Significant Synergistic Effect of Peroxisome Proliferator–Activated Receptor γ C-2821T and Diabetes on the Risk of Ischemic Stroke

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OBJECTIVE — To explore the relationship between the genetic polymorphisms of *PPARγ* (Pro12Ala, C1431T, and C-2821T) and the risk of ischemic stroke and to investigate whether these genetic polymorphisms of PPARγ would modify the risk of ischemic stroke among patients with hypertension or diabetes.

RESEARCH DESIGN AND METHODS — The case-control study was conducted with 537 ischemic stroke patients and 537 control subjects. A structured questionnaire was used to collect information on conventional cardiovascular risk factors and laboratory results. The genetic polymorphisms of *PPARγ* were determined by PCR–restriction fragment–length polymorphism.

RESULTS — A significant interaction was seen between the −2821C allele and diabetes but not between this allele and hypertension. A markedly elevated risk of ischemic stroke (odds ratio 9.7) was found in the subjects with diabetes and the −2821C allele compared with that in those without these two risk factors.

CONCLUSIONS — The −2821C allele of *PPARγ* was a strong predictor of ischemic stroke for diabetic patients.

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Peroxisome proliferator–activated receptor γ (PPARγ) is a ligand-dependent transcription factor involved in the regulation of lipid metabolism and inflammation (1–2). It is expressed in endothelium, vascular smooth muscle cells, macrophages, T-lymphocytes, and human atherosclerotic lesions (3–7). Indeed, PPARγ has been reported to play an important role in the progression of atherosclerosis, which is a well-known risk factor of stroke (8).

PPARγ was proved to prevent post-ischemic inflammation and neuronal damage in animal studies (9). It may serve as potential targets for treating ischemic stroke. Thus, we aimed to explore the relationship between genetic polymorphisms of *PPARγ* and the risk of ischemic stroke.

RESEARCH DESIGN AND METHODS

— A total of 537 ischemic stroke patients aged 30–95 years and confirmed with computed tomography or magnetic resonance imaging were recruited from the Department of Neurology at Chi-Mei Hospital, Lotung Poh-Ai Hospital, Wan-Fang Hospital, and Taipei Medical University Hospital between 2005 and 2007. A total of 1,636 subjects aged 30–95 years from the health examinations at Shin-Kong WHS Memorial Hospital and Wan-Fang Hospital between 2004 and 2007 were recruited as candidates for the control group. Among these candidates, 34 with a stroke history were excluded. A total of 537 control subjects were then randomly selected from 1,602 candidates and frequency matched by age (±2.5 years) and sex with case subjects. This case-control study was approved by the institutional review board for human subjects, and each subject provided written informed consent prior to the study. A structured questionnaire was used to collect information on conventional cardiovascular risk factors and laboratory results. The genetic polymorphisms of *PPARγ* (C-2821T, Pro12Ala, and C1431T) were determined by PCR–restriction fragment–length polymorphism. Details are described in the online appendix, available at http://care.diabetesjournals.org/cgi/content/full/dc09-0717/DC1. Student *t* tests, Mann-Whitney *U* tests, χ^2 tests, and logistic regression models were used as appropriate and performed with the SAS statistical software (version 9.1). Synergy index scores were used to evaluate interaction between two risk factors (10). Pairwise linkage disequilibrium between single nucleotide polymorphism markers was evaluated by Haploview (11).

RESULTS

— The occurrences of hypertension (75.6%), diabetes (45.9%), and *PPARγ* C-2821C genotype (12.3%) were more common in case than in control subjects, but the distribution of Pro12Ala and C1431T was similar between the two groups. The percentage of antihypertension drugs in ischemic stroke case subjects (66.7%) was similar to that in control subjects (66.6%). Of the ischemic stroke case subjects with diabetes, 54.1% had pharmacological treatment. The percentage of pharmacological treatment for diabetes in control subjects

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Table 1—*Adjusted ORs of ischemic stroke risk by hypertension, diabetes, and PPARγ −2821C allele*

Group of risk factors	Hypertension	Diabetes	PPARγ −2821C	Case/control subjects	OR (95% CI)*	Grouped OR (95% CI)*
Ref.	−	−	−	34/93	1.0	1.0
I	−	−	+	43/89	1.5 (0.7–3.4)	—
I	−	+	−	31/15	3.5 (1.2–10.0)†	—
I	+	−	−	101/129	2.7 (1.3–5.3)†	2.3 (1.2–4.3)‡
II	−	+	+	23/8	5.3 (1.5–18.3)†	—
II	+	−	+	112/126	2.3 (1.1–4.5)†	—
II	+	+	−	77/43	4.4 (1.8–10.4)‡	2.6 (1.3–5.1)‡
III	+	+	+	115/28	11.6 (5.0–26.9)§	9.6 (4.2–21.7)§

Data are *n* unless otherwise indicated. The reference group was the study subjects without hypertension, diabetes, and the *PPARγ* −2821C allele. $P_{trend} < 0.0001$ among groups I–III. *Adjustment for BMI, waist circumference, history of ever smoking, dyslipidemia, and pharmacological treatment for diabetes. †$P < 0.05$. ‡$P < 0.001$. §$P \leq 0.0001$. +, appearance of risk factor; −, absence of risk factor.

(41.5%) was less than that in case subjects. All genetic polymorphisms were in Hardy-Weinberg equilibrium. There was a high degree of linkage disequilibrium between Pro12Ala and C-2821T.

After adjusting for BMI, waist circumference, a history of ever smoking, dyslipidemia, hypertension, diabetes, and pharmacological treatment for diabetes, the odds ratio (OR) of *PPARγ* C-2821C genotype was not significant. Compared with nondiabetic subjects with T-2821T genotype, an increased risk of ischemic stroke was observed in TT genotype carriers with diabetes (OR 4.2; $P = 0.07$), and the OR drastically increased to 9.7 ($P = 0.008$) in C allele carriers but not in C allele carriers without diabetes. Thus, there was a significant joint effect of the *PPARγ* C-2821T polymorphism and diabetes (synergy − index = 2.7) on the risk of ischemic stroke. On the other hand, no interaction between hypertension and the *PPARγ* C-2821T polymorphism on the risk of ischemic stroke was found (synergy − index = 0.9).

The risk of ischemic stroke was estimated for each combination of hypertension, diabetes, and the *PPARγ* −2821C allele, using nonhypertension, nondiabetes, and non-2821C allele carriers as the reference group (Table 1). The ORs of ischemic stroke in hypertension alone or diabetes alone were 2.7 and 3.5, respectively. Furthermore, the OR increased to 5.3 in the subjects with diabetes and the *PPARγ* −2821C allele, which was higher than the risk in association with hypertension or diabetes alone. The greatest OR (11.6) was seen in the subjects with hypertension, diabetes, and the *PPARγ* −2821C allele. A trend test indicated that the risk of ischemic stroke increased along with the accumulating number of these three risk factors ($P < 0.0001$).

CONCLUSIONS — Our study concluded that there was no relationship between *PPARγ* Pro12Ala genotype and the risk of ischemic stroke. The same result was found by Zafarmand et al. (12), but the opposite conclusion was made by Lee et al. (13). The data of genetic polymorphism of *PPARγ* C-2821T in diseases were scarce. This novel genetic variant was first identified in the Pima Indian population from Arizona and was reported to associate with metabolic predictors of type 2 diabetes and obesity (14). In the present study, we found a joint effect of the *PPARγ* −2821C allele and diabetes on the risk of ischemic stroke. Adjusting for traditional cardiovascular risk factors and pharmacological treatment for diabetes, the risk of ischemic stroke in the −2821C allele carriers with diabetes was 9.7 times greater than that of the homozygous T allele carriers without diabetes. Higher transcriptional activity was found in the −2821T allele than in the −2821C allele by using the Dual Luciferase Reporter Assay in 3T3-L1 cells (14). This implied that PPARγ could present a protective effect on ischemic stroke. Wu et al. (9) used a rat model to prove PPARγ to be a critical factor for protection against neuronal apoptosis and cerebral infarction by the mediated 14-3-3ε protein. In addition, PPARγ was reported to be an important regulator of endothelial function in the cerebral circulation, especially under conditions of high fat–induced stress (15).

In the present study, we found that the risk of ischemic stroke in subjects with hypertension, diabetes, and the −2821C allele was 2.6 times higher than that in the subjects with hypertension and diabetes. Therefore, the combination of hypertension, diabetes, and the *PPARγ* −2821C allele was a strong predictor of ischemic stroke. Further studies in other populations will be very useful in establishing the contribution of this C-2821T polymorphism to ischemic stroke.

In conclusion, there was a strong interaction between the *PPARγ* −2821C allele and diabetes with regard to risk of ischemic stroke. Thus, the *PPARγ* −2821C allele was a strong predictor of ischemic stroke for diabetic patients, and PPARγ may serve as a potential target for treating ischemic stroke.

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