Association study in Alzheimer's disease of single nucleotide polymorphisms implicated with coffee consumption

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Received: 4/16/2015 – **Accepted:** 4/27/2015
DOI: 10.1590/0101-60830000000050

Abstract

Background: There is evidence from animal and *in vitro* models of the protective effects of caffeine in Alzheimer's disease. The suggested mechanisms through which caffeine may protect neurons against Alzheimer's disease pathology include the facilitation of beta-amyloid clearance, upregulation of cholinergic transmission, and increased neuronal plasticity and survival. Epidemiological studies support that Alzheimer's disease patients consume smaller amounts of coffee beverages throughout their lives as compared to age-matched cognitively healthy individuals. **Objective:** The aim of the present study was to determine whether the negative association between Alzheimer's disease and coffee consumption may be influenced by a common genetic predisposition, given the fact that the pattern of coffee consumption is determined by both environmental and genetic factors. **Method:** We conducted an *in silico* search addressing the association between genetic polymorphisms related to coffee consumption and the diagnosis of Alzheimer's disease. We further investigated the interactions between genes located in regions bearing these polymorphisms. **Results:** Our analysis revealed no evidence for a genetic association (nor interaction between related proteins) involving coffee consumption and Alzheimer's disease. **Discussion:** The negative association between Alzheimer's disease and coffee consumption suggested by epidemiological studies is most likely due to environmental factors that are not necessarily regulated by genetic background.

Yamamoto VJ et al. / Arch Clin Psychiatry. 2015;42(3):69-73

Keywords: Caffeine, Alzheimer's disease, SNP.

Introduction

More than 90% of new therapeutics developed for Alzheimer's disease (AD) have been unsuccessful[1]. The lack of an effective treatment contributed to increase the attention to life style and dietary interventions that could modify the etiology of the disease[2]. Epidemiological studies have indicated a negative association between coffee consumption and AD, suggesting that caffeine may have neuroprotective effects against cognitive decline[2-5]. This xanthine-derived substance, highly concentrated in coffee, is a stimulant to the central nervous system that may be beneficial against certain deficits associated with AD[3,6,7]. Putative mechanisms for caffeine neuroprotection in AD include the facilitation of beta-amyloid clearance[7,8], and the upregulation of cholinergic neurotransmission[9] and/or signalling pathways related to neuronal plasticity and survival[10-12]. Most of those effects are probably mediated the stimulation of adenosine receptors[13]. However, evidence of beneficial effects of caffeine in human cognition is largely derived from epidemiological associations, with scarce direct evidence for the protective effects of coffee/caffeine in patients with AD[14]. Coffee consumption is determined by both environmental and genetic factors. Environmental factors related to coffee consumption include mainly age and sex, but it is also influenced by geographical location, religious preferences and socioeconomic conditions. Great effort has been made to identify genetic factors involved in coffee intake. Meta-analyses of genome-wide association indicated some common genetic variants that influence coffee consumption, localized in CYP1A1/CYP1A2, NRCAM and AHR genes[15-17]. AD is a multifactorial neurodegenerative disorder resulting from the interaction between multiple genetic and environmental factors[18-20]. Since there are genetic factors associated with both AD and coffee consumption, one cannot rule out the possibility that the negative association observed in epidemiological studies are due to a common genetic background, or even interactions among related gene products. In the present study, our aim was to evaluate genetic associations of single nucleotide polymorphisms (SNP) related to coffee consumption in the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort, comprising patients with AD or mild cognitive impairment (MCI) and healthy age-matched controls. Also to investigate possible interactions among the products of genes associated with AD and those located near the coffee consumption SNP.

Methods

Sample

Data used in the preparation of this article were obtained from the ADNI database (adni.loni.ucla.edu). The ADNI was launched in 2003 by the National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies and non-profit organizations, as a $60 million, 5-year public-private partnership. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. Determination of sensitive and specific markers of very early AD progression is intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness, as well as lessen the time and cost of clinical trials.

The Principal Investigator of this initiative is Michael W. Weiner, MD, VA Medical Center and University of California – San Francisco. ADNI is the result of efforts of many co-investigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the U.S. and Canada.

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The initial goal of ADNI was to recruit 800 subjects but ADNI has been followed by ADNI-GO and ADNI-2. To date these three protocols have recruited over 1,500 adults, ages 55 to 90, to participate in the research, consisting of cognitively normal older individuals, people with early or late MCI, and people with early AD. The follow up duration of each group is specified in the protocols for ADNI-1, ADNI-2 and ADNI-GO. Subjects originally recruited for ADNI-1 and ADNI-GO had the option to be followed in ADNI-2. For up-to-date information, see www.adni-info.org.

A total of 757 individuals were genotyped using the Illumina Human Genome 610 Quad BeadChips in the ADNI database downloaded in November 2011. We excluded individuals with less than 65 years of age, since they might correspond to the early onset forms of AD. The most recent clinical data of those subjects, at the time, revealed 198 healthy controls (CT), 193 classified as MCI and 318 diagnosed with AD. All those individuals had less than 10% missing genotypes. There was no information regarding coffee consumption in the ADNI database at the time of download.

Genotype data of SNPs that had been strongly associated with coffee consumption[15-17] was retrieved from the ADNI database. From the chosen SNPs (rs12148488, rs2470893, rs2472297, rs2472304, rs382140, rs4410790, rs5751876, rs6495122, rs762551), only rs5751876 was not present in the ADNI database. The remaining SNPs passed our quality control with a call rate above 90% and minor allele frequency greater than 2%. rs16868941 was in Hardy Weinberg disequilibrium ($p < 0.10$; table 1) and was excluded from further analysis. Quality control analysis was performed with PLINK v1.07 and R software.

Firstly, we compared the genome location of the coffee consumption and AD SNPs from the top 10 associated SNPs in the repository of meta-analyses AlzGene (last updated 18th April 2011 – http://www.alzgene.org)[21].

We used the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; http://string-db.org)[22] to investigate known protein interactions and pathway enrichment among the coffee consumption genes (up to 50 kb of distance, if the SNP is not inside a gene) and the top 10 AD associated genes from Alzgene[21]. Significance for this analysis was set as $p < 0.05$ after FDR correction.

APOE alleles frequencies were retrieved from AlzGene meta-analysis and ADNI database. We used a chi-square test in LibreOffice Calc 3.5 (significance, $p < 0.05$) to compare the frequencies in control and AD individuals in those sets.

Association of each caffeine SNP among controls, MCI or AD was tested with Wald test for multinomial logistic regression controlled by age, gender, years of education and presence of the e4 allele of APOE. We also tested the model considering a dominant (MAF allele carrier vs non-carrier) or recessive (homozygous for the MAF allele vs other genotypes) pattern of inheritance for each MAF allele of each SNP. Significance was set as $p < 0.0018$, to correct for multiple comparisons with the conservative Bonferroni method. We also tested for association of controls, MCI or AD with caffeine SNP, age, gender, years of education and APOE genotype with a classification and regression tree (CART). These analyses were conducted with the aid of the R software version 2.15.2.

Results

Demographic data is summarized in table 2. The MCI group had significantly fewer females than control ($p = 0.010$) or AD ($p = 0.049$) groups. AD subjects on average had fewer years of education than controls ($p = 0.019$). There was no difference in APOE alleles frequencies between ADNI and AlzGene (table 1, $p > 0.90$). We observed a strong association of the e4 allele of APOE and AD tested with a general linear model controlled by age, gender and education compared to healthy controls ($p = 2.079 \times 10^{-17}$). Also, presence of APOE e4 in MCI was significantly different from both healthy controls ($p = 0.0005$) and AD patients ($p = 4.908 \times 10^{-7}$). No association was observed between the coffee consumption SNPs and AD, for the three different scenarios tested (general, dominant MAF allele and recessive MAF allele models; table 3). CART analysis showed no association of coffee consumption SNPs and the three groups after cross validation. However, if we exclude MCI from the analysis, rs6495122 appears as

Table 1. Coffee consumption SNPs – List of SNPs associated with coffee consumption, nearby genes (up to 50 kb, if the SNP is not inside a gene) and chromosome they are located – Minor allele frequency (MAF) and p value for Hardy-Weinberg equilibrium (HWE; In bold $p < 0.10$) for each SNP in the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort is also presented

SNP	Genes	Chr	MAF (%)	HWE	HWE Control	HWE MCI	HWE AD
rs12148488	PPCDC	15	G (49)	0.275	1.000	0.885	**0.033**
rs16868941	NCALD	8	A (19)	**0.063**	-	1.000	0.848
rs2470893	CYP1A1, CYP1A2, EDC3	15	A (28)	0.179	0.706	0.469	0.144
rs2472297	CYP1A1, CYP1A2, EDC3, CSK	15	T (21)	0.286	0.622	0.684	0.877
rs2472304	CYP1A2	15	G (37)	0.140	0.140	0.522	0.286
rs382140	LAMB4, NRCAM	7	A (17)	0.379	0.507	0.464	0.535
rs4410790	AHR	7	T (41)	0.940	0.659	**0.042**	0.347
rs5751876	ADORA2A	22	-	-			
rs6495122	CPLX3, ULK3, SCAMP2, MPI, LMAN1L, CSK	15	A (45)	0.825	0.774	**0.020**	**0.056**
rs762551	CYP1A2	15	C (27)	0.855	0.733	0.698	1.000

Table 2. Summarized demographic data of the ADNI individuals selected for this paper and APOE*e4 frequencies for ADNI and AlzGene cohort (# $p < 0.05$ in Fisher exact test when compared to either Healthy control or Alzheimer's Disease groups. * $p < 0.05$ in ANOVA, with post TUKEY-HSD when compared to Healthy control group. & $p < 0.05$ in Fisher exact test when compared to Healthy control)

	Healthy control	Mild cognitive impairment	Alzheimer's disease
Gender (% female)	45.9	33.2#	42.1
Age (mean ± SD)	79.2 ± 5.5	78.9 ± 6.6	79.3 ± 5.8
Age at diagnostics (mean ± SD)	-	-	75.0 ± 5.9
Years of education (mean ± SD)	16.0 ± 2.8	15.6 ± 3.2	15.2 ± 3.1*
Handedness (% right handed)	91.9	94.3	93.7
Ethnic background (% Caucasian)	91.4	90.0	91.8
APOE*e4 (%) ADNI	15	25#	42&
AlzGene	14	-	38&

a relevant factor to classify between controls and AD (Figure 1). Our analysis of protein interactions with STRING revealed no known interactions among coffee consumption associated proteins and the AD proteins. As can be seen in figure 2 the genes for AD and for coffee consumption had no significant interaction (with a confidence greater than 0.5 in STRING algorithm). Also, enrichment analysis of KEGG pathways showed only a significant (p = 0.038 after FDR correction) group of AD associated genes (PSEN1, PSEN2, APP and APOE).

Table 3. Association of AD and Coffee consumption SNPs in the ADNI cohort – Association of AD and Coffee consumption SNPs in the ADNI cohort was evaluated with a multinomial logistic regression model controlled by age, gender, years of education and presence of the e4 allele of APOE followed by Wald test – Dominant and Recessive columns, correspond to p value of the model, when testing for dominant (MAF allele carrier vs non-carrier) and recessive (homozygous for the MAF allele vs other genotypes) pattern of association. General is the p value with no pattern of inheritance considered. Significance was set as p < 0.0018, to correct for multiple comparisons with the conservative Bonferroni method

SNP			General	Dominant		Recessive	
				Vs Control	AD *vs* MCI	*Vs* Control	AD *vs* MCI
rs12148488	C: MCI: AD:	GG(23); GT(50); TT(27) GG(26); GT(51); TT(23) GG(27); GT(44); TT(29)	0.431	- 0.324 0.849	- 0.200	- 0.364 0.218	- 0.779
rs2470893	C: MCI: AD:	CC(57); CT(36); TT(7) CC(51); CT(43); TT(6) CC(50); CT(39); TT(11)	0.392	- 0.247 0.249	- 0.934	- 0.738 0.330	- 0.166
rs2472297	C: MCI: AD:	CC(69); CT(28); TT(3) CC(60); CT(34); TT(6) CC(58); CT(36); TT(6)	0.315	- 0.085 0.446	- 0.847	- 0.459 0.624	- 0.733
rs2472304	C: MCI: AD:	CC(19); CT(43); TT(38) CC(11); CT(47); TT(42) CC(17); CT(44); TT(39)	0.209	- 0.324 0.482	- 0.722	- 0.035 0.752	- 0.052
rs382140	C: MCI: AD:	CC(65); CT(30); TT(5) CC(68); CT(28); TT(4) CC(71); CT(26); TT(3)	0.741	- 0.559 0.174	- 0.444	- 0.953 0.698	- 0.652
rs4410790	C: MCI: AD:	AA(17); AG(47); GG(36) AA(17); AG(57); GG(26) AA(17); AG(45); GG(38)	0.049	- 0.011 0.782	- 0.012	- 0.859 0.758	- 0.901
rs6495122	C: MCI: AD:	GG(31); GT(48); TT(21) GG(28); GT(58); TT(14) GG(32); GT(44); TT(24)	0.024	- 0.629 0.594	- 0.280	- 0.142 0.306	- 0.010
rs762551	C: MCI: AD:	AA(49); AC(43); CC(8) AA(57); AC(38); CC(5) AA(49); AC(42); CC(9)	0.290	- 0.110 0.618	- 0.211	- 0.386 0.433	- 0.099

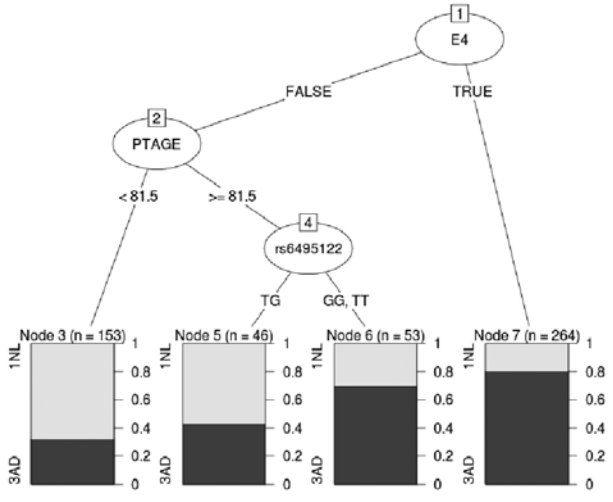

Figure 1. Coffee consumption SNP (rs6495122) improves the classification between healthy controls (1 NL) and Alzheimer's disease patients (3AD) in a classification and regression tree. Main factors for classification were presence of e4 allele of APOE (E4) and age (PTAGE).

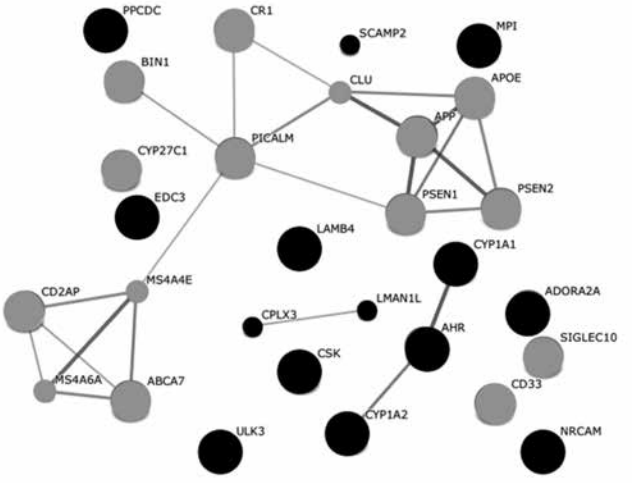

Figure 2. Network of interactions for the corresponding genes whithin 50 kb of the selected SNPs. Adapted from image generated with STRING with a confidence of 0.5 in June 2013. For better viewing we painted the nodes for Alzheimer's disease genes in gray and Coffee consumption in black. Stronger interactions are represented with thicker lines. Bigger nodes indicate those for which the protein structure has been elucidated.

Discussion

Our results suggest that it is unlikely that the negative association between coffee consumption and AD prevalence is due to a common genetic background, at least between the regions compared here. We found no association between coffee consumption SNPs and AD, also there was no correlation between genetic regions and gene products for those two characteristics.

Firstly, none of the evaluated coffee consumption SNPs[15-17] is genetically near any of the most associated SNPs for AD[21]. This rule out the possibility of linkage disequilibrium between them. Also, there was no significant association between coffee consumption SNPs and AD in general, dominant MAF allele and recessive MAF allele models in the ADNI cohort. Also, rs6495122 appears as a relevant factor for classification in CART only after APOE genotype and age, two already known risk factors for AD[19]. Lastly, we found no known interaction between the gene products considered for coffee consumption and AD, which might have suggested an indirect association. Cornelis *et al.* mentioned in his meta-analysis that the coffee consumption SNPs did not showed before in large AD GWAS studies[17]. This is the first attempt to investigate if there is a genetic correlation behind the evidence for a protective effect of coffee in AD. Larger cohorts with genetic and coffee consumption information might be required to further elucidate this question.

Experimental data suggests that caffeine derivates have neurobiological benefits on cognition and AD pathology. It probably results from the effects of these substances on signalling pathways related to AD pathophysiology and/or neuronal resilience.

Animal models suggested that caffeine administration was associated with reduction of both soluble and deposited amyloid-β (Aβ) in brain[7]. The hydrophobic nature of caffeine allows its rapid absorption through all biological membranes, rendering it is rapidly absorbed into the bloodstream and through the blood-brain barrier[23]. *In vitro* studies, showed that caffeine increases basal synaptic transmission, but does not affect LTP, at the same synapses[24]. However, the concentrations of caffeine required to exert these effects, are several orders of magnitude higher than the plasmatic concentration attained by ingestion of moderate amounts of coffee[17,25,26]. The only pharmacological mechanism known for caffeine in the low micromolar range is the antagonism of adenosine receptors, namely adenosine A1 and A2A receptors and maybe adenosine A3 receptors[13,16,27,28], leading to many downstream changes, including alterations in gene expression. The blockade of adenosine receptors confers neuroprotection against Aβ[6], but the underlying mechanisms by which caffeine reduces the relative risk for AD are not well elucidated. Adenosine A1 receptors in the cerebral cortex and hippocampus are primarily located on pre-synaptic terminals. Caffeine can block those receptors in cholinergic terminals which increases extracellular levels of acetylcholine, an important neurotransmitter for cognitive processing, dramatically decreased in the AD brain[9]. Another plausible hypothesis which could explain the decreasing level of Aβ is based on enhanced clearance of Aβ from the brain due to upregulation of P-glycoprotein (P-gp) induced by caffeine[8]. P-gp is an adenosine triphosphate (ATP) dependent transporter protein, which acts as an efflux pump. This transporter is located mainly in luminal membrane of brain capillary endothelium comprising the blood-brain-barrier, and is responsible for extrusion of drugs and toxins from the brain, including Aβ[29]. An increasing number of studies further suggest that alterations in expression and functional activity of P-gp contribute to the accumulation of Aβ in the brain, and lead to increased risk for developing AD[30]. A third possible mechanism by which caffeine could ameliorate AD prognosis is by acting in neuronal plasticity and survival. Caffeine induced both elongation of existing dendritic spines and new spine formation in primary cultured hippocampal neurons[11]. Also, long-term caffeine treatment in AD transgenic mice decreased pro-inflammatory cytokines such as TNFα and IFNγ in the brain[10]. Taken together with its beneficial effects on signal transduction[12], these results suggest an important role for caffeine in neuronal plasticity and survival. There are also other hypotheses about the mechanisms involved in neuroprotection by caffeine[31], however the exactly mechanisms are not completely understood[8].

There is no specific information of coffee consumption in the ADNI database. This is a limitation of the present analysis, since the evaluated SNPs might not correspond directly to coffee consumption in this sample. Also, we cannot rule out that the lack of significance might be due to a lack of power, because of the sample size. On the other hand, the ADNI sample had frequencies of APOE allele similar to the general population and we also replicated the strong association between APOE e4 allele and AD. These results indicate there were no sampling errors.

If we consider a less conservative threshold of $p < 0.05$, there are some observable differences in the MCI group compared to AD and/or healthy controls, rs4410790 (general and dominant models), rs6495122 (general and recessive models) and rs2472304 (recessive model). As mentioned, rs6495122 also appears as a relevant factor for the CART. Interestingly, the daily dose associated with cognitive protection is 3 to 5 cups of coffee. Depending on preparation and coffee type this dose corresponds to 210-1,100 mg of caffeine per day[5]. In comparison, the coffee consumption SNPs are associated with a change of less than 1 mg/day in a general model, but a maximum 44 mg per day difference between genotypes[17]. Given the effects magnitude, these coffee consumption genetic variants may not be a determinant factor in the estimated protective coffee dose. However, they might act as outcome modifiers as a result of the direct and indirect interaction with other genetic and environmental factors. Thus, it might be a good approach to introduce those SNPs as covariables in future studies of coffee and caffeine consumption, cognitive deficit and AD.

Acknowledgements

Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (National Institutes of Health Grant U01 AG024904). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: Alzheimer's Association; Alzheimer's Drug Discovery Foundation; BioClinica, Inc.; Biogen Idec Inc.; Bristol-Myers Squibb Company; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; GE Healthcare; Innogenetics, N.V.; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Medpace, Inc.; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Synarc Inc.; and Takeda Pharmaceutical Company. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are Rev November 7, 2012 facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Disease Cooperative Study at the University of California, San Diego. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of California, Los Angeles. This research was also supported by NIH grants P30 AG010129 and K01 AG030514.

The Laboratory of Neuroscience (LIM-27) receives financial support from FAPESP, Fundação de Amparo à Pesquisa do Estado de São Paulo, and ABADHS, Associação Beneficente Alzira Denise Hertzog da Silva.

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