Advancements in Genetic Engineering
ISSN: 2169-0111

Tadevosyan, et al., Adv Genet Eng 2015, 4:2
DOI: 10.4172/2169-0111.1000127

Research Article Open Access

Variations in Immediate-early Genes Encoding c-Fos, c-Jun and IER5 Transcription Factors are Associated with Ischemic Stroke

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Rec date: May 06, 2015, **Acc date:** Aug 13, 2015, **Pub date:** Aug 18, 2015

Abstract

Transcription factors make a great contribution in the regulation of mechanisms, which are involved in the pre- and post-stroke events. The main goal of the current study was to evaluate the potential association of single nucleotide polymorphisms FOS (rs7101, rs1063169), JUN (rs11688) and IER5 (rs6425663) with ischemic stroke in Armenian population. A total 161 patients with ischemic stroke and 165 controls were involved in this study. Transcription factors were genotyped using polymerase chain reaction with sequence-specific primers (PCR-SSP). Association of genotype, allelic and carriage frequencies with ischemic stroke was assessed using Pearson's Chi-square test. Multiple test corrected p values less than 0.05 were considered significant. The data obtained demonstrated a significant negative association between the FOS rs1063169*T, JUN rs11688*A and IER5 rs6425663*T minor alleles with ischemic stroke. On the other hand, the frequency distribution of rs7101*T minor allele of FOS gene and its carriage rate in the group of patients were significantly higher than in controls. Our results indicate that the minor alleles of FOS rs1063169, JUN rs11688 and IER5 rs6425663 SNPs can be considered as protective against ischemic stroke, whereas the minor allele of FOS rs7101 SNP represents a risk factor for this disease.

Keywords Ischemic stroke; Immediate-early genes; Transcription factors; Single nucleotide polymorphisms

Introduction

Ischemic stroke (IS) is a complex disorder caused by both environmental and genetic factors [1-3]. Promising studies have suggested that naturally occurring variations in synaptic neuronal plasticity, neuronal survival, and inflammation related genes may influence both stroke progression and poor functional recovery after stroke [2,4-10].

Immediate-early genes have important roles in processes such as brain development and learning, and play an essential role in cellular responses that contribute to long-term neuronal plasticity. Moreover, recent studies give an evidence of their contribution in multiple cardiovascular pathological processes, including atherosclerosis, intimal thickening after acute vascular injury, ischemic pathology, angiogenesis, allograft rejection, and cardiac hypertrophy. Among those genes are genes encoding transcription factors c-Fos (proto-oncogene c-Fos), c-Jun (Fos-binding protein p39), and Ier5 (immediate early response 5), which are the most important for neuronal responses [11-16].

Ier5 regulates activity-dependent functions or plasticity [17], and contributes to neuronal growth, differentiation, and development of neural network by mediating cell response to mitogenic signals [18].

The c-Fos is known to regulate the growth, differentiation, and migration of a variety of vascular and non-vascular cells, and to regulate matrix degradation [19]. It participates in molecular processes of learning and memory. Fleischmann et al. [20] demonstrated that in c-Fos-deficient mice both the long-term memory and N-Methyl-D-aspartate (NMDA) receptor-dependent synaptic plasticity are disturbed.

The c-Jun is an immediate interacting partner of c-Fos. Binding of c-Jun and c-Fos results in formation of transcription factor AP-1 (activated protein-1), representing c-Fos/c-Jun heterodimer [21,22]. AP-1 upregulates transcription of genes, which encode proteins involved in synaptic plasticity and long-term memory [23,24], biogenesis of synaptic vesicles [25], delivery of receptors to dendrites [26], as well as protein assembly in synaptic vesicle membrane [27]. In addition, AP-1 controls cellular activity, including cell differentiation, proliferation, and apoptosis [28-33].

Importantly, AP-1 is one of the mediators of proinflammatory cytokine production [34], and c-Fos is known as a regulator of cytokine production by activated mast cells [35]. This is particularly important in case of stroke, since a number of previous studies, including our own findings, suggested implication of alterations in inflammatory cytokine gene expression and blood levels in upregulated postischemic inflammatory response developed after stroke onset [36-41] and are associated with the risk of recurrent stroke [42].

A number of studies has shown profound increase of c-Fos and c-Jun expression in the brain after ischemia in animal models. In contrast to c-Fos and c-Jun, the role of IER5 has not been described yet. However, it has been shown that IER5 encodes a protein that acts as a regulator of cell proliferation [43], and thus may be involved in tissue remodeling after strokes.

All mentioned above has generated our interest in studying possible association of IS with genetic variations IER5, FOS and JUN in IS. In the present study we evaluated the potential association of IS with

rs7101, rs1063169, rs11688 and rs6425663 single nucleotide polymorphisms (SNPs) of FOS, JUN and IER5.

Methods

Study subjects

In total, 326 Caucasian individuals born and living in Armenia (161 acute IS patients and 165 controls) were enrolled in this study. First episode IS-affected subjects (female/ male: 82/79, mean age ± SD: 69 ± 9.7 years) were recruited from the Medical Clinic №2 of the Yerevan State Medical University, «Surb Grigor Lusavorich» Medical Center and «Armenia» Republican Medical Center.

Diagnosis of IS was based on clinical history and neurological examination and was confirmed by brain computer tomography (CT) imaging and basal laboratory tests. Stroke subtype was classified according to TOAST definitions [44]. Stroke severity was scored using the National Institutes of Health Stroke Scale.

Among IS patients involved in this study 58 had cardioembolic stroke, and 103 had large vessel atherothromboembolic stroke. Anatomically relevant CT hypodense areas in cortical-subcortical parts were detected in cerebral right hemisphere of 75 patients and left hemisphere of 93 patients.

Among IS patients 110 had hyperlipidemia, 82 had arterial hypertension, 31 had atrial fibrillation and 75 had coronary artery disease; 71 patients were nicotine-dependent (cigarette smokers), and 50 were alcohol consumers; 82 patients had positive family history of IS (50-maternal heredity, 19 - paternal heredity, 3 - both).

Age- and sex-matched healthy control subjects (HCS) with no family, past or present history of any serious neurological, acute cerebrovascular or cardiovascular disorders were recruited among the blood donors of the Erebouni Medical Center MH RA. Exclusion criteria for all study subjects included endocrine, oncological, inflammatory, autoimmune and metabolic disorders. All subjects gave their informed consents to participate in the study, which was further approved by the Ethical Committee of the Institute of Molecular Biology NAS RA (IRB #00004079).

Collection of blood samples and extraction of genomic DNA

Five mL of peripheral blood was obtained from antecubital vein into EDTA-vacutainers. Genomic DNA samples were isolated from the blood according to the standard procedure [45] and stored at -30°C until further use.

Selection of SNPs for FOS, JUN and IER5

The rs7101, rs1063169, rs11688 and rs6425663 polymorphisms of the FOS, JUN and IER5 were selected based on the tagging results obtained using HapMap database [http://hapmap.ncbi.nlm.nih.gov] with the selection criteria of r2 >0.8 and minor allele frequency (MAF) >0.2 by the tagger software [46].

Genotyping of FOS rs7101, rs1063169, JUN rs11688 and IER5 rs6425663 SNPs

All DNA samples were genotyped for FOS rs7101 (5′-UTR region), FOS rs1063169 (intron region), JUN rs11688 (synonymous codon) and IER5 rs6425663 (5′-UTR region) SNPs using sequence-specific primers (PCR-SSP) as described earlier [47]. All primers for PCR-SSP (Table 1) were designed using the genomic sequences in the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/). The presence/ absence of allele-specific amplicons in the PCR products was visualized in 2% agarose gel stained with ethidium bromide (0.5μg/ml) fluorescent dye (Figure 1). To check the reproducibility of results, randomly selected DNA samples of study subjects (10% of total) were genotyped twice.

Gene SNP ID (substitution)	Primer: nucleotide sequence
FOS rs7101 (C/T)	for ancestral allele: forward 5′- CTC-CTA-CCC-AGC-TCT-GCT-C-3′
	for mutant allele: forward 5′-CTC-CTA-CCC-AGC-TCT-GCT-T-3′
	constant: reverse 5′-TTG-ACA-GGC-GAG-CCC-ATG-C-3′
FOS rs1063169 (G/T)	for ancestral allele: reverse 5′-TCT-GAC-CTG-CAG-TTG-CAG-AC-3′
	for mutant allele: reverse 5′-TCT-GAC-CTG-CAG-TTG-CAG-AA-3′
	constant: forward 5′-GCA-CAT-CCG-TAA-CTG-GGA-G-3′
JUN rs11688 (G/A)	for ancestral allele: reverse 5′-TCC-GCC-TTG-ATC-CGC-TCC-3′
	for mutant allele: reverse 5′-TCC-GCC-TTG-ATC-CGC-TCT-3′
	constant: forward 5′-AAC-CCA-GGC-GCG-CTG-AGC-3′
IER5 rs6425663 (G/T)	for ancestral allele: reverse 5′-TGC-TCT-ACT-AAC-CTC-TCC-AC-3′
	for mutant allele: reverse 5′-TGC-TCT-ACT-AAC-CTC-TCC-AA-3′
	constant: forward 5′-AGC-CTG-CTC-CGC-GGG-AC-3′
Control Primers	forward 5'- TGC-CAA-GTG-GAG-CAC-CCA-A -3'

HLA-DRB1	reverse 5'- GCA-TCT-TGC-TCT-GTG-CAG-AT-3'

Table 1: Nucleotide sequences of primers used in PCR-SSP for genotyping of selected SNPs of FOS, JUN and IER5.

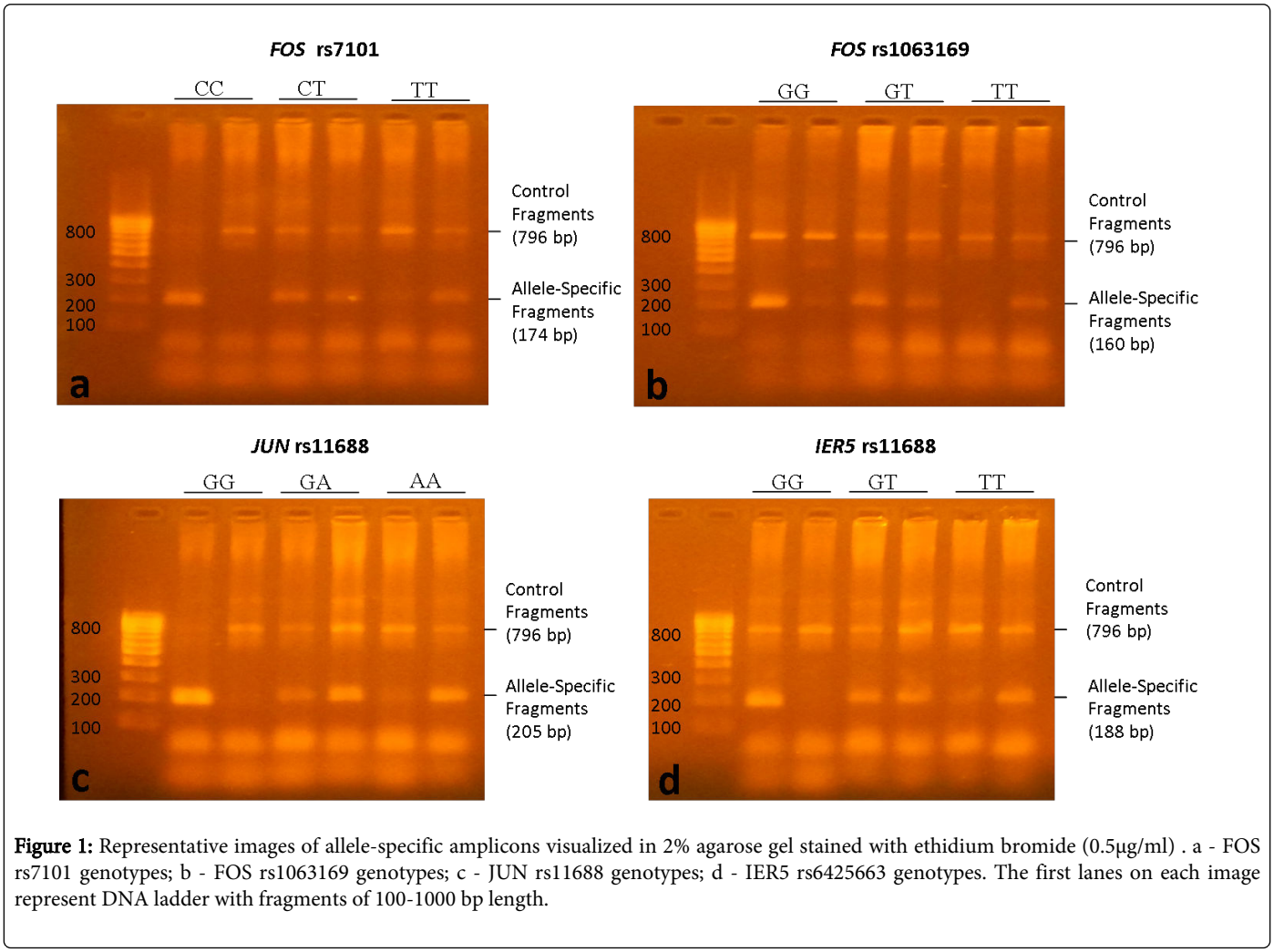

Figure 1: Representative images of allele-specific amplicons visualized in 2% agarose gel stained with ethidium bromide (0.5μg/ml) . a - FOS rs7101 genotypes; b - FOS rs1063169 genotypes; c - JUN rs11688 genotypes; d - IER5 rs6425663 genotypes. The first lanes on each image represent DNA ladder with fragments of 100-1000 bp length.

Statistical Analysis

The distributions of genotypes for all investigated SNPs were checked for correspondence to the Hardy-Weinberg (H-W) equilibrium. In order to find potential relevance of the selected SNPs to IS, their genotype, allele and phenotype (minor allele carriage rate) frequencies in IS patients and controls were compared. The calculations of allelic and phenotypic frequencies were based on the observed number of genotypes. The significance of differences in genotype, allele, and phenotype frequencies between patients and controls was determined using Pearson's Chi-square test. The odds ratio (OR), 95% confidence interval (CI), and P-value were calculated. P-values were adjusted by Bonferroni multiple correction approach. P-values < 0.05 were considered statistically significant. Statistical power for this study was calculated according to the protocol described elsewhere [48]. Statistical analysis was performed using GraphPad Prism 6.0 software (GraphPad Software Inc., USA).

Results

Distribution of FOS rs7101, rs1063169, JUN rs11688 and IER5 rs6425663 SNPs in patients with IS and HCS

The distribution of genotypes for the selected SNPs in both study groups complied with H-W equilibrium, since no significant differences were detected between the observed and expected genotype frequencies ($p>0.05$). The genotyping success rate was 100%, and rescreening of randomly selected DNA samples of study subjects (10% of total) gave identical results.

The obtained results demonstrated significant differences ($p<0.05$) of the allele frequencies and minor allele carriage rates for all studied SNPs (Table 2).

In case of FOS rs7101 SNP, such as frequency distribution of minor allele and its carriage rate in the group of IS patients were significantly higher than in HCS group (Table 2).

		IS	HCS	P (P correted)	OR	95% CI
FOS rs7101						
Genotypes	CC	37	72			
	CT	77	73			
	TT	47	20			
Minor allele frequency	T	0.53	0.34	<0.0001 (<0.0002)	2.18	1.59-2.98
Minor allele carriage frequency	T	0.77	0.56	<0.0001 (<0.0002)	2.59	1.61-4.19
FOS rs1063169						
Genotypes	GG	103	75			
	GT	50	70			
	TT	8	20			
Minor allele frequency	T	0.21	0.33	0.0003 (0.0006)	0.5	0.36-0.74
Minor allele carriage frequency	T	0.36	0.53	<0.0009 (<0.002)	0.47	0.30-0.73
JUN rs11688						
Genotypes	GG	93	45			
	GA	60	86			
	AA	8	34			
Minor allele frequency	A	0.24	0.47	<0.0001	0.35	0.25-0.49
Minor allele carriage frequency	A	0.42	0.74	<0.0001	0.27	0.17-0.36
IER5 rs6425663						
Genotypes	GG	63	18			
	GT	78	67			
	TT	20	80			
Minor allele frequency	T	0.34	0.69	<0.0001	0.26	0.19-0.36
Minor allele carriage frequency	T	0.55	0.89	<0.0001	0.19	0.11-0.34

Table 2: Distribution of genotypes, alleles and minor allele carriage rate for FOS rs7101, rs1063169, JUN rs11688 and IER5 rs6425663 SNPs in IS patients and HCS.

According to our data, the frequency distributions of minor alleles and their carriage rates for FOS rs1063169, JUN rs11688 and IER5 rs6425663 SNPs were significantly lower in the group of IS patients than in HCS group. This may suggests the protective effect of FOS rs1063169, JUN rs11688 and IER5 rs6425663 SNPs against the development of IS at least in Armenian population.

In case of FOS rs7101 SNP, the frequency distribution of minor allele and its carriage rate, were significantly higher in the group of IS patients than in HCS group. Our finding allows to speculate that minor allele of the rs7101 SNP may be considered as a risk factor for IS at least in Armenian population.

Though c-Fos, c-Jun and Ier5 are well characterized on the protein and mRNA levels in cardiovascular and oncological diseases, very little is known about their genetic variability. Thus, it was demonstrated that minor allele of FOS rs7101 SNP is protective against bladder cancer, whereas minor allele of FOS rs1063169 SNP associates with an increased risk of bladder cancer [49]. Also, in the genome-wide association study in Koreans FOS rs1063169 SNP demonstrated marginal association with Crohn's disease and low LD with rs4899554 SNP, which is intergenic between TMED10 and FOS and is located in one of 140 Chrohn's disease risk loci identified in Caucasians [50]. The selected SNPs of FOS gene were also studied in Chinese patients with high myopia, and the obtained data indicated that they are scarcely to

be important in predisposing humans to high myopia [51]. In case of JUN rs11688 SNP, Broyl et al presented its association with bortezomib-induced and vincristine-induced peripheral neuropathy [52]. At present no study reported association of IER5 polymorphisms and diseases.

Our study for the first time implicates genetic polymorphisms of immediate early genes with stroke development. These findings can be partially supported by the important role of these transcription factors in IS demonstrated in animal models of ischemia. Thus, on the mouse model it was shown that cerebral ischemia dramatically increased the expression of c-Fos and c-Jun proteins in the nervous system what lead to the ischemic neuronal damage. Moreover, a panel of genes, including FOS, was clearly able to identify acute IS patients from healthy subjects [53]. On the other hand, it has been shown that potentiation of early c-Fos expression after cerebral ischemia is associated with neuronal survival by up-regulation of AP-1 binding and expression of basic fibroblast growth factor, nerve growth factor, brain-derived neurotrophic factors, and neurotrophins that are essential for neuroprotection [54]. Thus, study of association between genetic polymorphisms and stroke may provide additional insight into the disease genetics and molecular pathophysiology.

Finally, it should be mentioned, that this study was performed by examining one distinct population. Consequently, the results obtained should be replicated in other populations and in larger groups.

Conclusion

In summary, our results, for the first time, demonstrated that IS are associated with single nucleotide variations in genes encoding c-Fos, c-Jun, and Ier5 transcription factors. These results suggested that FOS, JUN, and IER5 immediately response genes are among the candidate genes of IS.

Acknowledgements

We express our sincere thanks to the administration and medical staff of the Armenian Medical Centers MH RA for selection of patients and HCS for this study. The study was supported by the grant of the State Committee of Science of the Ministry of Education and Science RA (KT: #14A-1f74).

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