



Association between selected cholesterol-related gene polymorphisms and Alzheimer's disease in a Turkish cohort

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Abstract

Numerous genetic evidence has pointed out that variations in cholesterol-related genes may be associated with an Alzheimer's disease (AD) risk. We aimed to investigate the association between polymorphisms in several cholesterol-related genes [*APOA5* (rs662799), *APOC1* (rs11568822), *APOD* (rs1568565), *CH25H* (rs13500), *LDLR* (rs5930), *SORL1* (rs2282649)] and AD in a cohort of Turkish patients. The study group consisted of 257 AD patients (mean age: 75.9 years ± 10.4) and 414 controls (mean age: 62.2 years ± 13.1). Genotyping was performed by quantitative real-time polymerase chain reaction using hydrolysis probes. Our results showed that the 'TT' genotype of *CH25H* rs13500 polymorphism was significantly more frequent in the AD group ($p < 0.001$) and individuals carrying the *CH25H* 'T' allele had an increased risk for AD (OR 3.07, 95% CI 2.13–4.44, $p = 2.20 \times 10^{-9}$) independently from age, gender and *APOE* $\epsilon 4$ allele. Moreover, this risk was excessively increased (OR 14.04, 95% CI 6.99–28.23, $p = 9.78 \times 10^{-14}$) in the presence of *APOE* $\epsilon 4$ allele. The 'ins/ins' genotype of *APOC1* rs11568822 was significantly more frequent in the AD group compared to controls ($p = 1.95 \times 10^{-8}$). However, this increased AD risk in 'ins/ins' carriers was found to be dependent on their *APOE* $\epsilon 4$ carrier status. No significant associations were found in allele and genotype distributions of *APOA5*, *APOD*, *LDLR* and *SORL1* gene polymorphisms. Our results suggest that the association between *APOC1* 'ins/ins' genotype and AD risk can be explained by linkage disequilibrium with the *APOE* locus. *CH25H* rs13500 polymorphism is associated with an AD risk in the Turkish population and *CH25H* might have a role in the pathogenesis of AD together with, and independently from *APOE*.

Keywords Alzheimer's disease · Cholesterol metabolism · Polymorphism · Risk

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Introduction

Alzheimer's disease (AD), the most frequent type of dementia, is an irreversible and progressive neurodegenerative disorder characterised by decreased daily living activities and impaired cognitive abilities. AD aetiology is a complex process that results in a combination of genetic and environmental factors. Cholesterol metabolism plays an important role in the pathogenesis of the disease [15], and elevated serum cholesterol levels are known to increase the risk for developing AD [17, 19]. In vitro studies demonstrated that the cholesterol level and distribution within the plasma membrane could influence the processing of amyloid precursor protein in neurons [6, 32].

Since the discovery of the cholesterol related gene 'Apolipoprotein E' (*APOE*), as the major susceptibility gene for AD [33], it has been assumed that other genes in the cholesterol metabolism may also be a risk factor for the AD.

Plenty of genetic evidence points out that variations in cholesterol-related genes may be associated with an increased risk for AD [3, 11, 21, 35]. Therefore, in the selection of genes included in our study, we considered positive associations in AlzGene meta-analysis or knowledge of association with cholesterol and AD in the literature as well as the fact that they were not previously studied in the Turkish population. We selected the following six genes in order to test the association between selected polymorphisms of these genes and AD risk in the Turkish population. Apolipoprotein A5 (APOA5) has an important role in the regulation of lipoprotein homeostasis, by increasing the triglyceride hydrolysis in VLDL and converting VLDL to LDL, which consequently increases plasma cholesterol level [16]. Apolipoprotein C1 (APOC1) together with APOE, is involved in several biological processes, including neuronal reorganization and membrane remodelling via cholesterol transport and redistribution [23]. Apolipoprotein D (APOD) is the main component of the high-density lipoproteins (HDL) and transports small ligands with hydrophobic moiety such as phospholipids and cholesterol [25]. Cholesterol 25-hydroxylase (CH25H) catalyses the formation of 25-hydroxycholesterol from cholesterol and regulates the transcription of several genes involved in transport of cholesterol including APOE [18]. Low-density lipoprotein receptor (LDLR), one of the major APOE receptor in the brain, mediates the endocytosis of cholesterol-rich low-density lipoprotein (LDL) [34]. ‘Sortilin-related receptor 1’ (*SORL1*) gene encodes a multifunctional receptor which binds and mediates endocytotic uptake of lipoproteins like APOE-containing particles [34]. We aimed to investigate the association between single nucleotide polymorphisms (SNP) in these genes and AD in a cohort of Turkish patients. Therefore we have selected *APOA5* (rs662799), *APOC1* (rs11568822), *APOD* (rs1568565), *CH25H* (rs13500), *LDLR* (rs5930) and *SORL1* (rs2282649) gene polymorphisms that have been previously investigated in terms of association with AD.

Materials and methods

Patients and controls

The study population was comprised of 257 patients diagnosed with AD and 414 controls with no sign of neurological impairment at the time of examination. Participants were recruited from the Behavioral Neurology and Movement Disorders Unit outpatient clinic in Istanbul Faculty of Medicine, Istanbul University. All participants underwent detailed clinical and neuropsychological examination and, in most cases neuroimaging. The diagnosis of dementia was made according to the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer’s disease criteria [14]. Both

family members of the patients and different participants were recruited as controls in the study.

The study was approved by the Ethics Committee of Istanbul Faculty of Medicine, Istanbul University. Written and signed informed consent forms were obtained from the patients or the legally authorized representatives.

Genotyping

Genomic DNA from peripheral blood samples was isolated using the QIAmp DNA Maxi KIT (Qiagen, Germany) according to the kit protocol. Genotyping was performed by quantitative real-time polymerase chain reaction (RT-qPCR) method using hydrolysis probes in Real-Time PCR LightCycler 480 instrument (Roche Diagnostics, Germany). All the probes (FAM and YAK dye labeled) and the primers were purchased from the Integrated DNA Technologies (IDT, USA). A standard RT-qPCR reaction was performed using the LightCycler 480 Probes Master kit (Roche Diagnostics, Germany) in a 10 µl volume. The following RT-qPCR cycling conditions were used: pre-denaturation at 95 °C for 10 min, followed by 45 cycles of denaturation at 95 °C for 10 s and annealing at 56 °C for 30 s and extension at 72 °C for 1 s. The end-point analysis was performed for the discrimination of the alleles.

Statistical analysis

The genotype and allele distributions were compared using the Pearson Chi square test. Hardy–Weinberg equilibrium (HWE) was computed to the expected genotype distribution. The two-tailed t-test was used to compare continuous variables while Chi square test was used to compare categorical variables. Continuous variables are presented as mean ± standard deviation (SD) and dichotomous variables as percentage. Logistic regression models were used to derive maximum likelihood estimates of odds ratios (ORs) and related 95% confidence intervals (CIs), adjusted for age, gender and *APOE* ε4 carrier status (heterozygous, homozygous, non-carrier) as confounders. The age-at-onset in patients and age at examination in controls were used as age variable as a covariate. All statistical analysis was performed using SPSS version 21.0 software (IBM Corp., USA) and G*Power 3 (<http://www.gpower.hhu.de/>) software was used to calculate statistical power. The statistical significance level was considered as $p < 0.05$. The exact p-values with significance were calculated by R-program (v3.5.0).

Results

Comparative descriptive characteristics of AD and control groups are summarized in Table 1. As shown in the table, age was found to be significantly higher in AD than

in controls, and *APOE* ϵ 4 allele carriers were significantly more frequent in the AD group. The mean serum total cholesterol, high-density lipoprotein, low-density lipoprotein, and triglyceride levels in AD patients and controls were given in Supplementary Table 1.

The allele and genotype distributions of polymorphisms among AD and control groups are shown in Table 2; the genotyping success rate was over 95% for all polymorphisms. For all polymorphisms, the distributions of genotypes were in Hardy–Weinberg equilibrium.

The allele and genotype distributions of *APOC1* rs11568822 and *CH25H* rs13500 polymorphisms were significantly different between AD and controls ($p = 1.23e-07$, $p = 1.95e-08$ for *APOC1* rs11568822 and $p = 0.01e-11$, $p = 2.56e-13$ for *CH25H* rs13500, respectively). The distribution of *APOC1* rs11568822 genotypes in the AD group were 53%, 39.4% and 7.6% for the del/del, del/ins, ins/ins genotypes, respectively. The *APOC1* minor ‘ins’ allele frequency was 27.3% in AD patients while the frequency for the controls was 14% and ‘ins’ allele carriers had a 2.31-fold risk for developing AD. The distribution of *CH25H* rs13500 genotypes in the AD group was 54%, 40.9% and 5.1% for the CC, CT and TT genotypes, respectively. The *CH25H* minor T allele frequency for the AD patients was 25.6% while 10% for the controls and T allele carriers had a 3.10-fold risk for developing AD. No significant differences between AD and control groups were found in the allele and genotype distributions of *APOA5* rs662799, *APOD* rs1568565, *LDLR* rs5930 and *SORL1* rs2282649 polymorphisms (Table 2). Considering the age difference between AD patients and controls, we subset controls as age-matched (mean age: 75.9yrs) and age-unmatched (mean age 54.02 years) subgroups. As we compared the genotype distributions between AD patients and these subgroups, we found

that the significance level for each polymorphism did not change.

Logistic regression analysis revealed that, as shown in Table 3, after adjustment for age and gender (Model 1), the *APOC1* ins/ins carriers had a 3.28-fold risk ($p = 0.002$, OR 3.28, 95% CI 1.54–6.96) and del/ins carriers had a 2.38-fold risk ($p = 1.67e-06$, OR 2.38, 95% CI 1.67–3.40) for developing AD in comparison with del/del genotype carriers. In Model 2, which additionally comprised adjustment for *APOE* ϵ 4 carrier status, no significant risk associated with AD was found in the *APOC1* genotype carriers. In Model 1, the *CH25H* TT genotype carriers had a 29.29-fold risk ($p = 0.001$, OR 29.29, 95% CI 3.79–226.6) and CT carriers had a 2.95-fold risk ($p = 2.87e-09$, OR 2.95, 95% CI 2.07–4.22) for AD compared with CC genotype carriers. In Model 2, carrying the *CH25H* TT ($p = 0.001$, OR 31.66, 95% CI 4.03–248.8) or CT ($p = 1.73e-07$, OR 2.72, 95% CI 1.87–3.96) genotypes was significantly associated with AD risk. Because of the low number of individuals with the *CH25H* TT genotype, homozygotes and heterozygotes for the T allele (TT + CT) were combined together as a group of T allele carriers for logistic regression analysis. The *CH25H* T allele carriers had a 3.28-fold risk ($p = 3.01e-119$) in Model 1 and 3.07-fold risk ($p = 2.20e-09$) in Model 2 compared to CC carriers (Table 3).

We also examined the accumulative effect of *APOE* ϵ 4 allele with the *CH25H* T allele on AD risk (Table 4). As given in Table 4, individuals carrying the *CH25H* ‘T’ allele per se, had a 2.74 fold risk for AD (OR 2.74, 95% CI 1.79–4.18, $p = 3.26e-06$) and individuals carrying the *APOE* ϵ 4 per se, had a 3.19 fold risk for AD (OR 3.19, 95% CI 1.99–5.1, $p = 1.01e-06$) compared to individuals not carrying both *CH25H* T and *APOE* ϵ 4 alleles. However, this risk was excessively increased (OR 14.04, 95% CI 6.99–28.23, $p = 9.78e-14$) in individuals carrying both *CH25H* ‘T’ and *APOE* ϵ 4 alleles. Our sample size in this study was sufficiently powerful (> 80%) to detect differences between groups in the logistic regression analysis.

Table 1 Descriptive characteristics of AD patients and controls

| | AD patients (n=257) | Controls (n=414) | p-value |
|--|---------------------|------------------|-----------------|
| Gender, n (%) | | | |
| Female | 152 (59.1) | 226 (54.6) | 0.25 |
| Male | 105 (40.9) | 188 (45.4) | |
| Age, years | 75.9 \pm 10.4 | 62.2 \pm 13.1 | 2.2e–16 |
| AAO, years | 64.3 \pm 10.5 | – | |
| <i>APOE</i> ϵ 4 status, n (%) | | | |
| + | 104 (41.3) | 59 (14.4) | 2.57e–14 |
| – | 148 (58.7) | 350 (85.6) | |

Bold values indicate the significant associations

Continuous variables are presented as mean \pm SD and dichotomous variables as percentage. A t-test was used for comparison of means and χ^2 test for percentages

AD Alzheimer’s disease, n number of individuals, AAO age at onset, + means carrying at least one copy of *APOE* ϵ 4 allele, – means without carrying any copies of *APOE* ϵ 4 allele

Discussion

In this study, we have evaluated the effect of six polymorphisms in selected genes related to cholesterol metabolism on AD risk. So far, no genetic association studies have been conducted to investigate the role of these genes in a Turkish population with AD.

CH25H is located within the chromosome region linked with late-onset AD [4, 9]. Promoter polymorphisms in *CH25H* are associated with an increased risk for AD [18]. Although association was not shown to be significant in two of three studies dealing with rs13500 promoter polymorphism [27, 31], Papassotiropoulos et al. did find a significant

Table 2 Allele and genotype frequencies of polymorphisms in AD patients and controls

| Gene | SNP | MAF | Allele | Patients, n (%) | Controls, n (%) | OR (95% CI) | p-value | Genotype | Patients, n (%) | Controls, n (%) | p-value |
|-------|------------|----------|--------|-----------------|-----------------|-------------------------|-----------------|----------|-----------------|-----------------|-----------------|
| APOA5 | rs662799 | C=0.13 | C | 68 (13.7) | 100 (12.5) | 1.12 (0.80–1.55) | 0.52 | TT | 187 (75.4) | 308 (76.8) | 0.65 |
| | | | T | 428 (86.3) | 702 (87.5) | | | TC | 54 (21.8) | 86 (21.4) | |
| | | | Ins | 137 (27.3) | 114 (14) | 2.31 (1.75–3.05) | 1.23e-07 | CC | 7 (2.8) | 7 (1.8) | 1.95e-08 |
| APOC1 | rs11568822 | Ins=0.19 | Del | 365 (72.7) | 702 (86) | | | Del/del | 133 (53) | 306 (75) | |
| | | | C | 213 (42.3) | 347 (43.4) | 0.96 (0.76–1.19) | 0.69 | Ins/ins | 99 (39.4) | 90 (22.1) | |
| | | | T | 291 (57.7) | 453 (56.6) | | | TT | 19 (7.6) | 12 (2.9) | 0.49 |
| APOD | rs1568565 | C=0.43 | T | 291 (57.7) | 453 (56.6) | | | TC | 82 (32.5) | 134 (33.5) | |
| | | | C | 129 (25.6) | 82 (10) | 3.10 (2.29–4.2) | 3.01e-11 | CC | 127 (50.4) | 185 (46.3) | |
| | | | T | 129 (25.6) | 82 (10) | | | CT | 43 (17.1) | 81 (20.2) | 2.56e-13 |
| CH25H | rs13500 | T=0.16 | C | 375 (74.4) | 738 (90) | | | TT | 13 (5.1) | 1 (0.2) | |
| | | | A | 220 (42.5) | 324 (40.1) | 1.10 (0.88–1.38) | 0.39 | GG | 94 (37.6) | 145 (35.9) | 0.56 |
| | | | G | 298 (57.5) | 484 (59.9) | | | GA | 110 (44) | 194 (48) | |
| LDLR | rs5930 | A=0.41 | T | 182 (34.8) | 266 (33.8) | 1.05 (0.83–1.32) | 0.70 | CC | 125 (51.2) | 172 (43.7) | 0.13 |
| | | | C | 341 (65.2) | 522 (66.2) | | | CT | 91 (37.3) | 178 (45.1) | |
| | | | A | 220 (42.5) | 324 (40.1) | | | TT | 28 (11.5) | 44 (11.2) | |

Bold values indicate the significant associations

MAF was calculated based on all patients and controls. Odds ratios were calculated regarding the presence of the minor allele
AD Alzheimer's disease, CI confidence interval, MAF minor allele frequency, SNP single nucleotide polymorphism, OR odds ratio

Table 3 Association of APOC1 and CH25H polymorphisms with AD by logistic regression analysis

| Gene | SNP | Genotype | Model 1 | | Model 2 | |
|-------|------------|----------|---------------------------|-----------------|---------------------------|-----------------|
| | | | OR (95% CI) | p-value | OR (95% CI) | p-value |
| APOC1 | rs11568822 | del/del | Reference | | Reference | |
| | | del/ins | 2.38 (1.67–3.40) | 1.67e–06 | 1.14 (0.68–1.9) | 0.613 |
| | | ins/ins | 3.28 (1.54–6.96) | 0.002 | 0.52 (0.15–1.79) | 0.302 |
| CH25H | rs13500 | CC | Reference | | Reference | |
| | | CT | 2.95 (2.07–4.22) | 2.87e–09 | 2.72 (1.87–3.96) | 1.73e–07 |
| | | TT | 29.29 (3.79–226.6) | 0.001 | 31.66 (4.03–248.8) | 0.001 |
| | | CT+TT | 3.28 (2.31–4.66) | 3.01e–11 | 3.07 (2.13–4.44) | 2.20e–09 |

Bold values indicate the significant associations

APOC1 rs11568822 Model 1 comprised 637 subjects with 251 cases of AD and 386 controls, Model 2 comprised 630 subjects with 246 cases of AD and 384 controls. CH25H rs13500 Model 1 comprised 640 subjects with 252 cases of AD and 388 controls, Model 2 comprised 630 subjects with 246 cases of AD and 384 controls. In Model 1 age and gender were used as covariates, In Model 2 age, gender and APOE ϵ 4 carrier status were used as covariates

Table 4 Separate and accumulative AD risk for APOE ϵ 4 and CH25H T alleles

| APOE ϵ 4 allele | CH25H rs13500 T allele | OR (95% CI) | p-value |
|--------------------------|------------------------|--------------------|----------|
| + | + | 14.04 (6.99–28.23) | 9.78e–14 |
| + | – | 3.19 (1.99–5.1) | 1.01e–06 |
| – | + | 2.74 (1.79–4.18) | 3.26e–06 |
| – | – | Reference | |

All analyses were adjusted for age and gender

+ means carrying at least one copy of risk alleles, – means without carrying any copies of risk alleles

association of rs13500 ‘T’ allele with sporadic AD in 1282 AD patients and 1312 controls from five independent populations (French, Russian, USA, Swiss, Mediterranean) [18]. As each population was evaluated separately, this association was maintained in four populations but not in the French. Based on AlzGene meta-analysis performed with the data of the same study, it was observed that allelic OR varies among different populations and was highest in the Russian population (OR 2.19, 95% CI 1.02–4.71) whilst lowest in Mediterranean population (OR 1.77, 95% CI 1.03–3.04) [5]. In line with the findings of Papassotiropoulos et al., in our study, ‘T’ allele of CH25H rs13500 polymorphism was more frequent in patients and showed a significant association with AD. However, the T allele frequency (25.6%) for AD patients found in our study was pretty higher than the highest frequency (16%) [31] reported in all previous studies [18, 27]. The high T allele frequency found in our study can be thought to be due to the difference exist in allele frequencies between populations. However, the T allele frequency we determined for the control group was compatible with other populations [1]. Consistently, we found that the CT genotype carriers had an increased risk for AD, and in TT genotype

carriers this risk was excessively increased. Furthermore, while the presence of T allele per se had nearly threefold increased the risk of AD (OR 3.19, 95% CI 1.99–5.1), together with APOE ϵ 4 allele it increased the AD risk up to 14-fold (OR 14.04, 95% CI 6.99–28.23). On this basis, our results revealed that CH25H T allele is a risk factor for AD independently from age, gender and presence of APOE ϵ 4 allele, and even a stronger risk factor in the presence of APOE ϵ 4 allele. Since CH25H rs13500 polymorphism exhibits a large effect size in our study population, further investigations in the large discovery and replication samples are necessary to confirm this increased AD risk.

A large number of studies have investigated the association between the APOC1 rs11568822 insertion/deletion polymorphism and the risk of AD [10, 20, 22, 30, 37]. However, the results of these studies were inconsistent, partially due to the relatively small sample size and variety of ethnic groups. A meta-analysis performed by Zhou et al. found that APOC1 rs11568822 insertion allele was associated with an increased AD risk in Caucasians, Caribbean Hispanics and Asians, but not in African Americans [37]. In our study, we found that the ‘ins/ins’ genotype was significantly more frequent in AD group compared to controls and ‘ins/ins’ genotype carriers had an increased risk (OR 3.28, 95% CI 1.54–6.96) for AD. However, we failed to confirm this significant association in when adjusted for APOE ϵ 4 carrier status. Similar to our result, Zou et al. found no association between AD risk and APOC1 insertion allele in APOE ϵ 4 non-carriers. These findings could be explained by linkage disequilibrium (LD) between APOC1 and APOE locus due to the close proximity of these genes at Chromosome 19 apolipoprotein gene cluster.

APOA5 rs662799 polymorphism was found to be associated with fasting plasma lipids in a meta-analysis of 51,868 participants from different populations [36]. Our study was

the second study that investigates the association between this polymorphism and AD. Consistently with the findings of Barbosa et al. [2], in a cohort of Brazilian AD patients, no significant difference was found between genotype distributions among groups in our study.

The *APOD* gene is located adjacent to the region linked to AD [24]. Helisalmi et al. [8] have reported that the *APOD* rs1568565 polymorphism was associated with an increased AD risk in early-onset AD patients from Finland (age ≤ 65 years), but not in the late-onset AD (age > 65 years) or overall AD patients. However, this finding might be a consequence of the unequal distribution of genders in ≤ 65 and > 65 age groups and the relatively small number of early-onset AD patients. In our study, we did not find any significant association between *APOD* rs1568565 polymorphism and AD risk.

LDLR rs5930 polymorphism on exon 10 has previously been analysed in two different studies [7, 28]. Gopalraj et al. studied this SNP in two different USA series and found controversial results in terms of the association between AD risk and *LDLR* rs5930 polymorphism. They were unsure about the cause of this discrepancy but speculated that this might have arisen from the difference in ages of the AD patients and/or the types of studies in two series. However, Rodriguez et al. did not show any significant association of this SNP in a cohort of Spanish individuals, consistent with our finding that there is no significant association between *LDLR* rs5930 polymorphism and AD risk. Both in Rodriguez et al. and our study, the age of AD patients were similar to those of Gopalraj et al.'s, in which no association was found. Indeed, both Rodriguez et al.'s and our studies had larger sample sizes than the series of Gopalraj et al.'s.

Association of *SORL1* rs2282649 polymorphism with AD has been investigated in several studies [5, 12, 13, 26, 29]. The results of these studies, which were conducted in a Caucasian population, were inconsistent, but AlzGene meta-analysis did show a significant association between minor T allele and AD risk (OR 1.08, 95% CI 1.01–1.15) [5]. Although minor T allele frequency of *SORL1* rs2282649 polymorphism in our study (MAF = 0.34) is similar to that in other Caucasians populations (MAF = 0.30), we did not find any significant association in allelic and genotypic distributions among AD and controls.

This study reports an association of studied polymorphisms in cholesterol-related genes with the risk of AD in the Turkish population for the first time. In addition, our work includes an obviously larger sample size compared to previous similar studies. However, there are also some limitations while interpretation of our results: the small number of individuals carrying the TT genotype of *CH25H* rs13500 polymorphism leads to a wide confidence interval for OR. This wide confidence interval for OR results from the low minor 'T' allele frequency in our study as it is in

other populations. Although the age difference between AD patients and controls seems to be a limitation, both logistic regression analysis and comparison of patients with age-matched and age-unmatched controls, revealed that the age did not affect the significance of associations. Another stint is the small number of genes studied and their polymorphisms, which means that the effect of other polymorphisms in these genes and other genes associated with AD cannot be ruled out. Since we did not use ancestry-informative markers to test the ethnic homogeneity of our population, the possibility of a hidden population substructure should take into consideration.

In conclusion, our results suggest that:

1. The *CH25H* rs13500 polymorphism is associated with an increased AD risk in the Turkish population and co-occurrence of *CH25H* 'T' and *APOE* $\epsilon 4$ alleles is a strong risk factor for AD. Based on our overall results, it can be concluded that *CH25H* might have a role in the pathogenesis of AD together with, and independently from *APOE*.
2. Association between *APOC1* rs11568822 polymorphism and AD risk can be explained by linkage disequilibrium with the *APOE* locus.
3. *APOA5* rs662799, *APOD* rs1568565, *LDLR* rs5930 and *SORL1* rs2282649 polymorphisms may not be involved as a risk factor for AD in the Turkish population.

However, further studies in larger cohorts and in various populations are necessary to confirm the results reported here, since genetic variations vary among the populations of different ethnic and geographical origin.

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Compliance with ethical standards

Conflict of interest The authors have no actual or potential conflicts of interest.

References

1. Auton A et al (2015) A global reference for human genetic variation. *Nature* 526:68–74. <https://doi.org/10.1038/nature15393>
2. Barbosa FA, de Labio RW, de Oliveira SRV, Minett T, Bertolucci PH, de Arruda Cardoso Smith M, Payao SL (2006) Apolipoprotein A-V gene polymorphism -1131T>C and Alzheimer's disease. *J Alzheimers Dis* 10:365–369
3. Beecham GW et al (2014) Genome-wide association meta-analysis of neuropathologic features of Alzheimer's disease and related dementias. *PLoS Genet* 10:e1004606. <https://doi.org/10.1371/journal.pgen.1004606>

4. Bertram L et al (2000) Evidence for genetic linkage of Alzheimer's disease to chromosome 10q. *Science* 290:2302–2303. <https://doi.org/10.1126/science.290.5500.2302>
5. Bertram L, McQueen MB, Mullin K, Blacker D, Tanzi RE (2007) Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database. *Nat Genet* 39:17–23. <https://doi.org/10.1038/ng1934>
6. Ehehalt R, Keller P, Haass C, Thiele C, Simons K (2003) Amyloidogenic processing of the Alzheimer beta-amyloid precursor protein depends on lipid rafts. *J Cell Biol* 160:113–123. <https://doi.org/10.1083/jcb.200207113>
7. Gopalraj RK, Zhu H, Kelly JF, Mendiondo M, Pulliam JF, Bennett DA, Estus S (2005) Genetic association of low density lipoprotein receptor and Alzheimer's disease. *Neurobiol Aging* 26:1–7. <https://doi.org/10.1016/j.neurobiolaging.2004.09.001>
8. Helisalmi S et al (2004) Genetic variation in apolipoprotein D and Alzheimer's disease. *J Neurol* 251:951–957. <https://doi.org/10.1007/s00415-004-0470-8>
9. Kehoe P et al (1999) A full genome scan for late onset Alzheimer's disease. *Hum Mol Genet* 8:237–245
10. Ki CS, Na DL, Kim DK, Kim HJ, Kim JW (2002) Genetic association of an apolipoprotein C-I (APOC1) gene polymorphism with late-onset Alzheimer's disease. *Neurosci Lett* 319:75–78
11. Leduc V et al (2015) HMGCR is a genetic modifier for risk, age of onset and MCI conversion to Alzheimer's disease in a three cohorts study. *Mol Psychiatry* 20:867–873. <https://doi.org/10.1038/mp.2014.81>
12. Lee JH et al (2007) The association between genetic variants in SORL1 and Alzheimer disease in an urban, multiethnic, community-based cohort. *Arch Neurol* 64:501–506. <https://doi.org/10.1001/archneur.64.4.501>
13. Li Y et al (2008) SORL1 variants and risk of late-onset Alzheimer's disease. *Neurobiol Dis* 29:293–296. <https://doi.org/10.1016/j.nbd.2007.09.001>
14. McKhann GM et al (2011) The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 7:263–269. <https://doi.org/10.1016/j.jalz.2011.03.005>
15. Morris MC et al (2003) Dietary fats and the risk of incident Alzheimer disease. *Arch Neurol* 60:194–200
16. Nilsson SK, Heeren J, Olivecrona G, Merkel M (2011) Apolipoprotein A-V: a potent triglyceride reducer. *Atherosclerosis* 219:15–21. <https://doi.org/10.1016/j.atherosclerosis.2011.07.019>
17. Notkola IL et al (1998) Serum total cholesterol, apolipoprotein E epsilon 4 allele, and Alzheimer's disease. *Neuroepidemiology* 17:14–20. <https://doi.org/10.1159/000026149>
18. Papassotiropoulos A et al (2005) Cholesterol 25-hydroxylase on chromosome 10q is a susceptibility gene for sporadic Alzheimer's disease. *Neuro-degener Dis* 2:233–241. <https://doi.org/10.1159/000090362>
19. Pappolla MA et al (2003) Mild hypercholesterolemia is an early risk factor for the development of Alzheimer amyloid pathology. *Neurology* 61:199–205 doi. <https://doi.org/10.1212/01.Wnl.0000070182.02537.84>
20. Petit-Turcotte C et al (2001) Apolipoprotein C-I expression in the brain in Alzheimer's disease. *Neurobiol Dis* 8:953–963. <https://doi.org/10.1006/nbdi.2001.0441>
21. Picard C et al (2018) Alterations in cholesterol metabolism-related genes in sporadic Alzheimer's disease. *Neurobiol Aging* 66:180e181–180e189 <https://doi.org/10.1016/j.neurobiolaging.2018.01.018>
22. Poduslo SE, Neal M, Herring K, Shelly J (1998) The apolipoprotein CI A allele as a risk factor for Alzheimer's disease. *Neurochem Res* 23:361–367
23. Poirier J, Hess M, May PC, Finch CE (1991) Astrocytic apolipoprotein E mRNA and GFAP mRNA in hippocampus after entorhinal cortex lesioning. *Brain Res Mol Brain Res* 11:97–106
24. Price DL, Tanzi RE, Borchelt DR, Sisodia SS (1998) Alzheimer's disease: genetic studies and transgenic models. *Annu Rev Genet* 32:461–493 doi. <https://doi.org/10.1146/annurev.genet.32.1.461>
25. Rassart E, Bedirian A, Do Carmo S, Guinard O, Sirois J, Terrisse L, Milne R (2000) Apolipoprotein D. *Biochim Biophys Acta* 1482:185–198
26. Reiman EM et al (2007) GAB2 alleles modify Alzheimer's risk in APOE epsilon4 carriers. *Neuron* 54:713–720. <https://doi.org/10.1016/j.neuron.2007.05.022>
27. Riemenschneider M et al (2004) Association analysis of genes involved in cholesterol metabolism located within the linkage region on chromosome 10 and Alzheimer's disease. *Neurobiol Aging* 25:1305–1308. <https://doi.org/10.1016/j.neurobiolaging.2004.01.001>
28. Rodriguez E, Mateo I, Llorca J, Sanchez-Quintana C, Infante J, Berciano J, Combarros O (2006) Brief research communication - No association between low density lipoprotein receptor genetic variants and Alzheimer's disease risk. *Am J Med Genet B* 141B:541–543. <https://doi.org/10.1002/ajmg.b.30341>
29. Rogava E et al (2007) The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease. *Nat Genet* 39:168–177. <https://doi.org/10.1038/ng1943>
30. Scacchi R et al (1999) Plasma levels of apolipoprotein E and genetic markers in elderly patients with Alzheimer's disease. *Neurosci Lett* 259:33–36
31. Shibata N et al (2006) Association studies of cholesterol metabolism genes (CH25H, ABCA1 and CH24H) in Alzheimer's disease. *Neurosci Lett* 391:142–146. <https://doi.org/10.1016/j.neulet.2005.08.048>
32. Simons M, Keller P, De Strooper B, Beyreuther K, Dotti CG, Simons K (1998) Cholesterol depletion inhibits the generation of beta-amyloid in hippocampal neurons. *Proc Natl Acad Sci USA* 95:6460–6464
33. Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses AD (1993) Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci USA* 90:1977–1981
34. Wollmer MA (2010) Cholesterol-related genes in Alzheimer's disease. *Biochim Biophys Acta* 1801:762–773. <https://doi.org/10.1016/j.bbalip.2010.05.009>
35. Wollmer MA et al (2007) Association study of cholesterol-related genes in Alzheimer's disease. *Neurogenetics* 8:179–188. <https://doi.org/10.1007/s10048-007-0087-z>
36. Xu C, Bai R, Zhang D, Li Z, Zhu H, Lai M, Zhu Y (2013) Effects of APOA5 -1131T>C (rs662799) on fasting plasma lipids and risk of metabolic syndrome: evidence from a case-control study in China and a meta-analysis. *PLoS ONE* 8:e56216. <https://doi.org/10.1371/journal.pone.0056216>
37. Zhou Q et al (2014) Association between APOC1 polymorphism and Alzheimer's disease: a case-control study and meta-analysis. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0087017>

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