Gene 560 (2015) 200–204

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

PPARγ and IL-6 −174G>C gene variants in Croatian patients with ischemic stroke

A. Bazina [a], J. Sertić [b,c,*], A. Mišmaš [a], T. Lovrić [b], Z. Poljaković [a,b], D. Miličić [b,d]

[a] University Hospital Centre Zagreb, Department of Neurology, Neurological Intensive Care Unit, Zagreb, Croatia
[b] University of Zagreb School of Medicine, Zagreb, Croatia
[c] University Hospital Centre Zagreb, Department of Laboratory Diagnosis, Clinical Unit of Molecular Diagnosis, Zagreb, Croatia
[d] University Hospital Centre Zagreb, Clinic for Cardiovascular Diseases, Zagreb, Croatia

ARTICLE INFO

Article history:
Received 3 January 2015
Received in revised form 24 January 2015
Accepted 3 February 2015
Available online 7 February 2015

Keywords:
Ischemic stroke
Genes
Polymorphisms
Adults

ABSTRACT

Aim: Etiology of ischemic stroke (IS) is multifactorial and includes interaction of genetic and environmental factors. Different genes, their polymorphisms, host susceptibility, and inflammation processes play a role in IS development. The aim of this study was to evaluate the effect of *PPAR-γ* and *IL-6* gene variants on IS onset.
Material and methods: A total of 301 subjects (144 males, 157 females) participated in the study, 114 patients with IS and 187 healthy controls.
Results: Statistically significant predictors of IS were male gender (OR 7.13, 95% CI 2.92–17.39, $p < 0.001$), hypertension (OR 7.82. 95% CI 2.53–24.19, $p < 0.001$), lowered HDL cholesterol (OR 8.20, 95% CI 2.41–27.94, $p = 0.001$), elevated C-reactive protein (OR 5.26, 95% CI 1.92–14.41) and *IL-6* −174 GC (OR 2.44 95% CI 1.01–5.91, $p = 0.0048$) genotype. Males, compared to females, had 7 times higher odds for stroke. *IL6* −174G/C genotype increased the odds for IS for 2.4 times. PPARγ was not statistically significantly associated with stroke.
Conclusion: We can point to the *IL-6* −174G>C polymorphisms as candidate gene marker and risk factor for the prediction of ischemic stroke.

1. Introduction

According to the World Health Organization, stroke is a leading cause of disability and the third leading cause of death in the developed world, especially in the elderly population. About 85% of all strokes are ischemic in origin (Thrift et al., 2014). Etiology of ischemic stroke (IS) is multifactorial and includes interaction among genetic and environmental factors (Gao et al., 2006). Several different genes, their polymorphisms, host susceptibility, and inflammation processes play an important role in cerebrovascular disease (CVD) and IS development (Drake et al., 2011; Larsson et al., 2011; Ye et al., 2012). Evidence has shown that inflammatory processes and modifiable risk factors, including hypertension, smoking, physical activity, diabetes, hyperlipidemia, interact with genetic factors and contribute to stroke development (Humphries and Morgan, 2004). Literature data point to gene variants which can be associated with stroke (Traylor et al., 2012). Peroxisome proliferator activated receptor-γ (PPARγ) and interleukin-6 (IL-6) are the two important proteins that play a significant role in different processes and their gene variability could be considered as a predictive genetic marker for IS. PPARs (PPAR-α, PPAR-δ and PPAR-γ) are transcription factors that belong to the nuclear hormone receptor superfamily and regulate various genes involved in inflammation, glucose and lipid metabolism, adipogenesis, carcinogenesis, etc. (Sharma and Staels, 2007; Paracchini et al., 2005). *PPARγ* Pro12Ala variant (rs1801282) can modulate transcriptional activity and has a reduced affinity for the response element in target genes, which can result in less efficient stimulation of PPARγ target genes (He, 2009). Interleukin-6 is an inflammatory cytokine derived from diverse tissues that plays an important part in the acute inflammatory response and is a major inducer of hepatic fibrinogen and C-reactive protein (CRP) synthesis (Marso et al., 2006). Three variants have been identified in the *IL-6* gene promoter, of which −174G>C has been most widely studied since it affects the IL-6 transcription (Curti et al., 2011), and have been found associated with cytokine and metabolic modulation, impaired glucose and lipid homeostasis (Stephens et al., 2007). We hypothesized that the polymorphisms of this two analyzed genes: *PPAR-γ* and *IL-6* alone or in interaction with high level of acute inflammation phase protein CRP and in interaction with other modifiable risk factors might confer a higher

Abbreviations: IS, ischemic stroke; CVD, cerebrovascular disease; CRP, C-reactive protein; BMI, body mass index; PPARγ, peroxisome proliferator activated receptor-gamma; IL-6, interleukin-6; CT, computed tomography; DM, diabetes mellitus; TG, triglycerides; LDL, low density lipoprotein; HDL, high density lipoprotein; PCR, polymerase chain reaction.

* Corresponding author at: University of Zagreb, School of Medicine, Department of Medical Chemistry, Biochemistry and Clinical Chemistry, Šalata 3, 10 000 Zagreb, Croatia.
E-mail addresses: antonelabazina@gmail.com (A. Bazina), jadranka.sertic@kbc-zagreb.hr (J. Sertić), antonija.mismas@gmail.com (A. Mišmaš), tena.lovric@vip.hr (T. Lovrić), zdravka.po@gmail.com (Z. Poljaković), davor.milicic@mef.hr, davor.milicic@kbc-zagreb.hr (D. Miličić).

http://dx.doi.org/10.1016/j.gene.2015.02.003
0378-1119/

stroke risk than a single susceptibility gene. Based on this hypothesis, the aim of the present study was to evaluate the effect of *PPAR-γ* and *IL-6* variants on IS development.

2. Patients and method

This study was conducted at the University Hospital Centre Zagreb, Department of Neurology and Department of Medical Chemistry, Biochemistry and Clinical Chemistry, School of Medicine University of Zagreb, in a period between January 1, 2008 and January 1, 2012. A total of 301 subjects (144 males, 157 females) participated in the study, 114 patients with IS and 187 healthy controls. The study group was selected among patients admitted to our hospital under clinical presentation of IS, fulfilling the following enrollment criteria: 1) age 18–65 years and 2) computed tomography (CT) proven ischemic cerebral infarction. Exclusion criteria were: 1) diabetes mellitus (DM) and atrial fibrillation (AF) in earlier medical history, 2) hemorrhagic cerebral infarction on CT scan, and 3) age >65. Ischemic stroke was defined as a sudden loss of global or focal cerebral function persisted for >24 h. One hundred and eighty-seven subjects from the staff of our hospital with no known history of vascular disease were matched to the study group by sex and age and served as healthy controls. Both; the study and the control group were the same race and from the same geographic area and of the same social status. Blood samples for biochemical analyses (total cholesterol, triglycerides—TG, low-density lipoprotein—LDL, high-density lipoprotein—HDL, CRP) were collected after overnight fasting and analyzed by using routine laboratory methods. The following modifiable risk factors in the patient group were assessed: hypertension, hyperlipidemia, overweight, cigarette smoking and physical activity. In the case of participants' use of medications, corrections of medicated traits were made (Kraja et al., 2006). These variables were defined as follows: hypertension (systolic blood pressure ≥140 mm Hg and diastolic pressure ≥90 mm Hg in two separate measurements after the acute phase or use of an antihypertensive drug before recruitment); hyperlipidemia (cholesterol serum levels ≥5.0 mmol/L, LDL levels ≥3.0 mmol/L, TG levels ≥1.7 mmol/L, lowered HDL levels male <1.0; female <1.2 mmol/L or use of an antilipemic drug before recruitment); high CRP level >5.0 mg/L; overweight (high body mass index (BMI) >25 kg/m^2) (Wenger, 2014); current smoking habit (active smokers for past five years, including former smokers who had quit smoking 6 months before the study); physical activity: less than 30 min of daily activity 3–4 times a week (jogging, fast walking, cycling) (Wenger, 2014). Data about family history on stroke (stroke in first-degree relatives aged <55 years) were collected from each participant. Body mass index (BMI) was calculated as weight (kg) / height (m^2). All participants signed informed consent forms, and the study protocol was approved by the Ethics Committee of the University Hospital Center Zagreb.

2.1. Statistical data analysis

Departure of Hardy–Weinberg equilibrium was tested using the Markov chain method, implemented in Arlequin (Guo and Thompson, 1992; Excoffier and Lischer, 2010). Normality of distribution of continuous variables was tested by Kolmogorov Smirnov test. Median and interquartile ranges were used as measures of central tendency and variability since the distribution of age statistically significantly deviated from the normal one. Differences in medians for this continuous variable between two independent categories of nominal variables were tested using Mann–Whitney test, with AUC given as the standardized measure of size effect. AUC was calculated according to the formula: U / (m * n), where U is the result of the Mann–Whitney test, m and n are sizes of two samples. Differences between nominal variables were analyzed by Chi-square test, with Cramer's V as the standardized measure of size effect. The level of significance was set to 5% ($p < 0.05$), and all confidence intervals were given at the 95% level. Multivariate associations were tested by binary logistic regressions. All the analyses were carried out using SPSS 17.0 (SPSS Inc., Chicago, IL, USA) statistical software package.

2.2. Genotyping procedures

Genomic DNA was extracted from leukocytes using the salting out procedure (Miller et al., 1988). Genotyping of *PPARγ* Pro12Ala and *IL6* −174G>C was performed according to previously published methods. DNA was amplified in a 25 μL reaction volume by using polymerase chain reaction based methods (PCR) in a GeneAmp PCR System 9700 (Applied Biosystems, USA) (Božina et al., 2014; Sertic et al., 2009).

3. Results

3.1. Participants

Characteristics of study participants are given in Table 1. Compared to controls, patients were significantly older, had higher BMI, blood pressure, and TG level, lower HDL, and elevated CRP. Also, a higher

Table 1
Gender, age, and clinical and biochemical parameters by study groups.

n (%)	Group				P	ϕ
	Patients		Control			
Gender						
Male	79	(69.3)	70	(37.4)	<0.001	0.31
Female	35	(30.7)	117	(62.6)		
Total	114	(100.0)	187	(100.0)		
Age; median (IQR)	54	(51–57)	55	(50–61)	0.022*	
Body mass index						
Overweight (>25 kg/m^2)	69	(60.5)	69	(36.9)	<0.001	0.23
Not overweight	45	(39.5)	118	(63.1)		
Total	114	(100.0)	187	(100.0)		
Family history						
None	81	(71.1)	169	(90.4)	<0.001	0.25†
CVI	16	(14.0)	7	(3.7)		
Other heart disease	17	(14.9)	11	(5.9)		
Total	114	(100.0)	187	(100.0)		
Smoking						
Yes	37	(32.5)	38	(20.3)	0.492	
No	77	(67.5)	149	(79.7)		
Total	114	(100.0)	187	(100.0)		
Hypertension						
Yes	60	(52.6)	38	(20.3)	<0.001	0.33
No	54	(47.4)	149	(79.7)		
Total	114	(100.0)	187	(100.0)		
Total cholesterol						
Elevated (>5.0 mmol/L)	64	(56.6)	126	(70.4)	0.017	0.14
Normal	49	(43.4)	53	(29.6)		
Total	114	(100.0)	187	(100.0)		
LDL cholesterol						
Elevated (>3.0 mmol/L)	48	(43.6)	113	(64.8)	0.001	0.21
Normal	62	(56.4)	63	(35.2)		
Total	110	(100.0)	179	(100.0)		
Triglycerides						
Elevated (>1.7 mmol/L)	34	(30.9)	30	(16.8)	0.006	0.17
Normal	76	(69.1)	149	(83.2)		
Total	110	(100.0)	179	(100.0)		
HDL cholesterol						
Lowered (male <1.0; female <1.2 mmol/L)	40	(36.0)	11	(6.1)	<0.001	0.38
Normal	71	(64.0)	168	(93.9)		
Total	111	(100.0)	179	(100.0)		
C-reactive protein						
Elevated (>5.0 mg/L)	57	(50.0)	12	(10.5)	<0.001	0.43
Normal	57	(50.0)	102	(89.5)		
Total	114	(100.0)	114	(100.0)		

Abbrevations: P = two-tailed exact test of statistical significance; ϕ = Phi coefficient of association given as the standardized effect size only for statistically significant differences between patients and control group; IQR = interquartile range.

* Mann–Whitney *U* test.

† Contingency coefficient C.