Gene 539 (2014) 258–262

Contents lists available at ScienceDirect

Gene

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

Association of IL-6 − 174G > C and − 572C > G polymorphisms with risk of young ischemic stroke patients

Yang Xuan [a,*], Feng Lei [b], Li Changjiang [c], Li Yu [d]

[a] Department of Cardiology, Qingdao Municipal Hospital, Qingdao 266000, China
[b] Diagnostic Imaging Division, Qingdao Municipal Hospital, Qingdao 266000, China
[c] Intensive Care Unit, Qingdao Central Hospital, Qingdao 266000, China
[d] Zibo Municipal Hospital Authorities Internal Medicine, Zibo 255000, China

ARTICLE INFO

Article history:
Accepted 20 January 2014
Available online 29 January 2014

Keywords:
Interleukin-6
Ischemic stroke
Polymorphism
Risk

ABSTRACT

Aim: To investigate the association between interleukin-6 (IL-6) − 174G > C and − 572C > G polymorphisms and risk for ischemic stroke (IS) in young patients.
Methods: We genotyped IL-6 − 174G > C and − 572C > G in a case–control study of 430 young IS patients and 461 control subjects. An unconditional multiple logistical regression model was used to calculate the effects of IL-6 − 174G > C and − 572C > G polymorphisms on IS risk.
Results: Higher body mass index, diabetes, hypertension, obesity, and smoking were associated with risk of ischemic stroke. Multivariate regression analyses showed that subjects carrying the − 174CC genotype (OR = 1.69, 95% CI = 1.16–2.57) and C allele (OR = 1.37, 95% CI = 1.09–1.67) had a small but significant increased risk of IS. Similarly, those carrying the − 572GG genotype (OR = 2.12, 95% CI = 1.18–3.82) and G allele (OR = 1.43, 95% CI = 1.14–1.83) had a moderate increased risk of IS. We found the − 174G > C and − 572C > G polymorphisms interact with hypertension and obesity.
Conclusion: Our results suggest that polymorphisms in IL-6 − 174G > C and − 572C > G are associated with IS risk in young patients, and that these polymorphisms interact with hypertension, obesity and etiologic subtypes. These findings could be helpful in identifying individuals at increased risk for developing IS.

1. Introduction

Stroke is a common cerebrovascular disorder and has become one of the leading causes of death and disability worldwide. There are an estimated 15 million new cases of ischemic stroke (IS) each year worldwide, and among these patients, 5 million die and 5 million become permanently disabled (Ziganshina et al., 2010). IS accounts for 50% of strokes, and risk for it is greatly influenced by an individual's genetic background and various environmental factors (Dichgans and Markus, 2005; Venti et al., 2002).

Mounting evidence indicates that inflammatory processes play an important role in the development of IS, but the exact mechanism is still unclear (Lindsberg and Grau, 2003; van der Spuy and Pretorius, 2012). Pro-inflammatory cytokines are associated with IS, and variations in cytokine genes play an important role in altering the transcription profile to induce predisposition and penetrance and change the pattern of proinflammatory cytokine production (Hollegaard and Bidwell, 2006). Functional polymorphisms of inflammatory genes may thereby influence the incidence and outcome of IS.

Interleukin-6 (IL-6), a proinflammatory and immunoregulatory cytokine found in diverse tissues, such as fibroblasts, monocytes, adipocytes, and endothelial cells, plays a multifaceted role in the genesis and maintenance of the inflammatory response (Smith et al., 2004). The functional promoter polymorphism of the IL-6 gene seems to be associated with different levels of secreted protein, depending on the genotype. Two functional polymorphisms, − 174G > C (rs1800795) and − 572C > G (rs1800796), have been identified in the IL-6 promoter region, and the two genetic variants may influence inflammatory processes by altering gene regulation and protein expression (Morgan et al., 2006). Several recent studies have indicated that variants of the IL-6 gene are associated with risk for various cancers and autoimmune diseases (Gao et al., 2009; Gu et al., 2008; Xue et al., 2009). However, different meta-analyses have reported conflicting results regarding the association between variations of IL-6 and IS risk (Ma et al., 2011; Yin et al., 2013). We conducted a case–control study to investigate the association between IL-6 − 174G > C and − 572C > G polymorphisms and risk of ischemic stroke.

Abbreviations: IL-6, interleukin-6; IS, ischemic stroke; EDTA, ethylenediaminetetraacetic acid; DNA, deoxyribonucleic acid; PCR, polymerase chain reaction; CI, confidence interval; 95% OR, odds ratio; SD, standard deviation; HWE, Hardy–Weinberg equilibrium; BMI, body mass index; TC, total cholesterol; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; SPSS, Statistical Package for Social Science; TOAST classification, Acute Stroke Treatment classification.

* Corresponding author. Tel./fax: +86 13675328528.
E-mail address: yangxuan_qdh@163.com (X. Yang).

0378-1119/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.gene.2014.01.054

2. Materials and methods

2.1. Study population

We recruited Chinese patients with the first onset of IS from Qingdao Municipal Hospital between January 2011 and December 2012. All patients were confirmed by neuroimaging evidence with CT or MRI according to the World Health Organization's diagnostic criteria for IS. The subtype classification for the etiology of IS was based on the TOAST criteria (Johnson et al., 1995).

Patients with transient ischemic attacks, intracranial hemorrhage, postseizure palsy, brain tumors, or brain trauma were excluded from the study. Control subjects were recruited among patients seeking routine check-ups at the same hospital during the same time period as the cases. All patients and controls signed written informed consents. Our study was approved by the ethics committee of Qingdao Municipal Hospital.

A structured questionnaire was used to collect general information on the patients and control subjects, including age, sex, smoking and drinking habits, diabetes, and obesity.

2.2. Genotyping assays

Peripheral venous blood samples were drawn from each participant, and genomic DNA was prepared from 2 mL EDTA anticoagulant tubes. Genomic DNA was extracted using a TIANamp blood DNA kit (Tiangen Biotech, China). Genotyping of −174G > C and −572C > G was performed in a 384-well plate format on the Sequenom MassARRAY platform (Sequenom, San Diego, USA). Primers and probes of −174G > C and −572C > G were designed by Sequenom Assay Design 3.1 software (Sequenom, San Diego, CA, USA) (Table 1). Each PCR reaction (20 μL) contained 200 ng of DNA template, 200 μM dNTP, 1 unit of Taq DNA polymerase, and 200 μM primers as well as 1.5 mM $MgCl_2$. The amplification cycles consisted of 1 min at 98 °C for 3 min to activate Taq polymerase, 40 cycles of denaturation at 95 °C for 20 s, and annealing at 60 °C for 60 s. PCR products were verified by 1.0% agarose gel electrophoresis. Reproducibility was verified by repeat analysis of a randomly chosen subgroup of 10% of the subjects.

2.3. Statistical analysis

Statistical analysis was conducted using SPSS® version 11.0 (SPSS Inc., Chicago, IL, USA) for Windows®. Continuous and categorical variables were expressed as mean ± SD and *n* of subjects (%). Comparisons between cases and controls were made using the Student's t and χ^2 tests. The Hardy–Weinberg equilibriums of both groups were compared using the χ^2-test. Unconditional logistic regression was conducted to assess the effects of −174G > C and −572C > G polymorphism on IS risk after adjusting for potential confounding factors. The odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated to compare the proportions of the two SNPs in cases and controls. All *p*-values were two sided, and a *p*-value of <0.05 was considered statistically significant.

3. Results

Of the 457 IS patients screened, 430 were eligible and were included in the study, for a participation rate of 94.09%. For the control group, 502 people were screened and 461 recruited for the study, for a participation rate of 91.83%. There were 169 females and 261 males in the IS group, compared to 208 females and 253 males in the control group (Table 2). The average age of the IS patients was 45.4 ± 9.5, while the average age in the control group was 44.8 ± 10.1 years. IS patients had a significantly higher average BMI than did the control subjects ($p = 0.004$). IS patients also were more likely to have diabetes, hypertension, and obesity, and to smoke ($p < 0.05$). However, the levels of TG, TC, HDL-C, and LDL-C were not significantly different between the IS patient group and control group.

The genotype distributions of −174G > C in controls were in line with the Hardy–Weinberg equilibrium (HWE) ($p = 0.078$), but −572C > G deviated from HWE in that group (Table 3) (p value < 0.001). Genotype frequencies for −572C > G were significantly different between cases and controls ($\chi^2 = 6.91$, $p = 0.032$), while frequencies of −174G > C were not. Multivariate regression analyses showed that subjects carrying the −174CC genotype and C allele had a small but significant increased risk of IS, with adjusted ORs (95% CI) of 1.69 (1.16–2.57) and 1.37 (1.09–1.67), respectively. Similarly, we found that those carrying the −572GG genotype and G allele had a moderate increased risk of IS, with adjusted ORs (95% CIs) of 2.12 (1.18–3.82) and 1.43 (1.14–1.83), respectively.

In further analysis, carriers of −174G > C and −572C > G genotypes were classified into three subgroups based on demographic factors that could influence the risk of ischemic stroke (Table 4). The frequencies of the CC genotype of −174G > C and GG genotype of −572C > G were significantly higher in IS patients with hypertension than in those without (for −174G > C, $\chi^2 = 7.49$, $p = 0.024$; for −572C > G, $\chi 2 = 6.74$, $p = 0.034$). The frequencies of the CC genotype of −174G > C and GG genotype of −572C > G were significantly higher in patients with obesity than in those without (for −174G > C, $\chi^2 = 6.15$, $p = 0.046$; for −572C > G, $\chi^2 = 10.63$, $p = 0.005$). Similarly, the frequencies of the CC genotype of −174G > C and GG genotype of −572C > G were significantly higher in patients diagnosed as large-artery atherosclerosis when compared with those diagnosed as cardioembolism, small-vessel occlusion and stroke of undetermined etiology (for −174G > C, $\chi 2 = 14.85$, $p = 0.02$; for −572C > G, $\chi 2 = 15.28$, $p = 0.018$).

4. Discussion

IL-6, one of the most important mediators of inflammatory reactions associated with atherosclerotic disease, is likewise a key mediator of the inflammatory response to cerebral ischemia (Pola et al., 2003). One study found that the serum level of IL-6 was also significantly elevated in young patients with IS (Acalovschi et al., 2003). In this case–control study we analyzed the association between genetic polymorphisms of the IL-6 polymorphisms −174G > C and −572C > G and IS risk. Our main findings were that the −174 CC and −572 GG variants carried a significant increased risk of IS. The findings suggest that the functional promoter polymorphisms of −174G > C and −572C > G may be useful as a genetic susceptibility marker for ischemic stroke.

Ultimately, such genetic information could help in determining the IS's etiology and in identifying high-risk individuals who could be treated according to their individual genetic makeup. Many studies have examined the contribution of polymorphisms in genes for inflammatory cytokines to ischemic stroke, with consistent results. A previous study conducted in China reported that the IL-6 − 572C > G polymorphism

Table 1
Sequences of primers and probes used for polymerase chain reaction amplification of single nucleotide polymorphisms (SNPs).

	IL-6 − 174G > C (rs1800795)	IL-6 − 572G > C (rs1800796)
Probe 1	FAM-ACT TCG TGC ATG ACT TCA GC-MGB	FAM-GAGACGCCTTGAAGTAACTG–TAMRA
Probe 2	VIC-CTG ATT GGA ACC TTA TTA AG-MGB	VIC-AACCAAAGATGTTCTGAACTGA-TAMRA
Primer 1	5′-GGAGTCACACACTCCACCT-3′	5′-GGAGACGCCTTGAAGTAACTGC-3′
Primer 2	5′-CTGATTGGAAACCTTATTAAG-3′	5′-GAGTTTCCTCTGACTCCATCGCAG-3′