

# Angiotensin-Converting Enzyme Gene Polymorphism Is Associated with Vulnerability to Alcoholic Cardiomyopathy

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**Background:** Chronic alcohol abuse has a dose-dependent toxic effect on the myocardium, leading to alcoholic cardiomyopathy. The fact that only a minority of persons with chronic alcoholism have this condition suggests the possibility of a genetic vulnerability. In this context, polymorphism of the angiotensin-converting enzyme (ACE) gene has been implicated in cardiac dysfunction.

**Objective:** To compare the ACE genotypes of alcoholic persons who have cardiomyopathy with those of comparable alcohol abusers who have normal cardiac function.

**Design:** Case-control study over a 2-year period.

**Setting:** An academic tertiary referral hospital in Barcelona, Spain.

**Patients:** 30 alcoholic men with symptomatic cardiomyopathy and 27 alcoholic men with normal cardiac function.

**Measurements:** Ethanol intake, cardiac status, left ventricular ejection fraction (LVEF), and ACE gene polymorphism.

**Results:** The *DD* ACE genotype was present in 57% of alcoholic persons with an LVEF less than 0.50 and in 7% of those with normal cardiac function. Compared with persons who had an *I* allele, the odds ratio for development of left ventricular dysfunction in alcoholic persons with the *DD* genotype was 16.4.

**Conclusions:** Vulnerability to cardiomyopathy among chronic alcohol abusers is partially genetic and is related to presence of the ACE *DD* genotype. This finding demonstrates genetic susceptibility to alcohol-induced myocardial damage.

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Approximately 15% to 40% of all cases of dilated cardiomyopathy in western countries are related to alcohol abuse (1). Ethanol has a progressive and dose-dependent toxic effect on cardiac function, independent of malnutrition, thiamin deficiency, or electrolyte disturbances (2). We previously established a relationship between the total lifetime dose of alcohol consumed and the depression of left ventricular ejection fraction (LVEF); however, the quantity of alcohol consumed accounted for only about one third of the effect of alcohol ( $r^2 = 0.34$ ) (2). Of note, we have encountered alcoholic persons with high levels of alcohol consumption who have no evidence of myocardial impairment, whereas other alcoholic persons with less consumption display considerable loss of contractile capacity. In this context, we have previously suggested the possibility of a genetic vulnerability to the effects of alcohol on the myocardium (3, 4).

Researchers have recently focused on the possible importance of angiotensin-converting enzyme (ACE) as an influence on skeletal muscle performance (5, 6), cardiac hypertrophy (7-9), myocardial function (10, 11), and overall cardiac mortality (12). Therefore, we sought to evaluate the possibility that ACE gene polymorphism may play a role in the development of alcoholic cardiomyopathy. In this study, we compared alcoholic persons who have depressed LVEF with alcoholic persons who showed no evidence of cardiac dysfunction.

## METHODS

### Patients

Over a 2-year period (1998 to 1999), 41 men younger than 65 years of age with chronic alcoholism (according to

criteria of *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition [13]) and signs or symptoms of heart failure (New York Heart Association [NYHA] functional class II to IV [14]) presented to the emergency department at Hospital Clínic, Barcelona, Spain. We did not enroll 10 of these 41 patients: 2 persons with uncompensated liver disease, 5 persons with nonalcoholic causes of heart disease (4 with hypertension and 1 with ischemic heart disease), and 3 persons who declined to participate. We assigned the remaining 31 patients to group I. Of these 31 participants, 17 were admitted to the hospital's cardiology unit because of NYHA functional class III or IV disease; the other 14 patients, who had NYHA functional class II disease, were studied as outpatients. All participants reported consumption of at least 100 g of ethanol per day for 15 or more years and a total lifetime dose of greater than 15 kg of ethanol per kg of body weight. All participants had an LVEF lower than 0.50 and a cardiothoracic index higher than 0.5.

During the same 2-year period, 1325 asymptomatic persons with chronic alcoholism sought assistance for ending alcohol dependence at the hospital's outpatient alcoholism unit. Each week, the first two patients seen with a lifetime ethanol consumption similar to that of patients in group I were selected for the study. Of 165 such patients seen in the outpatient clinic, we excluded 24 from the study (19 had hypertension, 2 had coronary heart disease, 2 had uncompensated liver disease, and 1 had type 2 diabetes mellitus). Of the remaining 141 patients, all of whom agreed to participate in the study, 28 (20%) had an LVEF of 0.60 or greater and a cardiothoracic index less than 0.5. These patients made up group II. Participants in group I

**Context**

Although alcoholic cardiomyopathy is a dose-dependent phenomenon, the amount of alcohol consumed accounts for only one third of the effect of alcohol. Angiotensin-converting enzyme (ACE) gene polymorphism may help explain variations in cardiac vulnerability to alcohol.

**Contribution**

ACE genotypes of alcoholic persons with cardiomyopathy were compared with those of persons who had similar alcohol intake but no evidence of cardiomyopathy. Homozygotes with the *D* allele were 16.4 times as likely to have cardiomyopathy as those with the *I* allele.

**Implications**

Cardiac vulnerability to the adverse effects of alcohol appears to be partially under genetic control. Identification of alcoholic persons with the *DD* genotype may lead to early and novel treatments of cardiomyopathy.

—The Editors

were considered relatively vulnerable to the toxic effects of ethanol on the myocardium, and those in group II were considered relatively insensitive to these effects.

We excluded 1 patient in each group because of symptoms of alcohol withdrawal. Thus, 30 patients in group I and 27 participants in group II remained in the study. All participants gave informed consent for the various procedures. The institutional review board of Hospital Clínic, Barcelona, Spain, approved the study protocol. We did not explore ethnic or racial variables because all patients were white men of Spanish descent who lived with their families in or around Barcelona. Most were skilled laborers or office workers with a history of stable employment, and none were indigent. Approximately half the patients in each group had smoked 1 to 2 packs of cigarettes per day since

their second decade of life; none reported using illicit drugs.

**Laboratory and Nutritional Studies**

Blood samples were obtained 1 day after hospital admission to measure markers of alcohol intake and nutritional status. The latter was assessed as reported elsewhere (15). Patients were considered to have caloric malnutrition if they weighed less than 90% of their ideal weight or if their lean body mass was more than 10% below normal. Protein malnutrition was diagnosed when the patients had abnormal values for three of the following variables: hemoglobin level, lymphocyte count, total protein level, albumin level, prealbumin level, retinol-binding protein level, or transferrin level (15).

**Clinical and Cardiac Studies**

For each patient, one of two authors obtained a detailed history of ethanol intake and dietary habits using a structured questionnaire; this history was confirmed with a family member. The frequency and amount of ethanol intake were recorded. Life events, such as marriage, military service, and positions of employment, were used as “anchor points” to assist the patients’ recollection (the time-line follow-back method [16]). Withdrawal symptoms were evaluated according to the Clinical Institute for Withdrawal Assessment scale (17).

Past and present signs and symptoms of heart failure were evaluated, and NYHA functional class was determined according to the Goldman activity scale (14). Within 3 days after admission, when test results for blood alcohol were uniformly negative, the following cardiac examinations were performed: basal blood pressure, chest radiography, conventional electrocardiography, and determination of LVEF by technetium Tc-99m-pernechnetate radionuclide angiocardiology (Elscont SP4-HR, gamma-camera, Haifa, Israel) by using a standard technique of electrocardiography-gated equilibrium with an average of 400 to 450 cycles. Cardiac cycles with R-R intervals not

**Table 1. Epidemiologic Data and Cardiac Function in Alcoholic Persons with Cardiomyopathy or Normal Cardiac Function\***

Variable	Group I: Patients with Cardiomyopathy (n = 30)	Group II: Patients with Normal Cardiac Function (n = 27)
Mean age (range), y	52.8 ± 1.6 (34–64)	52.3 ± 1.5 (31–64)
Mean duration of alcoholism (range), y	26.1 ± 1.1 (15–45)	27.7 ± 1.2 (20–42)
Mean daily ethanol dose at admission (range), g/d	167 ± 6 (100–250)	173 ± 7 (140–300)
Mean daily ethanol dose (range), g/d	169 ± 5 (100–220)	173 ± 6 (140–280)
Mean total lifetime dose of ethanol (range), kg of ethanol per kg of body weight	23.2 ± 1.5 (15–40)	25.1 ± 1.0 (17–36)
Smoke ≥20 cigarettes/d, n (%)	19 (63)	17 (63)
Mean left ventricular ejection fraction (range), %	0.34 ± 0.02 (0.11–0.49)	0.65 ± 0.01 (0.60–0.75)†
New York Heart Association functional class, n (%)		
Class II	14 (46)	0 (0)
Class III or IV	16 (53)	0 (0)
Mean systolic blood pressure, mm Hg	127 ± 3	131 ± 2
Mean diastolic blood pressure, mm Hg	77 ± 3	76 ± 2
Cardiothoracic index	0.53 ± 0.01	0.46 ± 0.01

\* Data expressed with a plus/minus sign are the mean ± SE.

† P < 0.001 by using a two-tailed t-test.

Table 2. Laboratory and Nutritional Data in Alcoholic Persons with Cardiomyopathy or Normal Cardiac Function\*

Variable	Group I: Patients with Cardiomyopathy (n = 30)	Group II: Patients with Normal Cardiac Function (n = 27)
Percentage of ideal body weight, %	100.4 ± 1.9	104.6 ± 3.3
Tricipital skin fold thickness, cm	1.06 ± 0.11	0.93 ± 0.05
Lean body mass, kg	54.8 ± 0.91	55.8 ± 1.25
Hemoglobin level, g/dL	1460 ± 30	1430 ± 20
Lymphocyte count, × 10 <sup>6</sup> cells/L	1962 ± 118	2032 ± 130
Total protein level, g/dL	704 ± 15	685 ± 16
Albumin level, g/dL	429 ± 13	415 ± 11
Prealbumin level, mg/L	308 ± 32	295 ± 18
Retinol-binding protein level, mg/L	55 ± 6	53 ± 2
Transferrin level, g/L	2.73 ± 0.09	2.55 ± 0.12
Aspartate aminotransferase level, $\mu$ kat/L (IU/L)	0.76 ± 0.11 (45.6 ± 6.6)	1.04 ± 0.13 (62.9 ± 7.6)
Alanine aminotransferase level, nkat/L (IU/L)	698 ± 78 (41.9 ± 4.7)	896 ± 127 (53.8 ± 7.6)
$\gamma$ -glutamyl transpeptidase level, $\mu$ kat/L (IU/L)	3.42 ± 0.85 (205 ± 51)	3.11 ± 0.57 (187 ± 34)

\* Values are expressed as the mean ± SE.

within 10% of the average were rejected. The mean cardiac cycle was separated into 24 frames of a 64 × 64-pixel matrix, with a minimum of 3 300 000 counts collected in each frame. The LVEF was measured by using semiautomatic edge detection and counts with a varying region of interest. Persons who performed the examinations had no knowledge of the participants' history of alcoholism. To rule out ischemic heart disease, we evaluated all patients in group I using treadmill electrocardiography after they received treatment for heart failure. These patients were also studied by using echocardiography (Sonos 2500 instrument, Hewlett-Packard, Andover, Massachusetts). End-diastolic and end-systolic diameters, the shortening fraction, and the mass of the left ventricle were measured according to the standards of the American Society of Echocardiography (18).

### ACE Gene Polymorphisms

We extracted DNA from peripheral blood leukocytes, as described previously (19). The ACE *I/D* genotype was determined by polymerase chain reaction (PCR) using published primers 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3', which flank the polymorphic region (20). We performed PCR in a 25- $\mu$ L volume containing MgCl<sub>2</sub>, 1.5 mmol/L; KCl, 50 mmol/L; Tris-HCl, 10 mmol/L, at a pH of 8.3; 200  $\mu$ mol of each diethylnitrophenyl thiophosphate per L; 1- $\mu$ mol/L primers; *Taq* polymerase, 1 U (Boehringer Mannheim Biochemical, Mannheim, Germany); and genomic DNA, 100 ng. After an initial denaturation at 96 °C for 5 minutes, thermocycling consisted of denaturation at 94 °C for 30 seconds, annealing at 58 °C for 30 seconds, and extension at 72 °C for 1 minute for 35 cycles, followed by a final extension for 5 minutes. Amplification products were separated on 0.5% agarose gel/2.5% Nu Sieve (Amersham, Arlington Heights, Illinois) and visualized by ultraviolet transillumination after ethidium bromide staining. This primer pair produced the 480-base pair product (corresponding to the insertion, I) or a 194-base pair fragment (corresponding to the de-

letion, D). Participants were classified as having *II*, *DD*, or *ID* (heterozygous for insertion or deletion). In the past, *ID* heterozygotes have been mistyped as *DD* because of preferential amplification of the *D* allele and inefficiency in the amplification of the *I* allele. Therefore, all samples found to have the *DD* genotype were amplified with an insertion-specific primer pair that recognizes the inserted sequence 5'-TGG GAC CAC AGC GCC CGC CAC TAC-3' and 5'-TCG CCA GCC CTC CCA TGC CCA TAA-3' under identical PCR conditions except for an annealing temperature of 61 °C (20). This reaction yields a 335-base pair product in the presence of an *I* allele.

### Statistical Analysis

All variables are expressed as the mean (±SE). We analyzed differences between the two groups using a chi-square test, analysis of variance (ANOVA), and a two-tailed Student *t*-test. We evaluated the association between cardiomyopathy and the *DD* genotype using the Fisher exact test. Odds ratios and 95% CIs were calculated. Our analysis was performed by using the SPSS Statistical Analysis System, version 9.0 (SPSS, Inc., Chicago, Illinois), and Stat Xact software (Cytel Software Corp., Cambridge, Massachusetts).

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### RESULTS

All participants were normotensive. Patients in both groups were of similar age and reported similar amounts of tobacco and alcohol consumption (Table 1). Clinical and laboratory results showed no differences between the groups other than those associated with cardiac dysfunction (Tables 1 and 2). Electrocardiographic results for the patients in group I showed atrial fibrillation in 6 patients, conduction defects in 4 patients, and premature ventricular contractions in 9 patients. In group II, 1 patient had atrial

**Table 3. Epidemiologic Data in Alcoholic Persons, according to Angiotensin-Converting Enzyme Genotype\***

Variable	Patients with <i>II</i> Genotype (n = 12)	Patients with <i>ID</i> Genotype (n = 26)	Patients with <i>DD</i> Genotype (n = 19)
Age, y	51.2 ± 2.5 (31–62)	52.5 ± 1.5 (35–64)	53.7 ± 2.1 (34–64)
Duration of alcoholism, y	26.6 ± 1.9 (20–40)	27.0 ± 1.1 (16–42)	26.7 ± 1.6 (15–45)
Daily ethanol dose at admission, g/d	157 ± 5 (125–200)	179 ± 8 (100–300)	165 ± 7 (100–250)
Amount of ethanol consumed daily, g/d†	168 ± 6 (125–220)	170 ± 7 (100–280)	171 ± 7 (100–260)
Total lifetime dose of ethanol, kg of ethanol per kg of body weight	23.3 ± 1.5 (17–33)	24.5 ± 1.1 (17–40)	24.0 ± 1.9 (15–40)

\* Data are expressed as the mean ± SE (range). No statistically significant differences were observed among the groups, according to analysis of variance. ACE = angiotensin-converting enzyme.

† According to patient self-report at history-taking.

fibrillation, 2 had minor conduction defects, and 4 had premature ventricular contractions. The mean (±SE) LVEF was 33.6% ± 2.4% in group I and 64.9% ± 0.8% in group II. Group I had mean (±SE) values of 64.1 ± 2.2 mm for end-diastolic diameter, 50.2 ± 2.5 mm for end-systolic diameter, 305 ± 18 g for left ventricular mass, and 22.6% ± 1.4% for shortening fraction. We found no evidence of valvular disease or other structural cardiac abnormalities in any of these patients. In addition, results of the treadmill test showed no evidence of ischemic heart disease in any patient from group I.

Cirrhosis of the liver was diagnosed in 8 patients in group I (27%) and in 7 patients in group II (26%). However, none of the patients showed signs or symptoms of liver disease (Child–Pugh grade A) (21). Evidence of mild caloric malnutrition was observed in 5 (17%) patients in group I and 3 (11%) patients in group II; slight protein malnutrition was diagnosed in 3 (10%) patients in group I and in 4 (15%) in group II. No relation was observed between nutrition and the cardiac and genotype variables evaluated.

Patients were treated according to their NYHA functional class. For the patients in group I, we recommended abstinence from alcohol consumption and a dietary sodium intake of less than 100 mmol/d. In addition, patients in group I were treated with an ACE inhibitor (captopril, 25 mg three times daily). Six patients with NYHA class III or IV disease who presented with congestive heart failure received furosemide, 20 to 40 mg twice daily, and spirono-

lactone, 25 mg/d. The 7 patients with atrial fibrillation also received digoxin, 0.25 mg/d. No patient was treated with β-blockers or hydralazine.

The *I* and *D* alleles were identified in 36% and 65%, respectively, of the patients in group I and in 63% and 37%, respectively, of the patients in group II. Of the 57 total alcoholic participants in the study, 19 (33%) were homozygous for the ACE deletion allele (*DD*), 26 (45%) were heterozygous (*ID*), and 12 (21%) were homozygous for the insertion allele (*II*). This distribution of genotypes is similar to that of the general population of the Barcelona region (19) and of Spain in general (22).

Age and amounts of alcohol consumed were similar among the genotypes (Table 3). Seventeen of 19 (89%) patients with the *DD* genotype had cardiomyopathy. By contrast, 13 of the 38 (34%) participants who carried an *I* allele (*II* or *ID*) had impaired cardiac function. As shown in Table 4, more than half (57%) of the patients in group I were homozygous for the deleted version (*DD*) of the ACE gene, whereas among those in group II, only 7% had this variant (two-sided *P* value < 0.001). We observed the same pattern when we restricted the analysis to the 17 patients in group I with an LVEF lower than 0.40. Thus, compared with carriers of the *I* allele, alcoholic persons with the *DD* phenotype had an odds ratio of 16.4 (CI, 3.0 to 158) for the development of left ventricular dysfunction. Echocardiographic results showed that group I patients with the *DD* phenotype had somewhat greater end-systolic diameter, end-diastolic diameter, and left ventricular mass and had lower shortening fractions than group I carriers of an *I* allele, although the differences were not statistically significant.

**Table 4. Distribution of Angiotensin-Converting Enzyme Genotypes among Alcoholic Persons with or without Cardiomyopathy\***

Variable	Group I: Patients with Cardiomyopathy (n = 30)	Group II: Patients with Normal Cardiac Function (n = 27)
Mean LVEF	0.336	0.649
ACE genotype, n (%)		
<i>II</i>	3 (10)	9 (33)
<i>ID</i>	10 (33)	16 (59)
<i>DD</i>	17 (57)	2 (7)†

\* ACE = angiotensin-converting enzyme; LVEF = left ventricular ejection fraction.

† *P* < 0.001 for the difference in *DD* genotype between the groups I and II.

## DISCUSSION

We compared two groups of patients with chronic alcoholism—one with cardiomyopathy and one with normal cardiac function. No significant laboratory abnormalities other than modest elevations of hepatic enzyme levels were found in either group, and malnutrition was not a factor in cardiac impairment. We excluded patients with causes of cardiac dysfunction other than ethanol consumption (for example, coronary artery disease, heart valve disease, diabetes mellitus, or hypertension). Thus, the only

phenotypic difference that was evident between the two groups was the presence of alcoholic cardiomyopathy.

The distribution of ACE genotypes in the general population varies according to geographic location and race (23, 24). The distribution of ACE alleles among the alcoholic participants in this study was similar to that observed in Barcelona (19) and Spain (22), indicating that it is not involved in genetic or acquired susceptibility to alcoholism. However, this distribution in the general population does not necessarily apply to specific patient groups because the participants in the current study were selected according to their vulnerability to cardiomyopathy. We studied the distribution of the ACE gene polymorphism because it has been implicated in other cardiac disorders (25). Some alleles of this gene contain an inserted sequence (*I*), whereas in other alleles, the sequence is deleted (*D*). The *D* polymorphism has been associated with higher circulating levels of ACE and angiotensin II than those seen with the *I* variety (26, 27); however, local autocrine or paracrine effects may be of greater importance (25, 28). The *D* allele has also been linked to idiopathic cardiomyopathy (29, 30) and cardiac hypertrophy secondary to hypertension (31) or aortic stenosis (32). In patients homozygous for the *D* allele, regression of cardiac hypertrophy after valve replacement (33) or control of hypertension (33) is not as efficient as in patients with the *I* allele. The contribution of ACE polymorphism to the course of ischemic heart disease (11, 34, 35) or essential hypertension (19, 36) is still controversial.

In the current study, we have demonstrated an associated vulnerability to cardiomyopathy among chronic alcoholic persons homozygous for the *D* allele of the ACE gene. In terms of susceptibility to the cardiotoxic effects of ethanol, it appears that a single copy of the *I* allele is sufficient to eliminate the 16.4-fold excess risk associated with the *DD* genotype. In this context, the presence of the *I* allele has been shown to increase the efficiency of muscular contraction (6). However, even alcoholic carriers of the *I* allele may develop left ventricular dysfunction and should be advised that they are not invulnerable to this complication. The precise mechanism by which the absence of the *I* allele contributes to the previously demonstrated cardiotoxic effect of chronic alcohol abuse (2, 3) is a subject for further study. This demonstration of a genetic vulnerability to alcohol-induced myocardial damage suggests that alcoholic injury to other organs may also show a genetic influence. Because the sample of alcoholic persons we studied was ethnically homogeneous and did not include binge drinkers or indigent persons, further studies are needed to extend these findings to the general population of alcohol abusers. In addition, future studies should assess other genotypes related to the ACE system that may affect the development of cardiomyopathy.

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