Isolated and Joint Effects of Tobacco and Alcohol Consumption on Risk of Alzheimer's Disease

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ABSTRACT

The roles of smoking and alcohol on the development of Alzheimer's disease (AD) remain unclear. We performed a case-control study on the effects of both exposures before the age of onset of the disease in the cases (and same reference age for their age-matched controls) on disease risk. Interviews were conducted with population controls (n=246) and relatives of cases (n=176) identified through local Alzheimer's Disease Associations. Logistic regression models were built adjusting by gender, age, residence, education, economic situation, employment, and history of dementia in close relatives. Risk of AD was unaffected by any measure of tobacco consumption. Alcohol consumers showed a lower risk of AD than never consumers (adjusted odds ratio, aOR=0.53, 95% CI 0.32, 0.88), with differences by gender (women aOR=0.48, 95% CI 0.27, 0.84; men aOR=0.80, 95% CI 0.23, 2.80). Mean daily total consumption of alcohol and time consuming alcohol showed increasingly protective dose-response relationships in women. Lower AD risk was observed in alcohol drinkers of both genders who never smoked (aOR=0.37, 95% CI 0.21, 0.65). All these associations were independent of the presence of apolipoprotein E4 allele(s) in the cases. Although the sample was small for some analyses addressing these interactions, our results suggest a protective effect of alcohol consumption, mostly in non-smokers, and the need to consider interactions between tobacco and alcohol consumption, as well as interactions with gender, when assessing the effects of smoking and/or drinking on the risk of AD.

INTRODUCTION

The roles of smoking and alcohol consumption on the development of Alzheimer's disease (AD) remain controversial. Concerning tobacco, initial pooled analyses suggesting a protective effect [1,2] were later challenged by longitudinal and case-control studies finding increased risks [3-7], mostly in current or recent smokers. Interaction effects between smoking and the apolipoprotein E4 (ApoE4) allele have been suggested as well. First a protective effect of smoking on AD risk was observed only for ApoE4 carriers [8]; more recently, a significant increase in risk related to smoking was only evident for cases without any ApoE4 allele [4, 5, 7]. Dose-response relationships between smoking and AD are also elusive; some reports suggest complex non-linear shapes [9, 10], while others found linearly increasing risks [7]. In a prospective study [11], the highest risk of AD was observed for current smokers without any ApoE4 allele (adjusted OR aOR=4.32, 95% CI 1.28, 14.65), but there was no relationship between the magnitude of risk and pack-years smoked. Furthermore, former smokers at baseline with any ApoE4 allele showed decreased risk (aOR=0.27, 95% CI 0.08, 0.93), and AD risk decreased as the number of pack-years increased (p=0.02). A variety of mechanistic and methodological explanations have been proposed for these opposite findings [12-14]. As it has been suggested, it is clear that analyses must properly consider duration and intensity of smoking, time since quitting, and presence of ApoE4 alleles.

On the other hand, evidence of a potential relationship between alcohol consumption and AD is even scarcer than for smoking. After first reports found no association [1, 9, 10, 15], more recent results point to a protective effect of alcohol, mostly associated to moderate intakes and to wine consumption [16-18]. But differences according to type of alcohol beverage consumed have not always been observed [19]; and increased risks have also been reported for heavy

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alcohol consumption [20]. Analyses of interactions between alcohol consumption and ApoE4 alleles are scant and conflicting [18, 19]. Additionally, interactive effects of smoking and drinking have been observed, and they are supported by the fact that both alcohol and tobacco affect brain nicotinic cholinergic receptors. Smokers and drinkers tended to show lower risks of AD than subjects who were just smokers or just drinkers [21]. But antagonistic effects of tobacco and wine consumption have also been suggested for some dimensions of general cognitive decline [16].

We carried out a case-control study focused on occupational exposures as potential risk factors for AD. Detailed information on life-long tobacco and alcohol consumption was collected. In this report, we present results on the relationships between alcohol and tobacco consumption and AD risk, with special attention to controversial issues such as the influence of period, time and intensity of consumption before the age of disease onset, interactions with ApoE4, type of alcoholic beverages consumed, and joint effects of alcohol and tobacco.

MATERIALS AND METHODS

Design and selection of subjects

The protocol of the study was approved by the Ethical Committee of the University of Valencia (Spain). Cases were identified through local Alzheimer's Disease Associations (ADAs) in five towns of the region of Valencia (Valencia city, Alicante, Elda, Villena, and Alcoy). These locations were selected because of proximity and accessibility. ADAs are very common in Spain, having premises in almost all medium and big sized municipalities and with thousands of members, including AD patients and their relatives [22]. Local ADAs are mostly intended to give support, training, and information to patients and caregivers. A medical diagnosis of AD is

required for registration in an ADA, but medical histories of the patients were not accessible. All information regarding diagnosis, age of onset of the disease (age when the disease was first diagnosed), and symptoms of the disease was then obtained through personal interviews with caregivers.

To recruit cases, relatives or caregivers responsible for the AD patients registered in selected participating ADAs were randomly selected from available registries and invited by mail to participate in the study, introduced as a research on characteristics and antecedents of AD. Phone calls were later done to these same subjects to fix a meeting date to interview them. Interviews were attained with 95% of effective phone calls (5% of contacted persons refused to participate). Non-effective calls (31%) were mostly due to incorrect phone numbers (Figure 1).

Controls were randomly selected through Social Security registers, which in Spain cover almost 100% of the population. The aim was to have a 2:1 ratio of cases and controls, controls being matched to cases for gender, age at interview, and municipality of residence at interview. Matching for these variables was always accomplished in the selection of controls but the 2:1 ratio was not attained; nevertheless, all cases have at least one matched control. Eligible controls were invited by mail to participate in the research, introduced as a health study of the elderly. Eight percent of the letters were returned because of wrong or incomplete address. As with cases, phone calls were done in order to fix a date for personal interview. Twenty three percent of these calls were not valid, mostly because of incorrect number (59%) and non-answer (29%) after seven attempts at different times. Sixty one percent of located controls agreed to participate, and for 87% of them, the interview was completed (Figure 1).

Personal interviews

Next-of-kin of participating cases were personally interviewed (n=176). All controls were personally interviewed (n=246) and, when possible (n=108), independent interviews were also performed with a control's proxy selected upon his/her availability and close relationship with the control. Interviews were mostly carried out in the interviewees' homes, lasting between 25 and 35 minutes. Interviewers (n=8) were previously trained and used detailed consultation manuals. Interviewers were preferably located in the municipalities where cases and controls lived. The same interviewers were used for interviewing informants of cases, controls, and informants of controls. They were essentially unaware of the study hypotheses, although it was clear that AD and related circumstances were the focus, and informants of cases and controls were easily identified. Interviews were carried out between March 2001 and July 2003.

Tobacco and alcohol consumption assessment

Information in the structured questionnaire was organized in five sections: personal and family data, occupational history and exposures, lifestyles (tobacco and alcohol consumption), medical history (present and former health problems related to dementia), and social factors (education level and socioeconomic situation), all questions referring to antecedents or exposures with a potential relationship with AD according to available evidence. The questionnaire used in the study was previously piloted in order to test and improve its contents and structure [23].

Controls and informants of cases and controls were asked about the index subject consumption of tobacco and alcohol before the age of onset of the disease in the cases (and same reference age for their age-matched controls), including dates of starting and quitting, and average daily amounts and types of tobacco and alcoholic beverages. Average daily smoked

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cigarettes were considered for dose-response analysis. Information regarding amount and frequency of consumption of different categories of alcoholic beverages grouped according to alcohol content (beer, wine, and liquors) was collected and then transformed to mean daily total consumption of pure alcohol (in cubic centimetres, cc), with one unit (glass or cup) of beer or cider being equivalent to 8.5 cc of alcohol, one unit of wine or cava being equivalent to 12 cc of alcohol, and one unit of liquors being equivalent to 20 cc of alcohol. People in the categories "Never smokers" or "Never drinkers" reported null consumption of tobacco or alcohol throughout their life, respectively.

ApoE genotype ascertainment in cases

Apolipoprotein E (ApoE) genotype was assessed using PCR on blood samples from cases whose informants agreed to this test after signing informed consent. The technique and detailed results have been presented before [24]. In statistical analyses, ApoE genotype was dichotomized into presence of one ApoE4 allele (homozygotes and heterozygotes) or absence of any ApoE4 allele (i.e., any combination of ApoE2 and ApoE3 alleles).

Statistical analyses

Univariate analyses were performed as customary. Multivariate-adjusted odds ratios (aOR) and their 95% confidence intervals (CI) were computed through unconditional logistic regression [25]. Covariates included in the analyses were education level (categorized as less than primary education, primary education, and secondary-high education), life-long global economic situation (very good-good, and regular-bad-very bad), paid employment in the past (yes, no), history of dementia in close relatives (parents, brothers or sisters, children; yes, no), and gender,

age (age at onset of the disease in cases, and same age in matched controls), and municipality of residence. Main analyses were repeated stratifying by gender, ApoE4 (presence or absence of any ApoE4 allele), and AD onset before or after 65 years (early and late onset cases, respectively). The effects of joint consumption of tobacco and alcohol were investigated too. Additional analyses were performed excluding heavy alcohol drinkers, as heavy alcohol consumption is a known cause of dementia. Tobacco analyses were also repeated excluding subjects with antecedents of hypertension, cerebrovascular disease, or arteriosclerosis. Interactions between smoking and hypertension were explored too. In a previous analysis, good correlations were found between information elicited from controls and their proxy respondents [26]. Thus, for the present analysis we only used information directly obtained from controls (n=246) and from cases' informants (n=176). All data analyses were performed using Stata version 9.0 software (StataCorp LP, College Station, TX).

Statistical power in our data for the main associations observed was obtained based on the number of subjects in the different categories and the observed prevalence of exposure. These calculations were performed using the Power Calculator tool for unmatched case-control studies in OpenEpi software (www.openepi.com).

<mark>RESULTS</mark>

Interviews were completed with informants of 176 cases, of whom 141 (80%) were reported to show three main key symptoms related to AD diagnosis (cognitive impairment with gradual onset and progression, recent memory impairment, and impaired activities of daily living). ApoE genotyping was available for 113 cases (64%), 60 of whom (53%) were carriers of at least one ApoE4 allele.

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Table 1 shows the main characteristics of cases and controls. No differences were observed in gender, age, or residence. By contrast, the two groups differed in education, with more controls than cases in the upper and lower educational levels. Controls reported a better life-long economic situation than cases. Differences were also observed for depression, more frequent in cases, and for cardiovascular diseases, affecting controls with a higher frequency. Dementia in close relatives was reported more frequently in cases than controls.

Table 2 shows results for tobacco. No overall relationship was found with risk of AD. Neither were there associations seen with number of cigarettes smoked per day, number of years smoking, and time since quitting smoking before age at diagnosis. There was no evidence of interactions between tobacco and educational level, gender, familiar antecedents of dementia, or time of AD onset (data not shown). Association between smoking and AD risk was equally absent in ApoE4 carriers and non-carriers. Excluding subjects with antecedents of cardiovascular disease (hypertension, cerebrovascular disease, and/or arteriosclerosis, see Table 1) did not yield substantially different results (data not shown). Smoking cases showed slightly lower mean age at diagnosis than non-smoking cases (respectively, 67.9 and 69.8 years, 2 sided p=0.078).

A protective effect was observed for alcohol, with ever consumers showing lower risk of AD than never consumers (aOR=0.53, 95% CI 0.32, 0.88) (Table 3). This association was unambiguous only for women, and all remaining analyses are stratified by gender. Mean daily total consumption of pure alcohol (cc) showed an increasingly protective dose-relationship for women, but not for men. A strong protective association was also observed for women having drank for 40 years or more before reference age in cases and controls (aOR=0.22, 95% CI 0.08, 0.66), with evidence of dose-response relationship too. The relationship with time since quitting alcohol before reference age is uncertain (Table 3). No major differences were found among the

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three types of alcohol consumed (beer, wine, and liquors), or when dropping heavy drinkers (> 20 cc pure alcohol per day) (data not shown). Analysis considering only ApoE4 carrier cases showed only slightly stronger protective associations, mostly for women (ever vs. never consumers, both genders: aOR=0.42, 95% CI 0.19, 0.91; women: aOR=0.29; 95% CI 0.11, 0.77; men aOR=0.89, 95% CI 0.17, 4.55). Associations lost statistical significance in ApoE4 non-carriers, though they still pointed to the same protective effect (ever vs. never consumers, aOR=0.67, 95% CI 0.31, 1.43; women: aOR=0.70; 95% CI 0.31, 1.60; men aOR=0.23, 95% CI 0.03, 1.76). Separate analyses on early and late onset cases did not substantially vary from previous results.

Finally, interaction between tobacco and alcohol consumption was investigated. Unadjusted logistic regression model showed statistical significance for the interaction term between tobacco and alcohol (p=0.011), and this interaction term was also statistically significant in the model including our potentially confounding variables (p=0.003). Table 4 shows the results from adjusted analyses with the different combinations of this interaction. Although for some of the analyses the number of subjects was notably low, most consistent results across different combinations are seen for drinkers who never smoked, showing significantly reduced AD risk, both in men and women, when compared with subjects never drinking or smoking throughout life, independently of the presence of any ApoE4 allele in cases (information on ApoE was not available for controls).

DISCUSSION

We found that tobacco consumption did not affect risk of AD whereas alcohol had a protective effect, mostly in women. The lower AD risk was particularly evident among alcohol

drinkers who never smoked, both in men and women, and independently of ApoE4 alleles. Regarding analyses considering ApoE4 presence in cases, it should be noticed that we had no information on ApoE alleles in controls and p values are not adjusted for the potential multiple uses of data (cases carrying and not carrying ApoE4 are compared with the same subgroups of controls).

Although the low number of observations when addressing associations in some categories of exposure should be noticed, statistical power calculations with our data (based on the number of subjects and observed prevalence of exposure) was satisfactory for previously stressed main results: the power in our study to detect a significant difference for alcohol consumption between cases and controls at the significance level of 0.05 (95% confidence interval) was 90% (94% in women), and regarding the risk for ever drinkers and never smokers it reached 99%.

It has been argued that joint effects of alcohol and tobacco use on AD can be expected as both affect brain nicotinic cholinergic receptors [21]. Our data suggest that interactions between alcohol and tobacco do exist. It is likely that inappropriate assessment of joint effects of alcohol and tobacco in previous studies could explain divergent results. For example, in aged Spanish women as those included in our sample, tobacco consumption was still unusual, while moderate alcohol consumption is more frequent. In our study more women than men were recruited. If only joint analyses were carried out, some interactions between gender and tobacco and alcohol consumption could remain blurred. Again, for some combinations of gender, tobacco and alcohol consumption our study had a low number of subjects. It would hence be interesting to assess interactions between tobacco and alcohol in large prospective studies such as the cohort of British doctors [27].

A protective effect of smoking could be due to survival bias when prevalent cases are used if smokers with AD have relatively higher mortality rates than smokers without AD [14]. Increased mortality has been observed among smokers with dementia, but not among controls [28]. Besides, smoking could appear as a protective exposure if it delays onset of the disease [1]. However, in a sample of patients with AD followed to autopsy, active smokers were significantly younger at onset of AD and also significantly younger at death than never smokers [29]. In our data mean age at disease onset was slightly lower in smoking than in non-smoking cases, a result also reported in other studies [1]. Our analysis of tobacco consumption considering available information on previously suggested potential effect modifiers (duration and intensity of smoking, time since last smoking, age of onset of the disease, and ApoE4 allele) did not yield substantially different results.

Our results for alcohol consumption generally pointed to a protective effect, mostly for lifelong drinking women. Women in our sample were mainly light or moderate consumers (only four controls and one case reported to drink more than 20 cc alcohol/day). Few previous studies on alcohol and AD stratified by gender (*adjusting* by gender has been more usual). Again, different patterns of alcohol consumption by gender, and interaction with tobacco consumption could determine variability in the results found in different studies. Nonetheless, a protective effect of moderate alcohol consumption has been frequently reported [17-19]. Different effects according to type of alcohol beverages have also been reported, and it has been proposed that resveratrol, found in wine but not in other alcoholic beverages, could be responsible [17]. Our data allowed analysis considering consumption of three main categories of beverages (beer, wine, and liquors) but no differences were observed: as compared to non-drinkers, similar protective effects for consumers of the three beverages were observed. We observed protective

effects mostly for women with low alcohol consumption. Beneficial vascular effects of moderate drinking could be related to the potential protective effect of alcohol use on AD [30]. Also, moderate alcohol intake might favorably alter release of acetylcholine in the brain [31]. Antioxidant effects of alcohol, and particularly of wine, have also been proposed as potential mechanisms [18]. Again, survival bias could explain a protective effect of alcohol for dementia in prevalent cases. Yet, to the extent that all the analyses are referred to alcohol consumption before the age of disease onset, this potential bias should be minimized in our results.

Although the strong association between ApoE4 alleles and increased risk of AD is indisputable, the specific etiopathogenic role of ApoE4 is under discussion [32]. There is a clear need to further analyze interactions between environmental exposures and ApoE4 genotypes. In our analyses, the inclusion of cases with and without an ApoE4 allele did not substantially alter any of the results.

Some limitations of this study and their potential effects in observed results should be recognized. Our cases are not a representative sample of all people suffering from AD, but a selected sample of those reaching local Alzheimer's Disease Associations (ADAs). Medical record verification of diagnoses was not possible, and although medical diagnosis of AD is required for registration in an ADA and diagnosis was verified in the interviews with close relatives, some misclassification of disease in cases is possible, and, to an even lesser extent, it cannot be fully discarded for very early stages of the disease among controls. This problem commonly affects epidemiological studies of AD, as far as only autopsy is considered the gold standard for diagnosis of many dementias [33]. However, if some cases with other diseases than AD (most probably other dementias) are included in our sample of cases, and cases of AD (or other dementias at very early stages of evolution) are included in our sample of controls, and the

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associations with tobacco and alcohol consumption found in this study are real, this disease classification bias most probably would affect diluting observed associations.

All information on exposures was elicited using a questionnaire. Controls answered about their life-long tobacco and alcohol consumption, while proxies gave this same information for cases. If informants of cases reported real exposures in cases with a lesser frequency than controls did for their own exposures, a false protective effect could have occurred. Conversely, the effect of a potential recall bias among informants of cases would be a higher frequency of exposure reporting in cases than in controls, and spurious increased risks would then ensue. We are not able to check potential biases due to the retrospective nature of our study and to the use of informants for cases. We were only able to compare reports of exposure in informants of controls and controls themselves in a previous analysis [26]. For qualitative estimates of consumption of tobacco and alcohol, concordance indices between controls and their informants were generally high (>0.6, good-very good). However, lower concordance items were obtained for quantitative estimates addressing mainly the amount of alcohol or tobacco consumed in the past. This uncertainty on quantitative information could perhaps explain our non consistent results in analyses on dose-response relationships.

Regarding selection bias, with the potential of distorting the distribution of variables of interest in the sample of cases and controls being compared, although most of located cases' informants agreed to participate (Figure 1), 47% eligible and located controls refused. We did not have access to relevant information for non-participants, so a comparison between participants and non-participants was not possible. If tobacco or alcohol consumption is differently related to likelihood to participate in controls and cases, our results may have been altered in any direction. Besides, highly educated controls are more likely to participate, although

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more cases than controls completed primary level education. Tobacco and alcohol consumption could present different distributions in groups of people with different education level. But in adjusted analysis we always controlled by education and, more importantly, no major differences were found between crude and adjusted estimates.

With previous considerations in mind, we believe that our results add to existing evidence on the association between AD and tobacco and alcohol consumption, and strongly suggest the need to consider interactions between both exposures, as well as interactions with gender, to further clarify their effects on the risk of AD and the still largely unknown relationship of environmental factors with this disease.

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Table 1. Main Characteristics of Cases with Alzheimer's Disease (n=176) and Controls (n=246) when Interview was Performed. Case-Control Study on Risk Factors for Alzheimer's Disease, Spain, 2001-2003.

	Cases n (%)[a]	Controls n (%)[a]	p[b]
Gender			
Men	49 (27.8)	69 (28.0)	
Women	127 (72.2)	177 (72.0)	0.963
Age (years)			
< 60	15 (8.6)	21 (8.8)	
60-64	22 (12.6)	29 (12.2)	
65-69	46 (26.5)	70 (29.4)	
70-74	48 (27.6)	57 (23.9)	
75-79	31 (17.8)	43 (18.1)	
>=80	12 (6.9)	18 (7.6)	0.968
Residence			
Alcoy	81 (46.0)	92 (37.5)	
Alicante	10 (5.6)	21 (8.6)	
Elda	36 (20.5)	56 (22.9)	
Valencia	36 (20.5)	49 (20.0)	
Villena	13 (7.4)	26 (11.0)	0.325
Education			
< Primary level	65 (36.9)	129 (52.4)	
Primary level	91 (51.7)	76 (30.9)	
High school-university	20 (11.4)	41 (16.7)	<0.001
Reported economic status			
Very good - Good	64 (36.4)	115 (46.9)	
Regular – Bad – Very bad	112 (63.6)	130 (53.1)	0.030
Paid employment in the past			
Yes	145 (82.4)	204 (82.9)	0.885
Co-morbidity			
Depression	74 (42.5)	56 (22.8)	<0.001
Parkinson's disease	10 (5.8)	8 (3.3)	0.214
Hypertension	48 (27.6)	107 (43.7)	0.001
Cerebrovascular disease	29 (16.7)	28 (11.4)	0.123
Arteriosclerosis	27 (15.8)	56 (23.0)	0.073
Dementia in close relatives			
Yes	79 (47.6)	19 (7.8)	<0.001

[a]Percentages are estimated over total of valid answers for each item
[b]Two sided p values for χ^2 test

Table 2. Tobacco Consumption[a] and Risk for Alzheimer's Disease. Case-Control Study on Risk Factors for Alzheimer's Disease, Spain, 2001-2003.

	Cases n (%)	Controls n (%)	p[b]	cOR	95% CI	aOR	95% CI
Smoker							
Never	134 (76.1)	194 (78.9)		1		1	
Ever	42 (23.9)	52 (21.1)	0.507	1.17	0.74, 1.86	1.18	0.57, 2.45
Cigarettes/day[c]							
Non smokers	134	194		1		1	
<=10	14 (41.2)	22 (47.8)		0.92	0.46, 1.87	1.22	0.48, 3.11
11-20	15 (44.1)	14 (30.4)		1.55	0.72, 3.32	1.34	0.48, 3.70
>20	5 (14.7)	10 (21.8)	0.614	0.72	0.24, 2.17	0.90	0.20, 3.99
Time smoking[c]							
Non smokers	134	194		1		1	
<=20 years	7 (26.9)	10 (23.8)		1.01	0.38, 2.73	1.66	0.54, 5.09
21-40 years	13 (50.0)	13 (31.0)		1.45	0.65, 3.22	1.49	0.49, 4.55
>40 years	6 (23.1)	19 (45.2)	0.282	0.46	0.18, 1.17	0.62	0.18, 2.10
Quitting time[c]							
Non smokers	134	194		1		1	
<= 5 years	12 (33.3)	9 (25.7)		1.93	0.79, 4.71	2.14	0.68, 6.73
6-20 years	11 (30.6)	15 (42.9)		1.06	0.47, 2.38	1.30	0.45, 3.77
> 20 years	13 (36.1)	11 (31.4)	0.316	1.71	0.74, 3.93	2.34	0.79, 6.94

aOR: odds ratio adjusted by gender, age at diagnosis, residence municipality, education, economic status, paid employment in the past and family history of dementia; cOR: crude odds ratio; 95% CI: 95% confidence interval.

[a]All data on tobacco consumption are measured in the period before the age of onset of the disease in the cases (and same reference age for their age-matched controls)

[b]Two sided p values for χ^2 test

[c]Information on amounts and time periods smoking was not available for all smokers

Table 3. Alcohol Consumption[a] and Risk for Alzheimer's Disease. Case-Control Study on Risk Factors for Alzheimer's Disease, Spain, 2001-2003.

	Cases n (%)	Controls n (%)	p[b]	cOR	95% CI	aOR	95% CI
Drinker							
Both genders							
Never	95 (54.3)	93 (38.0)		1		1	
Ever	80 (45.7)	152 (62.0)	0.001	0.51	0.35, 0.76	0.53	0.32, 0.88
Women							
Never	84 (63.1)	81 (45.8)		1		1	
Ever	43 (33.9)	96 (54.2)	<0.001	0.43	0.27, 0.69	0.48	0.27, 0.84
Men							
Never	11 (22.9)	12 (17.7)		1		1	
Ever	37 (77.1)	56 (82.3)	0.483	0.72	0.29, 1.80	0.80	0.23, 2.80
cc alcohol/day[d]							
Women							
Non drinkers	84	81		1	<0.001[c]	1	0.005[c]
<1	19 (44.2)	28 (29.2)		0.65	0.34, 1.26	0.68	0.31, 1.50
1-12	14 (32.5)	38 (39.6)		0.36	0.18, 0.70	0.42	0.19, 0.94
>12	10 (23.3)	30 (31.2)	0.002	0.32	0.15, 0.70	0.37	0.15, 0.89
Men							
Non drinkers	11	12		1		1	
<1	4 (10.8)	8 (14.3)		0.55	0.13, 2.33	0.11	0.01, 1.71
1-12	9 (24.3)	15 (26.8)		0.65	0.20, 2.09	0.66	0.13, 3.36
>12	24 (64.9)	33 (58.9)	0.831	0.79	0.30, 2.10	1.06	0.28, 3.94
Time drinking[d]							
Women							
Non drinkers	84	81		1	<0.001[c]	1	0.003[c]
<=20 years	3 (21.4)	7 (14.0)		0.41	0.10, 1.65	0.33	0.06, 1.80
21-40 years	6 (42.9)	15 (30.0)		0.39	0.14, 1.04	0.48	0.16, 1.45
>40 years	5 (35.7)	28 (56.0)	0.001	0.17	0.06, 0.47	0.22	0.08, 0.66
Men							
Non drinkers	11	12		1		1	
<=20 years	1 (5.6)	0 (0.0)		--	, ,	--	, ,
21-40 years	8 (44.4)	10 (26.3)		0.87	0.25, 3.01	0.42	0.05, 3.42
>40 years	9 (50.0)	28 (73.7)	0.119	0.35	0.12, 1.06	0.47	0.07, 2.97
Quitting time[d]							
Women							
Non drinkers	84	81		1		1	
<= 5 years	17 (70.8)	65 (84.4)		0.25	0.14, 0.47	0.31	0.15, 0.62
6-20 years	6 (25.0)	3 (3.9)		1.93	0.47, 7.98	2.49	0.54, 11.43

> 20 years	1 (4.2)	9 (11.7)	<0.001	0.11	0.01, 0.86	0.10	0.01, 1.01
Men							
Non drinkers	11	12		1		1	
<= 5 years	17 (68.0)	47 (88.7)		0.39	0.15, 1.06	0.40	0.10, 1.55
6-20 years	7 (28.0)	4 (7.5)		1.91	0.44, 8.35	0.96	0.12, 7.59
> 20 years	1 (4.0)	2 (3.8)	0.056	0.55	0.04, 6.89	1.05	0.06, 18.30

aOR: odds ratio adjusted by gender (only in analysis for both genders), age at diagnosis, residence municipality, education, economic status, paid employment in the past and family history of dementia; cOR: crude odds ratio; c 95% CI: 95% confidence interval.
[a]All data on alcohol consumption are measured in the period before the age of onset of the disease in the cases (and same reference age for their age-matched controls)
[b]Two sided p values for χ^2 test
[c]Two sided p value for trend
[d]Information on amounts and time periods drinking was not available for all drinkers

Table 4. Risk for Alzheimer's Disease in Different Subgroups According to Alcohol and Tobacco Consumption[a]. Case-Control Study on Risk Factors for Alzheimer's Disease, Spain, 2001-2003.

	Cases n (%)	Controls n (%)	p[b]	aOR	95% CI
All					
Never smoker and never drinker	90 (51.4)	83 (33.9)		1	
Ever smoker, never drinker	5 (2.9)	10 (4.1)		0.21	0.05, 0.98
Ever drinker, never smoker	43 (24.6)	111 (45.3)		0.37	0.21, 0.65
Ever smoker and drinker	37 (21.1)	41 (16.7)	<0.001	0.85	0.36, 1.98
Men					
Never smoker and never drinker	7 (14.8)	4 (5.9)		1	
Ever smoker, never drinker	4 (8.3)	8 (11.7)		0.04	0.002, 0.70
Ever drinker, never smoker	8 (16.7)	21 (30.9)		0.12	0.02, 0.79
Ever smoker and drinker	29 (60.4)	35 (51.5)	0.154	0.26	0.05, 1.43
Women					
Never smoker and never drinker	83 (65.3)	79 (44.6)		1	
Ever smoker, never drinker	1 (0.8)	2 (1.1)		0.22	0.01, 3.38
Ever drinker, never smoker	35 (27.6)	90 (50.9)		0.39	0.22, 0.71
Ever smoker and drinker	8 (6.3)	6 (3.4)	0.001	1.95	0.51, 7.44
Only cases with at least one ApoE4 allele[c]					
Never smoker and never drinker	33 (55.0)	83 (33.9)		1	
Ever smoker, never drinker	2 (3.3)	10 (4.1)		0.32	0.05, 1.86
Ever drinker, never smoker	11 (18.3)	111 (45.3)		0.24	0.11, 0.52
Ever smoker and drinker	14 (23.4)	41 (16.7)	0.172	0.59	0.19, 1.85
Only cases without any ApoE4 allele[c]					
Never smoker and never drinker	23 (44.2)	83 (33.9)		1	
Ever smoker, never drinker	2 (3.8)	10 (4.1)		0.62	0.10, 3.72
Ever drinker, never smoker	15 (28.9)	111 (45.3)		0.49	0.24, 1.03
Ever smoker and drinker	12 (23.1)	41 (16.7)	0.001	0.91	0.29, 2.85

aOR: odds ratio adjusted by gender (except for analysis by gender), age at diagnosis, residence municipality, education, economic status, paid employment in the past and familial antecedents of dementia (except for analysis by ApoE4 allele); 95% CI: 95% confidence intervals.
[a]All data on tobacco and alcohol consumption are measured in the period before the age of onset of the disease in the cases (and same reference age for their age-matched controls)
[b]Two sided p values for χ^2 test

[c]ApoE genotype was available for 113 cases (60 ApoE4 carriers and 53 ApoE4 non carriers). Information on ApoE was not available for controls. P values are not adjusted for the potential multiple uses of data (cases carrying and not carrying ApoE4 are here compared with the same controls in each category).

FIGURE LEGEND

Figure 1. Selection Process for Cases (Figure 1a) and Controls (Figure 1b) in the Study. Case-Control Study on Risk Factors for Alzheimer's Disease, Spain, 2001-2003.

Figure 1a

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┌───────────────────┐
│        268        │
│    Invitation     │
│      letters      │
└───────┬───────────┘
        │
   ┌────┴──────────────┐
┌──┴────────────┐┌─────┴─────────────┐
│   83 (31%)    ││    185 (69%)      │
│Non effective  ││ Effective calls   │
│calls          ││                   │
│(no answer,    ││                   │
│wrong number)  ││                   │
└───────────────┘└───────┬───────────┘
                         │
                    ┌────┴──────────────┐
                 ┌──┴────────┐┌─────────┴─────┐
                 │  9 (5%)   ││  176 (95%)    │
                 │Did not    ││  Agreed to    │
                 │agree to   ││  participate  │
                 │participate││  Completed    │
                 │           ││  interview    │
                 └───────────┘└───────────────┘
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Figure 1b

753 Invitation letters	

60 (8%) Returned letters	**693 (92%)** Unreturned letters Personal phone calls

229 (23%) Non effective calls (no answer, wrong number)	**464 (67%)** Effective calls

181 (39%) Did not agree to participate	**283 (61%)** Agreed to participate

37 (13%) Did not complete interview	**246 (87%)** Completed interview