

An Association Study on *IL16* Gene Polymorphisms with the Risk of Sporadic Alzheimer's Disease

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Abstract

Background: Interleukin-16 (IL-16) is an important regulator of T cell activation and was reported to act as a chemoattractant agent. There are evidences that IL16 can control the neuroinflammatory processes in Alzheimer's Disease (AD). This study was performed to investigate the role or association of *IL16* polymorphisms, rs11556218 and rs4778889 with the risk of late-onset Alzheimer's disease (LOAD) in Iranian population.

Methods: Totally, 148 AD patients and 137 nondemented and age-matched subjects were recruited in this study. Genotyping of rs11556218 T/G and rs4778889 T/C polymorphisms was performed by PCR-RFLP method using the NdeI and AhdI restriction enzymes, respectively.

Results: Statistical analysis of rs11556218 genotypes showed a protective effect against AD in the heterozygote genotype ($p=0.001$, $OR=0.16$) as well as rs4778889 ($p=0.001$, $OR=0.23$). Frequency of rs11556218 allele T was higher in controls than patients ($p=0.001$, $OR=0.32$). However, there was no significant difference in the frequencies of rs4778889 alleles between the AD patients and controls.

Conclusion: Our results indicate that the rs11556218 and rs4778889 polymorphisms have a protective role in the development of sporadic AD in Iranian population.

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Keywords: Alzheimer's disease, Genetic association study, Interleukin-16, Polymorphism

Introduction

Alzheimer's Disease (AD), before all else described by Alois Alzheimer, as a main cause of dementia in the aged population and well-known as the most common type of dementia affects 1 out of 8 persons older than 65^{1,2}. Mendelian inheritance of 3 genes, *PSEN1*, *PSEN2*, and *APP*, is associated with an early-onset Alzheimer's disease (EOAD). Most cases of Alzheimer's disease are late-onset (LOAD) and are apparently affected by both of genetic and non-genetic factors². This form of AD is strongly associated with $\epsilon 4$ allele of *APOE* gene³. However, existence of some other causative genes responsible for LOAD is obviously expected because about half of LOAD patients do not bear mutations in *APOE* gene⁴. Inflammation is a response to remove the initial cause of cell injury⁵. Brain inflammation is one of the pathological markers of LOAD⁶. Inflammation occurs in pathologically susceptible areas of the AD brain, in the place in which the expression of acute phase proteins and proinflammatory cytokines is evident. Microglia, neurons, and as-

trocytes are mainly responsible for the inflammatory reactions⁵. Some studies referred to IL-16 as an immunoregulatory cytokine that contributes to the regulation of CD4 cell recruitment and activation at regions related to inflammation in correlation with distinct neurodegenerative disorders as well as AD^{7,8}. Previously, the association between inflammatory pathway genes, including *TNF α* , *CCR2-64I*, *CCR5 $\Delta 32$* and *CALHM1 P86L* polymorphisms with LOAD was studied^{9,10}. This study investigated whether the two polymorphisms of *IL16* gene, rs11556218 and rs4778889 are associated with the risk of LOAD in an Iranian population.

Materials and Methods

This research was performed in the Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran (2013-2014). Totally, 148 LOAD patients and 137 age-matched and unrelated healthy subjects from various old peoples' homes in

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Tehran, Iran were recruited. The diagnosis of cases was made according to DSM-IV criteria by an expert psychiatrist. In the control group, if they had any important neurologic or psychological disorder, they were excluded from the study. The control subjects were chosen based on extensive evaluation of their medical histories and physical conditions. The volunteers were included if they were older than 65 years and agreed to enter the study. Protocols were confirmed by Ethics Committee of the University of Social Welfare and Rehabilitation Sciences and all participants provided their informed consent.

Nearly, 5 ml of peripheral blood was collected from each subject in an EDTA-coated tube (0.2 ml, 0.5 M). DNA was extracted from the whole blood samples using a standard salting-out method¹¹. Genotyping of rs11556218 T/G and rs4778889 T/C polymorphisms was performed using PCR-RFLP technique. PCR amplification and genotyping of the fragment containing the rs11556218 T/G polymorphism was performed by a forward primer 5'-GCTCAGGTTACAGAGTGTTCATA-3' and reverse primer 5'-TGTGACAATCACAGCTTGCTTG-3', followed by digestion with the restriction enzyme NdeI (Fermentas, Germany). The rs4778889 T/C polymorphism was assessed by forward primer 5'-CTCCACACTCAAAGCCTTTTGTTCCTATG-3' and reverse primer 5'-ATCCACGCTGGTTCCTTTCGT-3', followed by digestion with the restriction enzyme AhdI (Fermentas, Germany).

The PCR reactions were carried out in the final volume of 25 µl containing: 10×PCR buffer (Roche, Germany), 2 mM MgCl₂ (Roche, Germany), 0.4 mM of each dNTP (Fermentas, Germany), 5 pmol of each primer, 50 ng template DNA, 1 U Taq DNA polymerase (Roche, Germany) and sterile distilled water up to 25 µl. Amplification conditions started with an initial denaturation step of 5 min at 94°C, followed by 30 cycles of 30 s denaturation (94°C), 30 s annealing (60°C for rs11556218 and 59°C for rs4778889) and 30 sec extension (72°C), finished by an ultimate extension for 5 min (72°C) and finally cooling to 4°C. All PCR products were subjected to electrophoresis on 2% agarose gel prepared in 1×TAE, stained with ethidium bromide and visualized by exposure to ultraviolet light.

The PCR products of *IL16* rs11556218 T/G and *IL16* rs4778889 T/C were digested with restriction enzymes NdeI and AhdI at 37°C overnight, respectively. DNA fragments were subjected to 8% polyacrylamide gel electrophoresis and stained with silver nitrate. Based on figure 1, the G allele of the rs11556218 produced two fragments 147 and 24 bp, whereas the T allele remained undigested (171 bp). In *IL16* rs4778889 T/C variant, the C allele was cut into two fragments 146 and 34 bp, while the T allele remained uncut (Figure 2).

The genotype frequencies in the cases and control subjects were analyzed using the chi square test. Logistic regression analysis was carried out to determine

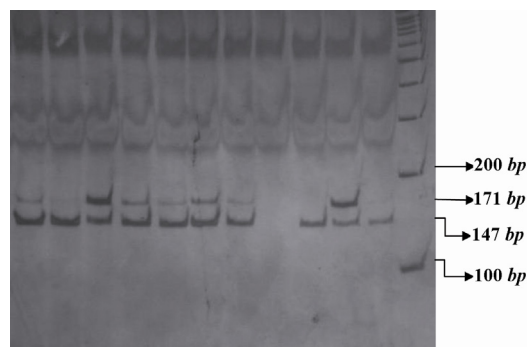


Figure 1. PCR-RFLP profile of *IL16* rs11556218 T/G polymorphism digested with NdeI restriction enzyme.

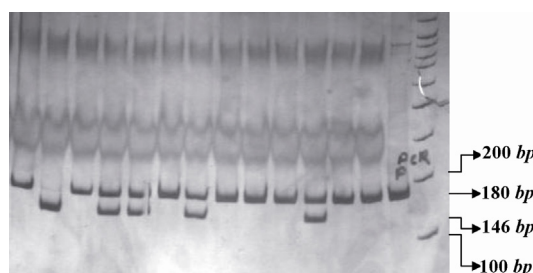


Figure 2. PCR-RFLP profile of *IL16* rs4778889 T/C polymorphism digested with AhdI restriction enzyme.

the relationship between LOAD and two polymorphisms (rs11556218 T/G and rs4778889 T/C) using SPSS software (Version 13, Chicago, IL, USA) and Open Epi (Open Source Epidemiologic Statistics for Public Health, Version 2.2.1, Atlanta, GA, USA). A p-value of less than 0.05 was considered statistically significant.

Results

The mean age of the AD group (78.39, SD±7.87) was not significantly different from the control group (77.07, SD±6.97). The distribution of age, sex, job, educational level and genetic background was almost the same in both groups (Table 1).

The highest and the lowest frequencies of LOAD were observed among the housewives and the farmers, respectively. People with academic education had the lowest incidence and uneducated individuals the highest frequency among the patients and control group, respectively. The subjects consisted of five different Iranian ethnicities in which the Fars was the most common population (Table 1).

The genotype and allele frequencies for the rs11556218 T/G polymorphism were analyzed in LOAD patients and controls. As summarized in table 2, the frequency of GG genotype was not different between the AD patients and the control group (Reference Group). Distribution of TG genotype frequencies showed significant differences between AD patients and control subjects (p=0.001, OR=0.16). Significant difference was observed between allele frequencies in

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Table 1. Comparison of mean age, sex, job, education level and ethnic groups between the AD and control groups using t-test and χ^2 test

Parameter	AD patients (n=146)	Controls (n=148)	p-value
Age	78.55±7.80	77.14±6.95 ^a	0.091
Sex (M/F) ^b	63/91	63/99	0.714
Jobs			
Housewife	82 (55.8%)	83 (56.2%)	0.938
Own business	34 (23.4%)	31 (21.0%)	
Worker	13 (9.2%)	13 (8.6%)	
Farmer	5 (3.2%)	5 (3.1%)	
Employee	12 (8.4%)	16 (11.1%)	
Education level			
Illiterate	61 (41.6%)	64 (43.2%)	0.427
Primary school	42 (29.2%)	44 (29.6%)	
Secondary school	24 (16.2%)	18 (12.3%)	
Diploma	16 (11.1%)	14 (9.3%)	
Academic	3 (1.9%)	8 (5.6%)	
Ethnic			
Fars	89 (61.0%)	94 (63.6%)	0.490
Turk	37 (25.3%)	37 (25.3%)	
Kurd	6 (3.9%)	3 (1.8%)	
Lor	1 (0.7%)	4 (2.5%)	
Gilak and Mazani	13 (9.1%)	10 (6.8%)	

a: Mean±SD, b: Male/Female

Table 2. The distribution of genotype and allele frequencies for the *IL16*-rs11556218 T/G and *IL16*-4778889 T/C polymorphisms

Genotype allele	<i>IL16</i> -rs11556218 T/G				<i>IL16</i> -4778889 T/C				
	Patients (n=137)	Controls (n=147)	p-value	Odds ratio	Genotype allele	Patients (n=146)	Controls (n=148)	p-value	Odds ratio
TT	2 (%1.5)	0	0.934	Undefined	TT	111 (%76)	95 (%64)		Ref*
TG	35 (%25.5)	100 (%68)	0.001	0.16 (0.1-0.28)	TC	7 (%5)	26 (%17.6)	0.001	0.23 (0.1-0.55)
GG	100 (%73)	47 (%32)		Ref*	CC	28 (%19)	27 (%18.4)	0.694	Not applicable
T	39 (%14)	100 (%34)	0.001	0.32 (0.21-0.49)	T	229 (%78.4)	216 (%73)	0.123	0.74 (0.51, 1.1)
G	235 (%86)	194 (%66)			C	63 (%21.6)	80 (%27)		

* Reference Group

Table 3. The distribution of genotype and allele frequencies for the *IL16*-rs11556218 T/G polymorphism stratified by sex

C	Male				Female			
	Genotype allele	Patients	Controls	p-value	Odds ratio	Patients	Controls	p-value
TT	1 (%1.6)	0	0.595*	Undefined	1 (%1.5)	0	0.99*	Undefined
TG	13 (%21.4)	33 (%68.75)	0.001	OR= 0.13 (0.05-0.3)	20 (%29.4)	61 (%71.8)	0.001	0.17 (0.08-0.33)
GG	47 (%77)	15 (%31.25)	Ref Group	1	47 (%69.1)	24 (%28.2)	Ref Group	1
T	15 (%12.3)	33 (%65.26)	0.001	OR= 0.26 (0.13-0.52)	22 (%16.2)	61 (%35.9)	0.001	0.34 (0.2-0.6)
G	107 (%87.7)	62 (%34.74)			114 (%83.8)	109 (%64.1)		

* Fisher exact test p-value

cases and controls ($p=0.001$, $OR=0.32$). Analysis of the data stratified by gender revealed that genotypic distribution in women group showed a significant association in the heterozygote genotype frequency in cases and controls as well as in men (Table 3).

There was no significant difference in the CC genotype of rs4778889 polymorphism; however, the difference in heterozygote genotype was significant ($p=$

0.001 , $OR=0.23$). There was no significant difference in the allele frequencies of rs4778889 polymorphism ($p=0.123$) (Table 4).

Analysis of the data stratified by gender revealed that neither female Alzheimer patients nor male Alzheimer patients had significant differences in genotype and allele frequencies compared with the female and male controls.

Table 4. The distribution of genotype and allele frequencies for the *IL16*-rs4778889 T/C polymorphism stratified by sex

C	Male			Female			
	Genotype allele	Patients	Controls	p-value	Patients	Controls	p-value
TT	52 (%80)	38 (%76)	Ref Group	52 (%75.4)	50 (%57.5)	Ref Group	1
TC	1 (%1.5)	3 (%6)	0.437	4 (%5.8)	22 (%25.3)	0.001	0.17 (0.06-0.54)
CC	12 (%18.5)	9 (%18)	0.957	13 (%18.8)	15 (%17.2)	0.696	Not applicable
T	105 (%80.8)	79 (%79)	0.7397	108 (%78.3)	122 (%68.9)	0.064	Not applicable
C	25 (%19.2)	21 (%21)		30 (%21.7)	55 (%31.1)		

Discussion

In the present study, *IL16* polymorphisms, rs11556218 and rs4778889 were associated with LOAD in an Iranian population. These findings suggest that rs11556218 and rs4778889 can be introduced as useful genetic hallmarks of AD, allowing for identification of related molecular pathways which protect us against LOAD. IL-16 and some other cytokines including IL-1, IL-6, IL-8, IL-11, IL-17, TNF α and TNF β are well-known as chronic inflammation cytokines. These cytokines operate as the regulators of systemic or tissue-specific inflammation¹².

Activated astrocytes and microglia are typically found in high quantities in the vicinity of neurons and plaques. This means that inflammation may be involved in LOAD, because glial cells regulate the innate immune response in the Central Nervous System (CNS). Activated astrocytes and microglia yield various proinflammatory signal molecules, including cytokines, growth factors, complement and adhesion molecules^{13,14}.

The *IL16* gene located on chromosome 15 containing 7 exons and 6 introns, is covering approximately 12.8 kb of chromosome 15¹⁵. The rs11556218 is identified within the intron, whereas rs4778889 is located in coding region. According to Genecards database, *IL16* gene is correlated with 84 different disorders containing LOAD. The rs11556218 T/G polymorphism has been shown to be associated with colorectal, gastric and nasopharyngeal cancers^{16,17}. In 2009, Xue *et al* showed that individuals carrying the G allele of rs11556218 and the C allele of rs4778889 are at a significantly higher risk of Systemic Lupus Erythematosus (SLE) as compared with those carrying the T allele of rs11556218 and the T allele of rs4778889, respectively. In 2007, Motta *et al* explained that the association between IL-16 plasma levels and LOAD was dependent on the disease progression. As mentioned before, inflammation has been reported to be involved in the pathogenesis of neurodegenerative disorders. Raised levels of IL-16 in the brain of AD patients confirm that the immune system may play an important role in progression of LOAD^{8,18}.

Conclusion

Our results confirm the relation between *IL16* polymorphisms and development of LOAD in Iranian population. Further studies with bigger sample sizes are required to confirm the present findings. The current study had some limitations. First, the collected sample size was not big enough. Secondly, LOAD is induced by many causative genes and environmental factors, none of which were regarded in this research. Finally, the present study shows that rs11556218 and rs4778889 are associated with LOAD risk in an Iranian population. Further large-scale studies are needed to clarify whether these *IL16* polymorphisms interact with non-genetic factors in the development of LOAD.

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