INVESTIGATION OF FIVE POLYMORPHIC DNA MARKERS ASSOCIATED WITH LATE ONSET ALZHEIMER DISEASE

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Alzheimer's disease is a complex neurodegenerative disorder characterized by memory and cognitive impairment and is the leading cause of dementia in the elderly. The aim of our study was to examine the polymorphic DNA markers CCR2 (+190 G/A), CCR5Δ32, TNF-α (-308 G/A), TNF-α (-863 C/A) and CALHM1 (+394 C/T) to determine the relationship between these polymorphisms and the risk of late onset Alzheimer's disease in the population of Eastern Azerbaijan of Iran. A total of 160 patient samples and 163 healthy controls were genotyped by PCR-RFLP and the results confirmed using bidirectional sequencing.

Statistical analysis of obtained data revealed non-significant difference between frequency of CCR5Δ32 in case and control groups. The same result was observed for TNF-α (-863 C/A) genotype and allele frequencies. Contrary to above results, significant differences were detected in frequency of TNF-α (-308 G/A) and CCR2-64I genotypes between the cases and healthy controls. A weak significant difference observed between allele and genotype frequencies of rs2986017 in CALHM1 (+394 C/T; P86L) in patient and control samples. It can be concluded that the T allele of P86L variant in CALHM1 & +190 G/A allele of CCR2 have a protective role against abnormal clinical features of Alzheimer's disease.

Key words: Alzheimer disease; CCR2; CCR5; CALHM1; TNF-α; Eastern Azerbaijan.

INTRODUCTION

Alzheimer's disease (AD), originally described by Alois Alzheimer in 1907, is the most common cause of dementia in the elderly. According to age at onset, two major types of AD are
generally differentiated: Early-onset (<60 years) familial AD (EOFAD) following Mendelian inheritance and late-onset AD (LOAD) or so-called “sporadic” cases with less apparent or no familial aggregation usually occurring later in life (≥60 years). However this traditional dichotomization is overly simplistic as there are cases of early-onset AD without evidence for Mendelian transmission while, conversely, LOAD is frequently observed with a strong familial clustering, sometimes resembling a Mendelian pattern (Bertram et al. 2010; Rubio-Perez and Morillas-Ruiz 2012).

Neurodegenerative disorders such as AD and also for many of the other neurodegenerative diseases, familial aggregation was already recognized as a salient feature decades before any of the underlying molecular genetics and biochemical properties were known. The cause of AD is principally genetic but also environmental, all mostly unknown. As a matter of fact, it was often only the identification of specific, disease-segregating mutations in previously unknown genes that directed the attention of molecular biologists to certain proteins and pathways that are now considered crucial in the development of the various neurodegenerative diseases (Bertram et al. 2012; Zlokovic 2011).

The etiology of LOAD is complex and it has strong genetic heterogeneity (Pastor and Goate 2004; Tuppo and Arias 2005). Furthermore, the genetic component of this form is itself complex and heterogeneous: complex because there is no single or simple model explaining the mode of disease transmission and heterogeneous because the gene mutations or polymorphisms may interact with one another and with environmental factors (Lambert and Amouyel 2011). Therefore studying of any candidate gene can help to identify the heterogeneous nature of LOAD in different populations.

The genetics of LOAD has taken impressive steps forwards in the last few years. To date, more than six-hundred genes have been linked to the disorder. However, only a minority of them are supported by a sufficient level of evidence (Olgiati et al. 2011). These studies identified several new susceptibility genes including Tumor necrosis factor-alpha (TNF-α), C-C chemokine receptor type 2 (CCR2), C-C chemokine receptor type 5 (CCR5) and Calcium homeostasis modulator 1 (CALHM1).

TNF plays a critical role in brain development, brain physiology, synaptic plasticity, sleep, circadian rhythm and normal behavior. Serum concentrations of TNF-α were found to be elevated in Alzheimer patients (Bonotis et al. 2008). The gene product is an important pro-inflammatory cytokine and activates the nuclear factor kappa B (NF-KB), a protein which activates the secretion of Apo lipoprotein E (APOE). APOE gene located on chromosome 19 is the only recognized susceptibility gene for this form of Alzheimer (Bales et al. 2000; Seshadri et al. 2010). Polymorphisms in the promoter region of TNF-α gene have been reported to increase the transcription rate of the gene and thus might influence the risk of AD (Feldmann and Maini 2003; Tedde et al. 2008). Amongst them the polymorphisms located on -308bp and -836bp have been shown to be associated with an increased and decreased transcriptional activity of the gene respectively (Fargion et al. 2004).

CCR2 signaling can mediate accumulation of microglia at sites affected by neuroinflammation. CCR2 and its main ligand CCL2 (MCP-1) might also be involved in the altered metabolism of Aβ (Harrises et al. 2012; Naert and Rivest 2012; Westin et al. 2012). The G to A single nucleotide polymorphism at position 190 of the CCR2 gene causes a change from valine to isoleucine at codon 64 (CCR2-64I) in the first transmembrane region of the protein resulting in an impaired protein product with lost function.
There is growing evidence that chemokines and their receptors are upregulated in reactive microglial cells in the brain tissue of affected individuals, and they may play a role in the recruitment and accumulation of microglial cells at Aβ sites in senile plaques. Indeed, some immunohistochemical studies have shown that the activated microglial cells in both control and patients present an increased expression of chemokine receptor CCR5, indicating to important role of CCR5 receptor in regulation of brain immune response in AD. Recently it has been suggested that CCR5Δ32 polymorphism has a protective effect towards some inflammatory diseases such as AD.

Tissue Info studies (SKRABANEK and CAMPAGNE 2001) and the Alzgene database (BERTRAM et al. 2007) have introduced the gene Calcium homeostasis modulator 1 (CALHM1; MIM# 612234), located about 1.6 Mb apart from LOAD marker D10S1671 on chromosome 10, as one of the candidate genes for LOAD (Bertram et al. 2000). CALHM1 is preferentially expressed in the hippocampus, a brain region which is affected early in the course of AD. The multipass transmembrane glycoprotein encoded by CALHM1, controls cytosolic Ca\(^{2+}\) concentrations in brain cells. In in vitro experiments, CALHM1 expression promotes calcium influx, and triggers a decrease in amyloid β levels, along with an elevation of soluble APPα levels that appears to be regulated by calcium influx (DRESES-WERRINGLOER et al. 2008). Substitution of proline with leucine at codon 86 (P86L) results in the SNP rs2986017 in CALHM1. A recent study has reported that this variant of CALHM1 increases the risk of AD by 40% (DRESES-WERRINGLOER et al. 2008). Functional studies have shown that the rs2986017 SNP results in decreased permeability to calcium ions and lowered calcium ion levels, ultimately leading to an increase in Aβ peptide (AQDAM et al. 2010).

Although many LOAD associated genes have been identified, the replication studies play a critical role in confirming the reported data, especially within different ethnic populations. The present study was conducted to reveal the possible association of polymorphisms of above mentioned genes in the population of eastern Azerbaijan of Iran. Similar results had been reported for a number of ethnicities when the present study was started; however no published data was present about the population investigated in our study.

**MATERIALS AND METHODS**

*Design, Setting, and Participant*

Reported information from investigations of genetic association of LOAD with SNPs located in the candidate gene regions was collected from a recent large genome-wide association study (GWAS) conducted by the Alzheimer Disease Genetics Consortium (ADGC). Our study included 160 AD patients (94 women & 66 men) and 163 healthy controls (95 women & 68 men). The age of patient group at onset was ranged within 65-99 years (mean age 76.06 ±7.75 years).

The samples for control group was selected from a distinguish lab by matching the same ethnicity to subject group with an age at onset ranging within 65-89 years (mean age 75.29±6.75 years). All Alzheimer subjects were diagnosed by expert clinicians according to the DSM-IV criteria (AMERICAN PSYCHIATRIC ASSOCIATION, 1994; THE DEMENTIA STUDY GROUP OF THE ITALIAN NEUROLOGICAL SOCIETY 2000). The sporadic form of the disease was ensured where no affected individuals were present in first degree relatives of subjects and the age of onset above 65 years.
**Gene selection**

We tested the genetic association of LOAD with three genes encoding the inflammatory pathway factors and with one other Calcium homeostasis modulator gene. The Tumor necrosis factor alpha, CCR2, CCR5 and CALHM1 genes have included in this study.

**Table 1. PCR primers complementary to the studied variation**

<table>
<thead>
<tr>
<th>Sequence name</th>
<th>primers</th>
<th>Allele size (basepairs)</th>
<th>Annealing condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR3A37</td>
<td>Forward: 5TCCGCCAGGAAAATCACCTTTTACCC3'</td>
<td>220</td>
<td>35 cycles 0.4°C for 1 min</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5ACGCGCTGTGCCCTCTTGC3'</td>
<td></td>
<td>56.4°C for 1 min</td>
</tr>
<tr>
<td>CCR2 V646</td>
<td>Forward: 5TCTGTGGAACACACATCTTTG3'</td>
<td>171</td>
<td>31 cycles 94°C for 1 min</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5GACATTTCCATGCCAAAG3'</td>
<td></td>
<td>55.8°C for 30 sec</td>
</tr>
<tr>
<td>TNFα (-308G/A)</td>
<td>Forward: 5GGAATAGGGTTTGGGAGGCTG3'</td>
<td>144</td>
<td>35 cycles 94°C for 1 min</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5TCGGATAGGAAATGGTTAG3'</td>
<td></td>
<td>56.4°C for 1 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72°C for 1 min</td>
</tr>
<tr>
<td>TNFα (-308G/A)</td>
<td>Forward: 5CCCTGGAGGAAAATGGTTAC3'</td>
<td>125</td>
<td>31 cycles 94°C for 1 min</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5CTACAGGCGCCTGCTCGCTAGG3'</td>
<td></td>
<td>52.1°C for 30 sec</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72°C for 15 sec</td>
</tr>
<tr>
<td>CALHM1</td>
<td>Forward: 5AAGAGGTTGGAAAGCGGAGG3'</td>
<td>141</td>
<td>31 cycles 94°C for 1 min</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5AAGGAAACGTTTCGCTG3'</td>
<td></td>
<td>54.1°C for 30 sec</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72°C for 1 min</td>
</tr>
</tbody>
</table>

**Sample Preparation, Genotyping and quality control of genotype data**

Blood specimens were collected in sterile tubes containing EDTA, and the DNA was extracted using the salting out method. The PCR reactions was prepared in a total volume of 25 µl, containing 0.1 micrograms of genomic DNA, 0.01 µg each of primers, 2.5 µl of 10×PCR buffer (670 mM Tris-HCl pH 8.8, 160 mM (NH4)2SO4, 0.1% Tween-20), dNTP mix (10mM each), 50 mM MgCl², Taq DNA polymerase (5000u/ml). The primer designing was carried out using an online program (Primer 3) and Ensembl Genome Browser for blasting. Cycles of PCR reactions were optimized employing the online software of mutation discovery. The primers and
PCR conditions for each of the polymorphic DNA fragments have been summarized in table 1. The PCR or RFLP products were fractionated on an 8% acrylamide gel and visualized flowing to staining by AgNO3.

Association analysis

The CCR5∆32 (32 bp deletion, nt794; chromosome 3p21) genotype was determined by PCR without RFLP. The PCR produced a 220bp product from the wild type allele and an 188bp product from the deleted allele. The conditions of RFLP reactions for each of the remaining polymorphisms have been shown in table 2.

Table 2: Digestion conditions of amplified DNA fragments

<table>
<thead>
<tr>
<th>Product name</th>
<th>NCBI SNP ID</th>
<th>Restriction enzyme</th>
<th>Digestion conditions</th>
<th>Size of Fragments after digestion(bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR2 (3p21)</td>
<td>rs1799864</td>
<td>BsefI</td>
<td>65°C for 1-16</td>
<td>171 for wild 152+19 for mutant</td>
</tr>
<tr>
<td>TNFa (-308G/A)</td>
<td>rs1800629 (6p21)</td>
<td>NcoI</td>
<td>37°C for 1-16</td>
<td>126 and 18 for wild 126 for mutant</td>
</tr>
<tr>
<td>TNFa (-863G/A)</td>
<td>rs1800630 (6p21)</td>
<td>TaIl</td>
<td>65°C for 12-16</td>
<td>125 for wild 104+21 for mutant</td>
</tr>
<tr>
<td>CALHM1 (10q24)</td>
<td>rs2986017 (10q24)</td>
<td>Alul</td>
<td>37°C for 1-16</td>
<td>122 and 19 for wild 144 for mutant</td>
</tr>
</tbody>
</table>

Heterogeneity study and gene-based multiple testing correction

The allelic and genotypic frequencies were obtained by direct counting. Data analysis was performed using SPSS16. Chi square and Fisher’s exact test were used to compare the genotypes. Statistical significance was set at P< 0.05. The odds ratio (OR) was calculated at 95% CI.

RESULTS

Case-control analysis, full sample

In this study the control group was matched by age, gender, race and education to the subjects. There was no major departure of the genotypes from the Hardy–Weinberg equilibrium. Table 3 shows the results obtained from statistical analysis of mentioned variables, indicating to non-significant differences between the two groups.

Only 1.3% of Alzheimer patients were homozygous for the CCR2-64I allele compared to 12.9% in normal controls, indicating to association of decreased frequency of CCR2-64I polymorphism with AD (Table 4).
Table 3. Comparison of mean age, sex and education levels between AD cases and control subjects using t-test and \( \chi^2 \) test analysis

<table>
<thead>
<tr>
<th>Education levels</th>
<th>All individuals</th>
<th>AD patients (n=160)</th>
<th>Healthy controls (n=165)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>76.06±7.75</td>
<td>75.29±6.72</td>
<td>0.344</td>
<td></td>
</tr>
<tr>
<td>Sex(f/m)</td>
<td>94/66</td>
<td>95/64</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>33.8%</td>
<td>31.5%</td>
<td>0.981</td>
<td></td>
</tr>
<tr>
<td>Primary school</td>
<td>21.9%</td>
<td>23.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary school</td>
<td>12.2%</td>
<td>11.7%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diploma</td>
<td>22.9%</td>
<td>23.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Academic</td>
<td>9.4%</td>
<td>11.0%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AD, Alzheimer disease; MA, minor allele; MAF, w, wild type; m, mutant; weighted-average minor allele frequency; OR, odds ratio; SNP, single-nucleotide polymorphism.

Table 4. Statically analysis results for association of AD with SNPs in CCR2, CCR5, TNFa and CALHM1 in Individuals

<table>
<thead>
<tr>
<th>Gene/SNP</th>
<th>MA</th>
<th>MAF</th>
<th>MA-OR (95% CI)</th>
<th>w/m + m/m frequencies</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR2</td>
<td>A</td>
<td>P &lt; 0.001</td>
<td>4.5 (2.78-7.29)</td>
<td>4.78 (2.83-7.94)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>rs1799864</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCR5</td>
<td>-</td>
<td>0.26</td>
<td>0.67 (0.33-1.35)</td>
<td>0.65 (0.32-1.35)</td>
<td>0.25</td>
</tr>
<tr>
<td>rs333</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNFa</td>
<td>A</td>
<td>P &lt; 0.001</td>
<td>0.08 (0.04-0.13)</td>
<td>0.067 (0.03-0.11)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>rs1800629</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNFa</td>
<td>A</td>
<td>0.27</td>
<td>0.68 (0.34-1.35)</td>
<td>0.65 (0.32-1.35)</td>
<td>0.25</td>
</tr>
<tr>
<td>rs1800630</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CALHM1</td>
<td>T</td>
<td>0.003</td>
<td>0.4 (0.22-0.74)</td>
<td>0.41 (0.22-0.79)</td>
<td>0.008</td>
</tr>
<tr>
<td>rs2986017</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AD, Alzheimer disease; MA, minor allele; MAF, w, wild type; m, mutant; weighted-average minor allele frequency; OR, odds ratio; SNP, single-nucleotide polymorphism.

a: \( P \) values and ORs estimated under an additive model using logistic regression with covariates (adjusted for age, and sex) in a cognitively normal controls. Generalized linear models were used to estimate case-control data, and generalized estimating equations were used to estimate family-based data.
Amplification of CCR5 (Δ32) by PCR demonstrated a single band of 220bp in individuals homozygous for the wild-type allele and two bands of 220 and 188bp in heterozygotes. No homozygous mutant subject was detected in this study. Table 4 shows non-significant difference between the case and control groups for the polymorphic region of the CCR5 gene.

The statistical analysis of patients, genotypes and allele frequencies (Table 4) revealed a significant difference in TNF-α -308 G/A genotype and allele frequencies between the AD patients and healthy subjects. Non-significant difference was detected between TNF-α -863 C/A genotype and allele frequencies between AD patients and healthy controls (Table 4).

The allele and genotype frequency distribution of P86L genotype were weakly significant between the two study groups (Table 4). As the difference between distribution of T allele (mutant) in case and control groups is significant, it indicates to linkage of this allele with AD.

DISCUSSION

On the basis of the data obtained in previous studies, the recruitment of microglial cells in senile plaques is induced by chemokines and their up regulated receptors in AD brain (AKIYAMA et al. 2000; BAJETTO et al. 2002; FELDMANN and MAINI 2003). Aβ deposition is associated with a local inflammatory response, which is initiated by the activation and migration of microglia in inflammatory sites (DEAN et al. 2000, SHARMA et al. 2012).

As CCR2 is located about 19Kb apart from CCR5 gene on chromosome 3, the co-segregation or independent assortment of the two markers was studied in case and control groups. CCR2-64I carriers were never found to be homozygous for the CCR5Δ32, confirming almost complete linkage disequilibrium of the two markers.

In our study population, occurrence of CCR2-64I polymorphism is decreased in AD patients indicating to association with low risk of developing the disease (P <0.001; OR=4.5, 95% CI: 2.78-7.29). The low frequency of the genotype 64I/64I in AD patients proves real protective effect of this polymorphism on AD (P<0.001; OR=4.78, 95% CI: 2.83-7.94). Our finding about this polymorphism covered the results of Galimberti et al carried out on Italian population (P=0.037; OR=0.65, 95% CI: 0.41–1.03). The conclusion drawn from this study is also comparable to those obtained by Huerta et al. performed on Spanish population (P>0.05; OR=1.06, 95% CI: 0.57–1.96) (HUERTA et al. 2004).

The statistical analysis of the data obtained in our study revealed non-significant difference in genetic distribution of CCR5 polymorphism between the case and control groups (P=0.25; OR=0.67, 95% CI: 0.33-1.35). Also, the haplotypes of CCR5Δ32/Δ32 were never observed either in AD or in control group. These observations are in agreement with the results reported for Italic ethnicity (P>0.05; OR=1.54, 95% CI: 0.97–2.45) (GALIMBERTI et al. 2004).

Most of the recent studies have shown fundamental molecular differences in CC chemokine functions according to origin of population. Regarding this, distribution of CCR2-64I polymorphism in our study population is in agreement with previously reported findings, while the frequency of the CCR5Δ32 allele emerged with a slight decrease compared to Caucasians. However, this mutation was shown to be significantly different in allelic distribution among Caucasians, Asian and Africans, even within close ethnic groups (WANG et al. 2003; FAVOROVA et al. 2002; Khorram Khoshid et al. 1996).
The -308 polymorphism has been associated with an increased transcriptional activity and TNF-α release, whereas the -863 polymorphism have been associated with a reduced transcriptional activity. In the present study the -308G/A polymorphism was shown to be a genetic marker for susceptibility to AD in Azeri Turk population ($P<0.001$; OR=0.067, 95% CI: 0.03-0.11). Our results are similar to the results came out for the other ethnic groups (MOHADDES et al. 2011). A report about the population of Southern China suggests that the -308G/A polymorphism of TNF-α gene might be a risk factor for AD in this ethnicity. Similar results have been reported for the Chilean population (Di et al. 2004). The association of -863 polymorphism with AD was not approved in the present study. This finding had been previously reported by others ($P=0.24$; OR=1.24, 95% CI: 0.86–1.85) (Di et al. 2004). Studies in Spain and Italy have also reported that there is no association between TNF-α -863C/A and AD.

Regarding to the different roles of -308 G/A and -863 C/A polymorphisms on the levels of TNF-α transcription, a negative association is expected between Alzheimer’s disease and -863 C/A polymorphism. However our results did not reveal such an association.

CALHM1 gene encodes a multipass transmembrane glycoprotein that controls cytosolic Ca$^{2+}$ concentrations. CALHM1 expression controls APP processing by interfering with extracellular Aβ accumulation. Also CALHM1 controls APP proteolysis in a Ca$^{2+}$ dependent manner. According to Calcium hypothesis of brain aging and Alzheimer’s disease neuropathological changes associated with AD is caused by changes in Ca$^{2+}$ homeostasis (KHACHATURIAN et al. 1989).

It had been shown that rs2986017 SNP in CALHM1 gene (P86L) is associated with increased risk of LOAD, significant dysregulation of Ca$^{2+}$ homeostasis and APP metabolism (DRESES-WERRINGLOER et al. 2008; SHOII et al. 2005). In a US study, the impact of rs2986017 on the risk of developing AD in 2043 AD cases and 1361 controls Distribution of the T allele was increased in AD cases as compared to controls in all studies, with odds ratios (ORs) ranging from 1.29 to 1.99 (DRESES-WERRINGLOER et al. 2008). In a similar study a significant association was detected between CALHM1-P86L polymorphism and AD in the ethnic Chinese (CUI et al. 2010). In a study carried out in Spain a weak evidence of association between P86L mutation and LOAD susceptibility was observed when a recessive model was applied ($P=0.24$; OR=1.38, CI: 1.01-1.89) (BOADA et al. 2010). However some other investigations performed on populations from other ethnic origins have reported that the difference between the above mentioned polymorphism and LOAD is non-significant (BERTRAM et al. 2008; INOUE et al. 2010; NACMIAS et al. 2010).

Our study corroborates the findings by different groups ($P=0.008$; OR=0.41, CI: 0.22-0.79) (BOADA et al. 2010; CUI et al. 2010; DRESES-WERRINGLOER et al. 2008). Even though our sample was much smaller than the original study, it showed an association with LOAD cases.

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ISPITIVANJA PET POLIMORFNIH DNK MARKERA VEZANIH SA KASNIM POČETKOM ALCHAJMEROVE BOLESTI

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Izvod

Cilj istraživanja je bio ispitivanje pet polimorfnih DNK markera: CCR2 (+190 G/A), CCR5Δ32, TNF-α (-308 G/A), TNF-α (-863 C/A) i CALHM1 (+394 C/T) i utvrđivanje odnosa polimorfnosti markera i rizika kasne pojave Alchajmerove bolesti u populaciji Irana – istočnog dela Azerbejdžana. U ispitivanju je uključeno ukupno 160 uzoraka obolelih pacijenata i 163 uzoraka zdravih osoba. Genotipiziranje je vršeno ispitivanjem polimorfizma (PCR – RFLP) a rezultati su potvrđeni dvosmernim sekvenciranjem. Statističkom obradom rezultata nisu utvrđene značajne razlike u učestalosti CCR5Δ32 unutar controlne grupe. Isto rezultat je dobijen za TNF-α (-863 C/A) genotip i frekvencu alela. Za razliku od navedenog utvrđene su statistički značajne razlike u frekvenci TNF-α (-308 G/A) i CCR2-64I genotipova bolesnika i kontrolnih uzoraka. Utvrdena je niska statistički značajna razlika između alela i frekvencije genotipa rs2986017 u CALHM1 (+394 C/T; P86L) između pacijenata i kontrolnih uzoraka. Zaključeno je da T alel P86L varijante u CALHM1 & +190 G/A alela CCR2 ima zaštitnu ulogu za pojavu abnormalnih osobina Alzhajmerove bolesti

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