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Association of metabolic gene polymorphisms with alcohol consumption in controls

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The objectives were to study the association between metabolic genes involved in alcohol metabolism (*CYP2E1 RsaI*, *CYP2E1 DraI*, *ADH1C*, *NQO1*) and alcohol consumption in a large sample of healthy controls. Healthy subjects were selected from the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens (GSEC). Subjects with information on both alcohol consumption and at least one of the studied polymorphisms were included in the analysis ($n=2224$). Information on the amount of alcohol consumption was available for a subset of subjects ($n=844$). None of the studied genes was significantly associated with drinking habits. A significant heterogeneity with age was observed when studying the association between *CYP2E1 RsaI* and alcohol drinking. *CYP2E1 RsaI* polymorphism was significantly associated with being a never drinker at older ages (odds ratio [OR] 2.4, 95% confidence interval [CI] 1.2–4.8; at ages above 68 years), while the association was reversed at ages below 47 years (OR 0.5, 95% CI 0.2–1.4). For subjects with detailed information on alcohol intake, no association between alcohol quantity and polymorphisms in metabolic genes was observed; subjects carrying the *NQO1* polymorphism tended to drink more than subjects carrying the wild-type alleles. Therefore, no significant association between *CYP2E1 RsaI*, *CYP2E1 DraI*, *ADH1C*, *NQO1* polymorphisms and alcohol consumption was observed in healthy controls.

Keywords: pooled analysis, epidemiology, diet.

Introduction

Alcohol is metabolized by several enzymes, including aldehyde dehydrogenase (ALDH), alcohol dehydrogenase (ADH), cytochrome P4502E1 (CYP2E1) and quinone oxidoreductase 1 (NQO1), which are responsible for the conversion of

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alcohol to acetaldehyde and acetic acid (Bosron *et al.* 1993, Crabb *et al.* 1993, Kunitoh *et al.* 1997, Lieber 1999). It has been suggested that two CYP2E1 polymorphisms, one in the 5′-flanking region (G-C at position 1259 — *Rsa*I), one localized in intron 6 (T-A at position 7668 — *DraI*) might affect the inducibility of the enzyme by alcohol (Hayashi *et al.* 1991, Badger *et al.* 1993, Takahashi *et al.* 1993, Tsutsumi *et al.* 1994), and may be linked to alcoholism (Iwahashi *et al.* 1993, 1998). However, recent studies do not confirm this association (Itoga *et al.* 2001, Okamoto *et al.* 2001, Pastorelli *et al.* 2001).

The hepatic enzyme ADH is a dimer composed of a random combination of three different subunits (α, β, γ) that are encoded by three closely linked loci on chromosome 4; *ADH1A* (previously *ADH1*), *ADH1B* (previously *ADH2*) and *ADH1C* (previously *ADH3*), respectively. The isozymes encoded by the *ADH1C*349 Ile* allele has been associated with faster metabolism of alcohol (Chen *et al.* 1996) and a more rapid production of acetaldehyde compared with the isozymes encoded by *ADH1C*349 Val*.

Two variants are known to occur at the *NQO1* locus, one of which (c609C > T) is associated with a loss of *NQO1* protein and enzyme activity (Traver *et al.* 1992, Ross *et al.* 1996). The polymorphic *NQO1* enzyme is a dimeric flavin adenine dinucleotide (FAD)-containing cytosolic protein that catalyses the two-electron reduction of a variety of quinone compounds including alcohol derivates.

Alcoholism is likely to have some genetic component. Both adoption and twin pair studies have shown that about 40–60% of the individual variation in alcohol preference and vulnerability to alcoholism are genetic in origin (Kendler *et al.* 1992, Heath *et al.* 1997). Several studies have shown that polymorphisms in certain genes (such as *ALDH* and *ADH*) are associated with alcoholism (Thomasson *et al.* 1991, 1993, Chen *et al.* 1996, Nakamura *et al.* 1996, Konishi *et al.* 2003), especially among Asian populations. However, to date no study has been performed on the role of metabolic genes on drinking habits in otherwise healthy subjects. It is possible that polymorphisms in genes involved in alcohol metabolism could critically modify sensitivity to alcohol and influence drinking habits by lowering the levels of alcohol consumption. This pattern has been suggested in individuals with the *ADH1C*349 Ile* allele (Higuchi *et al.* 1996). If an association exists between drinking habits and metabolic gene polymorphisms in healthy subjects, then case-control studies on metabolic gene and alcohol-related cancers should take into account the drinking status of the subjects under study, since drinking may act as a confounder of the association between a gene and a disease. In addition, case-case analyses assume the independence between exposure (in this case alcohol) and genetic polymorphisms among controls, but this independence has never been tested for alcohol and metabolic genes. Finally, the observation of any association between metabolic gene polymorphisms and drinking habits would be very useful for establishing preventive strategies against alcoholism.

The aim was to test for associations between polymorphisms in several metabolic genes (*CYP2E1 RsaI*, *CYP2E1 DraI*, *ADH1C*, *NQO1*) and alcohol consumption in a large set of healthy subjects selected from a pooled analysis (the GSEC study; Taioli 1999) designed to study gene–environment interaction and cancer.

Materials and methods

Study population

Healthy subjects were selected from the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens (GSEC), a collaborative project that collects information on metabolic gene polymorphisms and environmental exposures from both published and unpublished case-control studies. Investigators were contacted and asked to send their original data. For an explanation of the design of the study, see Taioli (1999).

Eleven studies were identified from the GSEC database, in which information on both alcohol consumption and *CYP2E1*, *ADH1C* or *NQO1* genotype was presented for healthy controls (table 1). Subjects without any information on the selected genes and subjects without information on alcohol consumption were excluded.

Alcohol consumption was defined as never, ex-, current drinkers. Subjects who never drank alcohol regularly or occasionally tried some alcoholic beverage over their lifetime were defined as never drinkers; ex-drinkers were subjects who quit drinking at least 6 months before entering the study. A new variable, ever drinkers, was created as the sum of ex- and current drinkers. This allowed us to add information on drinking from those studies that defined drinking status using only the variable 'ever drinker'. For some subjects (38%), there was also information on the total time of alcohol consumption and the amount of alcohol (g) usually ingested during a week. Information was available on the following polymorphisms: *CYP2E1 RsaI*, *CYP2E1 DraI*, *ADH1C* and *NQO1*. These genes were chosen because they are involved in alcohol metabolism and they are included in the GSEC project database.

Information on *CYP2E1 RsaI* genotype was available for 1499 subjects (67%), on *CYP2E1 DraI* genotype for 655 subjects (29%), on *ADH1C* genotype for 455 subjects (20%) and on *NQO1* genotype for 759 subjects (34%) (table 2). Information on other variables such as age, sex, race and smoking status were also available for almost all participants. Smoking status was defined as never, ex-, current, ever smokers, using the same definitions used for alcohol consumption.

Statistical methods

Drinking status was categorized as ever (ex- plus current plus ever) versus never drinkers; each polymorphism was categorized into two groups based on the absence or presence of the polymorphic allele (wild-type homozygote versus heterozygote plus variant homozygote). Crude and adjusted odds ratios (ORs) and 95% confidence interval (CI) were calculated for each genotype according to drinking status. ORs of being a non-drinker using ever drinker as the reference were calculated. The choice of drinkers as a reference group was based on the hypothesis that metabolic gene variants could produce toxic intermediates that would prevent people from drinking regularly, rather than the opposite. Data were adjusted for age, sex, race, institution and smoking status using multiple logistic regression models. For subjects with information on the amount of alcohol consumption, differences in means alcohol (g) ingested during a week were adjusted by age, sex, race, smoking status and institution with a multivariate linear model.

Results

The final sample consisted of 2224 subjects, 1097 (49.33%) of whom were Caucasians, 526 (23.65%) Latinos, 353 (15.87%) Asians, 143 (6.43%) African-Americans and 105 (4.72%) of other ethnic origin (table 2). The mean age was higher among subjects tested for *CYP2E1 DraI* than in subjects tested for other genes; almost all subjects tested for *ADH1C* were regular drinkers. The frequency distribution of polymorphisms in *CYP2E1 RsaI*, *CYP2E1 DraI* and *NQO1* in this sample was similar to that reported in the literature for Caucasians and Asians (Kelsey *et al.* 1997, Garte *et al.* 2001). The frequency of *ADH1C* polymorphism was similar to that reported in Caucasians (Borràs *et al.* 2000).

Table 3 shows the results on the presence of a polymorphism in each of the candidate genes and drinking habits; overall, there was no association between *CYP2E1 RsaI*, *NQO1*, *ADH1C* and drinking habits. A modest association between *CYP2E1 DraI* and alcohol disappeared after adjustment for age, gender, race, smoking status and institution. To avoid over adjustment, since race and institution often overlap, the analysis was repeated without the latter variable, obtaining similar results.

Table 1. Description of the papers included in the pooled analysis.

Reference	Number of subjects	Type of controls	Race	Country where the study was conducted	Details on drinking quantity	Polymorphisms under study
Bouchardy *et al.* (2000)	140	hospital controls	Caucasian	France	none	*CYP2E1 RsaI, CYP2E1 DraI, ADH1C*
Groppi *et al.* (1991)	39	healthy controls	Caucasian	France	none	*ADH1C*
Carere *et al.* (2002)	184	healthy controls	Caucasian	Italy	none	*CYP2E1 RsaI, NQO1*
Harty *et al.* (1997)	523	healthy controls	Latino	Puerto Rico	yes	*ADH1C*
Van Dijk *et al.* (2001)	132	hospital controls	Caucasian	the Netherlands	yes	*ADH1C*
Liu *et al.* (2001)	342	hospital and healthy controls	Caucasian and African-American	USA	none	*CYP2E1 RsaI*
Le Marchand *et al.* (1998)†, Chen *et al.* (1999)‡	454	healthy controls	Caucasian, Asian, other	USA	none	*CYP2E1 RsaI, CYP2E1 DraI, NQO1*
Morita *et al.* (1999), unpublished data	178 (14 unpublished)	healthy controls	Asian	Japan	none	*CYP2E1 RsaI*
Lewis *et al.* (2001)	164	hospital controls	Caucasian	UK	yes	*CYP2E1 RsaI, CYP2E1 DraI, NQO1*
Unpublished data	68	hospital controls	Caucasian, African-American, Latino	UK	yes	*CYP2E1 RsaI*

†Contains data on *CYP2E1*.
‡Contains data on *NQO1*.

Table 2. Summary of the data included in the analysis.

Gene	Number of studies	Number of subjects	Mean age ± SD	Percent drinkers	Percent males	Percent Caucasians	Percent subjects with polymorphism
CYP2E1 RsaI	7	1499	56.9 ± 13.5	59.8	88.8	59.7	15.7
CYP2E1 DraI	3	655	62.8 ± 10.9	57.6	67.6	57.6	26.6
ADH1C	4	455	59.5 ± 13.0	91.6	87.9	67.9	67.3
NQO1	3	759	58.8 ± 13.9	43.3	65.1	64.6	40.6

A significant heterogeneity with age was observed when studying a possible association between *CYP2E1 RsaI* and alcohol drinking. This is illustrated in figure 1, where the OR of being a never drinker with the *CYP2E1 RsaI* heterozygous plus homozygous variant was calculated according to quartiles of age, and adjusted for gender, race, smoking status and institution. The *CYP2E1 RsaI* variant was significantly associated with being a non-drinker at older ages (OR 2.4, 95% CI 1.2–4.8 at ages above 68 years), while the association was reversed at ages below 47 years (OR 0.5, 95% CI 0.2–1.4). Similar results were obtained when the data on age were stratified by ethnicity, although the numbers were very small in some subgroups. The OR for interaction between *CYP2E1 RsaI* and age was 1.4 (95% CI 0.9–2.0), significantly higher than the OR for *CYP2E1 RsaI* only (OR 0.5, 95% CI 0.3–0.8). An effect of gender was observed for *CYP2E1 DraI* polymorphism. After adjusting for age, race, smoking status and institution, males had a greater probability of being non-drinkers if they carried the variant allele (OR 1.9, 95% CI 1.2–3.1), while this effect was not observed in females (OR 0.8, 95% CI 0.3–1.7).

In a subset of subjects ($n = 844$) with detailed information on drinking quantity, the association between metabolic gene polymorphisms and alcohol (g) consumed weekly was analysed (table 4). No association between alcohol quantity and

Table 3. Association between drinking habits and polymorphisms in metabolic genes.

Drinking status	Total number with wild-type/ number with polymorphism	Crude Odds Ratio	Adjusted Odds Ratio*
CYP2E1 RsaI			
Ever	765/131	1.0 (reference)	1.0 (reference)
Never	499/104	1.2 (0.9–1.6)	1.2 (0.8–1.6)
CYP2E1 DraI			
Ever	305/72	1.0 (reference)	1.0 (reference)
Never	176/102	2.5 (1.7–3.5)	1.5 (1.0–2.3)
ADH1C			
Ever	139/278	1.0 (reference)	1.0 (reference)
Never	10/28	1.4 (0.7–3.0)	1.1 (0.5–2.5)
NQO1			
Ever	190/139	1.0 (reference)	1.0 (reference)
Never	261/169	0.9 (0.7–1.2)	0.8 (0.6–1.1)

*Odds ratios are adjusted for age, gender, race, smoking status and institution.

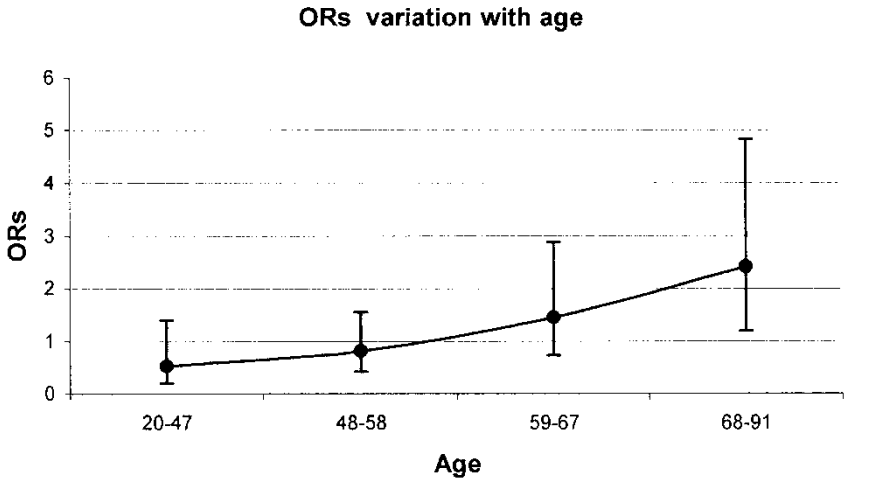

Figure 1. Effect of age on the association between *CYP2E1 RsaI* polymorphism and alcohol consumption. Odds ratios are adjusted for gender, race, smoking status and institution. p (Breslow–Day test for homogeneity) <0.0001.

polymorphisms in metabolic genes was observed, although subjects carrying the *ADH1C* or *NQO1* polymorphism tended to drink more alcohol (g) per week in comparison with subjects with the wild-type alleles.

Discussion

The aim was to assess if any association exists between polymorphisms in genes involved in alcohol metabolism and drinking habits. Studies on the association

Table 4. Association between genotype and the amount of alcohol consumed in a subsample of 844 subjects.

Genotype (number of subjects)	Grams alcohol/week (mean ± 95% confidence interval*)	p
CYP2E1 RsaI		
Wild-type (203)	91 ± 27	0.77
Heterozygous (14)	76 ± 68	
CYP2E1 DraI		
Wild-type (55)	65 ± 35	0.62
Heterozygous (8)	87 ± 100	
ADH1C		
Wild-type (85)	131 ± 34	0.56
Heterozygous (143)	149 ± 36	
Homozygous (34)	174 ± 93	
NQO1		
Wild-type (108)	77 ± 21	0.1
Heterozygous (34)	141 ± 104	
Homozygous (3)	0	

General Linear Model adjusted for age, gender, race, smoking status and institution.
* ± 1.96 SD/square root (N).

between genetic factors and drinking habits are very rare and controversial. Several studies found no association between *CYP2E1 RsaI*, *CYP2E1 DraI*, *ADH1C* polymorphisms and self-reported alcohol consumption (Nakamura *et al.* 1996, Chen *et al.* 1997, Neumark *et al.* 1997, Osier *et al.* 1998, Borràs *et al.* 2000, Itoga *et al.* 2001, Okamoto *et al.* 2001, Tamara *et al.* 2003), while others reported that *CYP2E1 DraI* polymorphism could have a protective function (Iwahashi *et al.* 1998) or that *CYP2E1 RsaI* could be a risk factor for alcoholism (Konishi *et al.* 2003) or for an excessive alcohol consumption (Sun *et al.* 1999, 2002).

The present analysis found no association between drinking habits and polymorphisms in *CYP2E1*, *ADH1C* and *NQO1*, and no significant association between weekly alcohol consumption and any of the studied genotypes. A reason for the observed lack of association between metabolic genes and regular alcohol consumption could lie in the great variability of self-reporting alcohol consumption, and a possible underestimation of the quantity of alcohol consumed during the week. One of the critical issues in any study on drinking habits is that there are no long-term biological markers of alcohol consumption. Some biological indices are used in clinical studies: median corpuscular volume, gamma-glutamyl transpeptidase and blood alcohol concentration, i.e. mg alcohol in 100 ml blood. The alcohol elimination rate, calculated from the decline in blood alcohol concentration after it reaches a maximum following absorption from the stomach and small intestine, shows a large between-individual variability in elimination rate that can result from a combination of both environmental and genetic factors.

Heterogeneity with age was observed when studying a possible association between *CYP2E1 RsaI* and drinking habits. This could suggest a selection, due to the fact that this polymorphism has been associated with several alcohol-related diseases such as hepatocellular carcinoma (Munaka *et al.* 2003), gastric cancer (Cai *et al.* 2001), upper aero digestive tract tumours (Bouchardy *et al.* 2000, Liu *et al.* 2001), hepatic lesions (Harada *et al.* 2001), cirrhosis, breast and stomach cancer (Vineis *et al.* 1999). Therefore, it is possible that older healthy subjects represent a selection of the population surviving such chronic diseases just because they have a certain polymorphism and consequently do not drink. The change in association between *CYP2E1 RsaI* and drinking with age has to be kept in mind when conducting case-control studies involving this gene and alcohol consumption.

One of the strengths of the present study is the inclusion of very large sample of healthy subjects. To the authors' knowledge, this is the largest study examining the *CYP2E1 RsaI* polymorphism and regular drinking habits. Another strength is the fact that this study refers to physiological alcohol consumption in a sample of healthy subjects and not, as in most published studies, the problem of alcoholism. Moreover, this is the first study to investigate a possible relationship between *NQO1* and regular alcohol consumption.

Unfortunately, the database did not allow for a more in-depth study of gene–gene interaction. For example, it did not include enough subjects with data on *ADH1C* and at least one polymorphism in another metabolic gene. Another limitation was the scarce available information on the quantity of alcohol consumed. Stratification of the data for ethnicity would have been useful, since some of the polymorphisms vary in frequency according to race, but this was not

possible due to small numbers for certain ethnic groups. Another issue is the standardization of the epidemiological data, since the subjects included in the study derived from the pooling of different studies conducted all over the world. To assess the degree of comparability of the various data sets, a formal request was mailed to each participant in the GSEC study to collect detailed information on criteria of inclusion of the subjects in their study, methods and techniques of data collecting, and laboratory methods used for identification of the genotype. However, one cannot rule out a certain heterogeneity in the epidemiological data. One possible limitation of the study is the inclusion of hospital controls, which may be hospitalized for conditions related to alcohol consumption. However, the reasons for hospitalization were checked in our data set and no hospital diagnosis was found that could be obviously linked to alcohol consumption.

In conclusion, none of metabolic genes included in this analysis is significantly associated with regular drinking habits. There is an age effect on the influence of *CYP2E1 RsaI* on drinking habits. Large databases with information on multiple genes should be further explored for an association between metabolic pathways and alcohol consumption in healthy subjects.

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