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Effect of Genetic Polymorphism +294T/C in Peroxisome Proliferator-Activated Receptor Delta on the Risk of Ischemic Stroke in a Tunisian Population

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Abstract PPARδ +294T/C polymorphism was investigated in diabetics, in normolipidemic healthy controls, in dyslipidemic and nondyslipidemic coronary artery disease patients but never in ischemic stroke patients. The aim of this study was to explore, for the first time, the relationship between the genetic polymorphism of PPARδ and the risk of ischemic stroke among patients with diabetes. The study group consisted of 196 patients with ischemic stroke and 192 controls. Plasma concentrations of total cholesterol, triglycerides, low-, and high-density lipoprotein did not differ significantly between subjects carrying the TT genotype and those carrying the CC/TC genotype in both ischemic stroke patients (with or without diabetes) and control groups. The +294C allele (CC + CT genotypes) as compared with TT genotypes was found to be higher in total ischemic stroke patients than in controls. On the other hand, no interaction between diabetes and PPAR +294T/C polymorphism on the risk of ischemic stroke was found (p=0.089). The PPARδ +294T/C polymorphism was associated with the risk of ischemic stroke in Tunisian subjects. This polymorphism has no influence on plasma lipoprotein concentrations and body mass index either in healthy subjects or in ischemic stroke patients with or without diabetes both in males and females.

Keywords PPARδ · Polymorphism · Diabetes · Ischemic stroke

Introduction

Stroke is a multifactorial disorder with major clinical manifestations and multiple etiologies and a significant cause of disability and death in developed countries (Hankey 2006; Dichgans 2007). The etiology of stroke is complex, involving both environmental and genetic risk factors (Schulz and Rothwell 2003; Kirshner 2009). Ischemic stroke (IS) is the major subtype of stroke, which accounts for over 50 % of the stroke cases. The cause of ischemic stroke is arterial thrombosis. Thrombosis can be caused by disorders in blood vessels, blood coagulation, and blood flow. Risk factors have profound effects on the structure and function of blood vessels. Many of the established risk factors alter vascular structure by promoting atherosclerosis and stiffening the arteries and by inducing the narrowing, the thickening, and the tortuosity of arterioles and capillaries (Allen and Bayraktutan 2008; Iadecola and Davisson 2008). Ischemic stroke may manifest in the form of thrombotic stroke (large vessel and small vessel types), embolic stroke (with/without known cardiac and/or arterial factor), systemic hypoperfusion (watershed or border zone stroke) or venous thrombosis. It is well known that there are multiple genetic factors associated with IS. Many studies have further shown that there is no single common genetic variant that could independently cause it. There are some unknown genes that contribute to IS aside from the common risk

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factors (Dichgans 2007). Epidemiological studies and animal models support the contribution of genetic factors to the development and progression of ischemic stroke (Flossmann et al. 2004; Dichgans 2007).

Peroxisome proliferator-activated receptor δ (also known as PPARβ or NR1C2) can be activated by endogenous compounds, which are thought to act as natural ligands. It is one of the most widely expressed members of the PPAR family. PPARδ has roles in metabolism: regulates adipogenesis, increases fatty acid oxidation and energy uncoupling, decreases insulin resistance, improves glycemic control, and elevates high density lipoprotein (HDL; Leibowitz et al. 2000; Dressel et al. 2003). The PPARδ gene, located on chromosome 6p21.2-p21.1, is involved in cellular transport, storage, and metabolism of lipids (Oliver et al. 2001). Other than its profound role in metabolism and fat homeostasis, increasing evidence suggests a role for PPARδ in various basic vascular processes such as apoptosis (Liou et al. 2006; Kim et al. 2009), survival, angiogenesis (Piqueras et al. 2007), and the control of inflammation (Rival et al. 2002; Welch et al. 2003). The role of PPARδ in inflammation depends on whether a ligand is bound or not to the receptor. Binding leads to the release of B-cell lymphoma gene 6 (BCL-6), which is a repressor of inflammatory response; this results in decreased expression of inflammatory cytokine genes, reduced inflammation, and subsequently possible decrease of atherosclerosis (Lee et al. 2003). PPARδ could be involved in the pathophysiology of atherosclerosis due to its pivotal role in lipid metabolism. HDL-C is believed to be protective against atherosclerosis. Synthetic ligands of PPARδ have been shown to increase HDL-C in monkeys and rodents and thus may have therapeutic potential to treat this disease. Implication of activation of the PPARδ receptor in this field has been demonstrated by the study of Oliver et al. in obese rhesus monkeys. Treatment of obese rhesus monkeys with the synthetic PPARδ agonist GW501516 resulted in an increase of high-density lipoprotein cholesterol (HDL) levels and a decrease of plasma triglycerides. The identification of synthetic PPARδ agonists has revealed an important role for this receptor in lipid metabolism. PPARδ agonists are likely to have beneficial effects on the lipid triad and the atherogenic particle composition through a mechanism that increases cholesterol flux from peripheral tissues (Oliver et al. 2001). To determine whether PPARδ could modulate atherosclerotic lesion progression, the effect of GW501516 on lesion development in apoE−/− mice was examined and an increase in HDL-C was also observed (Barish et al. 2008).

PPARδ/β is the highest expressed PPAR subtype in brain parenchyma and the cerebral vasculature (Iwashita et al. 2007).The role of PPARβ in brain repair was first addressed in a model of focal cerebral ischemia, with a middle cerebral artery occlusion. Compared with wild type, PPARβ-null mice exhibited a significant increase in the infarct size, suggesting that PPARβ exerts a neuroprotective activity (Arsenijevic et al. 2006; Pialat et al. 2007). Following middle cerebral artery occlusion (MCAO), increased cerebrovascular permeability and infarct size were detected in mice with specific deletion of PPARδ in vascular smooth muscle cells (VSMCs; Yin et al. 2011).

Additionally, PPARδ agonists appear to be protective after cerebral ischemia; rats given infusions of L-165041 or GW501516 had significantly attenuated ischemic damage 24 h after MCAO (Iwashita et al. 2007). In addition, PPARδ plays a vascular-protective role in ischemia-like insults via transcriptional repression of microRNA-15a (miR-15a), resulting in subsequent release of its post transcriptional inhibition of bcl-2 anti-apoptotic protein. Thus, regulation of PPARδ-mediated miR-15a inhibition of bcl-2 could provide a novel therapeutic strategy for the treatment of stroke-related vascular dysfunction (Yin et al. 2010). The pathogenesis of cerebral ischemia also involves the breakdown of matrix-cell adhesions, which results in the disruption of the blood brain barrier and ultimately neuronal death and brain damage. PPARδ may regulate these responses by decreasing activity of matrix metalloproteinase (MMPs), enzymes that degrade structural proteins in the extracellular matrix and that have been implicated in ischemia-induced parenchymal and vascular damage. PPARδ in vascular smooth muscle cells can prevent ischemic brain injury by inhibition of MMP-9 activation and attenuation of post-ischemic inflammation (Yin et al. 2011).

There has been particular interest in the polymorphism at the +294T/C position on the 5′-untranslated region of PPARδ and the effects of carrying the minor C allele. This polymorphism alters the affinity for binding of the SP1 transcription factor (Skogsberg et al. 2000), with carriers of the minor allele having higher transcriptional activity than the common T allele. Previous studies showed an association between the rare +294C allele and cardiovascular risk factors such as increased low-density lipoprotein cholesterol (LDL-C) levels, elevated apolipoprotein B levels, body mass index (BMI), and decreased serum levels of high-density lipoprotein cholesterol (HDL-C; Skogsberg et al. 2003b; Chen et al. 2004; Aberle et al. 2006a, b; Nikitin et al. 2010). However, some studies have yielded conflicting results (Skogsberg et al. 2003b; Gouni-Berthold et al. 2005; Robitaille et al. 2007; Jguirim-Souissi et al. 2010). PPARδ gene variants have been associated with obesity in some (Shin et al. 2004; Wang et al. 2004) but not all studies (Gouni-Berthold et al. 2005; Robitaille et al. 2006).

To the author's best knowledge, this is the first report to study on the genetic level the potential relationship between ischemic stroke and PPARδ +294T/C polymorphism. Therefore, the aim of the present study was to investigate, for the first time, a possible association of PPARδ +294T/C

polymorphism with plasma lipid and lipoprotein levels and with the risk of ischemic stroke in a Tunisian population and to explore whether this genetic polymorphism of PPARδ would modify the risk of ischemic stroke among patients with diabetes.

Materials and Methods

Subjects

Study subjects consisted of 196 ischemic stroke patients (117 diabetic and 79 nondiabetic), who were recruited from the emergency Department at Fattouma Bourguiba University Hospital in Monastir, Tunisia. Ischemic stroke was defined as the rapid development of focal or global cerebral function's disturbance, with symptoms lasting 24 h or longer, or leading to death, with no apparent cause other than vascular origin. Stroke subtype assignment was as per TOAST criteria. Diabetes was diagnosed according to the criteria of the American Diabetes Association (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus ADA 1998). Patients were considered diabetic if diabetes was previously known or was being treated with oral anti-diabetic medications and/or insulin. As controls, 192 healthy subjects consisting of blood donors were recruited from the same area as the patients (central Tunisia). Complete clinical history, including stroke risk factors, was taken for all participants. Controls with hyperlipidemia, cancer, history of diabetes, or severe obesity were excluded. The study sample consisted of serum for lipid profile assessment. Total serum cholesterol, HDL cholesterol, and triglycerides were measured by enzymatic methods. Low-density lipoprotein cholesterol levels were calculated according to the Friedewald formula (Friedewald et al. 1972). Hypertension was defined as systolic pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg in at least two separate measurements, or in the case of a hypertension history. Obesity was defined as BMI ≥30 kg/m^2. Subjects with plasma glucose ≥6.95 mmol/l in the fasting state or those receiving oral anti-diabetic drugs or insulin were defined as affected with diabetes. Hypercholesterolemia was considered present if total cholesterol serum levels were ≥5 mmol/l or if the subject was undergoing a treatment with cholesterol-lowering drugs.

PPARδ Genotyping

Genotyping of the PPARδ +294T > C polymorphism was performed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) using the restriction enzyme, BslI (10/μl; Nikitin et al. 2010). The PCR was performed with 100 ng genomic DNA. PCR amplification was performed using 5′-CATGGTATAGCACTGCAGGA A-3′ and 5′-CTTCCTCCTGTGGCTGCTC-3′ as the forward and reverse primer pairs, respectively. After electrophoresis on a 2.0 % agarose gel with 0.5 μg/mL ethidium bromide, the amplification products were visualized under ultraviolet light. After restriction enzyme digestion of the amplified DNA, the genotypes were identified by electrophoresis on 3 % agarose gel and visualized with ethidium bromide staining ultraviolet illumination. The PCR produced a 269-bp fragment including one Bsl I recognition site for the C allele. The C allele can be cleaved into a 167- and 102-bp fragment whereas the normal allele cannot be digested.

Statistical Analysis

Statistical analysis was performed on SPSS v.17.0 software. Data were expressed as mean±SD (continuous variables) or as percentages of total (categorical variables). The differences of anthropometric and biochemical parameters among the diabetic ischemic stroke patients, nondiabetic ischemic stroke patients and healthy controls were examined by unpaired Student's *t* test. A p value <0.05 was considered to be statistically significant. Genotype frequencies and Hardy–Weinberg equilibrium were compared by chi-squared test. The association between the +294T/C polymorphism and plasma lipids and lipoproteins and BMI was calculated using the Student *t* test with genotype as the group variable and BMI, total cholesterol, TG, LDL-C, and HDL-C as dependent variables. Comparisons between groups >2 were performed by one-way analysis of variance (ANOVA). To prevent generating subgroups too small for analysis, allelic variants were dichotomized in TT and TC/CC.

Results

Clinical and Biological Characteristics of the Study Population

Clinical characteristics of ischemic stroke patients and control groups are shown in Table 1. There were no differences in the mean age, BMI, and sex between the patients with or without diabetes and control groups. The results presented were obtained from the analysis of the whole population (men and women). All comparisons were re-done after dichotomization of men, and no differences in the results were revealed (data not shown).

Systolic blood pressure (SBP; $p<0.001$), diastolic blood pressure (DBP ($p<0.001$), glucose ($p<0.001$), and TG (triglycerides) level ($p=0.004$) were found to be higher in nondiabetic Ischemic stroke patient group than the controls, while HDL-C level ($p=0.55$), LDL-C level ($p=0.94$), and total cholesterol ($p=0.54$) were not significantly different

Table 1 Clinical and biological characteristics of the study population

Characteristics	Controls (n=192)	Groups		P values		
		DM(+) I stroke (n=117)	DM(−) I stroke (n=79)	P1	P2	P3
Age (years) mean±SD	61.69±7.0	64.23±12.31	62.46±10.02	0.20	0.47	0.22
Gender (male/female) [n%]	110/82 (57.29 %/42.70 %)	63/54 (53.84 %/46.15 %)	39/40 (49.36 %/50.63 %)	0.55	0.28	0.31
BMI (kg/m^2) mean±SD	26.29±4.28	25.74±4.05	26.19±3.34	0.29	0.86	0.84
Current smokers	(22) 11.45 %	33(28.20 %)	(15)18.98 %	0.031	0.55	0.72
Systolic blood pressure (mmHg)	123.20±12.78	153.19±29.29	162.37±34.98	<0.001	<0.001	<0.001
Diastolic blood pressure (mmHg)	77.88±7.06	89.08±15.5	84.74±16.28	<0.001	<0.001	<0.001
Systemic hypertension	0(0 %)	73(62.39 %)	62(78.48 %)	<0.001	<0.001	<0.001
Total cholesterol (mmol/l)	4.46±0.79	4.61±1.15	4.53±1.16	0.17	0.54	0.88
Triglycerides (mmol/l)	1.15±0.51	1.32±0.61	1.37±0.68	0.009	0.004	0.035
HDL cholesterol (mmol/l)	1.14±0.34	1.05±0.33	1.11±0.43	0.02	0.55	0.94
LDL cholesterol (mmol/l)	2.78±0.75	2.95±1.19	2.78±1.20	0.12	0.94	0.56
Glucose (mmol/l)	4.91±0.78	9.07±3.89	6.01±1.73	<0.001	<0.001	<0.001

Data are reported as means±SD or as number with percent in parentheses

DM(+) I stroke ischemic stroke patients with diabetes, *DM(−) I stroke* ischemic stroke patients without diabetes, *P1* control versus DM(+)I stroke, *P2* control versus DM(−) I stroke, *P3* DM(+)I stroke versus DM(−) I stroke, *BMI* body mass index, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol

(p>0.05). The diabetic ischemic patient group compared with controls, had a higher prevalence of conventional risk factors for stroke, including high levels of SBP, DBP, TG, smoking glucose, and lower level of HDL (p<0.05). On the other hand, LDL-C levels, total cholesterol were not significantly different (p>0.05).

There were no differences in the mean age, BMI and sex between the diabetic ischemic stroke patient group and the nondiabetic ischemic stroke patient group. Total cholesterol, LDL-C and HDL-C were not significantly different (p>0.05). TG was found to be higher in the nondiabetic ischemic stroke patient group than in the diabetic Ischemic patient group (p=0.035) while glucose was found to be higher in the diabetic ischemic stroke patient group than in the nondiabetic ischemic patient one (p<0.001; Table 1).

PPARδ +294T/C Genotype in Study Population

To assess whether this polymorphism had any effect on lipid and metabolic parameters, the baseline characteristics among the genotype groups in controls and in patients with or without diabetes were compared (Table 2). Plasma concentrations of total cholesterol, TG, LDL-C, and HDL-C did not differ significantly between subjects carrying the TT genotype and those carrying the CC/TC genotype in both ischemic stroke patients (with or without diabetes) and control groups (p 0.05). The effects of PPARδ polymorphism on serum lipid levels in ischemic stroke patients with and without diabetes mellitus in different sex groups were investigated and revealed no differences in the results (data not shown).

Body Mass Index

BMI was not different between subjects carrying the TT genotype and those with the CC/TC genotype in both ischemic stroke patients (with or without diabetes) and in control groups (p>0.05; Table 2).

PPARδ +294T/C Genotype Distribution

The distribution of genotypes in both ischemic stroke (diabetic and nondiabetic) and control groups satisfied the Hardy–Weinberg equilibrium. The frequencies of PPARD + 294TT, CC/TC genotypes were 0.564 and 0.435, respectively, among the diabetic ischemic stroke patients, 0.481 and 0.518, respectively among the nondiabetic ischemic stroke patients and 0.530 and 0.469, respectively among the control subjects. As shown in Table 3, genotype frequencies were significantly different between total ischemic stroke patients and controls. Indeed, the TC/CC genotype was significantly more frequent in total cases (diabetics and nondiabetics) compared with controls (p=0.007). The +294 C allele (CC + CT genotypes) as compared with TT genotypes was found to be higher in total ischemic stroke patient than in controls. The risk of stroke in subjects (with and without diabetes) carrying either one or two copies of the C allele was OR=1.76 (95 % CI=1.17–2.66). On the other hand, no interaction between diabetes and PPAR +294T/C polymorphism on

Table 2 Lipid and lipoprotein concentrations and BMI according to the PPAR δ +294T/C genotype

Parameter	DM(+) I stroke (n=117)		DM(−) I stroke (n=79)		Controls (n=192)		P values		
	TT	TC/CC	TT	TC/CC	TT	TC/CC	P1	P2	P3
TC (mM)	4.53±1.27	4.71±0.96	4.35±0.99	4.71±1.28	4.42±0.76	4.54±0.8	0.53	0.40	0.54
HDL-C (mM)	1.02±0.34	1.10±0.32	1.07±0.34	1.15±0.51	1.17±0.35	1.08±0.31	0.72	0.78	0.30
LDL-C (mM)	2.91±1.32	3±1.00	2.64±0.98	2.89±1.37	2.72±0.71	2.91±0.82	0.61	0.87	0.49
TG (mM)	1.31±0.67	1.32±0.53	1.26±0.65	1.46±0.70	1.12±0.50	1.19±0.54	0.15	0.20	0.13
BMI (kg/m^2)	25.23±2.57	26.47±5.47	26.77±3.54	25.66±3.11	26.13±3.98	26.58±4.80	0.46	0.51	0.65

P1 control versus DM(+) I stroke, *P2* control versus DM(−) I stroke, *P3* DM(+) I stroke versus DM(−) I stroke

the risk of ischemic stroke was found (p=0.089). While, there was an interaction between nondiabetic subjects and PPAR +294T/C polymorphism (p=0.008; Table 3).

Table 4 summarizes the distribution of +294T/C genotypes in stroke subtype (TOAST criteria). We also found no differences in the distribution of +294T/C genotypes among lacunar, atherosclerotic and cardio embolic stroke subtypes (p=0.22; Table 4).

Discussion

PPARδ is an ubiquitously expressed nuclear receptor that has been implicated in adipose tissue formation, brain development, placental function, wound healing, and atherosclerosis (Peters et al. 2000; Vosper et al. 2001; Barak et al. 2002; Michalik et al. 2001; Shi et al. 2002). It plays a role in lipid metabolism by stimulating fatty acid oxidation in the heart and the skeletal muscle cells (Gilde et al. 2003; Yilmaz-Aydogan et al. 2011). Newly developed synthetic ligands and genetically modified mouse models for PPARδ have rapidly advanced our understanding of the important roles of PPARδ in tissue development and repair and angiogenesis (Piqueras et al. 2007) and inflammation and metabolism (Leibowitz et al. 2000; Tan et al. 2001; Dressel et al. 2003; Tan et al. 2004).

T294C polymorphism (rs2016520) has been identified at position +294 in the 5′-UTR exon 4 of the PPARδ gene influencing the expression of PPARδ by changing the sequence of the DNA binding site for transcription factor Sp-1. Transient transfection assays revealed that the +294C allele has higher transcription activity suggesting a greater PPARδ activity (Skogsberg et al. 2003a).

To investigate the relationship between the genetic polymorphism of PPARδ and the risk of ischemic stroke and to explore whether this genetic polymorphism of PPARδ would modify the risk of ischemic stroke among patients with diabetes, we chose to investigate in a case–control study and for the first time, a group of ischemic stroke patients with and without Diabetes. This study is the first one conducted on the Tunisian population that shows the association between PPARδ +294C gene polymorphism and the risk of ischemic stroke. In this study, individual allele and genotype frequencies of PPARδ +294T/C polymorphism were significantly different between the study groups.

The results of the effects of PPARδ +294T/C polymorphism on the distribution of lipid/lipoprotein profiles are controversial. Our results showed that the presence of the C-allele had no effect on total cholesterol, TG, HDL-C, and LDL-C levels, both in cases (diabetics and nondiabetics) and controls. In addition, there was no association between the polymorphism and BMI both in males and females. These results were in accordance with Gouni-Berthold et al. (2005) both in diabetic and nondiabetic German controls and with Grarup et al. (2007) in which gender was not considered separately. A study conducted by our laboratory

Table 3 The distribution of PPAR δ +294T/C genotype in the study groups

PPARδ +294T/C genotypes	DM(+) I stroke (n=117)	DM(−) I stroke (n=79)	Controls (n=192)	Total patient (n=196)	P values			
					P1	P2	P3	P4
TT	66 (56.41 %)	38 (48.10 %)	128 (66.66 %)	104 (53.06 %)	0.089	0.008	0.30	0.007
TC/CC	51 (43.58 %)	41 (51.89 %)	64 (33.33 %)	92 (46.93 %)				

DM(+) I stroke ischemic stroke patients with diabetes, *DM(−) I stroke* ischemic stroke patients without diabetes, *P1* control versus DM(+) I stroke, *P2* control versus DM(−) I stroke, *P3* DM(+) I stroke versus DM(−) I stroke, *P4* controls versus total patient

Table 4 Distribution of PPAR δ T/C genotype with various subgroups of stroke

Stroke subtype	CC/CT	TT	P[a]
Lacunar	62[b] (67.39 %)	84 (80.76 %)	0.22
Atherothrombotic	3 (4.83 %)	3 (2.88 %)	
Cardioembolic	3 (4.83 %)	4 (3.84 %)	
Others	26 (28.26 %)	11 (10.57 %)	

[a] One-way ANOVA

[b] Number of subjects (percent of total)

had shown that the presence of the C allele had no effect on total cholesterol, TG, HDL-C, LDL-C, and BMI levels, both in CAD patient and in control groups (Jguirim-Souissi et al. 2010). There were no significant differences in the levels of TC, LDL-C and ApoB, and the genotypic and allelic frequencies of PPARδ +294T>C between nondrinkers and drinkers (Wei et al. 2011).

Raquel Villegas et al. (2011) found also no interaction between this gene and BMI in middle age Chinese women. Whereas other studies showed that the PPARδ +294T/C polymorphism was associated with modifications of serum lipid concentrations. In Swedish men, Homozygotes for the rare C allele had higher plasma LDL-C concentrations than homozygotes for the common T allele, while there were no associations with the HDL-C levels (Skogsberg et al. 2003a). Interestingly, the same group of investigators showed in another study in Scottish men from the WOSCOPS trial that the polymorphism had no effects on LDL-C concentrations but was associated with lower HDL-C levels ($p=0.049$; Skogsberg et al. 2003b). In another study of Aberle et al. (2006b), it was suggested that PPARδ had an involvement in the regulation of BMI and showed an association of the C-allele with plasma HDL-C concentrations in dyslipidemic women. The same group reported a trend toward higher VLDL-C and LDL-C levels in female +294C allele carriers. In addition, the same investigators also showed that this polymorphism was associated with lipid profiles in CHD females (Aberle et al. 2006a). In another study, it was demonstrated that female patients with metabolic syndrome (MS) and carrying the +294C allele had higher plasma HDL-C levels and lower total cholesterol/HDL-C ratio than those with homozygote for the common T allele. However, there was no association between PPARδ +294T/C and serum lipoprotein levels in males (Robitaille et al. 2007).

Another study was conducted in Russian population and it showed a relationship between the +294C allele and increased LDL-C levels in controls (Nikitin et al. 2010). This result was in accordance with Yilmaz-Aydogan et al. (2012) who reported that the PPARδ +294C allele was associated with increased serum LDL-C levels in nondiabetic CHD patients. The PPARδ +294C allele was also reported to be associated with body mass index, HDL-C, and was dependent on gender (Burch et al. 2010).

In the current study, we have reported for the first time that for the whole ischemic patient group (diabetics and nondiabetics), subjects carrying either one or two copies of the C allele had a 1.76-fold higher risk of stroke than subjects homozygous for T allele. The frequency of the CC/CT genotypes was found to be higher as compared with TT genotype in total ischemic stroke patients than in controls, with no differences between diabetics and nondiabetic patients. The effects of PPARδ variants on type 2 diabetes have been widely investigated; however, the results were inconsistent. Our results are consistent with a previous study of Koreans that sequenced the PPARδ gene and found no association between variants and T2D (Shin et al. 2004) and another study of 7495 middle age white people (Grarup et al. 2007). Similar to our results, no association between this polymorphism and T2D has been observed in a cross-sectional study of 402 cases and 436 controls (Gouni-Berthold et al. 2005) and no main gene effect was found for PPARδ with T2D among middle age Chinese women (Villegas et al. 2011). However, the PPARδ +294C allele was associated to higher fasting glucose in nondiabetic Korean subjects (Shin et al. 2004). In another study conducted in Shanghai, this polymorphism was associated with fasting glucose and insulin resistance in both normoglucose tolerant and diabetic Chinese subjects (Hu et al. 2006). These findings suggested a contribution of PPARδ to the risk of type 2 diabetes and related metabolic traits. Lu et al. (2012) found a marginal association of PPARδ-rs2016520 variant with combined IFG (impaired fasting glucose/type 2 diabetes), but not with the risk of type 2 diabetes and related traits.

In our study, we also found no differences in the distribution of +294T/C genotypes among lacunar, atherosclerotic, and cardio embolic stroke subtypes ($p=0.22$). Our findings suggest that the presence of the C-allele is an independent risk factor for ischemic stroke. Many factors such as population heterogeneity, ethnic stratification, population-specific linkage disequilibrium between markers and causal variants, sample size, variation in study design, confounding sampling bias, misclassification of phenotypes, and gene–gene and gene–environment interactions may contribute to conflicting results regarding associations (Cardon and Palmer. 2003; Colhoun et al. 2003). Our study suggests that the C allele is a risk factor for ischemic stroke and the effect of the polymorphism on stroke may be mediated through a mechanism other than lipid metabolism. It could be postulated that this PPARδ variant expresses variable effects in different study groups based on a variable interaction with other genetic and environmental factors or possibly through mechanisms involving inflammation.

Conclusions

Our study concluded that even the little sample size of our population, there was a relationship between PPARδ + 294T/C genotype and the risk of ischemic stroke. This effect was not mediated in our population through plasma lipid and lipoprotein levels. On the other hand, there was no significant joint effect of the PPARδ +294T/C polymorphism and diabetes on the risk of ischemic stroke. Authors hope that this study could provide a new approach for the study of ischemic stroke and merit further research.

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