Functional Genetic Variants of PPAR γ and PPAR α Encoding Peroxisome Proliferator-Activated Receptors and Susceptibility to Ischemic Stroke in Chinese Han Population

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Key Words

Ischemic stroke · Functional variants · Peroxisome proliferator-activated receptors · Han population

Abstract

Background: PPAR γ and PPAR α belong to a receptor family of ligand-activated transcription factors involved in the regulation of inflammation, cellular glucose uptake, protection against atherosclerosis and endothelial cell function. Through these effects, they might be involved with the ischemic stroke (IS). **Methods:** One thousand two hundred ninety-six subjects from the Chinese Han Population were chosen to assess the nature of the functional polymorphisms of PPARs and any links with IS. Multivariate logistic regression analysis was used to examine the association between PPAR γ and PPAR α genotypes and a diagnosis of IS. **Results:** Pro/Ala carriage may be associated with the decreased risk

of IS in Hans (OR 0.542, 95% CI 0.346–0.850). The 162Val allele frequency at the DNA-binding region of PPAR α was extremely rare in Chinese Han population. **Conclusions:** PPAR γ 12Pro/Ala resulting in an amino acid exchange in N-terminal sequence may be an independent protective factor for IS in the Chinese Han population. However, more populations are warranted to validate our findings.

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Introduction

Ischemic stroke (IS) is a major cause of morbidity and mortality in many developed and developing countries. High-density lipoprotein, homocysteine, troponin and fatty acid-related chronic low-grade inflammation and activation of the innate immune system are closely involved in the pathogenesis of IS [1–5]. Peroxisome pro-

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liferator-activated receptor γ and α (PPAR γ and PPAR α) play key roles in the regulation of the inflammation, adipocyte differentiation and lipid biosynthesis [6]. Through these effects, PPAR γ and PPAR α might play crucial roles in the development and progression of IS. It was proposed that Leu162Val polymorphism and PPAR γ Pro12Ala were associated with type 2 diabetes, atherosclerosis. However, their roles for IS have not yet been investigated.

In this study, we screened 648 patients with clinically definite IS and 648 age-, sex-, ethnically matched healthy controls from the Chinese Han subject panel for studying the association of PPAR α Leu162Val and PPAR γ Pro12Ala polymorphisms with IS.

Methods

Our study was approved by the local Institutional Review Board. A total of 648 typical Uyghur IS subjects were enrolled into the case-control study. The diagnosis of IS was established according to the World Health Organization, and Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification. The stroke subtypes were evaluated using the modified TOAST classification in total. Stroke was defined as a sudden onset of nonconvulsive and focal neurological deficit persisting for 24 h. Large vessel was diagnosed if the patient had the characteristics of infarction due to low flow or artery-to-artery embolism in the presence of disease in the ipsilateral arterial tree (extracranial or intracranial segments of carotid or vertebralbasilar arteries, or proximal middle cerebral arteries) and CT scan or MRI performed within 7 days of onset showed either an area of infarction in the territory of a large vessel compatible with the clinical symptoms or hemodynamically significant stenosis. Small vessel was diagnosed if the patient had one of the characteristic clinical lacunar syndromes and CT scan or MRI showed either normal or a deep focal infarction in the brain stem or subcortical region with a diameter of <1.5 cm. A cardioembolic condition was diagnosed if the patient had the clinical characteristics of abrupt non-progressive onset, palpitations at onset, decreased consciousness at onset or hemianopia without hemiparesis and magnetic resonance angiography, computerized tomographic angiography, CT scan or MR diffusion-weighted imaging showed either a risk cardioembolic source in a region compatible with the clinical symptom or some embolisms located in other organs. The undetermined was defined as a kind that could not be classified as the previous 3 subtypes. Concurrently, 648 age-, gender- and ethnically matched normal healthy controls were randomly selected. Subjects with a history of stroke, Alzheimer's disease, brain aneurysm and Parkinson's disease were excluded from the controls group. An epidemiological investigation was performed with a standardized questionnaire to record epidemiological features.

Genotyping of PPAR α Leu162Val and PPAR γ Pro12Ala was performed by TaqMan SNP genotyping assays using ABI 7500 (Applied Biosystems, Foster City, Calif., USA). Multivariate logistic regression model analysis was used to examine the association between Pro12Ala genotypes and a diagnosis of IS.

Results

The characteristics of patients and controls are summarized in online supplementary table 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000442306). The stroke subtypes were evaluated using the modified TOAST classification in total (large vessel [7.7%], small vessel [36.7%], cardioembolic [13.9%], and undetermined [41.7%]). TC, FBG, WHR, hypertension, history of heart diseases, and negative events may increase the risk of IS, with a trend for alcohol drinking, tea drinking and HDL to be protective factors for IS. In the additional multivariate logistic regression analysis, hypertension, diabetes, smoking, tea drinking, and WHR were also found to be independent predictors of IS among the Han population (online suppl. table 2).

Table 1 shows the distributions of the genotypes and allelic frequencies of PPAR α Leu162Val and PPAR γ Pro12Ala polymorphisms. There were no statistically significant difference in the distribution of PPAR α Leu162Val polymorphism between the patients and controls in the Han ethnic group ($p > 0.05$). The Val allele frequencies at the 162 DNA-binding domain of PPAR α were extremely low in the Chinese Han population. However, there were significant differences in the distribution of Pro12Ala polymorphism between the patients and controls in the Han group ($p < 0.01$). The PPAR γ Pro/Ala polymorphism was Hardy-Weinberg equilibrium in Han ethnic groups ($p > 0.05$). After adjustment for confounders, the PPAR γ Ala allele was associated with a decreased risk of IS, with an overall OR of 0.542 and 0.555 when assuming Additive and Dominant models of inheritance, respectively (all $p < 0.01$). Assuming disease prevalence between 0.5 and 1%, power analysis indicates that our sample size can reach >75% power to detect Pro12Ala with the concluded genotypic ORs at the nominal Type I error rate <0.05 under Additive and Dominant genetic models.

Figure 1A compares the PPAR α Leu162Val genotype frequency distribution between different ethnic groups based on the published research data and present study [7–10]. The PPAR α 162Leu/Val genotype was more frequent in the Danish, German, Czech, Tunisia, and Brazil population than among the Asian Hans and Japanese (all $p < 0.05$). The PPAR α 162Val/Val genotype was more frequent only in German and Tunisia population than in Hans and Japanese (all $p < 0.05$). The PPAR α 162Leu/Val genotype was absent in Hans and the Japanese. Meanwhile, the PPAR α 162Val/Val genotype was absent in Hans, Japanese, Germans and Brazils. Furthermore, we also analyzed the association of BMI, WHR, FBG of IS

Table 1. PPAR α Leu162Val and PPAR γ Pro12Ala genotype and allele distributions between cases and controls in the Chinese Han population

	Han		p value	Crude OR (95% CI)	p value	Adjusted OR* (95% CI)
	cases (n = 648)	controls (n = 648)				
PPAR α (162)						
Leu/Leu	648	648				
Leu/Val	0	0	–	–	–	–
Val/Val	0	0	–	–	–	–
PPAR α alleles						
Leu	1,296	1,296				
Val	0	0	–	–	–	–
MAF	0	0	–	–	–	–
PPAR γ (12)						
Pro/Pro	606	580				
Pro/Ala	40	67	0.007	0.571 (0.380–0.859)		
Ala/Ala	2	1	1.000	1.914 (0.173–21.167)	1.000	1.915 (0.172–21.170)
Pro/Ala + Ala/Ala	42	68	0.010	0.591 (0.396–0.883)	0.009	0.555 (0.356–0.864)
PPAR γ alleles						
Pro	1,252	1,227				
Ala	44	69	0.016	0.625 (0.425–0.920)	0.008	0.542 (0.346–0.850)
MAF, %	3.40	5.32				

MAF = Minor allele frequency. * Multivariate logistic regression analysis after adjustment for sex, age, hypertension, diabetes, smoking, alcohol drinking, tea drinking, BMI, WHR.

Fig. 1. A The PPAR α 162Leu/Val genotype frequency distribution between various populations. $\blacktriangle$ Compared with Han and Japanese populations on the Leu/Val genotype frequency; $p < 0.05$. $\blacklozenge$ Compared with Han and Japanese populations on the Val/Val genotype frequency; $p < 0.05$. **B** The association of BMI, WHR, FBG of IS patients with Pro12Ala genotypes in the Han (**a–c**) ethnic group. $\blacktriangle$ indicates $p > 0.05$ (Pro/Pro vs. Pro/Ala + Ala/Ala).

patients with Pro12Ala genotypes; however, we failed to find any significant association with any of these parameters in the Han population (all $p > 0.05$; fig. 1B).

Discussion

Taken together, we are the first to conclude that the 162Val allele frequency at the DNA-binding domain of human PPAR α was extremely low in the Chinese Han population not similar to some European and African populations. The PPAR α Leu162Val is unlikely to contribute significantly to a susceptibility to, or affect the progression of IS in Han population. Moreover, our findings lend support to the notion of an independent protective effect of the Ala allele of the PPAR γ Pro12Ala on the risk of IS in the Han population.

However, our study also has certain limitations. Although the current study includes a relative large sample

size in the Han population, rigorous methods used to diagnose IS, we used modified TOAST classification criteria which may be less comprehensive than ASCOD phenotyping [11] and lack of a validate population study to confirm our conclusions. Further explorations with larger, more ethnically diverse populations are warranted to better shed light on the functional properties of this receptor on the risk of IS, as well as the complicated pathophysiology mechanisms precisely. These findings may affect the diagnosis and treatment of IS.

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