JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
ORIGINAL ARTICLE

Association between Pro12Ala polymorphism and albuminuria in type 2 diabetic nephropathy

Yung-Nien Chen, Pei-Wen Wang, Shih-Chen Tung, Ming-Chun Kuo, Shao-Wen Weng, Chen-Kai Chou, Chih-Min Chang, Chia-Jen Tsa, Cheng-Feng Taso, Feng-Chih Shen, Jung-Fu Chen*

Division of Metabolism and Endocrinology, Department of Internal Medicine, Kaohsiung Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Kaohsiung, Taiwan

Keywords
Diabetic nephropathies, Peroxisome proliferator-activated receptor gamma, Polymorphism

***Correspondence**
Jung-Fu Chen
Tel.: +886-7-731-7123 ext. 8302
Fax: +886-7-732-2402
E-mail address:
rogershen1129@gmail.com

J Diabetes Investig 2020; 11: 923–929

doi: 10.1111/jdi.13208

ABSTRACT

Aims/Introduction: Diabetic nephropathy (DN) is a complication of diabetes mellitus that is characterized by the gradual loss of kidney function, which results in increased levels of albumin in the urine. The Pro12Ala polymorphism in the peroxisome proliferator-activated receptor-γ2 gene has been confirmed to improve insulin sensitivity, but its association with susceptibility to DN in patients with type 2 diabetes remains inconclusive.

Materials and Methods: To examine whether the Pro12Ala polymorphism leads to the development of DN, a case-control study was carried out in 554 patients with type 2 diabetes. The genotypes of Pro12Ala polymorphism of the peroxisome proliferator-activated receptor gamma 2 gene were analyzed by real-time polymerase chain reaction with TaqMan® probe genotyping assay in all patients.

Results: The mean age of the study population was 57.7 ± 8.8 years, with average diabetes duration of 12.8 ± 6.9 years. The prevalence of albuminuria was 43.5%. The frequency of genotype Pro12Pro, Pro12Ala and Ala12Ala genotype were 92.6%, 7.0%, 0.4% in our study population, and 90.4%, 8.9% and 0.7% in normal urinary albumin-to-creatinine ratio group, respectively. The *Ala* carriers (Pro12Ala + Ala12Ala) had significantly lower urinary albumin-to-creatinine ratio (15.0 vs 20.5 mg/g, $P = 0.001$) and better renal function (estimated glomerular filtration rate 81.8 [69.8–97.6] vs 78.7 mL/min/1.73 m^2 [61.6–96.2]; $P = 0.05$) compared with those with the genotype Pro12Pro. After adjustment for age, sex and other confounders, the odds ratio of albuminuria for the *Ala12* allele was 0.428 (95% confidence interval 0.195–0.940, $P = 0.034$]).

Conclusions: Our results suggest that the peroxisome proliferator-activated receptor gamma 2 *Ala12* variant has significant protective effects against albuminuria and DN.

INTRODUCTION

Diabetic nephropathy (DN) is a well-known microvascular complication of diabetes mellitus, and currently the primary cause of chronic kidney disease and end-stage renal disease (ESRD) globally[1]. DN is a syndrome characterized by the gradual loss of kidney function with pathological quantities of urine albumin excretion. For the timely intervention against DN, it is quite important to detect the increased urine albumin excretion as soon as possible. Epidemiological studies have shown that the prevalence rates of albuminuria range 19.5–49% among patients with type 2 diabetes mellitus[2–4]. Aging, sex, hypertension, hyperglycemia, abnormal lipid profile, smoking, insulin resistance and metabolic syndrome have been reported to be the risk factors for albuminuria[2]. In addition, increasing evidence shows that genetic factors play an important role in the development of DN[5].

Peroxisome proliferator-activated receptor gamma (*PPAR-γ*), a ligand-activated transcription factor, is a response for the regulation of numerous biological processes, such as cell proliferation, adipocyte cell differentiation and inflammation. Thiazolidinediones serve as *PPAR-γ* agonists, and improve blood sugar control in type 2 diabetes mellitus patients through the enhancement

Received 9 July 2019; revised 11 December 2019; accepted 2 January 2020

of insulin sensitivity[6]. Furthermore, *PPAR-γ* agonists ameliorate metabolic abnormalities of diabetes and consecutive DN[7]. The Pro12Ala polymorphism is the most frequent variation in the PPARγ gene and accounts for 4–14% of the population[8–14]. The Pro12Ala polymorphism is related to reducing the deoxyribonucleic acid (DNA) binding affinity and diminishing the transcriptional activity *in vitro*[15]. Furthermore, individuals who carried the *Ala* allele had significant elevation in insulin sensitivity[15], which might be a protective factor for DN[9,10,13,14,16–19].

Some studies supported that the *PPAR-γ*Pro12Ala polymorphism might contribute to reducing the risk of DN in type 2 diabetes mellitus patients[9,10,13,14,16–19], but some other studies suggested there was no significant association[8,20], thus leaving uncertainty about its role in diabetic renal disease. To further understand this issue, we carried out a case–control study with type 2 diabetes mellitus patients of ethnic Chinese backgrounds in southern Taiwan to test the association between *PPAR-γ*Pro12Ala polymorphism and albuminuria.

METHODS

Patients with type 2 diabetes mellitus (n = 581) followed up at least 1 year before enrollment were recruited from the outpatient clinic at Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan, between January 2017 and October 2017. After excluding 27 patients with anuria or oliguria due to ESRD under regular hemodialysis, a total of 554 participants were analyzed, and participants were divided into normoalbuminuria and albuminuria groups in October 2017 according to the following definition. Albuminuria was evaluated on the basis of the urine albumin-to-creatinine ratio (UACR) obtained from the initial voiding of urine in the morning. Normoalbuminuria (NA) was defined as a UACR <30 mg/g of the last two of three urine samples. Microalbuminuria (MA) was defined as an UACR of ≥30 and <300 mg/g, and macroalbuminuria (MAA) was defined as an UACR ≥300 mg/g in at least two urine samples in the past 3- to 6-month period[21]. Albuminuria was defined as the presence of MA or MAA. Diabetic retinopathy was evaluated through annual fundus photography by experienced diabetes doctors, and would be referred to ophthalmologists for abnormal findings.

All of the study participants received a standardized clinical and laboratory evaluation according to the standard clinical practice of our institution and recommendations from the Diabetes Association of Republic of China, Taiwan[22]. Information about alcohol use and smoking habits was obtained using questionnaires. Smoking habits are defined as positive for current smokers regardless of how much they smoked. Alcohol use was defined as positive for more than one drink per day for women and two drinks per day for men. Information regarding the use of antihypertensive medications, lipid-lowering agents, oral blood glucose-lowering agents and insulin treatments was collected from the electronic medical record systems at the hospital. Individuals were considered to have diabetes mellitus if they were taking diabetes medications or showed a hemoglobin A1c of ≥6.5% on repeated testing[23]. Hypertension was defined as either receiving antihypertensive medications or systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg[24]. The study protocol was approved by the Human Research Ethics Committee at our hospital, and all participants were given written-form informed consent.

Genotyping

Each participant's genomic DNA was extracted from leukocytes in peripheral blood samples. The *Pro12Ala* gene polymorphism was analyzed by real-time polymerase chain reaction (PCR). The single-nucleotide polymorphism (SNP) genotyping assays, also known as TaqMan assay, used to detect specific polymorphisms were purchased from Topgen Biotechnology (Kaohsiung City, Taiwan). SNP genotyping assays use TaqMan 5′-nuclease chemistry for amplifying and detecting specific polymorphisms in purified genomic DNA samples. All probes for quantitative PCR assays were 5′ labeled with FAM/VIC as a reporter and 3′ labeled with minor groove binder non fluorescent quencher as a quencher. A total of 20 ng of DNA was amplified using the TaqMan SNP assay. Preparation of quantitative PCR reaction followed the manufacturer's instructions (Topgen Biotechnology). The cycling parameters were as follows: (i) 5 min at 95°C; (ii) 30 s at 60°C; (iii) 40 cycles at 95°C for 3 s and then 60°C for 40 s; and (iv) 30 s at 60°C. Real-time PCR was carried out using 2X AceQ Probe High ROX qPCR Master Mix (Topgen Biotechnology) on StepOne Plus Real Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA). Allelic discrimination was called by StepOne software v2.3 (Applied Biosystems, Grand Island, NY, USA).

Statistical analysis

Patients' clinical and biochemical characteristics were presented as the mean ± standard deviation or percentages. The values of triglyceride, UACR, creatinine and estimated glomerular filtration rate (eGFR) were natural log-transformed due to the non-normal distribution. Data analysis was carried out using the statistical package for social sciences (SPSS) 25 software (IBM Corporation, Armonk, NY, USA). Statistical significance of the differences between the groups was determined by χ^2-tests for categorical variables, and unpaired Student's t-test for continuous variables. Binary logistic regression was used to describe the associations of variables with the presence of albuminuria controlling for potential confounders. In all statistical tests, $P < 0.05$ was considered statistically significant.

RESULTS

Clinical and laboratory characteristics of the study participants are shown in Table 1. The Pro12Pro genotype was significantly higher in the albuminuria group when compared with the group without albuminuria (95.4 vs 90.4%, P = 0.025). Compared with the patients without albuminuria, those patients with albuminuria have a significantly high percentage of hypertension (82.2% vs 56.2%, P = 0.001), poor renal function (eGFR 70.6 vs

Table 1 | Clinical and laboratory characteristics of study patients with and without albuminuria

Parameters	Normoalbuminuria	Albuminuria	P^*
Sample size (*n*)	313	241	–
Age (years)	60.8 ± 9.9	62.6 ± 9.5	0.033
Sex (female)	170 (54.3%)	96 (39.8%)	0.001
Genotype			
Pro/Pro	283 (90.4%)	230 (95.4%)	0.025
Pro/Ala + Ala/Ala	30 (9.6%)	11 (4.6%)	
Diabetes duration (years)	13.2 ± 7.5	14.3 ± 8.2	0.098
Hypertension (%)	176 (56.2%)	198 (82.2%)	0.001
SBP (mmHg)	137.5 ± 17.8	140.3 ± 20.3	0.085
DBP (mmHg)	77.9 ± 12.3	77.9 ± 11.5	0.945
Weight (kg)	70.3 ± 14.0	70.3 ± 13.6	0.983
BMI (kg/m^2)	26.8 ± 4.5	26.7 ± 4.0	0.701
Waist (cm)	90.0 ± 11.1	91.7 ± 9.9	0.063
Metabolic syndrome (%)	223 (71.2%)	194 (80.5%)	0.012
HbA1c (%)	7.4 ± 0.9	7.4 ± 1.1	0.284
UACR (mg/g)	9.4 (5.5–15.6)	105.7 (50.0–242.2)	0.001
Serum creatinine (mg/dL)	0.8 (0.7–1.0)	1.0 (0.8–1.3)	0.001
eGFR (mL/min/1.73 m^2)	86.1 (71.8–99.1)	70.6 (54.4–88.5)	0.001
Retinopathy (%)	61 (19.5%)	65 (27.0%)	0.037
Total cholesterol (mg/dL)	167.8 ± 29	168.2 ± 33.4	0.893
HDL (mg/dL)	48.5 ± 12.4	45.8 ± 13.7	0.017
LDL (mg/dL)	89.3 ± 21.9	90.3 ± 25.7	0.653
Triglyceride (mg/dL)	108.0 (75.0–156.0)	130.0 (86.3–188.0)	0.001
Smoking (%)	28 (8.9%)	26 (10.8%)	0.468
Alcohol consumption (%)	20 (6.4%)	15 (6.2%)	0.937
TZD (%)	59 (18.8%)	52 (21.6%)	0.427
Hypoglycemic treatment			
OADs (%)	263 (84.0%)	176 (73.0%)	0.007†
Insulin (%)	6 (1.9%)	6 (2.5%)	
Insulin + OADs (%)	42 (13.4%)	58 (24.1%)	
Diet control (%)	2 (0.6%)	1 (0.4%)	
Antihypertensive treatment			
ACEI/ARB (%)	139 (44.4%)	179 (74.3%)	0.001
Lipid-lowering drugs			
Statin (%)	228 (72.8%)	194 (80.5%)	0.036
Fibrate (%)	24 (7.7%)	23 (9.5%)	0.432

Data are expressed as the mean ± standard deviation, median (interquartile range) or *n* (%). ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor antagonist; BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OADs, oral antidiabetic drugs; SBP, systolic blood pressure; TZD, thiazolidinedione; UCAR, urine albumin-to-creatinine ratio. **P*-values were calculated by an unpaired Student's *t*-test or χ^2 analysis, or Fisher's exact test (†), sequentially.

86.1 mL/min/1.73 m^2, $P = 0.001$), higher triglyceride levels (130.0 vs 108.0 mg/dL, $P = 0.001$) and lower high-density lipoprotein cholesterol levels (45.8 vs 48.5 mg/dL, $P = 0.017$). In addition, more patients with albuminuria receive insulin injection, take angiotensin-converting enzyme inhibitor/angiotensin II receptor antagonist as antihypertensive treatment and use statin as lipid-lowering drugs.

As shown in Table 2, 513 (92.6%) patients were found to have the Pro12Pro (PP) genotype, 39 (7.0%) had the Pro12Ala (PA) genotype and two (0.4%) had the Ala12Ala (AA) genotype. The *Ala12* allele frequency was 4%. The Pro12Ala genotyping call rate was 100%. The distribution of Pro12Ala polymorphism in our study participants followed the Hardy–Weinberg equilibrium ($P = 0.184$). There was no significant difference in age, sex, bodyweight, diabetes duration, glycated hemoglobin A1c value, oral antidiabetic drugs, antihypertensive treatment and lipid-lowering drugs between individuals with and without the *Ala12* allele (Table 2). The Ala carriers (PA and AA) had significantly lower UACR (15.0 vs 20.5 mg/g, $P = 0.001$) and better renal function (eGFR 81.8 [69.8–97.6] vs 78.7 mL/min/1.73 m^2 [61.6–96.2], $P = 0.05$) compared with those with the Pro12Pro genotype. The PP genotype had clearly

Table 2 | Clinical and laboratory characteristics of patients classified according to peroxisome proliferator-activated receptor gamma Pro12Ala genotypes

Parameters	Pro/Pro (513)	Pro/Ala (39) + Ala/Ala (2)	*P**
Sample size (*n*)	513	41	–
Age (years)	61.6 ± 9.9	61.5 ± 7.8	0.984
Sex (female)	244 (47.6%)	22 (53.7%)	0.452
Diabetes duration (years)	13.6 ± 7.7	14.7 ± 8.7	0.374
Hypertension (%)	346 (67.4%)	28 (68.3%)	0.911
SBP (mmHg)	138.8 ± 18.9	137.3 ± 19.3	0.614
DBP (mmHg)	78.0 ± 12.0	76.0 ± 11.0	0.299
Weight (kg)	70.2 ± 13.9	71.3 ± 12.2	0.631
BMI (kg/m^2)	26.7 ± 4.3	27.3 ± 4.1	0.380
Waist (cm)	90.7 ± 10.6	91.4 ± 11.1	0.671
Metabolic syndrome (%)	388 (75.6%)	29 (70.7%)	0.484
HbA1c (%)	7.4 ± 1.0	7.4 ± 0.8	0.977
UACR (mg/g)	20.5 (8.5–87.4)	15.0 (4.7–54.9)	0.001
Serum creatinine (mg/dL)	0.9 (0.7–1.1)	0.8 (0.7–1.0)	0.011
eGFR (mL/min/1.73 m^2)	78.7 (61.6–96.2)	81.8 (69.8–97.6)	0.05
Retinopathy (%)	117 (22.8%)	9 (22.0%)	0.992
Total cholesterol (mg/dL)	168.3 ± 31.1	163.5 ± 30.0	0.342
HDL (mg/dL)	47.4 ± 13.4	45.7 ± 7.6	0.212
LDL (mg/dL)	90.0 ± 23.5	86.9 ± 25.4	0.427
Triglyceride (mg/dL)	117.0 (80.0–174.0)	109.0 (86.5–146.8)	0.404
Smoking (%)	53 (10.3%)	1 (2.4%)	0.165
Alcohol use (%)	33 (6.4%)	2 (4.9%)	1.0
TZD (%)	105 (20.5%)	6 (14.6%)	0.369
Hypoglycemic treatment			
OADs (%)	407 (79.3%)	32 (78.0%)	0.329†
Insulin (%)	11 (2.1%)	1 (2.4%)	
Insulin + OADs (%)	93 (18.1%)	7 (17.1%)	
Diet control (%)	2 (0.4%)	1 (2.4%)	
Antihypertensive treatment			
ACEI/ARB (%)	291 (56.7%)	27 (65.9%)	0.255
Lipid-lowering drugs			
Statin (%)	389 (75.8%)	33 (80.5%)	0.500
Fibrate (%)	46 (9.0%)	1 (2.4%)	0.239

Data are expressed as the mean ± standard deviation, median (interquartile range) or *n* (%). ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor antagonist; BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OADs, oral antidiabetic drugs; SBP, systolic blood pressure; TZD, thiazolidinedione; UCAR, urine albumin-to-creatinine ratio. **P*-values were calculated by an unpaired Student's *t*-test, chi-squared test mark analysis or Fisher's exact test (†), sequentially.

a higher percentage of DN (44.8% vs 26.8%, $P = 0.025$), as compared with PA and AA genotypes (Figure 1).

The distribution of *PPAR-γ* genotypes and their relationship with DN is shown in the Table 3. The frequency of genotype PP, PA and AA were 90.4%, 8.9% and 0.7% in the NA group; 94.7%, 5.3% and 0.0% in the MA group; and 98.0%, 2.0% and 0% in the MAA group, respectively. There is a linear-by-linear decrease in the association between ($P = 0.012$) NA, MA and MAA with *Ala* allele (Table 3), suggesting that the *Ala* allele might be a protective factor that shields an individual from the presence of albuminuria.

To clarify the contributions of the *PPARγ Pro12Ala* gene polymorphism to the risk of albuminuria, multivariate logistic regression analyses were carried out with the possible confounders. After adjustment for univariate parameters, including age, sex, genotype, diabetes duration, hypertension, systolic blood pressure, metabolic syndrome, eGFR, retinopathy, high-density lipoprotein, triglyceride, hypoglycemic treatment, the use of angiotensin-converting enzyme inhibitor/angiotensin II receptor antagonist and statin, the Ala12 variant of Pro12Ala polymorphism is a significantly protective factor for albuminuria (odds ratio 0.428, 95% confidence interval 0.195–0.940, $P = 0.034$; Table 4).

DISCUSSION

The present study demonstrated that the *Ala12* variant of the Pro12Ala polymorphism of the *PPARγ2* gene showed

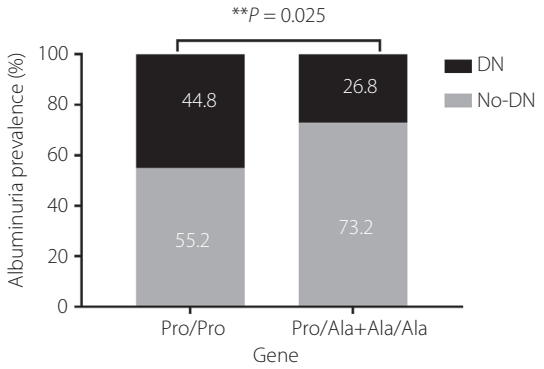

Figure 1 | Albuminuria prevalence of the peroxisome proliferator-activated receptor gamma Pro12Ala polymorphism in type 2 diabetes patients. The peroxisome proliferator-activated receptor gamma 2 Ala12 variant has significant protective effects against albuminuria and diabetic nephropathy. These findings suggest that genetic screening can help in the development of personalized therapies for diabetes. DN, diabetic nephropathy; No-DN, no diabetic nephropathy.

significant risk reduction with albuminuria after adjustments were made for the related risk factors. The *Pro12* allele is significantly associated with higher serum creatinine and UACR levels. In Caucasian populations[9,16,17], the *Ala12* allele had shown a protective effect against worsening albuminuria and DN. However, the protective role of the Pro12Ala polymorphism is not consistent in Asian studies, and Mori *et al.*[8] reported no significant difference in nephropathy prevalence between PP and PA + AA genotypes among the Japanese population. The present results are in agreement with the results from Li *et al.*[10] and Liu *et al.*[13], which reported the Ala12 variant significantly protects against DN in a central Han cluster of Chinese patients[25]. To the best of our knowledge, this is the first report of Pro12Ala polymorphism significantly related to a reduced risk of albuminuria for type 2 diabetes mellitus patients in Taiwan, mostly of southern Han Chinese ancestry[26].

The first evidence for the connection between the Pro12Ala polymorphism in the *PPARγ* gene and improving insulin sensitivity was reported by Deeb *et al.*[15] Furthermore, gene knockout mouse models confirmed that *PPARγ* plays an important role in regulating insulin sensitivity[27], which might be connected to the pathogenesis of albuminuria[28]. Currently, it is recognized that the Pro12Ala polymorphism plays an important role in the risk reduction of albuminuria in patients with type 2 diabetes mellitus [9,10,13,14,16–19]. In addition, the *Ala12* allele showed enhanced resistance of oxidative stress[29]. Oxidative

Table 3 | Distribution of peroxisome proliferator-activated receptor gamma genotypes in different categories of diabetic nephropathy

Diabetic nephropathy	*n*	Genotype				Allele		
		Pro12Pro (PP)	Pro12Ala (PA)	Ala12Ala (AA)	*P**	Pro (P)	Ala (A)	*P**
Normoalbuminuria	313	283 (90.4)	28 (8.9)	2 (0.7)	0.181	594 (94.9)	32 (5.1)	0.012†
Microalbuminuria	189	179 (94.7)	10 (5.3)	0 (0)		368 (97.4)	10 (2.6)	
Macroalbuminuria	52	51 (98.0)	1 (2.0)	0 (0)		103 (99.0)	1 (1.0)	

*Data are expressed as percentages. **P*-values were calculated by χ^2 analysis or the Mantel–Haenszel test for trends (†).

Table 4 | Logistic regression analysis for the risk of albuminuria

Parameters	Univariate analysis		Multivariate analysis*	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
PPAR-γ (Ala12)	0.451 (0.221–0.920)	0.025	0.428 (0.195−0.940)	0.034
Age	1.019 (1.001–1.037)	0.033	–	–
Male sex	1.796 (1.277–2.524)	0.001	1.795 (1.169−2.757)	0.008
Hypertension	3.584 (2.407–5.338)	0.001	–	–
Metabolic syndrome	1.666 (1.115–2.490)	0.012	–	–
eGFR	0.974 (0.967–0.982)	0.001	0.985 (0.976−0.994)	0.001
Retinopathy	1.526 (1.024–2.274)	0.037	1.725 (1.087−2.739)	0.021
HDL	0.983 (0.969–0.997)	0.017	–	–
Triglyceride	1.004 (1.002–1.007)	<0.001	1.003 (1.000−1.006)	0.040
Hypoglycemic treatment	1.395 (1.128–1.724)	0.007	1.348 (1.049−1.732)	0.020
ACEI/ARB use	3.614 (2.509–5.205)	<0.001	2.554 (1.331−4.900)	0.005
Statin use	0.650 (0.434–0.974)	0.036	–	–

*Adjusted for age, sex, genotype, diabetes duration, hypertension, systolic blood pressure (SBP), waist, metabolic syndrome, estimated glomerular filtration rate (eGFR), retinopathy, high-density lipoprotein (HDL), triglyceride, hypoglycemic treatment, the use of angiotensin-converting enzyme inhibitor (ACEI)/angiotensin II receptor antagonist (ARB) and statin.

stress is associated with hyperglycemia, insulin resistance and DN[30]. Thus, the Pro12Ala polymorphism might alleviate DN through the mechanism of improving insulin sensitivity and increasing resistance of oxidative stress.

The United Kingdom Prospective Diabetes Study revealed that independent risk factors for the development of albuminuria were male sex, increased waist circumference, triglyceride, low-density lipoprotein, glycated hemoglobin A1c, smoking and previous retinopathy[31]. Some studies have shown an association between albuminuria and hypertension[32,33]. In the present study, individuals with albuminuria are significantly associated with male sex, triglyceride and retinopathy, but not with higher waist circumference, low-density lipoprotein, glycated hemoglobin A1c and hypertension after adjustment for confounders. Furthermore, the *Pro12* allele is an additional risk factor.

A meta-analysis showed the *Ala* carriers have a lower chance of developing diabetic retinopathy among Caucasian type 2 diabetes mellitus patients, but not among Asian patients[18]. The ethnic differences might be related to the fact that the *Ala* allele is detected more often in Caucasians (14%), but is relatively lower in Asians (4% of Japanese and 4% of Chinese)[8–14]. The present study shows that the *Ala* carriers also are less likely to develop diabetic retinopathy compared with the *Pro12Pro* genotype, but is not statistically significant, partly due to the low frequency of *Ala* carriers in the present series. As a complex disease, diabetic retinopathy is a complication involving polygenic and environmental factors, and the contributing factor of the Pro12Ala polymorphism in the *PPARγ2* gene should require further studies to elucidate its role.

However, there were several limitations to the present study. First, this study was a case–control study; therefore, it could not explore causal relationships between the risk factors and the development of albuminuria and DM nephropathy. Second, those patients with ESRD were excluded from the study for anuria. Therefore, we could not survey the relationship between the polymorphism and ESRD risk.

In conclusion, the present study shows that the *PPAR-γ2 Ala12* variant is related to risk reduction of albuminuria among patients with type 2 diabetes mellitus. Further studies are still required to elucidate the role of this polymorphism for predicting and treating kidney dysfunction in type 2 diabetes mellitus.

ACKNOWLEDGMENTS

This work was supported by the Chang Gung Memorial Hospital under grant [CORPG8F1001]. We thank Hsin-Yi Chien, Chih-Yun Lin and the Biostatistics Center, Kaohsiung Chang Gung Memorial Hospital, for statistics work.

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Ogurtsova K, da Rocha Fernandes JD, Huang Y, *et al*. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res Clin Pract* 2017; 128: 40–50.
2. Parving HH, Lewis JB, Ravid M, *et al*. Prevalence and risk factors for microalbuminuria in a referred cohort of type II diabetic patients: a global perspective. *Kidney Int* 2006; 69: 2057–2063.
3. Yokoyama H, Sone H, Oishi M, *et al*. Prevalence of albuminuria and renal insufficiency and associated clinical factors in type 2 diabetes: the Japan Diabetes Clinical Data Management study (JDDM15). *Nephrol Dial Transplant* 2009; 24: 1212–1219.
4. Chen F, Yang W, Weng J, *et al*. Albuminuria: Prevalence, associated risk factors and relationship with cardiovascular disease. *J Diabetes Investig* 2014; 5: 464–471.
5. Pezzolesi MG, Krolewski AS. The genetic risk of kidney disease in type 2 diabetes. *Med Clin North Am* 2013; 97: 91–107.
6. Yki-Jarvinen H. Thiazolidinediones. *N Engl J Med* 2004; 351: 1106–1118.
7. Sarafidis PA, Stafylas PC, Georgianos PI, *et al*. Effect of thiazolidinediones on albuminuria and proteinuria in diabetes: a meta-analysis. *Am J Kidney Dis* 2010; 55: 835–847.
8. Mori H, Ikegami H, Kawaguchi Y, *et al*. The Pro12 –>Ala substitution in PPAR-gamma is associated with resistance to development of diabetes in the general population: possible involvement in impairment of insulin secretion in individuals with type 2 diabetes. *Diabetes* 2001; 50: 891–894.
9. Herrmann SM, Ringel J, Wang JG, *et al*. Peroxisome proliferator-activated receptor-gamma2 polymorphism Pro12Ala is associated with nephropathy in type 2 diabetes: The Berlin Diabetes Mellitus (BeDiaM) Study. *Diabetes* 2002; 51: 2653–2657.
10. Li LF, Liu LM, S. ZT, *et al*. Peroxisome proliferator activated receptor γ2 gene P12A polymorphism and type 2 diabetic nephropathy in Han population in Shanghai. *J Shanghai Jiaotong Univ (Med Sci)* 2008; 4: 376–379. [translated from Chinese into English]
11. Chang MH, Lindegren ML, Butler MA, *et al*. Prevalence in the United States of selected candidate gene variants: third national health and nutrition examination survey, 1991–1994. *Am J Epidemiol* 2009; 169: 54–66.
12. Hsieh MC, Lin KD, Tien KJ, *et al*. Common polymorphisms of the peroxisome proliferator-activated receptor-gamma (Pro12Ala) and peroxisome proliferator-activated receptor-gamma coactivator-1 (Gly482Ser) and the response to pioglitazone in Chinese patients with type 2 diabetes mellitus. *Metabolism* 2010; 59: 1139–1144.
13. Liu L, Zheng T, Wang F, *et al*. Pro12Ala polymorphism in the PPARG gene contributes to the development of diabetic nephropathy in Chinese type 2 diabetic patients. *Diabetes Care* 2010; 33: 144–149.
14. Zhang H, Zhu S, Chen J, *et al*. Peroxisome proliferator-activated receptor gamma polymorphism Pro12Ala Is associated with nephropathy in type 2 diabetes: evidence

from meta-analysis of 18 studies. *Diabetes Care* 2012; 35: 1388–1393.
15. Deeb SS, Fajas L, Nemoto M, *et al*. A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nat Genet* 1998; 20: 284–287.
16. Caramori ML, Canani LH, Costa LA, *et al*. The human peroxisome proliferator-activated receptor gamma2 (PPARgamma2) Pro12Ala polymorphism is associated with decreased risk of diabetic nephropathy in patients with type 2 diabetes. *Diabetes* 2003; 52: 3010–3013.
17. De Cosmo S, Motterlini N, Prudente S, *et al*. Impact of the PPAR-gamma2 Pro12Ala polymorphism and ACE inhibitor therapy on new-onset microalbuminuria in type 2 diabetes: evidence from BENEDICT. *Diabetes* 2009; 58: 2920–2929.
18. Ma J, Li Y, Zhou F, *et al*. Meta-analysis of association between the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-gamma2 gene and diabetic retinopathy in Caucasians and Asians. *Mol Vis* 2012; 18: 2352–2360.
19. Azab MM, Abdel-Azeez HA, Zanaty MF, *et al*. Peroxisome proliferator activated receptor gamma2 gene Pro12Ala gene polymorphism in type 2 diabetes and its relationship with diabetic nephropathy. *Clin Lab* 2014; 60: 743–749.
20. Liu G, Zhou TB, Jiang Z, *et al*. Relationship between PPARgamma Pro12Ala gene polymorphism and type 2 diabetic nephropathy risk in Asian population: results from a meta-analysis. *J Recept Signal Transduct Res* 2014; 34: 131–136.
21. KDOQI. KDOQI clinical practice guidelines and clinical practice recommendations for diabetes and chronic kidney disease. *Am J Kidney Dis* 2007; 49: S12–S154.
22. Diabetes Association of the Republic of China T. Executive summary of the DAROC clinical practice guidelines for diabetes care-2018. *J Formos Med Assoc* 2020; 119: 577–586.
23. Marathe PH, Gao HX, Close KL. American diabetes association standards of medical care in diabetes 2017. *J Diabetes* 2017; 9: 320–324.
24. James PA, Oparil S, Carter BL, *et al*. 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). *JAMA* 2014; 311: 507–520.
25. Xu S, Yin X, Li S, *et al*. Genomic dissection of population substructure of Han Chinese and its implication in association studies. *Am J Hum Genet* 2009; 85: 762–774.
26. Chen CH, Yang JH, Chiang CWK, *et al*. Population structure of Han Chinese in the modern Taiwanese population based on 10,000 participants in the Taiwan Biobank project. *Hum Mol Genet* 2016; 25: 5321–5331.
27. Duan SZ, Ivashchenko CY, Whitesall SE, *et al*. Hypotension, lipodystrophy, and insulin resistance in generalized PPARgamma-deficient mice rescued from embryonic lethality. *J Clin Invest* 2007; 117: 812–822.
28. Pilz S, Rutters F, Nijpels G, *et al*. Insulin sensitivity and albuminuria: the RISC study. *Diabetes Care* 2014; 37: 1597–1603.
29. Thamer C, Haap M, Volk A, *et al*. Evidence for greater oxidative substrate flexibility in male carriers of the Pro 12 Ala polymorphism in PPARgamma2. *Horm Metab Res* 2002; 34: 132–136.
30. Kashihara N, Haruna Y, Kondeti VK, *et al*. Oxidative stress in diabetic nephropathy. *Curr Med Chem* 2010; 17: 4256–4269.
31. Retnakaran R, Cull CA, Thorne KI, *et al*. Risk factors for renal dysfunction in type 2 diabetes: U.K. Prospective Diabetes Study 74. *Diabetes* 2006; 55: 1832–1839.
32. Pontremoli R, Sofia A, Ravera M, *et al*. Prevalence and clinical correlates of microalbuminuria in essential hypertension: the MAGIC study. Microalbuminuria: a Genoa Investigation on Complications. *Hypertension* 1997; 30: 1135–1143.
33. Jones CA, Francis ME, Eberhardt MS, *et al*. Microalbuminuria in the US population: third national health and nutrition examination survey. *Am J Kidney Dis* 2002; 39: 445–459.