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Research Article

Renin-Angiotensin A1166C Polymorphism and the Rrisk of Stroke

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Abstract

Stroke is the leading cause of death and disability in the world after the cancer and cardiovascular diseases. Genetic factors are the main players to get stroke. Renin-angiotensin system contains candidate genes and polymorphisms for causing stroke. There are reported associations between stroke and angiotensin II type-1 receptor g. 1166A > C polymorphism (rs5186). Therefore in this study this association was investigated for the east Iranian population. This study is based on 201 stroke patients and 220 controls. To predict the genetic risk of stroke allele and genotype frequencies of angiotensin II type-1 receptor rs5186 were analyzed in this population according to stroke subtypes, gender, age, hypertension, diabetes mellitus, high and low density lipoprotein and triglycerides. According to statistical analysis no significant difference was found between case and control groups. But there were a significant relevance between total cholesterol and stroke (p = 0.037). In this population angiotensin II type-1 receptor g. 1166A > C polymorphism did not increase the risk of stroke. The main reason for this study is complex nature of gene-environment interactions in the pathophysiology of this disease.

***Keywords*:** Stroke, Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP), Angiotensin II type-1 Receptor g. 1166A > C Polymorphism.

Introduction

Stroke has recognized as a multifactorial neurological disease, and is one of the most important causes of death and disability throughout the world (Deb Sharma and Hassan, 2010; Meschia et al., 2011). The question is that what is the molecular function of Stroke? And why people affected more and more with this disease. Each year 795000 people were affected a new or recurrent with stroke (Roger et al., 2011). Renin-angiotensin system (RAS) contains candidate genes for causing stroke (Hassan and Markus, 2000). This system is one of the most important physiological pathways that play a role in the maintenance of blood pressure (Harrison-Bernard, 2009). Angiotensin plays an important role in RAS pathway. Angiotensin I convert to angiotensin II by angiotensin converting enzyme (ACE).

At least there are two main receptors for angiotensin II: angiotensin II type I receptor (AGTR1 or AT1R) and angiotensin II type II receptor (AGTR2). Angiotensin II is a primary regulator of aldosterone secretion and acts as a vasoconstrictor by binding to angiotensin II type 1 receptor (Fyhrquist and Saijonmaa, 2008; Kobori et al., 2007). AT1R location is 3q23-25 as well as it contains more than 55 kb length and 5 exons between 59 to 2014 bp size ranges. First four exons encode a 5′untranslated region (Abdollahi et al., 2005; Guo et al., 1994). The activated receptor couples to G-protein and thus effect on intracellular messengers including phospholipase C, Ca^{2+} and protein kinase C (Carey and Siragy, 2003). There are lot polymorphisms in RAS pathway genes' (Gargano et al., 2009; Rupert et al., 2003; Wong et

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al., 2008). During the last years, there has been considerable debates over the association of angiotensin II type-1 receptor g. 1166A > C polymorphism and risk of stroke, myocardial infarction and hypertension (Brenner et al, 2005; Hahntow et al., 2010; Léon H Henskens et al., 2007; Lapierre et al., 2006; Rubattu et al., 2004; Takami et al., 2000). This polymorphism has been considered as a risk factor for stroke in several populations (Agachan al., 2003; Möllsten, Stegmayr et al., 2008; Szolnoki et al., 2006; Takami et al., 2000). In contrary, other studies have not pose AT1R 1166A > C as a risk factor for stroke (Hindorff et al., 2002; Zhang et al., 2010; Zhao et al., 2001).

Stroke incidence in Iran is considerably great than most western countries (Azarpazhooh et al., 2010). In this regard, in the present study a population based case-control study has been used to prospectively investigate the association of AT1R/1166A > C and stroke in east Iranian population.

Materials and Methods

Study population

In this study 201 randomly subjects were selected at the Ghaem hospital between March 2012 and December 2013 according to the following criteria: clinical symptoms of a stroke based on world health organization definition for stroke and ages between 20 and 70. In the cases group of this case-control study there were 86 males and 115 females. Stroke subtypes in subjects were determined by experienced neurologist according to the TOAST (Trial of ORG 10172 in Acute Stroke Treatment). To determine the type of stroke computed tomography (CT) scan and magnetic resonance imaging (MRI) was used. In the control group there were 96 males and 124 females (220 controls) without any history and clinical evidence of cerebrovascular disease. In control and stroke patient groups biochemical analysis were measured. Both groups were matched in age, sex. Stroke risk factors containing hypertension, diabetes, ischemic heart disease (IHD), low density lipoprotein (LDL), high density lipoprotein (HDL), triglyceride and cholesterol was analyzed. Patients by fasting blood glucose ≥ 126 mg/dl were diagnosed as diabetes mellitus. People with Hypertension were determined in Systolic blood pressure (SBP) ≥ 140 mmHg or diastolic blood pressure (SBP) ≥ 140 mmHg.

DNA Extraction

Venus blood was collected in tube containing ethylene diamine tetra acetic acid (EDTA). DNA was extracted from 200 µl of blood samples by PrimePrep Genomic DNA isolation kit from blood (catalog No K-2000; Genet Bio) and checked by 1% agarose gel. Blood samples were stored at -20ºC.

Genotyping

The AT1R/1166A > C polymorphism was identified using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The forward primer was 5′-AAAAGCCAAATCCCAC TCAA and the reverse primer was 5′-CAG GACAAAAGCAGGCTAGG (21). PCR was carried out with an Applied Biosystems 2720 thermal cycler. PCR amplification was performed in 25 µl reaction volume containing, 0.5 µl of DNA (40-80 ng), 0.4 µM of each primer, 0.25 mM of dNTPs, 1.5 mM $MgCl_2$ and 0.5 U *Taq* DNA polymerase. The PCR amplification conditions were as follows: initial denaturation at 96 ºC for 120s, followed by 35 cycles of 30s at 96 ºC, 30s annealing at 53 ºC, extension for 60s at 72ºC and final extension for 10 minutes at 72 ºC. PCR products with 432bp length were analyzed on 2% agarose gel. PCR products were digested by DdeI restriction endonuclease at 60 ºC overnight. Restriction fragment products were 58 and 374 bp for A allele and 58, 143 and 231 bp for C allele. Products were detected by electrophoresis on 3% agarose gel stained with ethidium bromide (Firure 1).

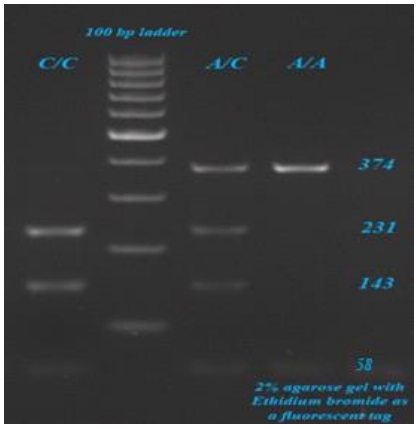

Figure 1. Restriction fragment products. 58 and 374 bp for A allele and 58, 143 and 231 bp for C allele.

Statistical analysis

The normality of numeric variables was assessed using Kolmogorov-Smirnov test and Deviation from Hardy-Weinberg equilibrium was tested by χ^2 test. Quantitative data were compared by Student's t-test and qualitative data such as genotypes and alleles were analyzed by the χ^2 and Fisher's exact test. Allele frequencies and genotype distribution

between case and control groups were compared by χ^2 test. Each stroke subtype was compared with their matched control subjects. Stroke risk factors were analyzed between both case and control groups. Two tailed pvalue of 0.05 was considered for data analyses.

Results

The demographic and clinical characteristics of the study population are shown in table 1.

Table 1. Clinical characteristics of study participants

Characteristics	Cases	Controls	*p*-value
Age (mean ± SD)	51.5 ± 13.8	50.3 ± 12.2	0.169
Sex (male/Female)	86/115	96/124	0.860
Diabetes mellitus (n, %)	57 (28.4)	45 (20.5)	0.055
LDL	127.0 ± 34.5	121.1 ± 32.1	0.094
HDL	40.8 ± 9.5	42.2±8.6	0.161
Triglyceride	140.2 ± 93.1	142.3 ± 86.6	0.796
Total Cholesterol	185.3 ± 45.9	190.3 ± 42.2	0.283

Stroke subtypes distribution in case group was: Ischemic 111 (55.2%), hemorrhagic 66 (32.8%) and other subtypes 24 (11.9%). Risk factors for stroke such as hypertension, diabetes mellitus, total cholesterol, high and low density lipoprotein and triglycerides were analyzed between case and control groups and there were no significant different between them ($p > 0.05$). The Hardy-Weinberg equilibrium was assessed in patient and control groups and both allele and genotype distribution was in accordance with it ($p > 0.05$). Alleles and genotypes frequency between stroke and control groups are presented in table 2, 3 and 4. There was no association between case and control groups in AT1R/1166A > C genotypes or allelic distribution.

Table 2. Alleles and genotypes frequency for male

	Male		*p*-value
Genotype and alleles	Stroke N= 87	Control N= 96	
AA n (%)	57 (65.5)	67 (69.8)	0.501
AC n (%)	27 (31.0)	25 (26.0)	0.425
CC n (%)	3 (3.4)	4 (4.2)	0.812
A n(%)	140 (80.9)	159 (82.8)	0.03
C n (%)	33 (19.1)	33 (17.2)	0.860

Table3. Alleles and genotypes frequency for female

	Female		*p*-value
Genotype and alleles	Stroke N=1 14	Control N= 124	
AA n(%)	76 (66.7)	84 (67.7)	0.897
AC n (%)	38 (33.3)	35 (28.2)	0.419
CC n(%)	0 (0.0)	5 (4)	0.03
A n (%)	190 (83.3)	203 (81.9)	0.8
C n (%)	38 (16.7)	45 (18.1)	0.537

Table 4. Total alleles and genotypes frequency

	Total		*p*-value
Genotype and alleles	Stroke N= 201	Control N= 220	
AA n (%)	133 (66.2)	151 (68.6)	0.589
AC n (%)	65 (32.2)	60 (27.3)	0.256
CC n (%)	3 (1.5)	9 (4.1)	0.110
A n (%)	331 (82.3)	362 (82.3)	0.110
C n (%)	71 (17.7)	78 (17.7)	0.589

In addition, the analysis was done in the subtypes of stroke, age, sex and other risk factors but except in one case there were no significant difference among patients and controls.

Discussion

In the present study the genotypic and allelic frequency of AT1R/1166A > C polymorphism in stroke patients and controls were examined. Neither genotype distribution nor the allelic frequency differed significantly between the case and control groups. Further subgroup analysis including stroke subtypes, gender, age, hypertension, diabetes mellitus, high and low density lipoprotein and triglycerides showed any direct association. Renin-angiotensin system plays a major role in blood pressure which is one of the most effective factors in stroke development. There are several polymorphisms in this pathway which all of them can be potentially a risk factor for stroke (Jia et al., 2014; Tsai et al., 2014). Among them AT1R/1166A > C is a candidate SNP which previously studied in different populations. A nested case-control study on 257 northern Sweden subjects who suffered a first ever stroke and 549 controls demonstrated AA genotype can increase risk of stroke (Möllsten et al., 2008). Henskens et al. have determined AGTR1 A1166C polymorphism is in a significant association with Silent white matter lesions (WMLs), as lesion volume was lowest in the presence of an AGTR1 C allele and CC genotype

(Léon HG Henskens et al., 2005). Rubattu et al. study supports the role of AT1R/1166A > C polymorphism in the development of ischemic stroke among Sardinia population. They assessed 215 cases and 236 controls in this population (Rubattu et al., 2004). In contrary, some other study demonstrated no association between this SNP and stroke. Szolonki et al. study on 308 patients and 272 neuroimaging alteration-free subjects showed AT1R/1166A > C polymorphism cannot be considered as a risk factor for stroke (Szolnoki et al., 2006). In a study of 800 African Americans and 1371 whites reported that this SNP is not associated with stroke (Hindorff et al., 2002). In a meta-analysis which has been performed by zhang et al. no significant association was found between A1166C polymorphism and ischemic stroke in Asian population (Zhang et al., 2010). In a nut shell, there are several studies demonstrating significant association between AT1R/1166A > C polymorphism and stroke but this study performed that this SNP cannot be considered as an independent risk factor for stroke in this population. It could be because of Iranian particular genetic context. It is worth nothing that, the biological relevance of the angiotensin II type-1 receptor g. 1166C polymorphism is unclear and further well-designed studies are needed to identify the biological cause of this relationship between angiotensin II type-1 receptor g. 1166C polymorphism and stroke.

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