J Mol Neurosci (2014) 54:780–786
DOI 10.1007/s12031-014-0377-5

Genetic Evidence for the Involvement of Variants at *APOE*, *BIN1*, *CR1*, and *PICALM* Loci in Risk of Late-Onset Alzheimer's Disease and Evaluation for Interactions with *APOE* Genotypes

Jalal Gharesouran · Maryam Rezazadeh · Aziz Khorrami · Morteza Ghojazadeh · Mahnaz Talebi

Received: 22 May 2014 / Accepted: 3 July 2014 / Published online: 15 July 2014

Abstract Alzheimer's disease (AD) is the most common form of dementia in older population. Growing evidence of genetic background that predisposes individuals to AD has been reported as the risk factors in recent years. The Department of Medical Genetics and the Immunology Research Centre investigated the distribution of 11 polymorphisms in 160 patients with late onset AD (LOAD) and in 163 healthy controls, using the sequencing technique. All participants were of Turkish Azeri ethnicity. We compared allele and genotype frequencies between the LOAD patients and control subjects using a chi-square or Fisher's exact test. Alleles and genotypes of APOE, PICALM rs3851179 and rs541458, and the BIN1 gene rs744373 polymorphism were significantly different between LOAD and control groups. The frequencies of the other investigated alleles were similar in the two groups. We also analyzed the association of *BIN1*, *CR1* and *PICALM* SNPs with LOAD in subgroups stratified by the presence or absence of the *APOE* ε4 allele. After adjusting for *APOE*, statistical analysis revealed that the association with PICALM rs541458 and BIN1 rs744373 were only significant among subjects without the *APOE* ε4 allele.

Keywords Alzheimer's disease · *APOE* · *BIN1* · *CR1* · *PICALM*

Introduction

Alzheimer's disease (AD) is a progressive age-related dementia with clinical features that include a decline in memory and an inability to perform the activities of daily living (Querfurth and LaFerla 2010; Stanford 2004). AD is classified into two clinical categories, early-onset AD (EOAD) and late-onset AD (LOAD) (Rogaev 1999). The symptoms of EOAD, which represents less than 5 % of all cases, appear before 60 years of age, and the disease tends to follow Mendelian patterns of inheritance. Early-onset AD is caused by highly penetrant single-gene mutations in one of three genes, amyloid precursor protein (APP) (Goate et al. 1991), presenilin 1 (Sherrington et al. 1995), and presenilin 2 (Rogaev et al. 1995). The late-onset form of AD, also called sporadic AD, is the most common form, and is diagnosed after 65 years of age. The genetics of LOAD are more complex than the genetics of EOAD and none of the mutations directly responsible for EOAD is involved in LOAD (Rao et al. 2014). The causes of LOAD are not yet completely understood, but a combination of several genetic, epigenetic, and environmental factors contribute to determining an individual's risk for the disease (Gatz et al. 2006; Zawia et al. 2009; Kwok 2010).

During the preclinical stage of AD, which can last a decade or more, affected people are free of symptoms, but toxic changes are taking place in the brain. Over time, neurons lose the ability to communicate with each other, causing

Jalal Gharesouran and Maryam Rezazadeh have equal contribution.

J. Gharesouran (✉)
Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
e-mail: Gharesouranj@tbzmed.ac.ir

J. Gharesouran · M. Rezazadeh · A. Khorrami
Department of Medical Genetics, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

M. Rezazadeh
Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran

M. Ghojazadeh
Liver and Gastrointestinal Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

M. Talebi
Neuroscience Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

neurological and physical changes; the condition of the patient deteriorates and eventually they die (Mei Sian and Sahadevan 2005; Van Rossum et al. 2010; Lazarczyk et al. 2012). Characterizing the various genetic and environmental causes of AD to better understand the mechanism of the disease is currently a major scientific challenge. Several genome-wide association studies (GWAS) have been carried out around the world, with the aim of identifying new AD association loci (Harold et al. 2009). In addition to the search for better ways to manage the symptoms of AD, scientists hope to develop ways of slowing or stopping the progression of AD.

Previous studies have identified a number of genes, in addition to *APOE* ε4, that may increase the risk of LOAD, including *BIN1*, *PICALM* and *CR1* (Harold et al. 2009) (Table 1). The *APOE* gene appears to act as a molecular chaperone for APP and it regulates APP conformation, aggregation and deposition. It has been shown that APOE and clusterin cooperate to suppress APP deposition and to regulate both the toxicity of Aβ and its conversion into insoluble forms (Koren et al. 2009). The bridging integrator 1 (BIN1) gene, also known as amphiphysin 2, is the second most important risk locus for LOAD after APOE. BIN1 is involved in endocytosis, inflammation, calcium homeostasis and apoptosis (Tan et al. 2013). The other gene locus shown to be associated with AD is phosphatidylinositol-binding clathrin assembly protein (PICALM), also known as clathrin assembly lymphoid-myeloid leukemia gene (CALM). *PICALM* is located at 11q23 and is ubiquitously expressed in all tissues, especially in neurons, where it is distributed in pre- and

Table 1 Investigated SNPs (PICALM, CR1, and BIN1), previously reported in different genome-wide association studies

SNP used in our study	Gene	Chromosome	Position	SNP cited in literature	Literature
rs3851179	*PICALM*	11q14	85546288	rs3851179	Harold et al. 2009; Lambert et al. 2009; Gyungah et al. 2010; Jones et al. 2010; Jun et al. 2010; Seshadri et al. 2010; Hu et al. 2011; Lee et al. 2011; Kamboh et al. 2012a, b; Shi et al. 2012
rs541458			85465999	rs541458	Jones et al. 2010; Lambert et al. 2011; Lee et al. 2011;
rs17159904			85497725	rs543293	Lee et al. 2011; Masoodi et al. 2013
			85477927	rs561655	Naj et al. 2011
rs12800974			85463935	rs17159904	Lee et al. 2011
			85536186	rs7941541	Lee et al. 2011
			85403635	rs12800974	Masoodi et al. 2013
rs3818361	*CR1*	1q32	205851591	rs3818361	Lambert et al. 2009; Jun et al. 2010; Kamboh et al. 2012a; Shi et al. 2012
rs6701713			205852912	rs6701713	Jones et al. 2010; Jun et al 2010;
			205758672	rs6656401	Lambert et al. 2009; Corneveaux et al. 2010; Kamboh et al. 2012a
rs1408077			205870764	rs1408077	Jun et al. 2010; Wijsman et al. 2011
			74124436	rs6701710	Wijsman et al. 2011
rs744373	*BIN1*	2q14	127611085	rs744373	Jones et al. 2010; Seshadri et al. 2010; Lambert et al. 2011; Shi et al. 2012
rs11554585			127556011	rs10194375	Lee et al. 2011
			127557567	rs13426725	Lee et al. 2011
			127589265	rs4663098	Lee et al. 2011
			127604351	rs11685593	Lee et al. 2011
			127544136	rs11554585	Masoodi et al. 2013
rs7561528			127604455	rs12989701	Hu et al. 2011
			127606107	rs7561528	Lee et al. 2011; Naj et al. 2011; Wijsman et al. 2011; Kamboh et al. 2012a, b;
rs2075650	*APOE*	19q13	50087459	rs2075650	Harold et al. 2009; Jones et al. 2010; Seshadri et al. 2010; Hostage et al. 2013; Shi et al. 2012

postsynaptic structures (Tebar et al. 1999). The PICALM gene encodes the 652 amino acid phosphatidylinositol-binding clathrin assembly protein involved in clathrin-mediated endocytosis (CME) (Harel et al. 2008).

Recently, it has been shown that BIN1 and PICALM proteins are involved in intracellular trafficking of proteins such as vesicle-associated membrane protein 2 (VAMP2) and neurotransmitters, through CME. The gene that encodes receptors for the complement peptides C3b, C4b, C3bi, and C1q, complement receptor 1 (CR1), also known as C3b/C4b receptor, and its variants are also associated with LOAD. CR1 has functions in the immune system and in the clearance of Aβ through phagocytosis (Krych-Goldberg and Atkinson 2001). The CR1 gene is located on Chromosome1 at locus 1q32. Various polymorphisms of CR1 can affect the expression of CR1 molecules on the cell surface (Khera and Das 2009). Systemic lupus erythematosus (SLE), rheumatoid arthritis, insulin-dependent diabetes mellitus (IDDM), and nephritic syndrome have been shown to be associated with CR1 gene variants (Khera and Das 2009). According to a recent GWAS, different markers within the CR1 gene have been found to be associated with susceptibility to LOAD in Caucasians (Lambert et al. 2009) (Table 1). The present case-controlled study included 160 LOAD cases and 163 ethnically and sex-matched healthy controls. We chose to genotype 11 previously investigated polymorphic sites (Table 1) to examine the possible relationship between these polymorphisms and susceptibility to LOAD disease in patients with a Turkish Azeri ethnic background.

Materials and Methods

Sample Preparation

A total of 160 unrelated LOAD patients of Turkish Azeri ethnicity, including 94 women and 66 men (age range 65–99 years, mean age 76.06±7.75 years), were enrolled in this study. All patients were from three provinces (East Azerbaijan, West Azerbaijan, and Ardabil) in northwest Iran. The patients were diagnosed by specialists between 2010 and 2013, based on DSM-IV diagnostic criteria (American Psychiatric Association 1994; The Dementia Study Group of the Italian Neurological Society 2000). We also included 163 ethnically and sex-matched healthy controls without AD or other mental disorders (95 women and 68 men; age range 65–89 years, mean age 75.29±6.75 years) (Gharesouran et al. 2013a; Gharesouran et al. 2013b). Informed consent was obtained from all participants prior to commencing the study.

Experimental Methods

Genomic DNA was isolated from EDTA-anticoagulated blood using the total DNA extraction kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Eleven fragments encompassing the above-mentioned polymorphisms were amplified by PCR using specific oligonucleotide primers. Each PCR was carried out in a total volume of 25 μL consisting of 12.5 μL of 2× PCR master mix, 0.75 μL of a 10 mol/L solution of each primer, 1 μL of genomic DNA (80 ng/μL), and 9.5 μL of H_2O. PCR products were purified with the High Pure PCR product purification kit (Roche, Mannheim, Germany) and were subjected to automated DNA sequencing using specific primers.

Data Analysis

Comparison of allele and genotype frequencies between LOAD patients and healthy controls was carried out using a chi-square test with Yates' correction or Fisher's exact test. Probability values of ≤0.05 were considered as statistically significant. The odds ratio (OR) and the 95 % confidence intervals (CI) were calculated when possible.

Results

The genotype frequencies in LOAD patients and healthy controls were found to be in the Hardy–Weinberg equilibrium (p=0.95 for patients and p=0.95 for healthy controls). Allele and genotype distributions in 7 of the 11 investigated polymorphisms showed no significant difference between LOAD patients and healthy controls in this ethnic group (Table 2). The allele and genotype distributions of PICALM rs3851179, PICALMrs541458, and the BIN1 rs744373 polymorphism were significantly different between the LOAD and control groups. The frequencies of the PICALMrs3851179, PICALM rs541458, andBIN1rs744373 minor alleles were 35.3, 19.3, and 12.8 %, respectively, in LOAD patients and 10.1, 7.3, and 5 %, respectively, in healthy controls. The frequencies of PICALM rs3851179, PICALM rs541458, andBIN1 rs744373 genotypes with at least one mutant allele (heterozygote or mutant homozygotes) were 55.6, 31.8, and 18.5 %, respectively, in LOAD patients and 19, 14, and 9.2 %, respectively, in healthy controls.

The frequency of the ε4 allele was 30 % in LOAD patients and 5.5 % in healthy controls, demonstrating an association of this allele with LOAD (Table 3). The frequency of APOE genotypes with at least one ε4 allele was 41.8 % in LOAD patients and 9.8 % in healthy controls. Our results demonstrated a statistically significant susceptibility to LOAD in patients who had the ε4/ε4, ε4/ε2or ε4/ε3genotype.

After adjusting for APOE, statistical analysis showed the association with PICALM rs541458, rs3851179, and BIN1 gene rs744373 was evident only among subjects without the APOE ε4 allele. The interactions of other SNPs with the APOE ε4 allele were not statistically significant (Table 3).

Table 2 Statistical analysis results for association of 11 investigated SNPs in PICALM, *BIN1*, *CR1*, and *APOE* in Iranian Azeri Turkish patients with LOAD and healthy controls

Gene	SNP	MA	MAF	MA-OR (95 % CI)	w/m+m/m frequencies	
					OR (95 % CI)	*p* value[a]
PICALM	rs3851179	G	$p<0.001$	4.8469 (3.1633–7.4264)	5.33 (3.236–8.802)	<0.001
	rs541458	T	$p<0.001$	3.023 (1.834–4.983)	2.848 (1.639–4.947)	<0.001
	rs12800974	T	$p=0.187$	1.954 (0.817–4.677)	1.713 (0.69–4.253)	0.342
	rs17159904	G	$p=0.596$	1.47 (0.552–3.91)	1.377 (0.466–4.062)	0.7518
CR1	rs6701713	G	$p=0.203$	0.5394 (0.2348–1.2392)	1.758 (0.746–4.143)	0.27
	rs3818361	T	$p=0.396$	1.857 (0.615–5.605)	1.663 (0.532–5.196)	0.548
	rs1408077	T	$p=0.3961$	1.857 (0.615–5.605)	1.445 (0.449–4.653)	0.74
BIN1	rs744373	T	$p<0.001$	2.847 (1.562–5.187)	2.276 (1.173–4.418)	0.02
	rs11554585	G	$p=0.5485$	1.328 (0.649–2.718)	1.371 (0.643–2.926)	0.5270
	rs7561528	G	$p=0.1482$	1.77 (0.879–3.592)	1.743 (0.840–3.61)	0.1846
ApoE	rs2075650	G	$p<0.001$	7.333 (4.307–12.484)	6.61 (3.617–12.109)	<0.001

AD Alzheimer disease, *MA* minor allele, *MAF* minor allele frequency, *w* wild type, *m* mutant, *OR* odds ratio, *SNP* single-nucleotide polymorphism

Discussion

The aim of this paper is to report the results of a case-controlled study of LOAD patients of Turkish Azeri ethnicity, performed in northwest Iran. We examined a total of 11 SNPs (one in APOE, four in PICALM, and three each in CR1 and BINI genes), those most widely implicated in LOAD in GWAS. All SNPs were in Hardy–Weinberg equilibrium in both LOAD patients and healthy controls.

The APOE gene was the first to be identified as an associated risk factor for LOAD (Saunders et al. 1993). This association was detected using genetic linkage in families with a history of AD, after the protein was detected in amyloid plaques (Strittmatter et al. 1993; Kuusisto et al. 1994). The APOE gene has since been mapped on chromosome 19. The protein coded by the gene functions as a chaperone, has antioxidant actions, plays a key role in brain lipid metabolism, and transports cholesterol, and other fats in the bloodstream (Barberger-Gateau et al. 2011; Mahley and Rall 1988). It exists in several isoforms with a number of known differences coded by distinct alleles. APOE has three prominent forms, which by convention are named ε2, ε3, and ε4. The ε4 allele has an arginine at positions 112 and 158 (of 299), the ε3 allele has a cysteine at position 112, and the ε2 allele has cysteine residues at positions 112 and 158 (Saunders et al. 1993). The different isoforms bind to different lipoproteins, but the ε4 variant of the APOE gene functions less efficiently in the delivery of cholesterol to neurons compared with APOE ε3, and APOE ε3 and ε2 have more stable protein folding compared with APOEε4. The ε4 variant has a dose-dependent effect on variation in the onset of LOAD and the average survival time after disease onset (Roses 1996).

We found significant differences in the APOE ε4 allele distribution between LOAD patients and healthy controls. The present study also verified a significant association between the PICALM (rs3851179 and rs541458) and BIN1 rs744373 alleles and their related genotypes with LOAD, confirming these polymorphisms as contributing factors for susceptibility to LOAD. There were no significant associations between other polymorphisms and LOAD. Our results agree with previous studies that have identified the APOE ε4 allele as the most important risk factor for AD (Schmechel et al. 1993; Blacker et al. 1997; Styczyñska et al. 2008). In the present study, individuals carrying the ε4 allele were 7.3 times more likely to develop AD than noncarriers (OR=7.333, 95%CI= 4.307–12.484). In a previous study in Iran, individuals carrying the ε4 allele were 6.5 times more likely to develop LOAD than non-carriers (OR=6.52, 95%CI=2.63–16.17) (Gozalpour et al. 2010). In our study, the frequencies of ε4 alleles and genotypes with at least one ε4 in patients (30 and 41.8 %, respectively) were higher than found in the previous study in Iran (12.7 and 23.2 %, respectively) (Gozalpour et al. 2010). In Gyungah et al. study on 7,070 cases with AD and 8,169 elderly cognitively normal controls, from12 different studies, including white, African American, Israeli-Arab, and Caribbean Hispanic individuals APOE ε4 was significantly associated with AD (ORs, 1.80–9.05) in all but not in one small white cohort and in the Arab cohort (Gyungah et al. 2010). Hostage et al. analyzed MR images and genetic data on 662 patients from the Alzheimer's disease Neuroimaging Initiative (ADNI) database—198 cognitively normal controls (CN), 321 mild-cognitive impairment (MCI) subjects, and 143 AD subjects to investigate dose-dependent effects of the e4 and e2 alleles on hippocampal volumes. This study showed there was a dose-dependent effect of the e4 allele on

Table 3 Association of AD with *PICALM*, *CR1*, and *BIN1* SNPs stratified by APOE 4 carrier status

Gene	Variation	ApoEε4	SNP level	Group		OR (95 % CI)	*P* value
				AD patients	Controls		
PICALM	rs3851179	+	−	27 (8.4 %)	18 (5.5 %)	−	<0.0001
			+	69 (21.5 %)	0 (0 %)		
		−	−	180 (56.25 %)	275 (84.3 %)	0.4909 (0.3011–0.8004)	0.0056
			+	44 (13.8 %)	33 (10 %)		
	rs541458	+	−	57 (17.8 %)	11 (3.3 %)	0.9627 (0.3436–2.6976)	0.841
			+	39 (12 %)	7 (2.1 %)		
		−	−	201 (62.8 %)	292 (89.6 %)	2.0883 (1.0763–4.052)	0.040663
			+	23 (7 %)	16 (5 %)		
	rs12800974	+	−	95 (29 %)	18 (5.5 %)	−	1
			+	1 (0.3 %)	0 (%)		
		−	−	210 (65.6 %)	300 (92 %)	0.4 (0.1649–0.9705)	0.0617
			+	14 (4.3 %)	8 (2.5 %)		
	rs17159904	+	−	91 (28 %.4)	17 (5.2 %)	−	1
			+	5 (1.6 %)	1 (%)		
		−	−	219 (……%)	302 (92.6 %)	−	1
			+	5 (1.6 %)	6 (1.8 %)		
CR1	rs6701713	+	−	89 (……%)	17 (5.2 %)	−	1
			+	7 (……%)	1 (%)		
		−	−	169 (……%)	285 (%)	0.5271 (0.1996–1.3921)	0.2899
			+	9 (2.8 %)	8 (2.5 %)		
	rs3818361	+	−	92 (28.7 %)	18 (5.5 %)	−	0.61
			+	4 (1.25 %)	0 (0 %)		
		−	−	219(68.4 %)	303(93 %)	0.7228 (0.2067–2.5271)	0.749
			+	5 (1.6 %)	5 (1.5 %)		
	rs1408077	+	−	93 (29 %)	17 (5.2 %)	1.8235 (0.1789–18.5837)	1
			+	3 (0.9 %)	1 (0.3 %)		
		−	−	218 (68.1 %)	304 (93.2 %)	0.4781 (0.1333–1.7144)	0.334
			+	6 (1.9 %)	4 (1.2 %)		
BIN1	rs744373	+	−	91 (28.4 %)	15 (4.6 %)		
			+	5 (1.56 %)	3 (1 %)		
		−	−	188 (58.75 %)	295 (90 %)	4.3453(2.2458–8.4078)	<0.001
			+	36 (11.25 %)	13 (4 %)		
	rs11554585	+	−	96 (30 %)	18 (5.5 %)	−	1
			+	0 (0 %)	0 (0 %)		
		−	−	206 (64.3 %)	294 (90 %)	0.7831(0.3824–1.6037)	0.6242
			+	18 (5.6 %)	14 (4.2 %)		
	rs7561528	+	−	92 (28.7 %)	16 (5 %)	2.875(0.4856–17.0228)	0.239
			+	4 (1.25 %)	2 (0.6 %)		
		−	−	206 (64.3 %)	296 (90.7 %)	2.1553(1. 0163–4.571)	0.064
			+	18 (5.6 %)	12 (3.6 %)		

AD Alzheimer disease, *APOE* apolipoprotein E, *CI* confidence interval, *OR* odds ratio, *SNP* single-nucleotide polymorphisms

hippocampal volume in AD (p=0.04) and MCI (p=0.02) (Hostage et al. 2013).

Apart from the APOE locus, GWAS have identified significant evidence for a novel susceptibility locus in the PICALM gene (rs3851179: $p=1.3\times10^{-9}$, OR=0.86; rs541458: $p=8.3\times10^{-10}$, OR=0.86) (Harold et al. 2009). This association was first observed in Caucasians in a two-stage GWAS. In another study, Lambert et al. identified association with the same alleles, supporting the PICALM locus as a susceptibility locus, but the levels of association (p=0.03 for rs3851179 and $p=3\times10^{-3}$for rs541458) differed from those reported by Harold et al. (Lambert et al. 2009). Another GWAS looked at the association between PICALM SNPs and AD but the association was not significant (p=0.071–0.086), although there was

a positive trend (Kamboh et al. 2012a, b). Xiaolan et al. designed a genome-wide association study in an independent set of 1,034 cases and 1,186 controls after coupling data with available GWAS datasets from the ADNI and GenADA and Genotype-Phenotype Alzheimer's disease Associations (GenADA), the associations in PICALM (rs3851179) was replicated ($p=0.006$) (Hu et al. 2011). PICALM (SNP rs3851179) confers risk for AD (OR, 0.89; 95 % CI, 0.84–0.94) in white individuals but not in the African-American, Arab and Hispanic populations in Gyungah et al. study. Evidence for association with PICALM greatly reduced after adjusting for the presence of at least one APOE ε4 allele. (Gyungah et al. 2010).

A possible association between the BIN1 gene and AD was initially identified in the GERAD1 (Genetic and Environmental Risk in AD Consortium 1) study (Harold et al. 2009). In a subsequent study, rs744373 and rs7561528 (located in the 5′ region, approximately 30 and 25 kb from the BIN1 coding region, respectively) were also found to be significantly associated with AD (Seshadri et al. 2010). In a three-stage meta-analysis (8,371 LOAD cases and 26,965 controls), after APOE, CLU, and PICALM, the strongest association with LOAD was found with variant rs744373, residing near BIN1 on chromosome 2 (OR=1.15, $p=1.6\times10^{-11}$) (Seshadri et al. 2010). In another study, BIN1 (OR=1.17, $p=0.02$) associations were successfully replicated in an independent Spanish sample (1,140 LOAD cases and 1,209 controls (Seshadri et al. 2010). Lambert et al. have replicated the association of BIN1 with the risk of AD in three European populations (rs744373, OR=1.26; 95 % CI 1.15–1.38; $p=2.9\times10^{-7}$) (Lambert et al. 2009).

Recently, rs744373 polymorphism has also been investigated in Chinese and Japanese populations. However, no significant association with AD was reported ($p=0.06$) (Ohara et al. 2012; Tan et al. 2012). We investigated the association of BIN1 with AD in the present case-controlled series and successfully replicated the association observed for the variant near BIN1 (rs744373).

Although the SNPs with the strongest association have varied in different studies in ethnically distinct populations, several independent candidate gene studies have replicated and confirmed these results (Table 1). On the other hand, for the remaining polymorphisms, contradictory to previous GWAS, our study found a lack of association between LOAD subjects and controls in either genotype or allele distribution in an Iranian Turkish Azeri population. In summary, our results suggest that these polymorphisms may not play a major role in the development of LOAD in the studied population. It is possible that the negative findings could be attributed to the small sample size with insufficient power to detect moderate association. In addition, association study results can be affected by specific gene–gene or gene–environment interactions in a population-specific manner. It is possible that the effect of these polymorphisms on AD risk is not large enough to be detected reliably by an investigation of our size, or that it is only specific to particular ethnic groups. To explore potential interactions with APOE, we re-evaluated the association of AD with the mentioned SNPs after adjusting for the presence of APOEε4.

Statistical analysis showed the association with PICALM rs541458, rs3851179, and BIN1 gene rs744373 was evident only among subjects without the APOE ε4 allele. These results suggest that the APOE and these SNPs antagonistically interact. The interactions of other SNPs with the APOE ε4 allele were not statistically significant.

For polymorphisms that did not have significant associations with AD, after stratification of the sample by APOE ε4status, statistical analysis showed that the lack of association was independent from the presence of APOE ε4.

References

American Psychiatric Association (1994) Diagnostic and statistical manual of mental disorders, 4th edn. American Psychiatric Association, Washington, DC

Barberger-Gateau P, Samieri C, Catherine F, Plourde M (2011) Dietary omega 3 polyunsaturated fatty acids and Alzheimer's disease: interaction with apolipoprotein E genotype. Curr Alzheimer Res 8(5): 479–491

Blacker D, Haines JL, Rodes L et al (1997) ApoE-4 and age at onset of Alzheimer's disease: the NIMH genetics initiative. Neurology 48: 139–147

Corneveaux JJ, Myers AJ, Allen AN et al (2010) Association of CR1, CLU and PICALM with Alzheimer's disease in a cohort of clinically characterized and neuropathologically verified individuals. Hum Mol Genet 19(16):3295–301

Gatz M, Reynolds CA, Fratiglioni L, Johansson B, Mortimer JA, Berg S (2006) Role of genes and environments for explaining Alzheimer disease. Arch Gen Psychiatry 63:168–174

Gharesouran J, Rezazadeh M, Ghojazadeh M et al (2013a) Association of CALHM1 gene polymorphism with late onset alzheimer disease. Middle East J Med Genet 2(2):50–54

Gharesouran J, Rezazadeh M, Mohaddes Ardebili SM M et al (2013b) Investigation of five polymorphic DNA markers associated with late onset alzheimer disease. Genetika 45(2):503–514

Goate A, Chartier-Harlin MC, Mullan M, Brown J et al (1991) Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature 349:704–706

Gozalpour E, Kamali K, Mohammd K et al (2010) Association between Alzheimer's disease and apolipoprotein E polymorphisms. Iran J Publ Health 39:1–6

Gyungah J, Adam CN, Gary WB (2010) Meta-analysis confirms CR1, CLU, and PICALM as Alzheimer disease risk loci and reveals interactions with APOE genotypes. Arch Neurol 67(12):1473–1484

Harel A, Wu F, Mattson MP, Morris CM, Yao PJ (2008) Evidence for CALM in directing VAMP2 trafficking. Traffic 9:417–429

Harold D, Abraham R, Hollingworth P et al (2009) Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease, and shows evidence for additional susceptibility genes. Nat Genet 41(10):1088–1093

Hostage AC, Choudhury K, Doraiswamy PM et al (2013) Dissecting the gene dose-effects of the APOE e4 and e2 alleles on hippocampal volumes in aging and Alzheimer's disease. PLoS ONE 8(2):e54483

Hu X, Pickering E, Liu CY et al (2011) Meta-analysis for genome-wide association study identifies multiple variants at the BIN1 locus associated with late-onset Alzheimer's disease. PLoS ONE 6(2):e16616

Jones L, Harold D, Williams J (2010) Genetic evidence for the involvement of lipid metabolism in Alzheimer's disease. Biochim Biophys Acta 1801:754–761

Jun G, Naj AC, Beecham GW, Wang L et al (2010) Meta-analysis confirms CR1, CLU, and PICALM as Alzheimer disease risk loci and reveals interactions with APOE genotypes. Arch Neurol 67:1473–1484

Kamboh MI, Minster RL, Demirci FY (2012a) Association of CLU and PICALM variants with Alzheimer's disease. Neurobiol Aging 33(3):518–521

Kamboh MI, Demirci FY, Wang X et al (2012b) Genome-wide association study of Alzheimer's disease. Transl Psychiatry 2:e117

Khera R, Das N (2009) Complement receptor 1: disease associations and therapeutic implications. Mol Immunol 46(5):761–772

Koren J, Jinwal UK et al (2009) Chaperone signalling complexes in Alzheimer's disease. J Cell Mol Med 13(4):619–630

Krych-Goldberg M, Atkinson JP (2001) Structure–function relationships of complement receptor type 1. Immunol Rev 180:112–122

Kuusisto J, Koivisto K, Kervinen K et al (1994) Association of apolipoprotein E phenotypes with late onset Alzheimer's disease: population based study. BMJ 309(6955):636–638

Kwok JB (2010) Role of epigenetics in Alzheimer's and Parkinson's disease. Epigenomics 2(5):671–682

Lambert JC, Heath S, Even G et al (2009) Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat Genet 41:1094–1099

Lambert JC, Zelenika D, Hiltunen M et al (2011) Evidence of the association of BIN1 and PICALM with the AD risk in contrasting European populations. Neurobiol Aging 32(4):756.e11–5

Lazarczyk MJ, Hof PR, Bouras C, Giannakopoulos P (2012) Preclinical Alzheimer disease: identification of cases at risk among cognitively intact older individuals. BMC Med 10:127

Lee JH, Cheng R, Barral S et al (2011) Identification of novel loci for Alzheimer disease and replication of CLU, PICALM, and BIN1 in Caribbean Hispanic individuals. Arch Neurol 68(3):320–328

Mahley RW, Rall SC (1988) Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 240:622–630

Masoodi TA, Al Shammari SA, Al-Muammar MN, Alhamdan AA, Talluri VR (2013) Exploration of deleterious single nucleotide polymorphisms in late-onset Alzheimer disease susceptibility genes. Gene 512(2):429–437

Mei Sian C, Sahadevan S (2005) Preclinical Alzheimer's disease: diagnosis and prediction of progression. Lancet Neurol 4:576–579

Naj AC, Jun G, Beecham GW (2011) Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. Nat Genet 43:436–441

Ohara T, Ninomiya T, Hirakawa Y et al (2012) Association study of susceptibility genes for late-onset Alzheimer's disease in the Japanese population. Psychiatr Genet 22:290–293

Querfurth HW, LaFerla FM (2010) Alzheimer's disease. N Engl J Med 362:329–344

Rao AT, Degnan AJ, Levy LM (2014) Genetics of Alzheimer disease. AJNR Am J Neuroradiol 35(3):457–458

Rogaev EI (1999) Genetic factors and a polygenic model of Alzheimer's disease. Genetika 35(11):1558–1571

Rogaev EI, Sherrington R, Rogaeva EA et al (1995) Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. Nature 376:775–778

Roses AD (1996) Apolipoprotein E alleles as risk factors in Alzheimer's disease. Annu Rev Med 47:387–400

Saunders AM, Strittmatter WJ, Schmechel D, George-Hyslop et al (1993) Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. Neurology 43:1467–1472

Schmechel DE, Saunders AM, Strittmatter WJ et al (1993) Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. Proc Natl Acad Sci 90:9649–9653

Seshadri S, Fitzpatrick AL, Ikram MA et al (2010) Genome-wide analysis of genetic loci associated with Alzheimer disease. JAMA 303(18):1832–1840

Sherrington R, Rogaev EI, Liang Y et al (1995) Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. Nature 375:754–760

Shi H, Belbin O, Medway C et al (2012) Genetic variants influencing human aging from late-onset Alzheimer's disease (LOAD) genome-wide association studies (GWAS). Neurobiol Aging 33(8):1849e5–1849e18

Stanford JW (2004) APOE genotype effects on Alzheimer's disease onset and epidemiology. J Mol Neurosci 23:157–165

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 90:1977–1981

Styczyñska M, Strosznajder JB, Religa D et al (2008) Association between genetic and environmental factors and the risk of Alzheimer's disease. Folia Neuropathol 46(4):249–254

Tan JT, Yu W, Zhang ZC et al (2012) Association of GWAS-linked loci with late-onset Alzheimer's disease in a northern Han Chinese population. Alzheimers Dement 9(5):546–553

Tan MS, Yu JT, Tan L (2013) Bridging integrator 1 (BIN1): form, function, and Alzheimer's disease. Trends Mol Med 19(10):594–603

Tebar F, Bohlander SK, Sorkin A (1999) Clathrin assembly lymphoid myeloid leukemia (CALM) protein: localization in endocytic-coated pits, interactions with clathrin, and the impact of overexpression on clathrin-mediated traffic. Mol Biol Cell 10:2687–2702

The Dementia Study Group of the Italian Neurological Society (2000) Guidelinesfor the diagnosis of dementia and Alzheimer's disease. Ital J Neurol Sci 21:187–194

van Rossum IA, Vos S, Handels R, Visser PJ (2010) Biomarkers as predictors for conversion from mild cognitive impairment to Alzheimer-type dementia implications for trial design. J Alzheimers Dis 20(3):881–891

Wijsman EW, Nathan DP, Yoonha C et al (2011) Genome-wide association of familial late-onset Alzheimer's disease replicates BIN1 and CLU and nominates CUGBP2 in interaction with APOE. PLoS Genet 7(2):e1001308

Zawia N, Lahiri DK, Cardozo-Pelaez F (2009) Epigenetics, oxidative stress and Alzheimer's disease. Free Radic Biol Med 46(9):1241–1249