

POTENTIAL PROTECTIVE ROLE OF SDF-1 AND CXCR4 GENE VARIANTS IN THE DEVELOPMENT OF DEMENTIA

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SUMMARY

Background: The aim of this study was to evaluate the role of polymorphisms of stromal cell-derived factor-1 (SDF-1) and chemokine receptor-4 (CXCR4) genes in dementia susceptibility in a Turkish population.

Subjects and methods: The study group included 61 dementia patients, while the control group comprised 82 healthy individuals. Gene polymorphisms of SDF-1 3'A G801A (rs1801157) and CXCR4 C138T (rs2228014) were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis.

Results: A significantly reduced risk for developing dementia was found for the group bearing an A allele for SDF-1 3'A polymorphism ($p=0.009$; $\chi^2=6.812$; OR=0.626; 95%CI= 0.429-0.913). The frequency of the CXCR4 TT and TC genotype was significantly lower in patients with dementia compared to controls ($p=0.028$; $\chi^2=5.583$; OR=0.215; 95%CI=0.05-0.914); ($p=0.027$; $\chi^2=4.919$; OR=0.484; 95% CI= 0.246-0.955). Additionally, combined genotype analysis showed that the frequency of SDF1 GA-CXCR4 CC was significantly lower in patients with dementia in comparison with those of controls ($p=0.049$; OR=0.560; 95% CI= 0.307±1.020).

Conclusions: Our study provides new evidence that SDF1 A and CXCR4 T alleles may be associated with a decreased dementia risk. The present study is important because to our knowledge, it is the first one to be conducted in a Turkish population to date, but we believe that more patients and controls are needed to obtain statistically significant results.

Key words: SDF-1 - CXCR4 - dementia

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INTRODUCTION

Chemokines are small secreted glycoproteins that display a wide variety of biological and pathological functions. Besides attracting and activating immune and non-immune cells, it has been suggested that chemokines and their receptors play important roles in the central nervous system (CNS) (Réaux-Le Goazigo et al. 2013). There are several different kinds of chemokines using different receptors and signal transduction pathways, but stromal cell-derived factor-1 (SDF-1)/ chemokine receptor-4 (CXCR4) couple have been shown to be one of the most important chemokine and chemokine receptor in the CNS development and NPC migration (Wu et al. 2012). SDF-1 is also known as chemokine ligand 12 (CXCL12), and it is the only known ligand for CXCR4 (Jiang et al. 2013). Both of them are constitutively expressed widely in the developing and adult nervous systems especially in primary sensory terminals and vesicles of spinal axonal boutons (Stumm et al. 2003, Miller et al. 2008, Wu et al. 2012).

The distribution of SDF-1 suggests that this chemokine may have many features in common with classical neuropeptides such as neuromodulation and gene regulation in particular neuronal populations (Wu et al. 2012, Guyon 2014). It has been shown that SDF-1/CXCR4 signaling mediates the migration of neuroblasts and

transplanted neural stem cells or bone marrow-derived cells to the area of brain injury after stroke (Cui et al. 2009, Wu et al. 2012). The chemokine CXCL12 which may also enhance the activity of GABA and glutamate on serotonergic neurons, so disruption of those systems are thought to be responsible for the mechanisms of some psychiatric disorders and dementia (Wu et al. 2009, Wu et al. 2012, Réaux-Le Goazigo et al. 2013). For instance, the expression of CXCR4 was previously shown to be enhanced under pathological conditions including stroke (Cui et al. 2009, Wu et al. 2012) and neurodegenerative diseases like HIV-associated dementia (HAD) and Alzheimer's disease (AD) (Smits et al. 2002, Cui et al. 2009, Xu et al. 2011, Wu et al. 2012).

SDF-1 is known to be located on chromosome 10q11.1 and it has a single nucleotide polymorphism (SNP) consisting of G to A (G>A) transition at position 801 relative to the start codon in the 3' untranslated region (3'UTR), (rs1801157) (Winkler et al. 1998) and its receptor CXCR4 is located on chromosome 2p2 and also a SNP resulting in a substitution of C to T (C>T) at codon 138 (rs2228014) that has an important regulatory function by affecting the expression of protein (Petersen et al. 2005, İşman et al. 2012). In our study, we had focused on two polymorphisms above and investigated a possible relation between those SDF-1 and CXCR4 polymorphisms and dementia in Turkish patients.

SUBJECTS AND METHODS

Study subjects

A total of 61 patients with dementia and 92 controls were recruited for this study. The mean age of the patients with dementia and healthy controls was 73.63 ± 7.387 and 74.05 ± 7.965 years, respectively. No significant differences were found between patients and controls in terms of median age ($p > 0.05$). The diagnosis of dementia was made according to the NINCDS/ADRDA criteria. Healthy subjects were choosed after careful examinations and defined as the individuals without having any of NINCDS/ADRDA dementia criteria. After obtaining written informed consent from the participants and approval of Istanbul University's Ethics Committee, blood specimens were collected in tubes containing EDTA.

DNA isolation and genotyping

A 10 mL sample of venous blood was collected from each subject into a test tube containing EDTA. Genomic DNA was extracted from peripheral whole blood according to a salting out technique (Miller et al. 1988).

SDF-1 3'A G801A (rs1801157) and CXCR4 C138T (rs2228014) polymorphisms were genotyped by the restriction fragment length polymorphism method. The polymerase chain reaction (PCR) method was used for amplification of SDF-1 gene using forward primer 5'-CAGTCAACCTGGGCAAAGCC-3' and reverse primer 5'-AGCTTTGGTCCTGAGAGTCC-3' and for CXCR4 gene using forward primer 5'-AACTTCCTATGCAAGGCAGT-3' and reverse primer 5'-TATCTGTCATCTGCCTCACT-3'. For SDF-1 genotyping, polymerase chain reaction (PCR) product was digested by MspI restriction enzyme (MBI Fermentas). After enzyme digestion, the wild-type GG genotype was identified with the presence of 201 and 101 bp products. The homozygote AA genotype was identified with the presence of 302 bp product. The heterozygote GA genotype had 302, 201, and 101 bp. For CXCR4 genotyping, PCR product was digested by

BccI restriction enzyme (MBI Fermentas). After enzyme digestion, the wild-type CC genotype was identified with the presence of 133 and 103 bp products. The homozygote TT genotype was identified with the presence of 236 bp product. The heterozygote CT genotype had 236, 133, and 103 bp. The PCR products were visualized by electrophoresis on 3% agarose gel. The relative size of the PCR products were determined by comparison of the migration of a 50-1000 bp DNA molecular weight ladder a permanent visual image was obtained using a UV illuminator. Two independent researchers checked all genotypes.

Statistical analysis

Statistical analysis were performed using the SPSS software package (revision 11.5 SPSS Inc., Chicago, IL, U.S.A.) data are expressed as means+SD. Limit of statistical significance was $p < 0.05$. Chi-square and Fischer's exact tests were used to assess the differences of genotype and allele frequencies in two groups. Comparison of intergroup demographic data was determined by using Student's t-test and Anova. Allele frequencies were performed according to gene counting method.

RESULTS

The frequency of gender was considerably different for the patients and controls (71.7% male, 28.3% female, for patients; 48.9% male, 51.1% female, for controls). There were significant differences with regard to gender in the study group ($p < 0.001$).

The allele and genotype frequencies for SDF-1 3'A G801A (rs1801157) and CXCR4 C138T (rs2228014) polymorphisms in patients with dementia and controls are given Table 1. We had significant differences for SDF-1 and CXCR4 genotype frequencies between patients with dementia and healthy controls. Frequencies of SDF-1 A allele in controls were higher than in patients and individuals with A allele seem to be protective

Table 1. Distribution of SDF-1 3'A G801A (rs1801157) and CXCR4 C138T (rs2228014) genotypes and alleles in patients with dementia and control group

SNPs	Controls, n=92		Patients, n=61		P value
	n	%	n	%	
SDF-1 G801A (rs1801157)					
GG	39	42.40	39	63.90	0.031 ^a
AA	17	18.50	6	9.80	
GA	36	39.10	16	26.20	
G allele	114	61.96	94	77.05	0.009 ^a
A allele	70	38.04	28	22.95	
CXCR4 C138T (rs2228014)					
CC	50	54.30	50	82.00	0.001 ^a
TT	14	15.20	2	3.30	
CT	28	30.40	9	14.80	
C allele	128	69.57	109	89.35	0.000 ^a
T allele	56	30.43	13	10.65	

Values are reported as number of patients (percentage of the total group); ^ap-Values < 0.05 denoted statistical significance

Table 2. Combined genotype frequencies of the SDF-1 3'A G801A and CXCR4 C138T polymorphisms among the patients with dementia and controls

SDF-1 3'A G801A	CXCR4 C138T	Controls, n (%)	Patients, n (%)	χ^2	p-value	OR (95 %CI) ^a
GG	CC	25 (43.9)	32 (56.1)	-	-	1
GG	CT	12 (66.7)	6 (33.3)	2.847	0.092	0.486 (0.204±1.161)
GG	TT	2 (66.7)	1 (33.3)	0.599	0.583	0.409 (0.039±4.273)
GA	CC	21 (65.6)	11 (34.4)	3.888	0.049 ^b	0.560 (0.307±1.020)
GA	CT	10 (76.9)	3 (23.1)	4.629	0.062	0.300 (0.09±0.998)
GA	TT	4 (80)	1 (20)	2.412	0.176	0.219 (0.026±1.855)
AA	CC	3 (33.3)	6 (66.7)	0.353	0.722	1.472 (0.403±5.405)
AA	CT	5	0	-	-	-
AA	TT	7	0	-	-	-

^aThe rest combined genotypes against the SDF-1 (rs1801157) GG and CXCR4 (rs2228014) CC genotypes

^bp-Values <0.05 denoted statistical significance; CI - confidence interval; OR - odds ratio

from dementia ($p=0.009$; $\chi^2=6.812$; $OR=0.626$; $95\%CI=0.429-0.913$). The individuals who had CXCR4 TT genotype and TC genotype had reduced risk dementia ($p=0.028$; $\chi^2=5.583$; $OR=0.215$; $95\%CI=0.05-0.914$); ($p=0.027$; $\chi^2=4.919$; $OR=0.484$; $95\%CI=0.246-0.955$). The CXCR4 T allele frequency was lower in patients with dementia compared with controls ($p<0.001$; $\chi^2=12.358$; $OR=0.394$; $95\%CI=0.221-0.705$).

We also analyzed gene-gene interactions by different combinations to evaluate the synergistic effect on dementia. The results are shown in Table 2. The combination of the SDF-1 (rs1801157) GG and CXCR4 (rs2228014) CC genotypes was the most frequent genotype, which was observed to be 56.1% and 43.9% in patients and healthy controls, respectively. Combined genotype analysis showed that the genotypes consisting of the combination of SDF1 GA-CXCR4 CC genotypes were significantly lower in the patients than in the control group ($p=0.049$, Table 2). While the other combined genotypes did not contribute to the risk of dementia relative to the SDF-1 GG genotype and the CXCR4 CC genotype.

DISCUSSION

Dementia is a general term for an impairment in mental ability severe enough to intervene with daily life (Wu et al. 2017). There are several pathological hallmarks (like senile plaques corresponding to extracellular accumulation of the amyloid β , accumulation of mononuclear phagocytes, neurofibrillary tangles, etc.) needed to confirm. There are more than four million people diagnosed as Alzheimer's disease (AD) in the United States each year and currently affects about 26 million people worldwide (Karaca et al. 2017). The most prominent feature of AD is dementia which means the progressive loss of cognitive functions (Karaca et al. 2017). Although it is such frequent, the etiology and pathogenesis of AD remain poorly understood and the molecular reason for the disease is not clear yet.

Dementia is not only specific to AD, for example, it is common in human immunodeficiency virus-1 (HIV-1)-infected cases, and it was found that as being a major co-receptor for HIV-1 entry. CXCR4 was playing an important role in HIV-mediated neuronal degeneration pathogenesis by affecting cell migration, virus-mediated neurotoxicity and neurodegeneration (Réaux-Le Goazigo et al. 2013, Guyon et al. 2014). In CXCR4 knockout mice, it was shown that disrupting the interneuron migratory paths were affecting both the regional and laminar distributions of interneurons (Zhu & Murakami 2012). It was also found that the ligand of CXCR4 and SDF-1 (CXCL12) was determining the migratory streams for neurons (Zhu & Murakami 2012). In a previous study, samples from AD patients were found to contain more CXCR4 mRNA levels than control samples (Laske et al. 2012). However, CXCL12 plasma concentrations were reported to decrease in early AD cases inversely correlating with CSF tau protein and also CXCL12 plasma level had a significant inverse correlation with dementia severity (Laske et al. 2012). In animal experiment with mouse model of AD, it has been shown that CXCL12 mRNA, protein, and receptor are downregulated, coinciding with cognitive deficits (Savarin et al. 2007, Laske et al. 2008). Accumulating data concluded that CXCL12-CXCR4 axis took roles in maintaining neural stem cells and initiating endogenous stem cell-based tissue repair (Li et al. 2012, Peng et al. 2012). Additionally, Parachikova et al had supporting results in animal models suggesting CXCL12/CXCR4 axis to contribute learning and memory functions (Parachikova et al. 2007).

Chemokine and chemokine receptor genes are highly polymorphic, and many of the polymorphisms affect the expression and functional characteristics of these molecules. Many studies have investigated the associations between the SDF-1 and CXCR4 polymorphisms and risk of various diseases, including lung cancer (Xu et al. 2015), cervical cancer (Roszak et al. 2015), gastric cancer (Ramzkhah et al. 2013), laryngeal cancer (Kruszyna et al. 2010), systemic lupus erythematosus (Warchol et al. 2010), colorectal cancer

(Chang et al. 2009), preeclampsia (Karakus et al. 2017), schizophrenia (Dasdemir et al. 2016), renal cell carcinoma (Cai et al. 2013) and inflammatory bowel disease (Mrowicki et al. 2014).

CONCLUSIONS

This was the first study about dementia and the polymorphisms of SDF-1 3'A G801A and CXCR4 C138T. We planned to detect whether the main polymorphisms were affecting the risk of having dementia or not. We found a strong relationship between A allele of SDF-1 and T allele of CXCR4; both were reducing the risk of having dementia. This SNPs seemed to be protective against dementia. In addition to SNP analyses, we investigated the association between dementia risk and combined genotypes in the SDF-1 and CXCR4 gene. Combined genotype analysis revealed that the frequencies of SDF-1 GA and CXCR4 CC genotypes were significantly lower in the patients as compared with those of controls.

It may be important to focus on those findings to be able to have an idea about dementia risk before getting older. In case this will be confirmed with further studies, those polymorphisms can be candidate markers for risk assessment of dementia in the future. The present study has some potential limitations, the major hurdle of the study was the relatively small sample size. Big-size studies such as focusing on gene and protein expression levels will be help us to understand the capability of mechanism role.

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Contribution of individual authors:

Burak Dalan: study design, first draft.

Ozlem Timirci-Kahraman: statistical analysis, first draft, approval of the final version.

Seda Gulec-Yilmaz: statistical analysis.

Emre Murat Altinkilic: study design.

Selvi Duman: study design.

Huseyin Ayhan: data collection.

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