Genetic variation of alcohol dehydrogenase type 1C (ADH1C), alcohol consumption, and metabolic cardiovascular risk factors: Results from the IMMIDIET study

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1. Introduction

Moderate alcohol consumption is associated with reduced coronary heart disease (CHD) risk [1] and mortality [2] as reported in large epidemiological studies and meta-analyses.

Variability in the individual ability to metabolize alcohol may be relevant in understanding its cardioprotective role [3]. Alcohol is oxidized to acetaldehyde, which is in turn oxidized by aldehyde dehydrogenase (ALDH) to acetate, by class I enzymes, codified by ADH1A, ADH1B and ADH1C (previously known as ADH3) genes, tandemly organized as a gene cluster on chromosome 4 [4].

ADH1B and ADH1C have polymorphisms that produce isoenzymes with distinct kinetic properties. Among white populations, variant alleles are relatively uncommon at the ADH1B locus but common at the ADH1C locus.

Keywords:
Alcohol
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The polymorphism (rs698) at the ADH1C locus has two alleles, namely gamma 1 and gamma 2 (γ1 and γ2), which differ by two amino acids: arginine vs. glutamine at position 271, and isoleucine vs. valine at 349, respectively [5]. Pharmacokinetic studies have shown that the homodimeric γ1 isoenzyme has a 2.5-fold higher maximal velocity of ethanol oxidation than the homodimeric γ2 isoenzyme [6]. Epidemiological studies have associated the ADH1C polymorphisms with alcohol-associated diseases, such as alcoholism (γ2γ2) [7], alcohol-related end-organ damage (γ1γ1) [8], and oropharyngeal cancer (γ1γ1) [9], but results are contrasting.

There is also evidence that ADH1C polymorphisms play a role in the cardioprotective effect of moderate alcohol consumption and in HDL regulation by alcohol [10]. However these results were not confirmed by subsequent studies [11–13].

We set-up a study (The IMMIDIET Project [14]) to evaluate dietary habits, including drinking habits, metabolic risk profiles for MI and the impact of dietary–gene interaction in determining such risk profile, in healthy male–female pairs from three European regions at different risk of myocardial infarction (MI) according to the MONICA study [15] and different consumption of alcoholic beverages.

In the framework of the IMMIDIET study, we investigated the distribution of ADH1C polymorphism in the three different European populations and whether the relation between alcohol consumption and metabolic risk factors for MI was associated with ADH1C variants.

2. Materials and methods

The IMMIDIET study [14,16], is a population-based cross-sectional study, comparing healthy male–female pairs from Abruzzo (Italy), Limburg (Belgium), and South–West (S–W) London (England), including both urban and non-urban areas. Mixed pairs living in Limburg, formed by a cross-cultural marriage between Italian and Belgian subjects, were also recruited, as a model of gene–environment interaction. The Abruzzo region was selected as a representative site of historical migration to Belgium while Limburg, the area in Belgium, as a typical site of immigrants hosting. Abruzzo and Limburg were also chosen as areas following Mediterranean and non-Mediterranean diet, respectively and at relatively lower and higher cardiovascular risk. SW London was taken as representative of Western-European diet type, at quite high cardiovascular risk, according to the MONICA data.

Apparently healthy pairs were male–female spouses or partners living together and recruited through local general practices. To protect against selection bias, the selection of eligible pairs was randomized in each centre. A computerized list of all eligible pairs in each practice was generated in advance and an invitation was made by letter and/or by phone call.

Subjects were examined in the framework of the practices, by research personnel, accurately trained. The recruitment strategies were carefully defined and standardized across the three recruiting centres.

Between October 2001 and October 2003, 1604 subjects (802 male–female pairs), aged 25–74 years were enrolled in the study. Five hundred and forty two Italian subjects (271 pairs), born and living in the Abruzzo region, with both partners from Abruzzo and all four grandparents born in the same region; 536 Belgian subjects (268 couples) born and living in Limburg, with both partner from Limburg, having all four grandparents born in the same region; 526 English subjects from 263 pairs, born and living in south-western England, with both English partner from the same area, having all four grandparents born in England; and 414 subjects from 207 pairs living in Limburg and formed by a member of Italian origin (born in Belgium from Italian immigrants) and their spouse of Belgian origin.

Exclusion criteria for all groups were: history of cardiovascular disease, diabetes mellitus, familiar hypercholesterolemia, malignancies, chronic diseases such as heart, liver or renal failure, hypothyroidism and epilepsy.

2.1. Biochemical measurements

Blood pressure and anthropometric measurements were described elsewhere [16,17]. Blood pressure was measured with an automatic device (OMRON-HEM-705CP; Omron Health Care Inc., Bannockburn, IL, USA) after the subject had been resting in seated position for 10 min. Hypertension was defined according to the WHO/ISH criteria. Body weight and height were measured on a standard beam balance scale with an attached ruler, with subjects wearing no shoes and only light indoor clothing. The body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m²). Waist circumference (WC) was measured according to the National Institutes of Health, National Heart, Lung and Blood Institute guidelines.

Blood samples were obtained between 07.00 and 10.00 from patients who had been fasting overnight and had refrained from smoking for at least 6 h, by using identical standardized protocol in each study centre. Venous blood samples were obtained with minimum stasis using 21 gauge butterfly needles in K2 EDTA vacutainers. Blood was centrifuged within 3 h from the collection. plasma or serum were separated in 500 μl aliquots and immediately stored at −80 °C. Biochemical analyses were performed in centralized laboratories after shipping of aliquots in dry ice. High sensitivity (hs)-CRP was measured in EDTA plasma samples by a latex particle enhanced immunoturbidimetric assay (IL Coagulation Systems on ACL9000, IL, Milan, Italy). Quality control was maintained using in–house plasma pool and internal laboratory standard at 2.59 and 6.19 mg l⁻¹.

Factor (F)VII:c levels were determined in citrated plasma, with a one-stage clotting assay (FVII deficient plasma Hemoliance, Instrumentation Laboratory, Lexington, MA, USA, and recombinant tissue factor–based thromboplastin Innovin®, DadeBehring, Marburg, Germany). Cholesterol, HDL-cholesterol, triglycerides and glucose were assessed in serum, by an automated analyzer (Roche Cobas Mira Plus, Roche Applied Science, Meylan, France). LDL-cholesterol was calculated according to the Friedewald formula [18].

2.2. Alcohol consumption

Alcohol consumption was assessed by validated food frequency questionnaires in Italy and England [19,20] that recorded the subjects mean consumption (in units) of wine, beer, cider and spirits for each day of the week. On the base of these questionnaires, we developed a new Belgian FFQ and an integrated multi-cultural FFQ for mixed couples (to be published under separate cover). The questionnaires were self-administered and checked by a dietitian for completeness and ambiguous answers. Intake of alcohol (expressed in ml of pure ethanol/week) was estimated from the average number of millilitres of ethanol in one unit of each type of alcoholic beverage: wine: (10% or 12% alcohol, v/v) = 12 cl serving; beer (5% alcohol) = 12 cl serving; beer (6 or 8% alcohol) = 25 or 33 cl serving; cider (5% alcohol) = 12 cl serving; spirits (20% or 40% alcohol) = 2 or 6 cl serving. For each gender, alcohol consumption was further classified into three groups: teetotallers, below and above the median.
2.3. Genotyping

The ADH1C Ex8-56A>G (rs698) was assessed using the TaqMan or 5′-nuclease assay (Applied Biosystems Division). The ADH1C polymorphism (rs698) was genotyped by Real-Time-PCR (TaqMan SNP Genotyping Assay, C_26457410_10). The rs698 polymorphism was genotyped in a total of 1947 individuals. ADH1C genotypes were available for 535 Italians, 507 Belgians, 502 English, and 403 subjects from Belgian/Italian mixed couples. A 10% random sample was genotyped twice in a blinded manner without any discrepancies between genotype score. The genotyping success rate was 99.9%.

Subjects were classified into three categories according to the presence of γ2 (slow) allele: 11 homozygotes, 12 heterozygotes and 22 homozygotes.

2.4. Statistical analysis

The power to detect gene–environment interaction was calculated using the program QUANTO (http://hydra.usc.edu/gxe). Based on a sample size of almost 900 individuals for each sex, α = 0.05, two-sided, under various values for main (genetic and environmental) and interaction effects, the power to detect gene–environment interaction in our study was constantly >80%.

Standard statistical genetic methods were used to verify whether the assumption of Hardy–Weinberg equilibrium was respected. Differences in allele and genotype frequencies were determined by χ² statistics. Results were expressed as numbers of subjects and (percentage) or as mean ± standard deviation (S.D.). Bivariate statistical comparisons were assessed by the analysis of variance (ANOVA) or by χ². The association of alcohol intake (independent continuous variable) with CAD risk factors (dependent continuous variables) was analyzed using multivariate analysis of variance (Procedure GLM in SAS). To evaluate whether ADH1C genotypes modify the association between alcohol and CAD risk factors, interaction effects (i.e. differences in slopes) were evaluated adding appropriate interaction terms in multivariate ANOVA. We re-fitted all the models including the square of alcohol intake and the corresponding term for interaction with genotype as additional independent variables. In this way the models include linear and quadratic terms for alcohol, and may fit a non-linear J-shaped trend. We also categorized alcohol consumption, and evaluated nested models that examined interaction across categories. Categories were defined as: 0 gr/d, 0–24 gr/d, 24–48 gr/d, >48 gr/d, for the reference level, first, second and third level of intake, in males, and 0 gr/d, 0–12 gr/d, 12–24 gr/d, >24 gr/d in females.

CRP, triglyceride, glucose and homocysteine levels were log transformed to normalize their positively skewed distribution. Two-tailed p-values <0.05 were considered statistically significant.

All computations were carried out using the SAS statistical package (Version 9.1.3 for Windows. SAS Institute Inc., Cary, NC, SAS Institute Inc., 1989)

3. Results

Both in the total population and within each group, the distribution of rs698 was in Hardy–Weinberg equilibrium (p > 0.05). The distribution of rs698 by country is shown in Table 1. There was a statistically significant difference in the allelic distribution across the population group studied (Table 1). The Italian subjects (living either in Italy or in Belgium) showed a lower prevalence of gamma 1 homozygous as compared to Belgians or English.

Men were more frequently alcohol drinkers and the average alcohol units per week consumed among drinkers were substantially higher in men than in women (p<0.0001) in all countries.

There was no statistically significant difference in the intake of alcohol in men by country (p = 0.11). In contrast, the intake of alcohol was significantly different in women from different European regions (p < 0.001), being highest in English women. The intake of alcohol did not vary in relation to ADH1C genotypes (multivariate ANOVA adjusted for country, age, sex, tobacco, social status and total energy intake, both in men and in women p = 0.56 and p = 0.50, respectively). Moreover, there was no association between alcohol consumption and ADH1C genotype in the different regions (Table 2).
In multivariate analysis adjusted for country, age, smoking habits, social status and energy intake, BMI, waist circumference, waist-to-hip ratio, blood pressure, HDL-cholesterol, cholesterol levels, triglyceride levels were positively associated with alcohol intake in men; while FVII:ag levels were inversely associated.

A significant impact of ADH1C genotypes on the interaction between alcohol consumption and BMI or waist circumference was found (Table 3A). The associations of alcohol with waist-to-hip ratio, blood pressure, HDL-cholesterol, total cholesterol levels, triglyceride levels and FVII:ag were independent on ADH1C genotypes.

The inclusion of quadratic terms (alcohol × alcohol and genotype × alcohol × alcohol) never improved the fit of the model, indicating that when a correlation between alcohol and biomarkers exists, a linear relationship is appropriate, both as a whole and within each genotype.

The use of category of alcohol intake and corresponding interaction with genotype closely reproduced the previous findings, and no additional interactions were found. The unique statistically significant interactions were found for BMI and waist circumference, in males. The strength both of association between biomarker and alcohol categories and interaction by genotypes, increased when first, second and third category of intake were compared with the reference level. These findings indicate that BMI and waist circumference increase linearly with alcohol, and that interaction-by-genotype effect is more evident when the higher category of alcohol intake was contrasted with the lower (Fig. 1a and b).

In women, HDL-cholesterol was positively associated with alcohol intake and negatively associated with LDL-cholesterol and FVII:ag; all these associations were independent from ADH1C genotypes (Table 3B).

We repeated all the analyses pooling men and women and further adjusting for sex. Despite the increase in sample size, and adequate statistical power, all the by genotype-interactions non-statistically significant in analyses separate by sexes remain statistically non-significant. In particular, the association between HDL-C and alcohol seems to be modified by genotype in both sexes and in pooled analysis ($\beta = 0.20 \pm 0.03$; $\beta = 0.14 \pm 0.03$ and $\beta = 0.008 \pm 0.05$ in 11, 12, and 22 genotype, in pooled analysis), but the term for interaction remains non-statistically significant ($p = 0.082$, in pooled analysis) (Fig. 1c, in males).

4. Discussion

We have assessed ADH1C polymorphism (rs698) in three European population samples at different risk of MI and at different dietary and drinking habits. The distribution of genotypes in the whole population did not differ from those already described in previous studies [21]. However, both men and women of Italian origin living either in Limburg or in Abruzzo showed a lower prevalence of gamma 1 homozygous as compared to people of Belgian or English origin. Allele frequency and genotype distribution of rs698 polymorphism in the Italian sample were similar to those already found by our group in a similar population by using a different genotyping method.

Among men the percentage of alcohol consumers and the mean amount of alcohol consumed per day did not differ by country. Women from SW-London drank more than women from either Limburg or Abruzzo, however such difference was not dependent on the different ADH1C polymorphism distribution. Indeed, there was no genetic influence on the propensity to drink alcohol either in the whole population or in individual sub-samples. Difference in allele frequency by country might reflect a micro-heterogeneity among European populations [22] as already described for other potentially functional polymorphisms [23]. Previous studies showed that genetic variations in ADH genes were related to alcohol consumption, however this was more evident for ADH2 gene, while ADH1C polymorphism, as in our study, played a small if any role in affecting the risk for alcoholism in Europeans [21,24]. The genetic variants ADH1B (ADH2) and ALDH2 also modulate the rate of alcohol metabolism; furthermore both genes are not ideal for this study because the rare alleles are uncommon in Caucasian population [24].

Beside a different pattern of alcohol drinking between men and women, we also found differences in the association between alcohol drinking and cardiovascular risk factors. In particular, in men alcohol intake was independently associated with higher levels of BMI, waist circumference, waist hip ratio, blood pressure, HDL and cholesterol, while only the association with higher HDL and lower LDL and FVII:ag levels was apparent in women. However, in women, there was a non-significant trend to a negative association between alcohol and BMI and WC. Such sex difference in the association between alcohol and anthropometric variables were already described in previous studies [23].

In men a strong interaction was observed between the ADH1C genotype ($\gamma 1/\gamma 2$) and the level of alcohol consumption in relation to the BMI and the waist circumference levels.

In particular, BMI and waist circumference increased with alcohol consumption in men who were heterozygous or homozygous for the $\gamma 2$ allele, while decreased in $\gamma 1/\gamma 1$ homozygous. These findings suggest that the effects attributable to alcohol consumption are more expressed in $\gamma 2$ carriers.

A similar trend was observed in separate analyses by country; however, the small sample size of separate groups did not allow to reach any statistical significanced (data not shown).

Since the predominant function of alcohol dehydrogenase type IC is to metabolize alcohol, our findings are consistent with the hypothesis that in gamma 2 carriers a slower rate of alcohol clearance is associated with a greater effect of alcohol on metabolic pathways and disease risk.

Alcohol consumption was suggested to increase HDL levels by various mechanisms: increase in lipoprotein lipase activity [26] or in synthesis and secretion of apolipoprotein A-I [27]. We confirmed these data both in men and in women, and in pooled analysis; however, no evidence of an interaction effect between alcohol consumption and ADH1C variants on HDL-cholesterol levels could be found. Our results are in line with those of other population-based studies that found no interaction between alcohol consumption and ADH1C polymorphism on HDL-cholesterol concentrations [11–12,28–29]. We observed that in $\gamma 2$ carriers...
Table 2

The geometric mean alcohol units per weeks in relation to ADH1C genotypes.

<table>
<thead>
<tr>
<th>Italy (gr alcohol/die)</th>
<th>Belgium (gr alcohol/die)</th>
<th>England (gr alcohol/die)</th>
<th>Mixed (gr alcohol/die)</th>
<th>Total (gr alcohol/die)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± S.E.M.</td>
<td>p</td>
<td>Mean ± S.E.M.</td>
<td>p</td>
<td>Mean ± S.E.M.</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(269)</td>
<td>(253)</td>
<td>(248)</td>
<td>(202)</td>
<td>(972)</td>
</tr>
<tr>
<td>11</td>
<td>17.69 ± 1.48</td>
<td>0.40</td>
<td>19.32 ± 1.79</td>
<td>0.38</td>
</tr>
<tr>
<td>12</td>
<td>19.13 ± 1.61</td>
<td>0.08</td>
<td>17.05 ± 1.81</td>
<td>0.09</td>
</tr>
<tr>
<td>22</td>
<td>118 ± 5.46</td>
<td>0.05</td>
<td>21.05 ± 2.54</td>
<td>0.07</td>
</tr>
<tr>
<td>Total</td>
<td>16.2 ± 2.85</td>
<td>0.05</td>
<td>19.14 ± 2.05</td>
<td>0.40</td>
</tr>
<tr>
<td>Women</td>
<td>(266)</td>
<td></td>
<td>(254)</td>
<td>(201)</td>
</tr>
<tr>
<td>11</td>
<td>3.94 ± 0.61</td>
<td>0.93</td>
<td>7.23 ± 0.93</td>
<td>0.97</td>
</tr>
<tr>
<td>12</td>
<td>4.12 ± 0.75</td>
<td>0.08</td>
<td>7.08 ± 0.90</td>
<td>0.08</td>
</tr>
<tr>
<td>22</td>
<td>4.49 ± 1.46</td>
<td>0.03</td>
<td>7.55 ± 1.60</td>
<td>0.03</td>
</tr>
<tr>
<td>Total</td>
<td>4.18 ± 0.94</td>
<td>0.05</td>
<td>7.29 ± 1.17</td>
<td>0.13</td>
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Multivariate ANOVA adjusted for age, tobacco, social status and energy intake.

Table 3A

Association between alcohol intake, genotypes of ADH1C and CAD risk factors in men.

<table>
<thead>
<tr>
<th>Risk factors for CAD</th>
<th>Beta ± S.E.</th>
<th>p1</th>
<th>ADH1C genotypes</th>
<th>p4</th>
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<td></td>
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</tbody>
</table>

Multivariate analysis adjusted for age, country, genotype, tobacco, social status, and energy intake. BMI: body mass index; WC: waist circumference; W/H ratio: waist-to-hip circumference ratio.

1. p for alcohol.
2. p for genotype.
3. p for differences.

Table 3B

Association between alcohol intake, genotypes of ADH1C and CAD risk factors in women.

<table>
<thead>
<tr>
<th>Risk factors for CAD</th>
<th>Beta ± S.E.</th>
<th>p1</th>
<th>ADH1C genotypes</th>
<th>p4</th>
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</tbody>
</table>

Multivariate analysis adjusted for age, country, genotype, tobacco, social status, and caloric intake. BMI: body mass index; WC: waist circumference; W/H ratio: waist-to-hip circumference ratio.

1. p for alcohol.
2. p for genotype.
3. p for differences.
HDL-cholesterol levels increase with alcohol less than in γ1. Although non-statistically significant, this trend is opposite to that seen in the Physicians’ Health Study, in which HDL-cholesterol levels increased with alcohol steeper in γ2 than in γ1 carriers [10].

5. Conclusions
A marked, significant interaction between the ADH1C genotype and alcohol consumption in relation to BMI and waist circumference was found in men from three different European origins. Men homozygous for the gamma 2 allele who drank daily had a substantial increase in both BMI and waist values. In contrast, ADH1C variants did not interact with the effects of alcohol intake on HDL, blood pressure or total cholesterol levels.

Identifying genetic variants in Europeans populations that may influence alcohol metabolic clearance and its impact on cardiovascular risk factors, might help differentiating national campaigns against alcohol consumption and better defining the different benefit/risk balance related to either moderate or heavy alcohol consumption.

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Appendix A. European collaborative group of the IMMIDIET project

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References


