Pro12Ala Polymorphism in the *PPARG* Gene Contributes to the Development of Diabetic Nephropathy in Chinese Type 2 Diabetic Patients

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OBJECTIVE — Oxidative stress is a major contributing factor in the development of diabetic nephropathy. Peroxisome proliferator–activated receptor γ heterozygous mice and Pro12Ala polymorphism in *PPARG* exhibited increased resistance to oxidative stress. Smoking increases the production of reactive oxygen species, which accelerates oxidative stress under hyperglycemia. To determine whether the Pro12Ala polymorphism, alone or in combination with smoking, contributes to the development of diabetic nephropathy, a case-control study was performed in 760 Chinese patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS — Among patients, 532 had diabetic nephropathy with microalbuminuria ($n = 245$) or overt albuminuria ($n = 287$), and 228 did not show either of these symptoms but had had diabetes for ≥10 years and were not undergoing antihypertension treatment.

RESULTS — After adjustment for confounders, the Pro/Pro genotype was significantly associated with diabetic nephropathy (odds ratio 2.30 [95% CI 1.18–4.45], $P = 0.014$); smoking was also an independent risk factor for diabetic nephropathy (1.99 [1.08–3.68], $P = 0.029$). In addition, we identified possible synergistic effects; i.e., the high-risk group (smokers with the Pro/Pro genotype) showed 4.52 times higher risk (1.78–11.48, $P = 0.002$) of diabetic nephropathy than the low-risk group (nonsmokers with the Pro/Ala genotype) in a multiple logistic regression analysis controlled for the confounders.

CONCLUSIONS — Our results indicated that the Pro/Pro genotype and smoking were significant independent risk factors for diabetic nephropathy. The possible synergistic effects of genotype and smoking may aggravate oxidative stress and contribute to the development of diabetic nephropathy.

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Diabetic nephropathy is a leading cause of end-stage renal disease in developed countries. A recent epidemiological study indicated that albuminuria was present in 49.6% of Chinese type 2 diabetic patients aged >30 years and living in the Shanghai urban area (1). Although the causes of diabetic nephropathy are not fully understood, the familial aggregation of the disease and the disproportionate prevalence among specific ethnic minority groups suggest that genetic factors may influence the risk of developing the disease (2). The *PPARG* gene located on chromosome 3 has been linked to the risk of developing diabetic nephropathy. The peroxisome proliferator–activated receptor (PPAR)-γ2 is a transcription factor formed by an alternative mRNA splicing pathway, and it regulates the transcription and expression of numerous target genes, which have been shown to be involved in adipocyte differentiation, lipid and glucose metabolism, and atherosclerosis (3). The Pro12Ala polymorphism, a Pro-to-Ala exchange that results in the substitution of proline with alanine at codon 12, was associated with reductions in both DNA binding and transcriptional activity in vitro, and Ala12 carriers showed significant improvement in insulin sensitivity (4). This beneficial effect of lower PPAR-γ activity on insulin sensitivity in humans can be replicated in PPAR-γ heterozygous (PPAR-$\gamma^{+/-}$) mice (5). The Pro12Ala polymorphism has also been associated with diabetic nephropathy in Caucasians (6–8). Recent studies have indicated that the Ala12 allele–mediated improvement in insulin sensitivity may involve enhanced suppression of lipid oxidation, which permits more efficient glucose disposal (9). Moreover, adipose tissue–specific PPAR-γ2 heterozygous mice and human Ala12 allele carriers show increased resistance to oxidative stress (9,10). Oxidative stress resulting from overproduction of reactive oxygen species (ROS) under hyperglycemic conditions has been suggested to contribute to the development and progression of diabetic nephropathy (11). Smoking increases the production of ROS (12), consequently accelerating oxidative stress under hyperglycemic conditions. Smoking is also known to increase urinary albumin excretion and is predicted to lead to faster progression of nephropathy in patients with type 2 diabetes (13). A number of epidemiological studies have provided evidence that interactions between genetic and nongenetic risk determinants contribute to the development and progression of diabetic nephropathy

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(14). However, previous studies have shown that the prevalence of Pro12Ala varies greatly among populations, and the prevalence in Asians is much lower than that in Caucasians (15). In addition, the Pro12Ala polymorphism was found to be associated with type 2 diabetes in Caucasians, but not in Chinese (16,17). In this study, we investigated the influence of the *PPARG* Pro12Ala polymorphism on the risk of diabetic nephropathy and determined whether this polymorphism and smoking showed a synergistic effect on the development of type 2 diabetic nephropathy in Chinese patients.

RESEARCH DESIGN AND METHODS

We selected type 2 diabetic subjects (n = 760) of Chinese Han ethnicity who were inpatients at the Department of Endocrinology and Metabolism and the Department of Nephrology at Shanghai Jiaotong University Affiliated Sixth People's Hospital between January 2005 and October 2008. Type 2 diabetes was diagnosed according to the 2003 American Diabetes Association diagnostic criteria for diabetes, and subjects were divided into no diabetic nephropathy and diabetic nephropathy groups according to their 24-h albumin excretion rates (AERs). The no diabetic nephropathy group (n = 228) consisted of patients who had had type 2 diabetes for at least 10 years, were not receiving antihypertension treatment, and did not show albuminuria (AER <30 mg/24 h). After ruling out urinary tract infection, hematuria, nephritis, and other conditions (18), the diabetic nephropathy group was further subdivided into a microalbuminuria group (n = 245, 300 mg/24 h > AER ≥ 30 mg/24 h), and an overt albuminuria group (n = 287, AER ≥300 mg/24 h), with AER determined in at least two consecutive overnight samples collected over a 3- to 6-month period. Diabetic retinopathy was evaluated for all patients simultaneously by an experienced ophthalmologist. In the diabetic nephropathy microalbuminuria group, all of the patients were confirmed to have coexistent diabetic retinopathy.

All of the patients underwent a standardized clinical and laboratory evaluation. Homeostasis model assessment of insulin resistance (HOMA-IR) and homeostasis model assessment of β-cell function (HOMA-β) were calculated by using the formulae described by Matthews et al. (19). Information on smoking habits was obtained using questionnaires. Patients who had smoked at least 1 cigarette/day for at least 1 year at the time of study recruitment were stratified into the smoking group, and patients who did not smoke or had given up smoking for >1 year at the time of study recruitment were stratified into the nonsmoking group. This study was approved by the institutional review board of Shanghai Jiaotong University Affiliated Sixth People's Hospital. Written informed consent was obtained from all of the participants.

Genotyping

Genomic DNA was extracted from 5 ml of peripheral blood by using the conventional phenol/chloroform method. Mismatch PCR and restriction fragment-length polymorphism for *Hae*III digestion were used for genotyping analysis of the Pro12Ala polymorphism by using previously established procedures (20). For the genotyping analysis, PCR products with a length of 155 bp were digested to two fragments with sizes of 132 and 23 bp, which were for the Pro12 allele, and the complete 155-bp fragment was for the Ala12 allele. To confirm that detection of this C→G nucleotide substitution, which resulted in Pro12Ala (CCA→GCA) by PCR-restriction fragment-length polymorphism analysis, is reproducible, we performed PCR-based direct sequencing analysis for each study subject.

Statistical analysis

Because there were no differences between the genotype frequencies of the microalbuminuria and overt albuminuria groups, we combined these two groups into a diabetic nephropathy group for further data analyses. The clinical and laboratory values are expressed as means ± SD or as medians (interquartile range). Comparisons of the clinical and laboratory parameters between the diabetes with and without diabetic nephropathy groups as well as those between the genotypic groups were performed with unpaired Student t tests and χ^2 analysis, as appropriate. Skewed distribution data such as those for the duration of diabetes, fasting plasma insulin level, HOMA-β, HOMA-IR, triglyceride level, AER, and serum creatinine level were logarithmically transformed before analysis and are presented as medians (interquartile range). $P < 0.05$ was considered significant. To evaluate the independent contributions of the Pro12Ala polymorphism and smoking to the risk of diabetic nephropathy, we performed multivariate logistic regression analysis of type 2 diabetic patients with diabetic nephropathy (microalbuminuria and overt albuminuria grouped together) by using type 2 diabetic patients without diabetic nephropathy as the control subjects; the analyses included possible confounders (sex, age at diagnosis of diabetes, diabetes duration, hypertension, triglyceride level, total cholesterol level, and A1C). Odds ratios (ORs) and 95% CIs were calculated. SPSS (version 10.0; SPSS, Chicago, IL) was used for data analysis and processing.

We used SAS (version 9.1.3; SAS Institute, Cary, NC) to calculate sample size and power. Results of the pilot study, which enrolled 94 no diabetic nephropathy and 165 diabetic nephropathy patients, on association between Pro12Ala and diabetic nephropathy showed that the OR of the high-risk genotype Pro/Pro to the low-risk genotype Pro/Ala was 2.21. Thus, we decided to use an OR of 2.0 to calculate sample size. Other assumptions used to calculate sample size were that *1*) the prevalence of the high-risk genotype of *PPARG* was 85%, *2*) the sample size proportion between the no diabetic nephropathy and diabetic nephropathy groups was 1:2, and *3*) the power was 80% ($\alpha = 0.05$); therefore, the total sample size needed was 732. In our study, 760 patients including 228 patients without diabetic nephropathy and 532 patients with diabetic nephropathy were enrolled. With use of the current sample, the power to find an OR of 2.2 was 82.2% ($\alpha = 0.05$); therefore, the sample size was considered to be adequate.

To investigate the possible combined effects of the genotype and smoking on the risk of diabetic nephropathy, we used multivariate logistic regression analysis with stratification on the basis of genotype (Pro/Pro or Pro/Ala) and smoking status (smokers or nonsmokers). A low-risk genotype and no history of smoking were considered as reference groups. If the combined effect of the two factors is 0, then there is no sign of interaction between the factors; consequently, there is no departure from additivity. We used the following formula to calculate the combined effect: $1 + OR_{A+B+} - OR_{A+B-} - OR_{A-B+}$, where 1 is the effect of the reference (21).

RESULTS

In comparison with the patients without diabetic nephropathy, the patients with diabetic nephropathy showed significant differences in several

Table 1—*Clinical and laboratory characteristics of type 2 diabetic patients with and without diabetic nephropathy*

	Without diabetic nephropathy	With diabetic nephropathy	*P**
n	228	532	—
Age (years)	65.0 ± 9.6	64.1 ± 12.8	0.328
Age at diagnosis of diabetes (years)	49.7 ± 10.4	54.3 ± 13.0	<0.001
Sex (male/female)	101/127	281/251	0.033
Diabetes duration (years)	13.0 (11.0–17.8)	10.0 (4.0–15.0)	<0.001
Hypertension (%)	129 (56.6)	391 (73.5)	<0.001
Systolic blood pressure (mmHg)	138.1 ± 18.9	143.2 ± 20.2	0.001
Diastolic blood pressure (mmHg)	80.5 ± 10.3	82.7 ± 10.5	0.008
Fasting plasma glucose (mmol/l)	9.1 ± 3.9	9.0 ± 3.5	0.802
A1C (%)	9.2 ± 4.2	8.9 ± 2.7	0.437
Fasting plasma insulin (mU/l)	14.7 (9.2–21.0)	14.8 (9.8–22.8)	0.381
HOMA-β	63.8 (34.5–118.4)	62.6 (34.1–137.6)	0.653
HOMA-IR	5.3 (3.3–8.2)	5.7 (3.3–8.6)	0.446
Triglyceride level (mmol/l)	1.3 (0.9–1.9)	1.6 (1.1–2.5)	<0.001
Total cholesterol level (mmol/l)	4.8 ± 1.2	4.9 ± 1.4	0.125
Retinopathy (%)	98 (43.0)	284 (53.4)	0.008
Serum creatinine (μmol/l)	64.0 (55.0–78.0)	84.0 (64.0–129.0)	<0.001
Smoking (%)	19 (8.3)	88 (16.5)	0.003
Hypoglycemic treatments			
Insulin (%)	111 (48.7)	275 (51.7)	0.467
OHA (%)	63 (27.6)	152 (28.6)	
Insulin + OHA (%)	54 (23.7)	105 (19.7)	

Data are means ± SD, medians (interquartile range), or *n* (%). Values of HOMA-β, HOMA-IR, and fasting plasma insulin were calculated for no diabetic nephropathy ($n = 63$) and diabetic nephropathy ($n = 152$) patients, who were not receiving insulin therapy. *P values were obtained by an unpaired Student t test or χ^2 analysis, as appropriate. OHA, oral hypoglycemic agent.

clinical and laboratory characteristics in this study (Table 1). The crude analysis revealed that the risk of developing diabetic nephropathy was significantly increased by smoking (OR 2.18 [95% CI 1.30–3.68], $P = 0.003$).

As shown in Table 2, with the exception of the Pro/Ala patients who had a lower AER level than the Pro/Pro patients ($P = 0.001$), there were no differences between the Ala12 carriers and noncarriers with regard to clinical and laboratory characteristics. All Ala12 carriers were heterozygotes (Pro12Ala); there were no Ala/Ala homozygotes in the population sample studied. The genotypic distribution of the Pro12Ala polymorphism in each group was in Hardy-Weinberg equilibrium ($P > 0.05$) (Fig. 1). However, because there were no significant differences between the genotype distributions in the microalbuminuria and overt albuminuria groups, we combined these samples into a diabetic nephropathy group, as described in RESEARCH DESIGN AND METHODS (Fig. 1). The Pro/Ala genotype and the Ala12 allelle frequency in the diabetic nephropathy group were clearly lower than those in the no diabetic nephropathy group (6.2 vs. 12.7% for the Pro/Ala genotype and 3.1 vs. 6.4% for the Ala allele; $P = 0.003$ for both of the parameters) (Fig. 1).

When the risks of microalbuminuria and overt albuminuria nephropathy were separately analyzed using multivariate logistic regression analysis with adjustment for possible confounders, the Pro/Pro genotype was significantly associated with overt albuminuria nephropathy (OR 2.76 [95% CI 1.22–6.19], $P = 0.014$). There was a tendency for association with microalbuminuria nephropathy that was not statistically significant (1.94 [0.90–4.14], $P = 0.089$). In unadjusted analyses, the Pro/Pro genotype was significantly associated with diabetic nephropathy (2.20 [1.30–3.73], $P = 0.003$) (Fig. 1).

To evaluate the independent contributions of the polymorphism and smoking to the risk of diabetic nephropathy, multivariate logistic regression analyses of type 2 diabetic patients with and without diabetic nephropathy were performed with the possible confounders. We obtained the following values for the individual confounders: sex (OR 0.92 [95% CI 0.62–1.34], $P = 0.649$), age at diagnosis of diabetes (1.02 [1.00–1.04], $P = 0.021$), diabetes duration (0.91 [0.88–0.94], $P < 0.001$), hypertension (2.34 [1.56–3.49], $P < 0.001$), triglyceride level (1.59 [1.18–2.14], $P = 0.002$), total cholesterol level [1.10 [0.94–1.29], $P = 0.222$), A1C (0.97 [0.92–1.03], $P = 0.281$), and smoking (1.99 [1.08–3.68], $P = 0.029$); the Pro/Pro genotype significantly increased the risk of diabetic nephropathy (2.30 [1.18–4.45], $P = 0.014$).

To study the possible interaction between the polymorphism and smoking, we used a variable that stratified the participants according to the genotype and smoking status in a multivariate logistic regression analysis that was adjusted for the confounders. The high-risk group (smokers with the Pro/Pro genotype) had a 4.52 times higher risk of diabetic nephropathy (95% CI 1.78–11.48) than the low-risk group (nonsmokers with the Pro/Ala genotype) ($P = 0.002$), and the departure from additivity was 1.40, indicating a possible synergistic interaction between genotype and smoking in patients with diabetic nephropathy (Fig. 2). Except for the synergistic interaction between smoking and genotype, no synergistic effects were found between smoking and other covariates that were associated with diabetic nephropathy.

CONCLUSIONS — In our study, the Pro/Pro genotype showed significant risk associations with diabetic nephropathy, when adjustments were made for other risk factors. The association with the Pro/Pro genotype seemed stronger in patients with overt albuminuria nephropathy than in those with microalbuminuria nephropathy. Despite a larger sample, subdividing the patients into microalbuminuria and overt diabetic nephropathy groups will lead to a greater risk of statistical instability, but we cannot exclude the possibility that what we see is an association with overt diabetic nephropathy and that other factors, stronger than the polymorphism, are more important for the progression from no diabetic nephropathy to microalbuminuria nephropathy. These results for Chinese patients are in agreement with the findings for Caucasian patients, which were reported by Herrmann et al. (6), Caramori et al. (7), and Pollex et al. (8), who found that the frequencies of the Ala12 allele in German, Brazilian, and Oji-Cree type 2 diabetic patients with diabetic nephropathy were lower than the

Table 2—*Clinical and laboratory characteristics of type 2 diabetic patients classified according to their* PPARG *Pro12Ala genotypes*

	Pro/Pro	Pro/Ala	*P**
n	698	62	—
Age (years)	64.4 ± 11.6	64.0 ± 12.9	0.842
Age at diagnosis of diabetes (years)	53.1 ± 12.2	51.3 ± 14.3	0.286
Sex (male/female)	355/343	27/35	0.275
Diabetes duration (years)	10.0 (6.0–15.0)	11.0 (8.8–17.0)	0.163
Hypertension (%)	479 (68.6)	41 (66.1)	0.685
Systolic blood pressure (mmHg)	141.9 ± 20.2	139.5 ± 16.0	0.286
Diastolic blood pressure (mmHg)	82.1 ± 10.5	81.5 ± 10.0	0.696
Fasting plasma glucose (mmol/l)	9.0 ± 3.6	9.5 ± 3.8	0.473
A1C (%)	9.0 ± 3.3	8.9 ± 2.3	0.763
Fasting plasma insulin (mU/l)	14.6 (9.2–22.0)	16.8 (12.4–23.5)	0.140
HOMA-β	62.5 (33.8–128.0)	72.1 (50.3–189.9)	0.242
HOMA-IR	5.4 (3.2–8.6)	5.9 (3.8–10.4)	0.211
Triglyceride level (mmol/l)	1.2 (0.9–1.6)	1.1 (0.8–1.6)	0.303
Total cholesterol level (mmol/l)	4.9 ± 1.3	5.1 ± 1.4	0.129
Retinopathy (%)	354 (50.7)	28 (45.2)	0.402
AER (mg/24 h)	80.8 (17.8–377.9)	21.4 (5.8–159.3)	0.001
Serum creatinine (μmol/l)	76.0 (60.0–105.8)	68.0 (57.0–104.0)	0.515
Smoking (%)	97 (13.9)	10 (16.1)	0.628
Hypoglycemic treatments			
Insulin (%)	351 (50.3)	35 (56.5)	0.414
OHA (%)	197 (28.2)	18 (29.0)	
Insulin + OHA (%)	150 (21.5)	9 (14.5)	

Data are means ± SD, medians (interquartile range), or *n* (%). Values of HOMA-β, HOMA-IR, and fasting plasma insulin were calculated for patients with Pro/Pro ($n = 197$) and patients with Pro/Ala ($n = 18$) respectively, who were not receiving insulin therapy. *P values were obtained by an unpaired Student t test or χ^2 analysis, as appropriate. OHA, oral hypoglycemic agent.

corresponding frequencies in patients without diabetic nephropathy.

The Pro12 allele is more common in the Chinese population than in Caucasian populations (95 vs. 88%) (15,17), and we observed a stronger significant association with diabetic nephropathy. However, the observed stronger genetic association with diabetic nephropathy confirms the earlier findings, and this association can be observed across ethnicities, which highlights the importance of this polymorphism in the development of diabetic nephropathy. However, previous studies reported that the Ala12 allele provided resistance to type 2 diabetes in Caucasians, but not in Chinese, thus reflecting an ethnic genetic heterogeneity of type 2 diabetes at this locus (16,17).

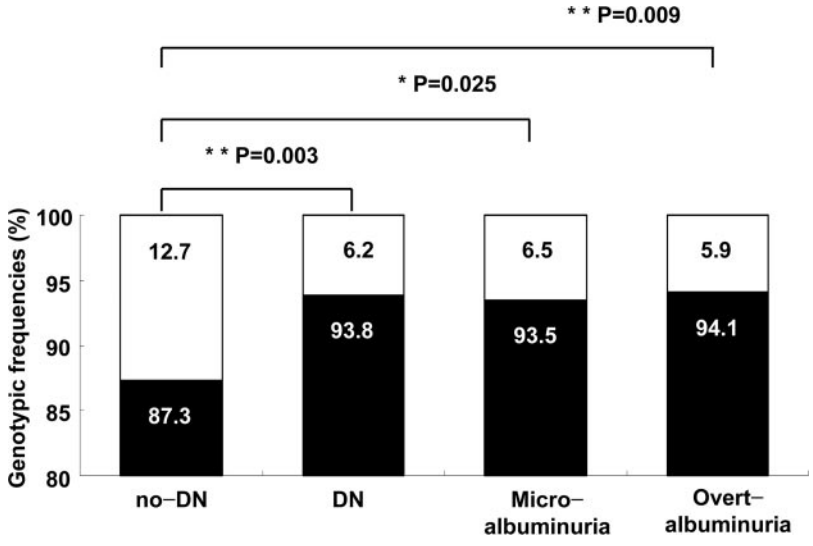

Figure 1—*Genotypic frequencies of the* PPARG *Pro12Ala polymorphism in type 2 diabetic patients with and without diabetic nephropathy. no-DN, no diabetic nephropathy; DN, diabetic nephropathy.* ■*, Pro/Pro;* □*, Pro/Ala.*

Smoking was also an independent risk factor for the development of diabetic nephropathy in the crude analyses as well as in the analyses performed after adjustment for possible confounders. However, smoking was a weaker risk factor for microalbuminuria nephropathy (OR 1.70 [95% CI 0.863–3.351], $P = 0.125$) and a stronger risk factor for macroalbuminuria nephropathy (2.36 [1.21–4.59], $P = 0.012$), supporting the oxidative stress hypothesis (11).

In the present study, we observed that both homozygosity for the Pro12 allele of the *PPARG* Pro12Ala polymorphism and smoking were associated with a significant increase in the risk of diabetic nephropathy, even after adjustments for possible confounders. Moreover, we also detected a possible synergistic effect of these two factors on diabetic nephropathy; i.e., the high-risk group (smokers with the Pro/Pro genotype) showed 4.52 times higher risk of diabetic nephropathy than the low-risk group (nonsmoking patients with the Pro/Ala genotype). The adipose tissue–specific PPAR-γ2 Ala12 carriers exhibit increased resistance to oxidative stress (9), and smoking increases the production of ROS (12). Thus, the aggravation of oxidative stress under hyperglycemic conditions may reflect the possible synergistic effects of the combination of the Pro/Pro genotype and constant smoking on the development of diabetic nephropathy.

The present case-control study was performed using a relatively larger sample size than previous studies and was statistically well powered, involving two carefully characterized groups of type 2 diabetic patients with and without diabetic nephropathy. We identified two independent risk factors, i.e., Pro/Pro genotype and smoking, and we found that these two risk factors showed synergistic effects on the development of diabetic nephropathy in Chinese type 2 diabetic patients, supporting the idea that an interplay between genetic and nongenetic risk determinants contributes to the development and progression of diabetic nephropathy (14). The interaction between the genetic risk factor, the *PPARG* gene, and the lifestyle-related risk factor,

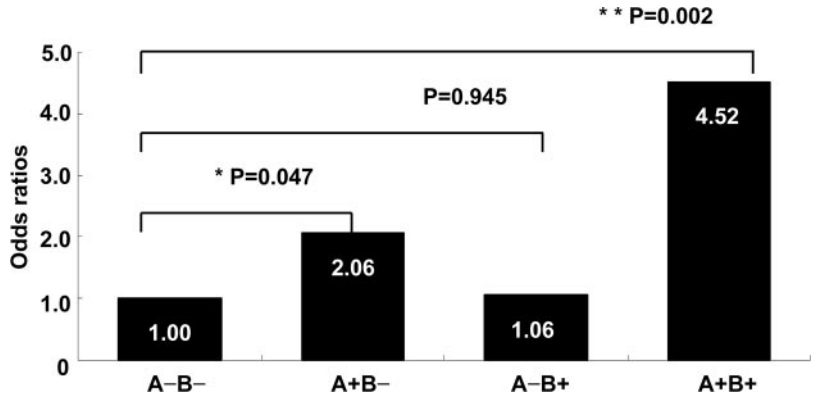

Figure 2—*ORs of diabetic nephropathy versus no diabetic nephropathy in different combinations of* PPARG *Pro12Ala genotype and smoking status in a multivariate logistic regression analysis. The ORs were adjusted for sex, age at diagnosis of diabetes, diabetes duration, hypertension, triglyceride, total cholesterol, and A1C levels.* A: *Pro/Pro genotype.* B: *Smoker. A−B−, nonsmokers without the Pro/Pro genotype, who were considered as the reference group for determining* P *values and ORs (95% CI). A+B− vs. A−B−, OR 2.06 (95% CI 1.01–4.20); A−B+ vs. A−B−, 1.06 (0.20–5.52); A+B+ vs. A−B−, 4.52 (1.78–11.48).*

smoking, is an interesting finding; however, these are preliminary findings, and they should be confirmed by studies in other ethnic groups. This is the first China- or Asia-specific report on the effects of the higher prevalence of the *PPARG* Pro/Pro genotype and the interaction between this genotype and smoking in the development of diabetic nephropathy in type 2 diabetic patients. A large population study in Japanese type 2 diabetic patients, which showed that the prevalence of this genotype in the Japanese patients was similar to that observed in Chinese patients, did not show any effects of the genotype on diabetic nephropathy. However, the results of that study were influenced by the standard used for defining diabetic nephropathy, i.e., AER >10 μg/ml (22).

The mechanisms by which the *PPARG* Pro12Ala polymorphism contributes to diabetic nephropathy have still not been clarified. Previous studies have suggested that Ala12 carriers show significant improvement in insulin sensitivity (4); this suggestion implies that non-Ala carriers, i.e., patients who are homozygous for the Pro12 allele, show increased insulin resistance, which contributes to the development of diabetic nephropathy. However, we could not detect the association between the Ala12 allele and the insulin resistance–related clinical parameters; this finding is consistent with the results of the study by Li et al. (17) on diabetic Chinese Hans. In addition, the latest studies indicated that mice that were heterozygous for the adipose tissue–specific PPAR-γ2 and human carriers of the Ala12 allele showed increased resistance to oxidative stress (9,10). The enhanced oxidative stress tolerance is associated with significant upregulation of antioxidant genes and a significant increase in the adipose tissue of FoxO3a, a transcription factor that is known to regulate the clearance of ROS (10). The enhanced oxidative stress tolerance of the Ala12 carrier also implies that the Pro/Pro genotype increases the production of ROS, which accelerates oxidative stress, causing an increase in glomerular albumin permeability, and the degree of proteinuria correlates with the progression of glomerulosclerosis and tubulointerstitial fibrosis (23). Oxidative stress can cause insulin resistance, which is a consequence as well as a potential cause of diabetic nephropathy (24).

In summary, our results indicated that smoking and the Pro/Pro genotype of the Pro12Ala polymorphism in the *PPARG* gene were significant independent risk factors for diabetic nephropathy. The possible synergistic effects between the genotype and smoking implied that a positive interaction between genetic and nongenetic factors may aggravate oxidative stress and contribute to the development of diabetic nephropathy in Chinese type 2 diabetic patients.

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