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Association of -1382A>G CCL11 gene variant with ischemic stroke, its subtypes and hemorrhagic stroke in a South Indian population

Sitara Roy[1], Satrupa Das[1], Anjana Munshi[2], Subhash Kaul[3], Akka Jyothy[4],

[1] Department of Molecular Biology, Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Begumpet; Dr. NTR University of Health Sciences, Vijaywada, Andhra Pradesh, India

[2] Department of Molecular Biology, Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Begumpet; Centre for Human Genetics, School of Health Sciences, Central University of Punjab, Bathinda, Punjab, India

[3] Nizams Institute of Medical Sciences, Punjagutta, Hyderabad, India

[4] Department of Molecular Biology, Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Begumpet, India

Correspondence Address:
Anjana Munshi
Centre for Human Genetics, School of Health Sciences, Central university of Punjab, Bathinda, Punjab
India

Abstract

Background: CCL11 (Eotaxin-1) is an important inflammatory cytokine belonging to the CC family of chemokines associated with a number of infection or inflammation-related diseases such as atherosclerosis and stroke. We investigated the association of CCL11 gene polymorphism rs4795895-1382A>G with ischemic and hemorrhagic stroke. **Materials and Methods:** Six hundred and twenty ischemic stroke patients, 620 age- and sex-matched healthy controls, and 220 hemorrhagic stroke patients, 220 age- and sex-matched healthy controls were included in the present study. The CCL11 gene polymorphism rs4795895-1382A>G was determined using PCR-RFLP technique. **Results:** We found a statistically significant difference in the genotypic distribution between ischemic stroke patients and controls (For GG vs. AA, χ^2 = 7.604; P < 0.001, Odds ratio = 2.793; 95% CI = 1.308-5.9). For GG vs. AA + AG, χ^2 = 44.8, P < 0.001, Odds ratio = 2.382 (95% CI = 1.842-3.081). A significant difference was observed in the frequency of G and A alleles in patients and controls (For G vs. A, χ^2 = 43.26; P < 0.001, Odds ratio = 2.127; 95% CI = 1.693-2.672). Statistically significant difference was observed in the genotypic distribution between hemorrhagic stroke patients and controls (For GG vs. AG, χ^2 = 26.78; P = 0.001, Odds ratio = 3.5; 95% CI = 2.162-5.824). A significant difference was observed in the frequency of G and A alleles in patients and controls (For G vs. A, χ^2 = 41.98; P = 0.001, Odds ratio = 4.1; 95% CI = 2.61-6.44). **Conclusion:** The results of the present study show that the GG genotype is a significant risk factor for ischemic as well as hemorrhagic stroke. Further, the frequency of the GG genotype was observed to be higher in hemorrhagic stroke patients in comparison with ischemic stroke. Evaluating the association with ischemic stroke subtypes, a significant association was observed with intracranial large artery atherosclerosis and lacunar stroke.

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Full Text

Introduction

Stroke is one of the most common causes of mortality and adult disability worldwide. [1] Commonly, stroke is a multi-factorial

disorder that is influenced by both genetic and environmental factors. [2],[3],[4],[5],[6],[7],[8],[9],[10],[11] In India, stroke is a major health problem and ischemic stroke is the most common type of stroke. [12],[13] Although the exact etiology and mechanisms of stroke have not been well understood, studies have confirmed that high blood pressure, poor diet, smoking, obesity, and lack of physical activities are common risk factors associated with 80% of all strokes. [14] However, these conventional risk factors do not completely account for the overall risk of stroke. In addition, genetic factors have been suggested to play a major role in the stroke risk. Several studies show that inflammatory processes play a major significant role in pathophysiology and etiology of stroke. [15] Atherosclerotic stroke is a result of chronic inflammation involving pathways of immune regulation, thrombosis, and cellular adhesion. [16],[17] Inflammatory mechanisms are also involved in the progression of intracerebral hemorrhage (ICH). After ICH, prominent inflammatory responses occur, which are characterized by the production of inflammatory mediators such as the cytokines. [18] Inflammatory molecules and gene variants coding for inflammatory mediators significantly contribute to the development and progress of cerebrovascular and cardiovascular diseases. [19] The present study investigates the association of CCL11gene polymorphism rs4795895-1382A>G with ischemic stroke, its sub-types and hemorrhagic stroke.

Eotaxin-1 also known as C-C motif chemokine 11 [CCL11] and eosinophil chemotactic protein is an inflammatory cytokine that is involved in the allergic response by selectively engaging eosinophils by inducing their chemotaxis. [20],[21],[22] Inflammatory chemokine CCL11 is involved in the formation and rupture of cerebral aneurysms. It plays a very significant role as chemoattractants for leukocytes directing them towards sites of tissue inflammation. [23] The human CCL11 gene is located on chromosome number 17q21.1-q21, spans approximately 8 kb and consists of 3 exons. CCL11 operates mainly through CCR3 a G-protein-coupled receptor with seven transmembrane spanning domains. [24],[25],[26],[27] CCR3 receptor is present on several cells including eosinophils, basophils, mast cells, dendritic cells, brain microglial cells, and endothelial cells. [25],[27],[28],[29], [30] Several CC chemokines have been identified in injured arteries and in the atherosclerotic plaque. [31] It has been observed that CCL11 antigen is found in smooth muscle cells of human atheroma [32] and its level is upregulated in the media of ischemic rat aorta. [33] Recent studies indicate that CCL11 induces chemotaxis of human microvascular endothelial cells [34] and plays a role in number of chronic inflammatory diseases. [35] Elevated plasma levels of CCL11 gene have been observed in patients with advanced atherosclerosis. [36] A single nucleotide change of adenine to guanine at 1382 position located in the 5′ flanking region of CCL11 gene was reported to be significantly associated with stroke in Chinese Hans population. [37] Until date, there are no reports on the association of this polymorphism with stroke in Indian patients. Therefore, the present study was carried out to investigate the association of CCL11 gene polymorphism rs4795895-1382 A>G with ischemic stroke, its subtypes, and hemorrhagic stroke in a South Indian population from Andhra Pradesh.

Materials and Methods

Six hundred and twenty ischemic stroke patients (males:females = 431:189) and 220 hemorrhagic stroke patients (males:females = 158:62) presenting with new stroke evaluated in the neurology department of Nizam's Institute of Medical Sciences, Hyderabad (A.P, India) between August 2007 and July 2013 were included in the study. The study was approved by the ethical committee of the study hospital and of the institution. All patients were examined by a qualified stroke neurologist. Ischemic and hemorrhagic strokes were differentiated by computed tomography (CT) scans and magnetic resonance imaging (MRI). All patients underwent CT scan as well as MRI. Patients with major cardiac, hepatic, renal, endocrinological disorders, skeletal disorders, and cancerous diseases were excluded from this study. The ischemic stroke was classified into subtypes according to the TOAST classification. [38] As control group, 620 healthy individuals (males:females = 428:192) and 220 healthy individuals (males:females = 430:180) matched for sex and age for comparison with ischemic patients and hemorrhagic patients respectively were recruited from the same demographic area. The controls had no clinical evidence of any cerebrovascular disease. Information on demographic features and risk factors was collected by using a structured questionnaire. Hypertension, alcohol use, diabetes, and smoking were defined as reported previously (39). Subjects included in the study were above the age group of 18 years. All samples were collected only after obtaining the written informed consent.

DNA isolation and genotyping

Five milliliters of blood was collected in EDTA-coated tubes and genomic DNA was extracted from blood samples using standard phenol-chloroform method. The rs4795895-1382A>G polymorphism in CCL11 gene was analyzed by using PCR and RFLP technique. The primers used for the amplification of the CCL11 bearing the polymorphism-1382A>G are forward: 5′ TCCTGCTCTTACCCTAGCAGA 3′ and reverse 5′ TGCCCATCTAGGATAATTGGTC 3′. The amplified 231-base pair (bp) PCR product was digested with MvaI restriction enzyme (Fermentas Fast digest) by incubating at 37°C for 5 min followed by separation of fragments on 2% agarose gel. Most common homozygous (GG) genotype shows the presence of a fragment of 231 bp, heterozygous (AG) shows 106 bp and 125 bp bands, and rare homozygous genotype (AA) was detected as 106-, 125-, and 231-bp bands.

Statistical analysis

Hardy-Weinberg equilibrium was tested for the CCL11 gene rs4795895-1382A>G polymorphism. Association between genotypes with ischemic and hemorrhagic stroke was examined by odds ratio with 95% confidence interval (CI) and Chi-square analysis using Open EPI6 software (Open Epi Version 2.3.1 from Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA 30322, USA). Allelic frequencies were calculated according to the number of different alleles observed and the total number of alleles examined. Statistical significance was defined as $P < 0.05$.

Results

Six hundred and twenty ischemic stroke patients, 620 age- and sex-matched controls, 220 hemorrhagic stroke patients, and 220 healthy controls from the same demographic area were included in the study. All patients belonged to a South Indian population from Andhra Pradesh. The clinical characteristics of the ischemic stroke patients and controls are given in [Table 1]. Mean age was 49.4 years for ischemic patients and 49.08 years in controls. Risk factor profile of the ischemic patients revealed hypertension in 57.8%, diabetes in 44.7%, smoking in 43.81%, alcohol use in 33.1%, and family history of stroke in 26.8% subjects. In the control group, 32.1% had hypertension, 28.8% were diabetic, 31.9% smokers, 25.8% were alcohol users, and 11.7% had a family history of stroke. The plasma levels of LDL and TG were significantly elevated ($P < 0.001$) whereas HDL levels were significantly reduced in ischemic stroke patients when compared to controls ($P < 0.001$). The demographic and clinical data of the hemorrhagic stroke patients and controls have been summarized in [Table 2]. The mean age of hemorrhagic stroke patients was 53.8 years and that for controls was 54.7 years. Profiles of patients for the various risk factors revealed hypertension in 55.85%, diabetes in 33.04%, smoking in 38%, alcohol use in 42%, and family history of stroke in 5% of patients. In the control group, 41% had hypertension, 28.02% were diabetic, 30% smokers, 29% were alcoholic, and only 2% had family history of stroke. Hemorrhagic stroke patients also had significantly higher serum triglycerides ($P < 0.001$).{Table 1} {Table 2}

The genotypic distribution of CCL11 rs4795895-1382A>G polymorphism and frequency of A and G alleles in ischemic stroke patients and controls has been given in [Table 3]. There was a statistically significant difference in the genotypic distribution between patients and controls. The frequency of GG genotype and G allele was significantly high in ischemic stroke patients in comparison with the controls. (For GG vs. AA, $\chi2 = 7.604$; $P < 0.001$, Odds ratio = 2.793; 95% CI = 1.308-5.9). For GG vs. AA + AG, $\chi2 = 44.8$; $P < 0.001$, Odds ratio = 2.382; 95% CI = 1.842-3.081). A significant difference was observed in the frequency of G and A alleles in patients and controls (For G vs. A, $\chi2 = 43.26$; $P < 0.001$, Odds ratio = 2.127; 95% CI = 1.693-2.672). We performed multiple logistic regression analysis with a forward stepwise selection controlling all other confounding factors and GG genotypes of the CCL11 gene. It was observed that the GG genotype is a significant risk factor for ischemic stroke. Examining the association of Eotaxin-1 gene polymorphism rs4795895-1382A>G with ischemic stroke subtypes classified according to TOAST classification, we found significant association with intracranial large artery atherosclerosis ($P < 0.001$, odds ratio = 9.298; 95% CI = 5.432-16.32) and small artery occlusion ($P < 0.001$, odds ratio = 9.798; 95% CI = 4.876-19.62) [Table 4]. Intracranial large artery atherosclerosis is the most common subtype in the study population as reported previously. [39] The genotypic distribution of CCL11 rs4795895-1382A>G polymorphism and frequency of G and A alleles in hemorrhagic patients and controls has been given in [Table 5]. A significant difference was observed in the genotypic distribution between patients and controls. The frequency of the GG genotype was significantly higher in patients in comparison with controls. For GG vs. AG, $\chi2 = 26.7$; $P < 0.001$, Odds ratio = 3.54; 95% CI = 2.126-5.824). A significant difference was observed in the frequency of G and A alleles in patients and controls (For G vs. A, $\chi2 = 41.98$; $P < 0.001$, Odds ratio = 4.1; 95% CI; 2.61-6.44). The distribution of -1382A>G genotypes, in ischemic stroke patients, hemorrhagic stroke patients and controls has been depicted graphically in [Figure 1] and [Figure 2]. Further, we compared the ischemic stroke patients with the hemorrhagic stroke group for genotypic distribution. The frequency of GG genotype was significantly high in hemorrhagic stroke group in comparison with Ischemic stroke group (For GG vs. AG, $\chi2 = 4.088$; $P = 0.001$, Odds ratio = 1.59; 95% CI = 1.012-2.499) [Table 6].{Figure 1}{Figure 2}{Table 3}{Table 4} {Table 5}{Table 6}

Discussion

In this study, we assessed the relationship of rs4795895 SNPs in CCL11 gene involved in inflammatory response, with the risk of ischemic stroke and hemorrhagic stroke in a South India population from Andhra Pradesh. An association of CCL11 gene rs4795895-1382A>G polymorphism (GG genotype, G allele) was observed with the risk of ischemic as well as hemorrhagic stroke. A case-control study carried out by Zhao et al. (2012) including 1224 ischemic stroke patients and 1163 controls from a

Chinese Han population showed that CCL11 rs4795895 gene polymorphism was significantly associated with ischemic stroke. [37] However, we did not find AA bearing individuals in the hemorrhagic group. This is in agreement with a previous study carried out in Chinese population, where A allele was the rare allele. [37] Several previous studies have reported the significant role of CCL11 gene in inflammation and in the pathogenesis of coronary artery disease in various ethnic groups. [23],[40],[41] There are various mechanisms through which the Eotaxin gene relates to the risk of stroke. It could be because it plays a prominent role in the development of atherosclerosis as studies indicate that the expression of the Eotaxin gene is higher in human atherosclerosis. [32] Eotaxin also plays a vital role in the progression of atherosclerosis as it is prominently expressed in endothelium and vascular smooth muscle cells in human atheroma. [42],[43] Circulating eotaxin levels have been found to be increased in patients with coronary artery disease and elevated levels of eotaxin could be involved in the development of inflammatory responses in heart and brain. [44] To the best of our knowledge, this study is the first to demonstrate the association of CCL11 genetic polymorphisms -1382A>G with the risk of ischemic and hemorrhagic stroke in a South Indian population from Andhra Pradesh. However, the exact functional significance of this (rs4795895) polymorphism to ischemic and hemorrhagic stroke remains to be further elucidated and CCL11 gene polymorphism could signify promising therapeutic targets in the prevention of stroke. In conclusion, the present study shows that -1382A>G polymorphism of CCL11 gene is associated with risk of ischemic and hemorrhagic stroke in the South Indian population from Andhra Pradesh. Evaluating the association with ischemic stroke subtypes a significant association was observed with intracranial large artery atherosclerosis and lacunar stroke.

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