were shown in Table S2, S3, and S4. When the patients with multiple aPL positivity (n = 15) were compared with the aPL negative patients (n = 53) as shown in Table S2, multiple aPL positivity shows nearly significant association with arterial/venous thrombosis (OR 3.52, 95% CI 1.07–11.58, P = 0.055), but no significant association with increased pregnancy morbidity risk (OR 3.71, 95% CI 0.22–63.19, P = 0.395). When the patients with single aPL positivity (n = 31) were compared with the aPL negative patients (n = 53) as shown in Table S3, single aPL positivity shows no significant association with arterial/venous thrombosis (OR 0.59, 95% CI 0.19–1.86, P = 0.365) nor significant association with increased pregnancy morbidity risk (OR 0.98, 95% CI 0.95–1.02, P = 1.000).When the patients with multiple aPL positivity (n = 15) were compared with the patients with single aPL positivity (n = 31), multiple aPL positivity shows significant association with arterial/venous thrombosis (OR 5.94, 95% CI 1.47–23.97, P = 0.014), but no significant association with increased pregnancy morbidity risk (OR 1.07, 95% CI 0.94–1.22, P = 0.326).

Discussion

Antiphospholipid antibodies (aPL) are a heterogeneous group of autoantibodies that are found in 1%–5% of the general population [1]. In previous studies, 30%–55% of SLE patients were found positive for aPL [1, 10-15];

Table S4. Outcome parameters between multiple aPL positivity and single aPL positivity patients checked for three aPL (n = 46)

	Multiple aPL positivity (n = 15)	Single aPL positivity (n = 31)	OR (95% CI)	*p* value
Arterial/venous thrombosis	53.3% (8/7)	16.1% (5/26)	5.94 (1.47-23.97)	0.014
Pregnancy morbidity	6.7% (1/14)	0.0% (0/31)	1.07 (0.94-1.22)	0.326

on individual investigation of each aPL, the prevalence range of positive LA test, aCL, and anti-β2-GPI antibodies was 11.0%–31.1%, 17.0%–55.3%, and 5.7%–44.5%, respectively [1, 10-15]. Some studies have found that the combination of SLE and aPL is worrisome, since aPL positivity has been shown to increase the risk of thrombosis in patients with lupus [8, 10, 19, 21, 23, 25]. In the present study, we evaluated the frequencies as well as isotype distribution of LA, aCL, and anti-β2-GPI; the most prevalent immunological features [aCL IgG, 27.3%; aCL IgM, 26.3%; LA, 18.2%; anti-β2-GPI IgG, 10.1%; anti-β2-GPI IgM, 6.1%] were similar to those found in previous studies [1, 10-15]. The aim of this study was to determine the impact of aPL profile on thrombosis and pregnancy morbidity in SLE patients.

Patients with SLE have a higher than expected incidence of vascular events and thrombotic risk, which is not completely explained by the traditional vascular risk factors [33, 34]. In the study by Urowitz, et al., the prevalence of vascular events (arterial/venous thrombosis) in SLE patients was 10%–30% [10, 35], which is similar to that in the present study (26.3%). On the contrary, the prevalence of aPL-positive patients with SLE who developed arterial/venous thrombosis was lower than the study by Love, et al., which reported that approximately 40% of aPL-positive patients with SLE developed arterial/venous thrombosis in comparison with 10%–20% of aPL-negative patients with SLE ($p<0.001$) [10, 20]; which may be due to different population study or different disease activity. There is no clear consensus on the association between individual aPL positivity and arterial/venous thrombotic events in SLE patients. According to a meta-analysis by Wahl et al. [26], patients with SLE and LA are at approximately six times (OR 5.6, 95% CI 3.8–8.2) greater risk of venous thromboembolism, while patients with SLE and aCL are at approximately two times (OR 2.2, 95% CI 1.5–3.1) greater risk of venous events. However, the systemic reviews by Galli et al. found LA as the aPL most strongly related to thrombosis [4, 36]; however, they found no association between thrombosis and aCL. Similarly, in a previous study, presence of aCL alone was not associated with thrombosis even in patients with medium-to-high titers (aCL > 40 GPL or MPL units) [37]. In prior studies, isolated anti-β2-GPI showed a weak association with clinical manifestations of APS [36, 38]. However, other studies have found no association of isolated LA or anti-β2-GPI antibodies with elevated thrombotic risk [37, 39, 40]. In the present study, only anti-β2-GPI IgM showed a significant association with arterial/venous thrombosis in univariate analysis. However, the association was not statistically significant after adjusting for age, gender, smoking habit, and hypertension in multivariate analysis. Neither LA, aCL IgG, nor aCL IgM showed a significant association with arterial/venous thrombosis; this lack of association was likely attributable to the relatively small sample size. On the other hand, combined aPL positivity has been shown to be associated with an increased risk of thrombosis [5, 25, 37]. Notably, in previous retrospective and prospective studies, patients with three positive aPL tests were shown to be at an increased risk of thrombosis or pregnancy morbidity (odds ratios for thrombosis: 5–33) [5, 37, 41, 42]. In the present study, multiple aPL positivity was significantly associated with venous/arterial thrombosis. However, triple positivity and double positivity showed no significant association with increased vascular thrombotic risk despite the fact that triple positivity was nearly significant, probably due to the relatively small sample size. Single aPL positivity showed no significant association with increased vascular thrombotic risk, which is generally consistent with the results of previous studies [5, 37, 39, 40]. Notably, 7 of the 31 (22.6%) patients with single aPL positivity were persistently positive for aPL antibodies and 13 of the 15 (86.7%) patients with multiple aPL positivity were persistently positive for aPL antibodies in the present study, which is generally consistent with the prior studies [8, 43] that individuals with multiple positive aPL tend to have more stable antibody levels on repeated determinations.

In previous studies, the reported frequency of pregnancy morbidity in SLE patients with and without aPL was 25%–47% and 0%–38%, respectively [16, 22]; these figures are much higher than those found in the present study (2.1% and 1.8%, respectively). Evidence pertaining to the role of different single aPL positivity has been inconsistent; some studies have suggested LA [44-46], while others have suggested anti-β2-GPI antibodies [47-49] as the more relevant single risk factor for pregnancy outcome. In the NOH-APS study of almost 1,600 non-thrombotic women with obstetric APS (pregnancy morbidity only), triple positivity was not associated with increased risk [50]. In the present study, we found no significant association of multiple aPL positivity or single aPL positivity with pregnancy morbidity; this was largely attributable to the small sample size ($n = 2$). In the present study, one patient with multiple aPL positivity (triple positivity) experienced pregnancy morbidity (premature birth before the 34th week of gestation due to placental insufficiency) while another aPL-negative patient experienced recurrent

incomplete abortion.

Some limitations of our study should be acknowledged. This was a retrospective study with a relatively small event numbers since only patients with aPL profile analysis were included. Simultaneous tests for aPL such as LA assay, aCL IgM/IgG, and anti-β2-GPI IgM/IgG were rarely performed as part of routine clinical investigation for SLE patients during the study reference period. Furthermore, aPL-positive patients presenting with thrombosis usually have one or several additional acquired factors for thrombosis such as hypertension, smoking, hypercholesterolemia, or estrogen use. However, some factors, such as hypercholesterolemia, or estrogen use, were not included in the analysis. Nevertheless, the present study supports the concept of risk stratification of Taiwanese lupus patients based on the aPL profile. Multiple aPL positivity was associated with approximately 5.7 times greater risk of venous or artery thromboembolism; however, single aPL positivity showed no significant association with the risk of thrombotic events. In conclusion, we suggest routine aPL profile analysis to better stratify the thrombotic risk of Taiwanese SLE patients.

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抗磷脂抗體特徵對於台灣紅斑性狼瘡患者的臨床影響

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目的：本研究目的為評估抗磷脂抗體特徵對台灣紅斑性狼瘡患者的臨床影響。

方法：回顧性研究單一醫學中心99位紅斑性狼瘡患者的抗磷脂抗體特徵。將99位紅斑性狼瘡患者分成多重抗磷脂抗體陽性、單一抗磷脂抗體陽性、抗磷脂抗體陰性等族群，使用卡方檢定與多元羅吉斯回歸模型評估抗磷脂抗體特徵與動靜脈血栓事件、懷孕併發症的相關性。

結果：99位紅斑性狼瘡患者可區分為抗磷脂抗體陽性（n = 46）、抗磷脂抗體陰性（n = 53），其中抗磷脂抗體陽性者可再區分為多重抗磷脂抗體陽性（n = 15）、單一抗磷脂抗體陽性（n = 31）。卡方檢定顯示紅斑性狼瘡患者中具有多重抗磷脂抗體陽性者與動靜脈血栓顯著相關（OR 4.19, 95% CI 1.34-13.11, P = 0.021）。納入年齡、性別、抽煙、高血壓等因素進行羅吉斯迴歸分析亦顯示多重抗磷脂抗體陽性與紅斑性狼瘡患者的動靜脈血栓事件顯著相關（OR 5.72. 95% CI 1.59-20.60, P = 0.008）。而單一抗磷脂抗體陽性則並未顯示與紅斑性狼瘡患者的動靜脈血栓事件或懷孕併發症顯著相關。

結論：多重抗磷脂抗體陽性與台灣紅斑性狼瘡患者的動靜脈血栓事件顯著相關。

關鍵詞：紅斑性狼瘡、抗磷脂抗體、血栓、懷孕併發症

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Original Article

Polymorphisms of rs2231142 in the ABCG2 gene in the Taiwanese gout population

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Objectives: Genome-wide association studies in Caucasian populations have identified multiple gene loci associated with gout, including the single nucleotide polymorphism (SNP) rs2231142 in ABCG2. However, this association is not clear in the Taiwanese population.
Methods: A case-control study was performed to investigate the association between the ABCG2 rs2231142 polymorphism and gout in the Taiwanese population.
Results: A total of 178 study participants including 78 gout patients and 100 age- and sex-matched control subjects were included in this study. Compared with the controls, gout patients exhibited a higher frequency of the AA genotype (35.9% cases vs. 9.0% controls) and the A allele (61.5% cases vs. 29.5% controls) at SNP rs2231142. The odds ratios for the AA genotype and the CA genotype (compared to the CC genotype) were significant: 15.56 for the AA genotype (95% confidence interval (CI): 5.65–42.81, $p < 0.001$) and 4.88 for the CA genotype (95% CI: 2.18–10.93, $p < 0.001$). The odds ratio (OR) for the A allele (compared to the C allele) was also significant at 3.82 (95% CI: 2.46–5.96, $p < 0.001$).
Conclusion: The A allele of rs2231142 was associated with an increased risk of gout. The SNP rs2231142, particularly the AA genotype, is associated with increased susceptibility to gout in the Taiwanese population.

Key words: gout, rs2231142, SNP, polymorphism, Chinese, Taiwanese

Introduction

Hyperuricemia, defined as a serum urate level over 6.8 mg/dL (405 μM), predisposes individuals to gout, which clinically manifests as acute gouty arthritis attacks, chronic tophaceous gout, uric acid urolithiasis, and monosodium urate gouty nephropathy [1-3]. Global studies have found an increase in mean serum urate levels in both sexes over the past four decades [1-5]. Gout has been referred to as the king of diseases and the disease of kings. Gout is an increasingly common rheumatic disease and affects more than eight million Americans [4]. Gout affects approximately 1-2% of men in Japan, 2.5% of the UK adult population, 3.9% of American adults, 6.4% of the New Zealand Maori people, 9.7% of men and 2.9% of women in the Australian Aboriginal population, and 4-6% of the Taiwanese population [4, 5].

In terms of pathogenesis, gout is a disease of purine metabolism or renal excretion of uric acid. Several molecules are associated with hyperuricemia and gout in various populations [7-18]. Genome-wide association studies in Caucasian populations have identified multiple genetic loci associated with gout and hyperuricemia, including the single nucleotide polymorphism (SNP) rs2231142, which causes an amino acid change from glutamine to lysine at position 141 (Q141K) of the membrane transporter gene *ABCG2* [8]. This loss-of-function mutation in ABCG2, the gene encoding ATP-binding cassette, subfamily G, member 2, is associated

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with serum uric acid levels and gout in Asians, Europeans, African Americans, and Americans [7-18]; however, individual genetic association studies examining the relationship between ABCG2 polymorphisms and gout have yielded inconsistent results. For example, association of the minor allele of rs2231142 with gout was observed in New Zealand Pacific Islander samples but not in New Zealand Maori samples [13, 14, 44]. Moreover, less is known about this association in the Taiwanese population. Furthermore, the severity of gout in Taiwan is increasing, and the age of onset of gout is now much earlier than that found in previous studies [4, 6]. Current evidence indicates that Asian Pacific Polynesia was populated over a 5,000-year period by populations migrating from Taiwan [19, 20, 44]. Given the very high prevalence and early onset of gout in Taiwanese people [4, 6], we analyzed whether the rs2231142 (Q141K) polymorphism in *ABCG2* confers a strong risk for gout in case–control samples drawn from the Taiwanese population. We also review the association between *ABCG2* 2231142 (Q141K) and the risk of gout in the present study population and different races/ethnicities.

Patients and Methods

All enrolled patients were examined at the Chang Gung Memorial Hospital (CGMH) rheumatology outpatient clinic from February 2013 to September 2014. We conducted a matched case-control study that included 178 subjects (78 patients with gout as the case group and 100 age- and sex-matched subjects as the control group). This study was approved by the Institutional Review Board of Chang Gung Memorial Hospital (CGMH IRB 101-4659A3). Written informed consent was obtained before conducting this study in all cases. The diagnosis of gout was based on the 1977 American College of Rheumatology diagnostic criteria [21]. Information regarding the medical histories, conditions, and family histories of the subjects was obtained from a medical interview of each subject at the time of enrollment. Clinical parameters were recorded, and blood biochemistry tests were conducted for both groups.

All blood specimens were sent to the clinical laboratory at CGMH, which is certified by the College of American Pathologists. External quality control for laboratory data was provided by participation in the international program of the College of American Pathologists and the National Quality Control Program conducted by the Taiwanese government. Serum creatinine and uric acid levels were measured using a Clinical Analyzer 7600 system (Hitachi High-Technologies, Tokyo, Japan). Genomic DNA was isolated from the peripheral blood lymphocytes of each patient using a QIAamp DNA Blood Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The rs2231142 (Q141K) polymorphism in *ABCG2* was genotyped using TaqMan SNP Genotyping Assays with the ViiA 7 Real-Time PCR System (Applied Biosystems). To confirm the genotyping results, PCR-amplified DNA samples were selected and examined via DNA sequencing. Amplicons were gel purified using a QIAquick gel purification kit (Qiagen, Valencia, CA, USA). DNA sequencing analysis was performed on an ABI PRISM 3130 genetic analyzer (Applied Biosystems, Foster City, CA, USA).

Categorical variables are expressed as percentages and were analyzed by a chi-square test or Fisher's exact test, as appropriate. Continuous variables are expressed as the mean ± SD. Genotype and haplotype analyses were utilized to determine the disease odds ratios (ORs). Multivariate logistic regression analysis was also used to analyze the data. All statistical analyses were performed using SPSS software version 17.0. (SPSS Inc., Chicago, IL, USA). P-values < 0.05 were considered statistically significant.

Results

A total of 78 gout patients and 100 gout-free normal controls were recruited from Chang Gung Memorial Hospital. The clinical features of the individuals enrolled in the study are summarized in Table 1. The average age of the patients with gout was 53.2 ± 13.2 years (range: 20–78); In total, 73 (93.6%) of the patients were men,

Table 1. Clinical and biochemical profiles of gout patients and gout-free controls.

	Gout patients (n = 78)	Controls (n = 100)	p-value
Male, n (%)	73 (93.6%)	86 (86.0%)	0.104
Age (years)	53.2 ± 13.2 (range, 20–78)	51.2 ± 12.5 (range, 27–81)	0.305
Uric acid (mg/dL)	9.3 ± 1.7	5.3 ± 1.1	<0.001
Creatinine (mg/dL)	1.06 ± 0.26	0.81 ± 0.17	<0.001

Table 2. Genotype and allele frequencies for rs2231142 in gout patients (n=78) and controls (n=100).

Group	Samples (n)	Genotype frequency			Allele frequency	
		A/A	C/A	C/C	A	C
Gout	78	28 (35.9%)	40 (51.3%)	10 (12.8%)	96 (61.5%)	60 (38.5%)
Control	100	9 (9.0%)	41 (41.0%)	50 (50.0%)	59 (29.5%)	141 (70.5%)
		OR (A/A: C/C) = 15.56 (5.65–42.81)*			OR: 3.82 (2.46–5.96)*	
		OR (C/A: C/C) = 4.88 (2.18–10.93)*				

For alleles of rs2231142 (C for cytosine; A for adenine), allele A is the minor allele; OR: odds ratio; * $p < 0.001$

Table 3. Genotype and allele frequencies for rs2231142 in gout patients and controls, stratified by gender.

		Samples (n)	Genotype frequency			Allele frequency	
			A/A	C/A	C/C	A	C
Male	Case	73	25 (34.2%)	38 (52.1%)	10 (13.7%)	88 (60.3%)	58 (39.7%)
	Control	86	9 (10.5%)	36 (41.9%)	41 (47.7%)	54 (31.4%)	118 (68.6%)
Female	Case	5	3	2	0	8	2
	Control	14	0	5	9	5	23
			Males only:				
			OR (A/A: C/C) = 11.39 (4.07–31.86)*				
			OR (C/A: C/C) = 4.33 (1.89–9.91)*				

For rs2231142, allele A is the minor allele; OR: odds ratio; * $p < 0.001$

and 5 (6.4%) were women. Patients with gout and the control patients did not differ statistically in mean age or gender (mean age: 53.2 ± 13.2 cases vs. 51.2 ± 12.5 controls, $p = 0.305$; gender: 93.6% male cases vs. 86.0% male controls, $p = 0.104$) (Table 1). As expected, the gout patients had much higher serum uric acid levels than the controls (9.3 ± 1.7 mg/dL cases vs. 5.3 ± 1.1 mg/dL controls, $p < 0.001$). Gout patients also had higher serum creatinine levels than controls (1.06 ± 0.26 mg/dL cases vs. 0.81 ± 0.17 mg/dL controls, $p < 0.001$).

Compared with the controls, gout patients exhibited a higher frequency of the AA genotype (35.9% cases vs. 9.0% controls) and the A allele (61.5% cases vs. 29.5% controls) at SNP rs2231142 (Table 2). The A allele (compared to the C allele) was significant associated with an increased risk of gout (odds ratio 3.82, 95% CI: 2.46–5.96, $p < 0.001$). The ORs for the AA genotype and the CA genotype (compared to the baseline of the CC genotype) were significant: 15.56 for the AA genotype (95% CI: 5.65–42.81, $p < 0.001$) and 4.88 for the CA genotype (95% CI: 2.18–10.93, $p < 0.001$).

In addition, we tested whether the association between gout susceptibility and genotype at rs2231142 would remain significant after stratifying by gender. The frequency of the A allele appears to be higher in males with gout than in male controls (60.3% cases vs. 31.4% controls; OR: 3.32, 95% CI: 2.09–5.26, $p < 0.001$) (Table 3). Compared to a baseline of the C allele, the OR in males for the A allele was 3.32 (95% CI: 2.09–5.26, $p < 0.001$). The ORs for the AA genotype and CA genotype were 11.39 (95% CI: 4.07–31.86, $p < 0.001$) and 4.33 (95% CI: 1.89–9.91, $p < 0.001$), respectively (Table 3). We also presented the rs2231142 genotypes of gout patients stratified by gender and age of onset of gout (Table 4). Further subgroup analyses of female cases and those with early onset of gout (<30 years old) were not performed given the small sample size of the study population.

Table 4. Genotype and allele frequencies at rs2231142 in gout patients and controls, stratified by gender and age of onset.

		Samples (n)	Genotype frequency			Allele frequency	
			A/A	C/A	C/C	A	C
Gender	Male	73	25	38	10	88 (60.3%)	58 (39.7%)
	Female	5	3	2	0	8 (80.0%)	2 (20.0%)
Age of onset (year)	≤ 30	26	9	16	1	34 (65.4%)	18 (34.6%)
	> 30	52	19	24	9	62 (59.6%)	42 (40.4%)

Discussion

Gout is a disorder of purine metabolism or the renal excretion of uric acid, which is the final product of endogenous and dietary purine metabolism in humans. Gout is a common metabolic disorder with high heritability [4, 6, 22-27]. The prevalence of gout is highest in the New Zealand Maori people and the aboriginal people of Taiwan [4]. The prevalence of gout in the Taiwanese population has increased markedly in recent decades [4]. Recent genome-wide association studies (GWASs) of both serum uric acid and gout identified several transporter genes, such as *ABCG2*, the adenosine triphosphate (ATP)-binding cassette (ABC), subfamily G, member 2 gene. *ABCG2* is located in a gout-susceptibility locus (MIM 138900) on chromosome 4q. The rs2231142 polymorphism in *ABCG2* represents a missense mutation that leads to a glutamine-to-lysine amino acid substitution (Q141K) in exon 5 [9]. Genetic variants of *ABCG2* influence serum uric acid levels and to participate in the pathogenesis of gouty arthritis [7-18, 28-34, 44]. Recent functional studies have shown that *ABCG2* codes for a urate transporter [35-37]. Associations between the causal *ABCG2* variant (rs2231142) and both uric acid levels and gout were confirmed in samples of Caucasian [7, 8, 12, 18, 28-31, 44], Japanese [15, 16, 32-33], Han Chinese [9, 10, 17], African American [7], American [7, 12], and New Zealand Pacific Islander populations [44]. However, results of another study showed no association between the rs2231142 SNP and gout risk in the New Zealand Maori population [44]. In the present study, we detected a higher frequency of the AA genotype and the A allele at rs2231142 in Taiwanese gout patients than in controls (35.9% vs. 9.0% by genotype; 61.5% vs. 29.5% by allele). The A allele at rs2231142 is associated with increased gout susceptibility in the Taiwanese population. Moreover, we showed that the risk of developing gout was much greater for individuals with the AA genotype (OR 15.56) than for those with the CA genotype (OR 4.88).

Current evidence indicates that New Zealand Polynesia was populated over a 5,000-year period from Taiwan [19, 20,44]. The prevalence and incidence of hyperuricemia and gout are extremely high in both the New Zealand Maori and Taiwanese aboriginal populations [4]. The present study results however differ from those of a study of the New Zealand Maori population, which showed no association between the rs2231142 SNP and gout risk [44]. Because the Pacific Austronesian population, including the Taiwanese aboriginal population, has a remarkably high prevalence of gout and hyperuricemia, it is possible that founder effects have occurred across the Pacific region [26]. In population genetics, the founder effect is the loss of genetic variation that occurs when a new population is established by a very small number of individuals from a larger population. As a result of the loss of genetic variation, the new population may be distinctively different, both genotypically and phenotypically, from the parent population from which it is derived.

Previous studies have employed various genetic models to analyze the association between the rs2231142 SNP in *ABCG2* and gout risk, with varying or conflicting results [13, 14, 44], especially with regard to the role that sex plays in the association [10, 12, 14, 28]. Studies have shown that the associations between rs2231142 and uric acid and gout are stronger in males than females, suggesting that sex modifies this association [7, 10, 12, 14, 28]; however, the evidence has been inconsistent [12, 31,44]. For example, in Phipps-Green's study [44], no difference in the strength of the association between rs2231142 and gout was found between male and female samples from Eastern Polynesian or Caucasian populations, whereas Zhang's study identified a significantly stronger association between rs2231142 and gout in males than in females in European American populations but no significant gender differences in African American, Mexican American, or American Indian populations [12]. In the present study, our data confirmed that males with the A allele at rs2231142 had a higher risk of gout than those with the C allele. Subgroup analyses showed that gender may have an impact on the association between the susceptibility of gout and the polymorphism. However, the limited sample size of female gout patients in the present study precluded further analysis. Moreover, in previous studies, the rs2231142 genotype influenced the age of onset of gout [16]. However, the sample size of the present study was insufficient for analyzing such an association.

In addition to gender and age of onset, the strength of the association between rs2231142 and gout has been found to vary with ethnicity [8, 12-14, 44]. For example, association of the minor allele of rs2231142 with gout was observed in Pacific Islander samples but not in Maori samples [44]. The common causal *ABCG2* variant rs2231142 leads to elevated uric acid levels and an increased prevalence of gout. More specifically, the A allele at rs2231142 is associated with an increased risk of gout [7,8,12-13,15-18,28-33]. However, the associations between rs2231142 and serum uric acid

Table 5. The association of the ABCG2 gene rs2231142 polymorphisms and gout risk in various studies

Study [ref]	Year	Ethnicity/ country	A allele (n)		C allele (n)		Risk allele frequency %		OR	95% CI	*p*
			Gout	Control	Gout	Control	Gout	Control			
Wang [42]	2014	Asians China	156	182	214	440	42.2%	29.3%	1.76	1.35–2.30	<0.001
Zhang [43]	2014	Asians China	155	174	139	468	52.7%	27.1%	3	2.25–4.00	<0.001
Li [41]	2011	Asians China	181	152	219	318	45.3%	32.3%	1.73	1.31–2.28	0.001
Amanda 1 [44]	2010	Maori New Zealand	38	41	318	383	10.7%	9.7%	1.12	0.70–1.78	0.64
Amanda 2 [44]	2010	Pacific Islander New Zealand	152	44	194	174	43.9%	20.2%	3.1	2.59–4.59	<0.001
Amanda 3 [44]	2010	Caucasian New Zealand	102	141	320	975	24.2%	12.6%	2.2	1.66–2.93	<0.001
Zhang [45]	2012	Asians China	95	136	125	336	43.2%	28.8%	1.88	1.35–2.62	0.002
You [46]	2013	Asians China	134	75	174	245	43.5%	23.4%	2.52	1.79–3.55	<0.001
Ye [47]	2012	Asians China	116	58	88	146	56.9%	28.4%	3.32	2.20–5.01	<0.001
Yamagishi [32]	2010	Japanese	42	2409	48	5347	46.7%	31.1%	1.94	1.28–2.95	0.002
Lee [48]	2019	Taiwan	698	8739	816	19563	46.1%	30.9%	1.89	1.70–2.10	<0.001
Chen [49]	2018	Taiwan	723	1152	771	2988	48.4%	27.8%	2.43	2.15–2.75	<0.001
Zheng [2]	2016	China	165	100	145	180	53.2%	35.7%	2.05	1.47–2.85	<0.001
Matsuo [33]	2009	Japanese	149	490	169	1240	46.9%	28.3%	2.23	1.75–2.85	<0.001
Stark [30]	2009	German	186	323	1168	2781	13.7%	10.4%	1.37	1.13–1.66	0.001
Present study	2014	Taiwan	96	59	60	141	61.5%	29.5%	3.82	2.46–5.96	<0.001

For rs2231142, allele A is the minor allele; OR: odds ratio

and gout have not been established in Maori populations [44]. We examined whether the association between rs2231142 and gout is observed in a Taiwanese population given that Asians have a high reported prevalence of the risk allele [38]. The rs2231142 variant is found with low frequency in individuals of African (1–3%), African American (2%–5%), European (11%–14%), Hispanic (10%), Middle Eastern (13%), or Eastern Polynesian descent (9%), but it is found at high frequency in individuals of Western Polynesian (29%), Japanese (31%-35%), or Chinese (35%) descent [17, 38]. In the present study, we found that the A allele frequencies at rs2231142 were 29.5% in Taiwanese controls and 61.5% in Taiwanese gout patients, which is similar to that previously reported in Han Chinese [10, 17, 39, 40] and Japanese [32] populations. Table 5 presents the association of the ABCG2 gene rs2231142 polymorphisms and gout risk in various studies [2,30,32,33,41-49].

The study has some limitations. The study results are limited by the small number of gout cases. Moreover, several epidemiologic studies have demonstrated that environmental factors (e.g., alcohol intake) and genetic predisposition (gender, ethnicity, etc) together contribute to elevated urate levels in gout [14,17]. We were unable to perform separate analyses for patients with different levels of disease severity (i.e., BMI, creatinine, serum uric acid level, alcohol consumption, and tophi), which has been reported in previous studies [12,14,17,32,34,43,49,50]. Another limitation is that the number of cases included in the subgroup analyses was relatively small. Nonetheless, our findings remain consistent with studies of other populations, highlighting their robustness. Future studies should incorporate a larger sample size to verify the present findings across more diverse populations.

In conclusion, the genetic variation at rs2231142 (Q141K) in *ABCG2*, encoding a uric acid transporter, is associated with gout in diverse populations. Our study emphasizes the significance of this common causal variant in the Taiwanese population. The present study showed the association between rs2231142 on 4q22 in *ABCG2* and gout in a Taiwanese population. The A allele and, in particular, the AA genotype were associated with increased susceptibility to gout in Taiwanese individuals.

Disclosures

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Conflict of interest statement

The authors report no conflicts of interest.

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台灣痛風人群中ABCG2基因rs2231142的多態性

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目的：在白種人族群中的全基因組關聯研究中確定了與痛風相關的多個基因位點，包括ABCG2中的單核苷酸多態性（SNP）rs2231142。但是，這種關聯在台灣人群中還不清楚。

方法：進行病例對照研究，以研究台灣人群ABCG2基因rs2231142多態性與痛風之間的關係。

結果：總共178名研究參與者包括78名痛風患者和100名年齡和性別相配的匹配對照受試者。與對照組相比，痛風患者在SNP rs2231142處表現出更高頻率的AA基因型（35.9%病例對9.0%對照）和A等位基因（61.5%病例對29.5%對照）。AA基因型和CA基因型的勝算比（與CC基因型相比）很明顯：AA基因型為15.56（95%CI：5.65-42.81，p <0.001），CA基因型為4.88（95%CI：2.18–10.93，p <0.001）。A等位基因（與C等位基因相比）的比值比（OR）也很明顯，為3.82（95%CI：2.46-5.96，p <0.001）。

結論：rs2231142的A等位基因與痛風的風險增加有關。SNP rs2231142（尤其是AA基因型）與台灣人群對痛風的敏感性增加有關。

關鍵詞：痛風，rs2231142，SNP，多態性，中文，台灣人

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Acknowledgments. Individuals or grants that have substantive contribution to this study may be acknowledged.

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Books:

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