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Original Article

Vitamin D Receptor (VDR) Polymorphisms and Late-Onset Alzheimer's Disease: An Association Study

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Abstract

Background: Late-onset Alzheimer's disease (AD), a genetically heterogeneous neurodegenerative disorder, is the most common form of dementia in people over 65 years old. The role of vitamin D in neuropsychiatric and neurodegenerative disorders such as AD has been supported by epidemiologic investigations and animal models, as well. We examined the association of the *vitamin D receptor* (*VDR*) gene polymorphisms and late-onset AD in an Iranian population.

Methods: This study was performed in Tehran, Iran from 2007 to 2008. Totally, 145 AD patients and 162 age-matched unrelated healthy controls were included. The genotype and allele frequencies for the VDR polymorphisms, ApaI (G>T; rs7975232) and TaqI (C>T; rs731236), were determined in the case and control subjects PCR-RFLP analysis. Logistic regression analysis was performed to assess the effect of mutant genotype or allele in the study groups.

Results: The statistical analyses showed significant differences neither in genotype nor in allele frequencies of the ApaI and TaqI polymorphisms between the case and control groups.

Conclusion: It seems that the ApaI and TaqI polymorphisms are not associated with the risk of late-onset AD in Iranian population.

Keywords: Alzheimer's Disease, Vitamin D Receptor, Polymorphism, Association Study, PCR-RFLP, Iran

Introduction

Alzheimer's disease (AD), the most common cause of dementia in the elderly, is a neurodegenerative disorder characterized by progressive cognitive defects such as memory loss, apraxia and personality changes (1, 2). Late-onset Alzheimer's disease, a type of AD, is the most common form of late-onset dementia affecting people over 65 years old (3). AD is a genetically heterogeneous condition; it has many etiologies, but one pathogenesis (4). Early-onset AD, also known as familial AD, is caused mutations in the *amyloid precursor protein* (*APP*), *presenilin 1* (*PS1*) and *presenilin 2* (*PS2*) genes (5). The *apolipoprotein E* (*APOE*) gene, on chromosome 19, is the only confirmed susceptibility locus for the late-onset form (6-8). However, 50% of late-onset AD patients do not carry the APOEε4 allele (9, 10). Numerous studies showed that there is strong evidence for linkage of late-onset AD and another locus on chromosome 12q13 (11-14). The *vitamin D receptor* (*VDR*) gene, located on chromosome 12q13, is a genetic risk factor for late-onset AD (5,

13, 15, 16). The *VDR* gene consists of 14 exons and it spans approximately 75 kb of genomic DNA (17). The VDR gene encodes a nuclear receptor with pluripotent effects (18); it is able to specifically bind DNA response elements when bound to ligand (19). It is expressed in the human brain, with the highest expression in the hypothalamus and in the large neurons of the substantia nigra (20). *VDR* is the receptor for 1, 25-(OH) 2 D3, the active metabolite of vitamin D, and mediates biological actions of vitamin D (21). Epidemiological studies have indicated that vitamin D insufficiency is a risk factor for AD (22, 23).

Given that *VDR* is the major mediator for vitamin D's actions, a few studies evaluated the role of *VDR* polymorphisms in Alzheimer disease (5, 15, 16, 24). We investigated the association of the *VDR* gene polymorphisms with sporadic AD in an Iranian population**.**

Materials and Methods

Subjects

This case-control study was carreid out in the Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran (2007-2008). Totally, 156 Alzheimer's patients and 161 age-matched and unrelated healthy controls from several old peoples' homes in Tehran were recruited. Diagnosis of the Alzheimer's disease was confirmed by a psychiatrist, and the control subjects were selected based on comprehensive evaluation of their medical histories and physical conditions. The participants included if they were older than 65 years and in agreement to enter the study. The main inclusion criterion in the case group was the diagnosis of AD according to DSM-IV criteria. In the control group, if the participants had any serious neurologic or psychological disorder they were excluded from the study. The participants/ guardians were asked about some personal and baseline information. Additionally, ethnicity, job, educational level and gender were considered as co-variables. We obtained informed consent from all participants or guardians **.**

DNA Extraction and Genotyping

Genomic DNA was extracted using the salting out method from 5 ml of peripheral blood samples which collected in tubes containing 200 µl EDTA (0.5 M), as an anti-clotting factor, and stored at -20°C until DNA extraction. We studied two polymorphisms in the human *VDR* gene including ApaI (G>T) in intron 8 (rs7975232), and TaqI (C>T) in exon 9 (rs731236).

Genotyping of the *VDR* polymorphisms were performed using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) analysis. Both ApaI (G>T) and TaqI (C>T) polymorphisms were assessed by a forward primer 5'- CCGGTCAGCAGTCATAGAGG -3' and reverse primer 5'-GAATGGGCTGGGTGGA-TAG-3', followed by digestion with the restriction enzymes ApaI and TaqI (New England BioLabs, USA), respectively.

The PCR reactions carried out in final volume of 25 µl containing: 10× PCR Buffer (Roche, Germany), 4.5 mM $MgCl_2$ (Roche, Germany), 0.4 mM of each dNTP (Fermentas, Germany), 10 pmol of each primer, 30 ng template DNA, 1U Taq DNA polymerase (Roche, Germany) and sterile distilled water up to 25 µL. Amplification conditions start with an initial denaturation step of 5 min at 94 °C, followed by 30 cycles of 30 sec denaturation (94 °C), 20 sec annealing (62 °C) and 30 sec extension (72 °C), ended by a final extension for 5 min (72 °C) and finally cooling to 4°C.

All PCR products were subjected to electrophoresis on 1.5% agarose gel prepared in 1× TAE, stained with ethidium bromide and visualized by exposure to ultraviolet light. Briefly, the PCR products of *VDR* were digested with the two restriction enzymes ApaI and TaqI at 37 °C overnight. DNA fragments were subjected to 8% polyacrylamide gel elect-rphoresis and stained with Silver Nitrate. The ApaI G allele produced two fragments 91 and 453 bp, whereas the ApaI T allele remained uncut (544 bp). The TaqI T allele was cut into two fragments 176 and 368 bp, while the TaqI C allele produced three fragments 167, 176 and 201 bp.

Statistical Analysis

The data was analyzed using SPSS ver. 11.5 (SPSS, Chicago, Ill., USA). Chi-square and independent sample *t* tests were performed to compare sex, job, education level, genetic background and mean age between the study groups. Logistic regression analysis was performed to assess the effect of mutant genotype or allele in the study groups and related Odds Ratio (OR) and 95% confidence interval (CI) reported. *P* values less than 0.05 were considered as significant. The results were also reported by the *ApoE* ε4 allele.

Result

The distribution of important potential confounders in the study groups were similar (Table 1). The mean age of case and control groups were 78.5 (SD=7.8) and 77.4 (SD=7), respectively. The most frequent ethnicities in the study population were Fars and Azari. Approximately, 2% of AD patients had academic education.

Table 1: Comparison of mean age, sex, jobs, education levels and genetic backgrounds between the study groups

		AD Patients (n=145)	Control Subjects (n=162)	*P* Value
Age		78.55 ± 7.80[a]	77.14 ± 6.95	0.091
Sex (M/F)[b]		63/91	63/99	0.714
Previous Job	Housewife	55.8%	56.2%	0.938
	Own Business	23.4%	21.0%	
	Worker	9.2%	8.6%	
	Farmer	3.2%	3.1%	
	Employee	8.4%	11.1%	
Education Levels	Illiterate	41.6%	43.2%	0.427
	Primary School	29.2%	29.6%	
	Secondary School	16.2%	12.3%	
	Diploma	11.1%	9.3%	
	Academic	1.9%	5.6%	
Genetic Background	Fars	61.0%	63.6%	0.490
	Azari	25.3%	25.3%	
	Kurd	3.9%	1.8%	
	Lor	0.7%	2.5%	
	Gilak and Mazani	9.1%	6.8%	

[a] Mean ± S.D./ [b] Male/Female

Totally, 145 AD patients and 162 healthy controls were analyzed using PCR-RFLP methods. Genotype and allele distributions for the ApaI and TaqI polymorphisms are summarized in Tables 2 and 3. There were no statistically significant differences in the genotype distributions or allele frequencies of the ApaI (G>T) and TaqI (C>T) polymorphisms between the cases and controls. Subgroup analysis of the genotype and allele frequency by *ApoE* ε4 allele (ε4 positive or negative) also did not show significant statistical differences (Tables 2 and 3). Then, the AD patients and controls were subdivided into men and women groups. However, no significant differences were found in allele frequencies of the ApaI (P values were 0.677 and 0.523 for men and women, respectively) and TaqI (P value were 0.874 and 0.817 for men and women, respectively) between the subgroups.

Table 2: Genotype distributions of the TaqI (C>T) and ApaI (G>T) polymorphisms in the study groups (Totally and according to *ApoE* ε4 allele)

Genotype Study Group	Alzheimer Number (%)	Control Number (%)	Alzheimer Number (%)(ε4+)	Control Number (%)(ε4+)	Alzheimer Number (%)(ε4-)	Control Number (%)(ε4-)	*P* Value	*P* Value (ε4+)	*P* Value (ε4-)
CC	64(44.1)	76(46.9)	7(33.3)	15(57.7)	57(46)	61(44.9)	Reference Group		
CT	64(44.1)	65(40.1)	12(57.2)	10(38.5)	52(41.9)	55(40.4)	.483	.132	.965
TT	17(11.8)	21(13)	2(9.5)	1(3.8)	15(12.1)	20(14.7)	.914	.266	.571
GG	29(20)	28(17.3)	3(14.3)	3(11.5)	26(21)	25(18.4)	Reference Group		
GT	65(44.8)	78(48.1)	8(38.1)	11(42.3)	57(46)	67(49.2)	.488	.735	.546
TT	51(35.2)	56(34.6)	10(47.6)	12(46.2)	41(33)	44(32.4)	.695	.843	.757

Table 3: Allele distributions of the TaqI (C>T) and ApaI (G>T) polymorphisms in the study groups (Totally and according to *ApoE* ε4 allele)

Allele Study Group	Alzheimer Number (%)	Control Number (%)	Alzheimer Number (%)(ε4+)	Control Number (%)(ε4+)	Alzheimer Number (%)(ε4-)	Control Number (%)(ε4-)	*P* Value	*P* Value (ε4+)	*P* Value (ε4-)
C	192(66.2)	217(67)	26(61.9)	40(76.9)	166(66.9)	177(65.1)	Reference Group		
T	98(33.8)	107(33)	16(38.1)	12(23.1)	82(33.1)	95(34.9)	.840*	.132	.965
G	123(42.4)	134(41.4)	14(33.3)	17(32.7)	109(44)	117(43)	Reference Group		
T	167(57.6)	190(58.6)	28(66.7)	35(67.3)	139(56)	155(57)	.791**	.735	.546

*statistical power: 4.2% /**statistical power: 4.55%

Discussion

Late-onset AD is a genetically heterogeneous condition which affected by both genetic and environmental factors and characterized by a progressive decline in cognitive function (4). To date, hundreds of potential and possible candidate genes for late-onset AD have been described in the literature (25-27). However, the *APOE* gene is the only well-established susceptibility factor for late-onset AD (26). Genome-wide association studies have confirmed that there is strong evidence for linkage of late-onset AD and a susceptibility locus on chromosome 12q13 (11-14). It has been shown that the *VDR* gene, on chromosome 12q13, is a genetic risk factor for late-onset AD (5, 13, 15, 16).

In the current study, we evaluated association of the two *VDR* polymorphisms, ApaI (in intron 8) and TaqI (in exon 9), with the risk of late-onset AD in an Iranian population. Our results showed that neither allele nor genotype frequencies for the ApaI and TaqI polymorphisms were significantly different between the AD cases and controls**.** The calculated statistical power for allele analysis were very low so the study sample size may not be sufficient to show the difference between study groups, but p values are very far from the significant level and it seems that the role of theses polymorphisms are not very prominent; to show the lack of association we will need a very large sample size to achieve an acceptable power. The result of the recent study can be used for ongoing meta-analysis in this regard.

Luedecking-Zimmer et al. examined the first association study between late-onset AD and VDR polymorphism (24). They assessed the FokI polymorphism (rs10735810) and found no evidence for association in a North American Caucasian population (24).

To our knowledge, there are 3 studies which reported positive results for association between the *VDR* polymorphisms and late-onset AD (5, 15 and 16). Ours results are not in accordance with those of Gezen-AK et al. (15), who reported positive associations between the ApaI and TaqI poly-

morphisms and late-onset AD in a Turkish population. They found that the frequency of heterozygote genotype for the ApaI was significantly higher in AD patients than controls (15). According to their data, genotypic distributions of ApaI in the cases (excess of heterozygotes, P = 0.001) and genotypic distributions of TaqI in the controls (excess of homozygotes, P = 0.04) were not in Hardy-Weinberg equilibrium). Lehmann et al. showed that the presence of the ApaI T allele and TaqI G allele were associated with the risk of AD, particularly in people under 75 years old (16). However, their results were on the contrary with those of Gezen-Ak et al. (15) who found a relative excess of heterozygote genotype in AD patients for ApaI polymorphism.

Recently, Wang et al.(5) assessed the association of late-onset AD cases with a total of 80 single nucleotide polymorphisms (SNPs) within the promoter and coning region as well as intron boundaries of the *VDR* gene. They identified the most significant association at a promoter SNP rs11568820 (also known as CDX2) which is located in the transcription factor Cdx-2 binding site. The T allele (risk-allele) at rs11568820 was associated with lower *VDR* promoter activity (5). They showed that overexpression of VDR and vitamin D treatment suppresses *APP* promoter activity and suggested that *APP* is a novel target gene for VDR signaling. This could provide a molecular mechanism for the genetic association between VDR and late-onset AD risk (5).

Conclusion

It seems that the two *VDR* polymorphisms, ApaI and TaqI, are not associated with the risk of late-onset AD in Iranian population. Additional association studies may help to further elucidate the function of these polymorphisms in AD.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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