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# Variation of the butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) genes in coronary artery disease

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ABSTRACT

Butyrylcholinesterase (BChE) and acetylcholinesterase (AchE) are two enzymes of the cholinergic system putatively involved in coronary artery disease (CAD). We investigated two single nucleotide polymorphisms (SNPs) of the genes encoding these enzymes to determine whether some allele or genotype might represent a factor of risk or protection for CAD onset. AChE rs2571598 and BChE rs1803274 (the so-called K-variant) SNPs were investigated in a sample of 199 patients and 199 healthy subjects. No significant results were obtained for BChE, whereas for AChE the A allele was found significantly more frequent in patients than in controls (0.437 vs. 0.332; $p = 0.002$). The crude Odds Ratio (OR) for CAD conferred by carrying the A allele was 1.76 (95% confidence interval [CI] 1.17–2.65). Stratification of the sample by gender revealed that the statistical significance was limited to female, where the crude OR associated with the A allele was 3.26 (95% CI 1.58–6.73). The lipidic pattern was also tested and related to variation of the two SNPs. In this case, an at limits significant result ($p = 0.03$) was obtained for BChE, whose A allele (the K variant) in patients was found associated with higher plasma concentrations of high density lipoprotein-cholesterol.



## 1. Introduction

Coronary artery disease (CAD) is recognized as a complex disease whose onset is attributable to environmental, lifestyle and genetic factors. Research on genes and variation in their sequence has focused chiefly on those underlying the pathogenetic mechanisms of the disease. Many genes have been directly and convincingly implied, others are less so [1].

The variation in some genes of the cholinergic system, such as those encoding butyrylcholinesterase (gene BChE, chromosome 3q26.1–q26.2) and acetylcholinesterase (gene AChE, chrom. 7q22.1) [http://www.genatlas.org] has been largely studied in relation to the onset of Alzheimer's disease (AD) [2–5], since the levels of these enzymes are found increased in the brains of AD patients.

These enzymes seem to have a role in CAD as well. Acetylcholinesterase hydrolyzes acetylcholine (ACH) which, in postmenopausal female CAD patients, causes the release of the endothelium-derived relaxing factor and has been reported to exert an indirect dilatatory effect and a direct constrictive effect after exposure of the coronary arteries to 17ß-estradiol [6]. Moreover, CAD is considered a disease in which low-grade systemic inflammation is common. In other low-grade systemic inflammatory conditions such as diabetes mellitus, hypertension, insulin resistance, and hyperlipidemia, the plasma and tissue activities of the enzymes butyrylcholinesterase and acetylcholinesterase have been found elevated [7].

Butyrylcholinesterase is an enzyme that contributes to cholinesterase activity and though it is present in several different tissues its physiological function is not yet clear. However, it is believed to be involved in the lipid metabolism since the serum BChE has been found significantly associated to triglyceride levels, and to LDL and HDL cholesterol. Studies on animals revealed that BChE could have a role in the regulation of lipoprotein metabolism [8–12].

A substantial reduction in the catalytic activity of plasma BChE has been reportedly associated with the A allele of the Ala539Thr polymorphism of the BChE gene [13]. However, it is noteworthy that this pattern is not reproducible in the brain because Tasker et al. [14], examining autopsy brain tissues, did not observe a reduction in BChE activity associated with the K variant, at difference of what reported in serum. Variation in the BChE gene, and especially the polymorphic K-variant (the A allele of the Ala539Thr polymorphism) investigated in combination with other genes in CAD onset, has been shown to be associated with the disease onset [15]. To our knowledge, no such data are present in the literature as concerns the AChE gene; therefore, we were interested in examining these two genes in order 1) to verify the trend previously reported for BChE in a further CAD sample, 2) to investigate for the first time the presence of any association with

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the AChE gene, and 3) to study the relationships between the variation of these genes and the plasma lipid profile.

## 2. Materials and methods

### 2.1. Subjects

The patient sample was 199 Italian Caucasian individuals coming mostly from Central and Southern Italy, who had sustained a myocardial infarction (94%) or had angina pectoris (6%), recruited from those consecutively attending the S. Giovanni–Addolorata Hospital of Rome. The control group came from the same area and consisted of 199 Italian Caucasian subjects free of symptoms and clinical signs of CAD, and free of diabetes, hypertension and hypercholesterolemia. The characteristics of the two groups were shown in Table 1. CAD was diagnosed on the basis of the patients' enzyme and electrocardiographic profiles. Patients that were taking lipid lowering drugs at the time of the study were excluded. Blood samples were drawn in EDTA as anticoagulant after overnight fasting.

### 2.2. DNA analysis

The technique of Miller et al. [16] was used for the extraction of high molecular-weight DNA from whole blood. The two SNPs studied were AChE rs2571598 and BChE rs1803274 (the so-called K-variant). AChE rs2571598 is an intronic SNP, whereas BChE rs1803274 is a point mutation at nucleotide 1615 (GCA to ACA) leading to replacement of an alanine by a threonine at codon 539 (Ala539Thr). The AChE SNP was investigated in genomic DNA by means of a PCR technique according to Cook et al. [2], and BChE rs1803274 according to Tilley et al. [17]. The PCR products, after digestion with Hpy188 I and Mae III, were electrophoresed on agarose gel containing ethidium bromide. The apolipoprotein E gene (APOE) polymorphism has been previously typed [18].

### 2.3. Lipid measurements

Plasma total cholesterol (TC) and HDL-C were assayed by an enzymatic method (Sera-Pak Bayer) using a BM/Hitachi 717, and TG by a Boehringer–Mannheim enzymatic method. LDL-cholesterol (LDL-C) was calculated according to the Friedewald formula [19].

### 2.4. Statistical analysis

The allelic frequencies were calculated by the gene-counting method. The $\chi^2$ test was used to verify the agreement of the observed genotype frequencies with those expected according to the Hardy–Weinberg equilibrium. The differences in frequencies between patients and controls were analyzed by a $\chi^2$ test. Logistic regression analysis was used to evaluate the impact of each polymorphism on CAD risk, after entering gender, age, APOE genotypes, TC, HDL-C, and TG levels as covariates, since most of these variables have often been found associated with a major CAD risk. In Table 1 we reported the APOE allele frequencies related to patients and controls which, in this case, had very similar frequency distributions.

Given that stratifications of the sample were performed, the Bonferroni's correction for multiple comparisons was taken into account. Analysis of variance (ANOVA) was performed to compare the mean lipid levels associated with different AChE and BChE genotypes. Triglyceride data, since they were abnormally distributed, were transformed into natural logarithms prior to the analyses.

## 3. Results

Table 2 reports the genotype and allele frequencies of the AChE and BChE polymorphisms in the overall samples of CAD patients and normal controls. The observed genotype frequencies in each case fitted the ones expected according to the Hardy–Weinberg equilibrium, as can be seen from the HWE $p$ values. In the whole sample, the AChE A allele was significantly more frequent in patients than in controls (0.437 vs. 0.332; $p=0.002$). The crude Odds Ratio (OR) for CAD conferred by carrying the AChE A allele was 1.76 (95% CI 1.17–2.65). The frequency of the BChE A allele, the so-called K variant, was lower in patients than in controls (0.171 vs. 0.226; $p=0.07$), so that no risk was associated with this allele that was protective though at non significant level (OR = 0.67; 95% CI = 0.44–1.03).

Logistic regression analysis was used to correctly estimate the risk associated with the AChE genotypes. Table 2 reports the ORs from the logistic regression in the overall sample.

In the logistic regression model, the inclusion of the interactions between AChE and APOE, age, and gender revealed that only the interaction with gender was significant (data not shown; $p=0.04$). So the sample was stratified by gender and the results shown in Table 3. The A allele was significantly more frequent in female patients than in female controls (0.500 vs. 0.306; $p<0.001$) and the crude OR conferred by carrying the A allele was 3.26 (95% CI 1.58–6.73). Stratification of the sample by gender necessitated application of the Bonferroni's correction for multiple comparisons. This diminished the significance of the difference in AChE, which remained statistically significant, nonetheless.

No particular patterns were observed for BChE even when the sample was stratified as for AChE, since all the comparisons were not significant.

The lipid pattern of CAD patients and healthy controls are illustrated in Table 1. Patients had significantly higher TG levels ($p<0.001$) and lower HDL-C levels ($p=0.001$) than controls. The effects of the two polymorphisms on plasma lipid levels were investigated. In the

**Table 1**
Characteristics of CAD patients and control subjects.

| | CAD patients | Control subjects | $p$ value |
|---|---|---|---|
| $n$ | 199 | 199 | |
| Age ± S.D. | 71.0 ± 12.2 | 73.4 ± 10.9 | 0.034 |
| Age range | 31–98 | 44–98 | |
| Gender | Male 142 (72%)<br>Female 57 (28%) | Male 101 (50.7%)<br>Female 98 (49.3%) | <0.001 |
| TC (mg/dl) | 190.1 ± 44.5 (197) | 184.6 ± 44.4 (199) | 0.217 |
| HDLC (mg/dl) | 38.1 ± 11.1 (197) | 43.2 ± 13.3 (191) | <0.001 |
| LDLC (mg/dl) | 120.4 ± 38.0 (197) | 115.5 ± 44.9 (191) | 0.240 |
| TG (mg/dl) | 158.1 ± 69.0 (197) | 133.8 ± 58.6 (199) | <0.001 |
| APOE alleles | | | |
| e*2 | 0.071 | 0.073 | |
| e*3 | 0.844 | 0.847 | |
| e*4 | 0.084 | 0.080 | 0.980 |

**Table 2**
Genotype and allele frequencies of AChE (RS 2571598) and BChE (RS 1803274) in CAD patients and controls (whole sample).

| | Genotypes | | | | Alleles | | |
|---|---|---|---|---|---|---|---|
| AChE | GG | GA | AA | $n$ | G | A | HWE ($p$) |
| Patients (%) | 63 (0.32) | 98 (0.49) | 38 (0.19) | 199 | 0.563 | 0.437 | 0.99 |
| Controls (%) | 88 (0.45) | 86 (0.44) | 22 (0.11) | 196 | 0.668 | 0.332 | 0.89 |
| BChE | GG | GA | AA | $n$ | G | A | HWE ($p$) |
| Patients (%) | 121 (0.68) | 53 (0.30) | 4 (0.02) | 178 | 0.829 | 0.171 | 0.52 |
| Controls (%) | 117 (0.59) | 74 (0.37) | 8 (0.04) | 199 | 0.774 | 0.226 | 0.38 |

AChE A allele positivity: crude OR 1.76 (95% CI 1.17–2.65); adjusted OR by logistic regression 1.88 (95% CI 1.20–2.95).
BChE A allele positivity: crude OR 0.67 (95% CI 0.44–1.03); adjusted OR 0.73 (95% CI 0.46–1.15).