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Butyrylcholinesterase K variant and the APOE-ε4 allele work in synergy to increase the risk of coronary artery disease especially in diabetic patients

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Abstract We have previously shown that butyrylcholinesterase-K (BCHE-K, G1615A/Ala539Thr) variant increases the risk of coronary artery disease (CAD). In addition, we have found that the presence of APOE-ε4 allele augments the risk of CAD in patients with type II diabetes mellitus (T2DM/CAD). Here we explored the concomitant presences of two alleles of the BCHE-K and APOE-ε4 in increasing the risk of CAD or diabetes in T2DM patients with or without CAD and CAD patients without T2DM. This case–control study comprised 631 subjects undergoing their first coronary angiography. They were matched and randomly assigned into four groups: type II diabetic patients with no sign of CAD (T2DM), type II diabetic patients with CAD/ND (T2DM/CAD), CAD patients with no sign of diabetes (CAD/ND), and healthy individuals (NCAD/ND). BCHE-K variant and APOE genotypes were detected by PCR-RFLP and serum lipid level was measured enzymatically. We found that BCHE-K and APOE-ε4 allele act synergistically to increase the risk of CAD in both T2DM, non-diabetic and total CAD (TCAD = T2DM/CAD + CAD/ND) individuals. The level of synergy 1.5 and 1.2 fold are higher in CAD patients (OR = 4.5; $P = 0.011$) with T2DM than the non-diabetic CAD patients (OR = 3.07; $P = 0.024$) and TCAD patients (OR = 3.74; $P = 0.018$), respectively. The CAD subjects with and without T2DM and TCAD patients carrying both APOE-ε4 allele and BCHE-K had significantly lower plasma HDL-C (P values = 0.008, 0.047, and 0.036, respectively) and higher plasma LDL-C (P values = 0.025, 0.048, and 0.04, respectively), than that of the control carriers both APOE-ε4 and BCHE-K. We have found that BCHE-K and APOE-ε4 allele not only act synergistically to increase the risk of CAD, particularly in T2DM subjects in population from western Iran, who have high levels of LDL-C and low levels of HDL-C, suggesting that a specific therapeutic intervention should be considered for these particular groups of patients.

Keywords Butyrylcholinesterase K variant · Apolipoprotein E · Genetics · Type 2 diabetes mellitus · Coronary artery disease

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Introduction

Type 2 diabetes mellitus (T2DM), coronary artery disease (CAD) and Alzheimer's disease are common age-related disorders that result from genetic and/or environmental [1–3]. T2DM is a disease with a high incidence and prevalence throughout the world. It is associated with important chronic diseases such as CAD, retinopathy, neuropathy and nephropathy [1] with CAD being the most common complication and a major cause of mortality in T2DM [4]. Type two diabetic patients usually have a high level of small and

dense LDL particles, which are more susceptible to oxidation. The presence of these particles increases the risk of atherosclerosis even in the absence of low concentration of LDL-C [2, 4].

Apolipoprotein-E (APOE) and butyrylcholinesterase-K variant (BCHEK, G1615A) gene polymorphism may influence the metabolism of lipids and lipoproteins in CAD and diabetic patients [5–11]. Previously, we as well as other investigators have shown that individuals carrying APOE-ε4 allele has a higher tendency to develop CAD [1, 5–7, 9, 10] In addition, BCHE-K variant gene is also implicated as a risk factor associated with T2DM [8], type I diabetes and CAD [6, 7, 11, 12]. In addition, it has been shown that individuals carrying BCHE-K variant gene are at a higher risk of developing T2DM [12], type 1diabetes and CAD [5, 6, 11, 12]. The relationship between the BCHE-K variant and APOE-ε4 allele in prevalence of CAD in patients with T2DM has yet to be established.

We have recently demonstrated that there is a synergistic association between BCHE-K and APOE-ε4 allele in promoting the risk of Alzheimer's disease in Tehran, Iran [13]. Here, we have found that BCHE-K and APOE-ε4 allele act synergistically to increase the risk of CAD, particularly in T2DM subjects in population from western Iran.

Materials and methods

Subjects

The target population for this case–control study consisted of 118 unrelated T2DM patients (56 males and 62 females; mean age 57.2 ± 7.9 years), 162 unrelated individuals consisting of 89 males and 73 females (mean age 56.3 ± 8.5 years) with angiographic documented CAD and without diabetes (CAD/ND), 172 unrelated T2DM subjects (95 males and 77 females; mean age 57.2 ± 7.9 ! years) having angiographically-proven CAD (greater than 50% diameter stenosis of one or more major coronary vessel; T2DM/CAD), and 179 unrelated control subjects (88 males and 91 females; mean age 55.7 ± 12.9 years), consisting of non-diabetic individuals, as assessed according to their fasting blood sugar level, who were angiographically evaluated for suspected CAD but found to have a normal coronary arteries. The control groups were matched for sex and age with T2DM, CAD/ND and T2DM/CAD patients. The subjects were assessed and referred to the Cardiology Division of the Imam Ali Hospital of the Kermanshah University of Medical Science and were selected from individuals who had undergone their first coronary angiography for evaluating the presence and extent of CAD. Only patients undergoing elective angiography were included in order to avoid the influence of stress situations. Patients undergoing coronary angiography for diseases such as valvular or congenital heart disease and restrictive or dilated cardiomyopathy were all excluded. The presence of diabetes in patients was confirmed using World Health Organization criteria [14]. The study was approved by the Ethics Committee of the Kermanshah University of Medical Sciences and was in accordance with the principles of the Declaration of Helsinki II and an informed written consent was obtained from each individual before participation.

DNA analysis

Genomic DNA was extracted from peripheral blood leukocytes using phenol chloroform extraction method [15]. BCHE-K (*G1615A*) variant and APOE polymorphisms were detected by PCR-RFLP as previously described [16, 17].

Chemical analysis

Total serum cholesterol (TC), triacylglycerol (TG), serum LDL-C and HDL-C levels were measured by the standard enzymatic method (Pars Azmon kit, Iran), using an automated RA-1000 (Technician, USA).

Statistical analysis

The SPSS statistical software package version 11.5 was used for the statistical analyses. The allelic frequencies were calculated by the gene counting method. The χ^2-test was used to verify the agreement of the observed genotype frequencies with those expected according to the Hardy–Weinberg equilibrium. Genotype and allele frequencies of BCHE and APOE were compared between T2DM, CAD, T2DM/CAD and control groups using χ^2- test. Odds ratios (OR) as estimates of relative risk for disease were calculated and 95% confidence intervals obtained by SPSS logistic regression. The interaction between the BChE-K and APOE-ε4 allele was determined using logistic regression model. The correlation values of plasma HDL-C, LDL-C, TG and TC level with the BCHE-K and APOE-ε4 allele between patients and controls were calculated using linear regression and an unpaired *t* test. Two-tailed Student's *t* test and ANOVA analysis were also used to compare quantitative data. Statistical significance was assumed at the $P < 0.05$ level.

Results

The age, gender, distribution of genotypes and alleles of BChE-K, APOE frequencies and lipid parameters found in

Table 1 Age, sex, lipid parameters levels (mg/dl) and the distribution of BCHE and APOE genotypes and alleles in T2DM/CAD, CAD, T2DM patients and in control subjects in a population from west of Iran

	T2DM/CAD patients ($n = 172$)	CAD patients ($n = 162$)	T2DM ($n = 118$)	Total CAD ($n = 334$)	Control subjects ($n = 179$)
Age (years)	57.2 ± 7.9	56.3 ± 8.5	56.8 ± 9.5	56.9 ± 8.2	55.5 ± 129
Sex (M/F)	95/77	89/73	56/62	184/150	88/9,001
BCHE genotypes					
BCHE GG	103 (60%)	110 (67.9%)	80 (67.8%)	213 (63.8%)	137 (76.5%)
	($\chi^2 = 8.8$, $df = 2$, $P = 0.003$)	($\chi^2 = 7$, $df = 2$, $P = 0.008$)	($\chi^2 = 2.4$, $df = 2$, $P = 0.1$)	($\chi^2 = 2.4$, $df = 2$, $P = 0.1$)	
BCHE GA	61 (35%)	46 (29.5%)	35 (29.7%)	107 (32%)	40 (22.3%)
	($\chi^2 = 8.7$, $df = 1$, $P = 0.003$)	($\chi^2 = 2.1$, $df = 1$, $P = 0.15$)	($\chi^2 = 2.2$, $df = 1$, $P = 0.13$)	($\chi^2 = 6.4$, $df = 1$, $P = 0.011$)	
BCHE AA	8 (4.7%)	6 (3.7%)	3 (2.5%)	14 (4.2%)	2 (1.1%)
	($\chi^2 = 5.3$, $df = 1$, $P = 0.021$)	($\chi^2 = 2.9$, $df = 1$, $P = 0.09$)	($\chi^2 = 1.1$, $df = 1$, $P = 0.3$)	($\chi^2 = 4.6$, $df = 1$, $P = 0.032$)	
BCHE (GA+AA)	69 (40.1%)	52 (32.1%)	38 (32.2%)	121 (36.2%)	41 (24.8%)
	($\chi^2 = 11.2$, $df = 1$, $P = 0.001$)	($\chi^2 = 3.2$, $df = 1$, $P = 0.07$)	($\chi^2 = 2.8$, $df = 1$, $P = 0.097$)	($\chi^2 = 8.7$, $df = 1$, $P = 0.003$)	
BCHE alleles					
G	268 (77.9%)	266 (82.1%)	195 (82.6%)	534 (79.9%)	314 (87.7%)
A	76 (23.3%)	58 (17.9%)	41 (17.4%)	134 (20.1%)	44 (12.3%)
	($\chi^2 = 11.9$, $df = 1$, $P = 0.001$)	($\chi^2 = 4.2$, $df = 1$, $P = 0.04$)	($\chi^2 = 3$, $df = 1$, $P = 0.08$)	($\chi^2 = 9.8$, $df = 1$, $P = 0.002$)	
APOE genotypes					
ε2ε2 + ε2ε3	35 (20.3%)	31 (19.1%)	26 (22%)	66 (19.8%)	32 (17.9%)
	($\chi^2 = 1.6$, $df = 1$, $P = 0.2$)	($\chi^2 = 0.3$, $df = 1$, $P = 0.5$)	($\chi^2 = 1.3$, $df = 1$, $P = 0.26$)	($\chi^2 = 1.1$, $df = 1$, $P = 0.3$)	
ε2ε4	3 (1.7%)	–	–	3(0.9%)	2 (1.1%)
ε3ε3	91 (52.9%)	99 (61.1%)	69 (58.5%)	190 (56.9%)	119 (66.5%)
	($\chi^2 = 6.5$, $df = 1$, $P = 0.011$)	($\chi^2 = 1.4$, $df = 1$, $P = 0.24$)	($\chi^2 = 2.4$, $df = 1$, $P = 0.12$)	($\chi^2 = 4.7$, $df = 1$, $P = 0.031$)	
ε3ε4	31 (18%)	28 (17.3%)	20 (16.9%)	59 (17.7%)	24 (13.4%)
	($\chi^2 = 3$, $df = 1$, $P = 0.08$)	($\chi^2 = 1.2$, $df = 1$, $P = 0.27$)	($\chi^2 = 1.1$, $df = 1$, $P = 0.28$)	($\chi^2 = 2.6$, $df = 1$, $P = 0.1$)	
ε4ε4	12 (7%)	4 (2.5%)	3 (2.5%)	16 (4.8%)	2 (1.1%)
	($\chi^2 = 9.5$, $df = 1$, $P = 0.002$)	($\chi^2 = 1$, $df = 1$, $p = 0.3$)	($\chi^2 = 1.1$, $df = 1$, $P = 0.29$)	($\chi^2 = 5.5$, $df = 1$, $P = 0.019$)	
ε3ε4+ε4ε4	43 (32.1%)	32 (24.4%)	23 (25%)	75 (28.3%)	26 (17.9%)
	($\chi^2 = 7.5$, $df = 1$, $P = 0.006$)	($\chi^2 = 1.8$, $df = 1$, $P = 0.18$)	($\chi^2 = 1.7$, $df = 1$, $P = 0.19$)	($\chi^2 = 5.4$, $df = 1$, $P = 0.02$)	
APOE alleles					
ε2	39 (11.5%)	31 (9.6%)	26 (11%)	70 (10.6%)	40 (11.3%)
	($\chi^2 = 0.4$, $df = 1$, $P = 0.58$)	($\chi^2 = 0.4$, $df = 1$, $P = 0.6$)	($\chi^2 = 0.2$, $df = 1$, $P = 0.97$)	($\chi^2 = 0.3$, $df = 1$, $P = 0.95$)	
ε3	244 (72.2%)	257 (79.3%)	184 (78%)	501 (75.7%)	286 (80.8%)
	($\chi^2 = 0.7$, $df = 1$, $P = 0.008$)	($\chi^2 = 0.3$, $df = 1$, $P = 0.63$)	($\chi^2 = 0.7$, $df = 1$, $P = 0.4$)	($\chi^2 = 3.5$, $df = 1$, $P = 0.06$)	
ε4	55 (16.3%)	36 (11.1%)	26 (11%)	91 (13.7%)	28 (7.9%)
	($\chi^2 = 11.7$, $df = 1$, $P = 0.001$)	($\chi^2 = 2.8$, $df = 1$, $P = 0.047$)	($\chi^2 = 1.6$, $df = 1$, $P = 0.2$)	($\chi^2 = 7.5$, $df = 1$, $P = 0.036$)	

Table 1 continued

	T2DM/CAD patients ($n = 172$)	CAD patients ($n = 162$)	T2DM ($n = 118$)	Total CAD ($n = 334$)	Control subjects ($n = 179$)
Lipid parameters					
LDL-C	94 ± 29 ($P < 0.001$)	87 ± 22 ($P = 0.32$)	101 ± 30 (p < 0.001)	91 ± 26 ($P < 0.001$)	82 ± 18
HDL-C	43.5 ± 6.8 ($P < 0.001$)	48.2 ± 7 ($P < 0.001$)	45.2 ± 7.1 ($P < 0.001$)	45.8 ± 7.3 ($P < 0.001$)	49 ± 5
TG	210 ± 66 ($P < 0.001$)	187 ± 49 ($P < 0.001$)	196 ± 70 ($P < 0.001$)	199 ± 59 ($P < 0.001$)	178 ± 30
TC	187 ± 33 ($P < 0.001$)	181 ± 29 ($P = 0.129$)	196 ± 40 ($P < 0.001$)	184 ± 31 ($P = 0.004$)	176 ± 23

T2DM, CAD/ND, T2DM/CAD and TCAD(T2DM/CAD + CAD/ND) patients and in control subjects are reported in Table 1. The distribution of the BChE-K and APOE genotypes were found to differ significantly in CAD subjects with (CAD/T2DM; $\chi^2 = 12.6$, $P = 0.002$ and $\chi^2 = 14$, $P = 0.015$, respectively) and without (CAD/ND; $\chi^2 = 4.5$, $P = 0.048$ and $\chi^2 = 12.9$, $P = 0.024$, respectively) diabetes and TCAD(($\chi^2 = 10.2$, $P = 0.006$ and $\chi^2 = 1.8$, $P = 0.011$, respectively) compared to control by Hardy–Weinberg equilibrium. In addition, the BChE-K (G1615A) and APOE-ε4 allele frequencies (as calculated using the actual allele number) in the T2DM/CAD subjects were found to be 1.9 ($P = 0.001$) and 2 ($P = 0.001$) times, in CAD/ND subjects to be 1.5 ($P = 0.04$) and 1.4 ($P = 0.047$) times and in TCAD patients to be 1.6 ($P = 0.002$) and 1.7 ($P = 0.036$) times higher than that of the control group, respectively. However, the distribution of the APOE and BChE alleles in T2DM were not significantly different from that of the control group.

Computation of the OR (95% confidence interval) as an estimate of relative risk for CAD showed that subjects with the BChE-K and APOE-ε4 alleles in the CAD/T2DM subjects were found to be 2.02 ($P = 0.001$) and 2.3 ($P = 0.001$) times, in CAD/ND patients to be 1.56 ($P = 0.003$) and 1. 6 ($P = 0.018$) times and in TCAD individuals 1.8 ($P = 0.002$) and 1.86 ($P = 0.007$) times more likely to suffer from CAD (Table 2).

As shown in Table 3, logistic regression analysis as defined by Lehmann et al. [18] and Raygani et al. [19], demonstrated a strong and significant interaction between BCHE-K and APOE-ε4 in TCAD individuals ($\chi^2 = 13.8$, $P = 0.004$) specially, in those with T2DM ($\chi^2 = 16$, $P = 0.001$). The APOE-ε4 and BChE-K variant increased risk of CAD in T2DM patients by 2.1 ($P = 0.022$) and by 2.1 ($P = 0.003$) times, in TCAD subjects by 1.7 ($P = 0.05$) and by 1.9 ($P = 0.006$) times, respectively (Table 3). The odds ratio for possession of both risk factors BChE-K and APOE-ε4 were found to be 3.07 ($P = 0.024$) in CAD/ND patients and in TCAD individuals 3.74 ($P = 0.018$) and it increased to 4.5 ($P = 0.001$) for the CAD subjects with T2DM.

The effects of presence (+) or absence (−) of APOE-ε4 and/or BChE-K allele on plasma lipid and lipoprotein concentration were also investigated (Table 4). Our results showed a significant correlation between possession of both APOE-ε4 and BChE-K allele and serum lipid profile in all study groups. The CAD patients with or without T2DM, TCAD patients and diabetic patients without CAD who carried both APOE-ε4 and BCHE-K allele had significantly higher LDL-C (P values = 0.025, 0.048, 0.04 and 0.007, respectively) and lower HDL-C (P values = 0.008, 0.047, 0.036 and 0.001, respectively) levels compared to both APOE-ε4 and BChE-K carrier control subjects.

Table 2 Odd ratio of BCHE and APOE genotypes and alleles with respect to GG or G, ε3/ε3 or ε3, irrespectively in T2DM/CAD, CAD, T2DM patients and in control subjects separately in each group

	T2DM/CAD patients ($n = 172$) ORs (95% CI)	CAD patients ($n = 162$) ORs (95% CI)[a]	T2DM ($n = 118$) ORs (95% CI)[a]	Total CAD ($n = 334$) ORs (95% CI)[a]	Control subjects $n = (179)$
BCHE genotypes					
GG	Referent group ($n = 110$)	Referent group ($n = 80$)	Referent group ($n = 80$)	Referent group ($n = 213$)	Referent group ($n = 137$)
GA	1.43 (0.88–2.3, $P = 0.15$, $n = 46$)	1.5 (0.78–2.5, $P = 0.13$, $n = 35$)	1.5 (0.78–2.5, $P = 0.13$, $n = 35$)	1.72 (1.13–2.6, $P = 0.012$, $n = 107$)	($n = 0$)
AA	3.7 (0.74–18.9, $P = 0.11$, $n = 6$)	2.6 (0.42–15.7, $P = 0.3$, $n = 3$)	2.6 (0.42–15.7, $P = 0.3$, $n = 3$)	4.5 (1.09–20.1, $P = 0.049$, $n = 14$)	($n = 2$)
GA+AA	1.52 (0.96–2.5, $P = 0.07$, $n = 52$)	1.56 (0.9–2.6, $P = 0.096$, $n = 38$)	1.56(0.9–2.6, $P = 0.096$, $n = 38$)	1.85 (1.3–2.8, $P = 0.003$, $n = 121$)	($n = 41$)
BCHE alleles					
G	Referent group ($n = 268$)	Referent group ($n = 266$)	Referent group ($n = 195$)	Referent group ($n = 534$)	($n = 314$)
A	2.02 (1.4–3.1, $P = 0.001$, $n = 76$)	1.56 (1.02–2.4, $P = 0.04$, $n = 58$)	1.5 (0.95–2.4, $P = 0.086$, $n = 41$)	1.8 (1.3–2.6, $P = 0.002$, $n = 134$)	($n = 44$)
APOE genotypes					
ε3ε3	Referent group ($n = 91$)	Referent group ($n = 99$)	Referent group ($n = 69$)	Referent group ($n = 190$)	Referent group ($n = 119$)
ε2ε3	1.44 (0.85–2.5, $P = 0.2$, $n = 35$)	1.17 (0.7–2.1, $P = 0.6$, $n = 31$)	1.4 (0.78–2.5, $P = 0.27$, $n = 26$)	1.3 (0.8–2.1, $P = 0.29$, $p = 0.3$)	($n = 32$)
ε3ε4	1.7 (0.93–3.1, $P = 0.08$, $n = 31$)	1.4 (0.8–2.6, $P = 0.27$, $n = 28$)	1.44 (0.75–2.8, $P = 0.28$, $n = 20$)	1.54 (0.91–2.6, $P = 0.1$, $n = 59$)	($n = 24$)
ε4ε4	7.8 (1.7–36, $p = 0.008$, $n = 12$)	2.4 (0.5–13.5, $P = 0.3$, $n = 4$)	2.6 (0.4–15.2, $P = 0.32$, $n = 3$)	5 (1.13–22, $P = 0.034$, $n = 16$)	($n = 2$)
ε3ε4 + ε4ε4	2.17 (1.2–3.8, $P = 0.007$, $n = 43$)	1.5 (0.85–2.6, $P = 0.018$, $n = 32$)	1.52 (0.8–2.8, $P = 0.19$, $n = 19$)	1.8 (1.1–3, $P = 0.021$, $n = 75$)	($n = 26$)
APOE alleles					
ε3	Referent group ($n = 222$)	Referent group ($n = 230$)	Referent group ($n = 153$)	Referent group ($n = 501$)	Referent group ($n = 286$)
ε2	1.14 (0.7–1.8, $P = 0.58$, $n = 33$)	0.86 (0. 6–1.4, $P = 0.56$, $n = 28$)	1.01 (0.6–1.7, $P = 0.97$, $n = 15$)	0.98 (0.67–1.5, $P = 0.98$, $n = 70$)	($n = 40$)
ε4	2.3 (1.4–3.7, $P = 0.001$, $n = 45$)	1.6 (0.85–2.4, $P = 0.018$, $n = 36$)	1.44 (0.82–2.5, $P = 0.2$, $n = 18$)	1.86 (1.2–2.9, $P = 0.007$, $n = 91$)	($n = 28$)

[a] ORs were calculated using standard logistic regression controlling age and sex

Table 3 Carrier odds ratios in T2DM/CAD, CAD, T2DM and TCAD patients and in control subjects separately in each group

BCHE-k	APOE-ε4	T2DM/CAD patients ORs[a] (95% CI)	CAD patients ORs[a] (95% CI)	T2DM ORs[a] (95% CI)	TCAD ORs[a] (95% CI)	Control subjects
−	−	Referent group	Referent group	Referent group	Referent group	Referent group
		(n = 77, 44.8%)	(n = 86, 53.1%)	(n = 63, 53.4%)	(n = 163, 48.8%)	(n = 116, 64.8%)
	+	(n = 53, 30.8%)	(n = 44, 27.2%)	(n = 33, 28%)	(n = 97, 29%)	(n = 34, 19.2%)
		2.1 (1.3–3.6, P = 0.003)	1.6 (0.96–2.7, P = 0.07)	1.64 (0.94–2.7, P = 0.08)	1.9 (1.2–3, P = 0.006)	
		χ^2 = 8.9[b], df = 1, P = 0.003	χ^2 = 3.2[b], df = 1, P = 0.073	χ^2 = 3[b], df = 1, P = 0.08	χ^2 = 7.6[b], df = 1, P = 0.006	
−	+	(n = 30, 17.4%)	(n = 23, 14.2%)	(n = 17, 14.4%)	(n = 53, 15.9%)	(n = 22, 12.4%)
		2.1 (1.21–4.45, P = 0.022)	1.4 (0.8–2.7, P = 0.27)	1.4 (0.74–2.7, P = 0.32)	1.7 (1.08–3, P = 0.05)	
		χ^2 = 5.3[b], df = 1, P = 0.022	χ^2 = 1.1[b], df = 1, P = 0.29	χ^2 = 0.97[b], df = 1, P = 0.32	χ^2 = 4[b], df = 1, P = 0.047	
+	+	(n = 12, 7%)	(n = 9, 5.6%)	(n = 5, 4.2%)	(n = 21, 6.3%)	(n = 4, 2, 3%)
		4.5 (1.4–14.5, P = 0.011)	3.07 (1.2–123, P = 0.024)	2.3 (0.6–8.9, P = 0.22)	3.74 (1.3–11.2, P = 0.018)	
		χ^2 = 7.5[b], df = 1, p = 0.006	χ^2 = 3.8[b], df = 1, p = 0.049	χ^2 = 1.6[b], df = 1, p = 0.21	χ^2 = 6.2[b], df = 1, p = 0.012	
		χ^2 = 16[c], df = 3, P = 0.001	χ^2 = 7.8[c], df = 3, P = 0.049	χ^2 = 4.3[c], df = 3, P = 0.22	χ^2 = 13.8[c], df = 3, P = 0.004	(n = 4, 2, 4%)

[a] ORs were calculated using standard logistic regression controlling age and sex

[b] Compared distribution between corresponding alleles in control group with CAD/T2DM, CAD/ND, T2DM and TCAD

[c] Compared distribution of the APOE-ε4,BCHE-K (−, −), APOE-ε 4, BCHE-K (−, +), APOE-ε 4, BCHE-K (+, −) and APOE-ε 4, BCHE-K (+,+) between control subjects with each group CAD/T2DM, CAD/ND, T2DM and TCAD separately

These data suggest that the individuals with the presence of both BCHE-K and APOE-ε4 allele have a district plasma lipid profile synergistically increase the risk of CAD in the population in western part of Iran and their presence intensifies the risk of CAD in the T2DM.

Discussion

The results of several studies suggest that BChE-K variants and APOE-ε4 allele are genetic factors that may increase the risk of developing CAD and T2DM in individuals [1, 2, 6–12]. However, to the best of our knowledge, no information is available about the clinical significance of the relationship between the presence of BCHE-K variants and APOE-ε4 allele in increasing risks of CAD in T2DM patients. In the present case-control study we examined the relationship between these two risk factors and increasing risks of CAD in T2DM patients. We found that BCHE-K variants and APOE-ε4 allele act in synergy to increase the risk of CAD in T2DM. The presences of BChE-K variants increase the risk of CAD in patients carrying APOE-ε4 allele and have T2DM by 4.5 fold and those without T2DM by 3.07 times and TCAD by 3.74 times. These data also indicate that T2DM patients carrying both BCHE-K variants and APOE-ε4 allele are more susceptible to CAD than the non-diabetic patients. Consistent with our previous data [9–11] and data obtained from different ethnic groups around the world [1, 2, 5–8, 12, 18–25], this study indicates that the presence of either BCHE-K, APOE-ε4 allele is sufficient to increase the risk of CAD in T2DM individuals by 2.02, and 2.3 times and in non-diabetic patients by 1.56, and 1.6 times and in TCAD subjects by 1.8, and 1.86 times respectively. However, several investigators were not able to find an association between the APOE alleles (-ε4 alleles) and BCHE-K variants and the onset of CAD and T2DM in the population that they were investigating [1, 26, 27]. These suggest that the association between BCHE and APOE polymorphism and the risk of CAD in diabetic and non-diabetic patients varies in different populations and must be assessed individually.

BCHE-K is a significant risk factor for CAD in diabetic and non-diabetic patients. It acts in synergy with APOE-ε4 to increase the risk of developing CAD in T2DM patients by 4.5 fold ($P = 0.011$) and in non-diabetic individuals by 3.07 times ($P = 0.024$) and in TCAD subjects by 3.74 times ($P = 0.018$).

Interestingly, our results showed that individuals carrying both APOE-ε4 and BCHE-K allele have a distinct plasma lipids profile and carrier of these alleles with low levels of HDL-C and with high levels of LDL-C may be more susceptible to CAD and myocardial infarction, especially diabetic patients. Low level of HDL-C and high

Table 4 The lipid parameters levels (mg/dl) related to APOE ε4 BCHE-K alleles in T2DM/CAD, CAD/ND, T2DM and total CAD patients and in control subjects separately in each group

APOE and BCHE alleles		CAD/T2DM patients				CAD patients				T2DM patients			
		LDL-C	HDL-D	TG	TC	LDL-C	HDL-C	TG	TC	LDL-C	HDL-C	TG	TC
APOE-ε4 BCHE-K													
−	−	82 ± 21	45.2 ± 5.5	188 ± 50	174 ± 26	83.5 ± 8.4	49.3 ± 8.5	178 ± 19	187 ± 15	91.2 ± 18.5	47.2 ± 4.7	169 ± 51	182 ± 28
		$P = 0.25^{a}$	$P = 0.001^{a}$	$P = 0.009^{a}$	$P = 0^{a}$	$P = 0.1^{a}$	$P = 0.8^{a}$	$P = 0.8^{a}$	$P = 0.049^{a}$	$P = 0.001^{a}$	$P = 0.019^{a}$	$P = 0.5^{a}$	$P = 0.009^{a}$
−	+	89 ± 20	45.3 ± 6	200 ± 53	183 ± 22	86 ± 26	48.8 ± 6.5	195 ± 69	179 ± 39	96.5 ± 36	47 ± 7.3	212 ± 73	192 ± 41
		$P = 0.07^{a}$	$P < 0.001^{a}$	$P = 0.06^{a}$	$P = 0.28^{a}$	$P = 0.4^{a}$	$P = 0.6^{a}$	$P = 0.27^{a}$	$P = 0.8^{a}$	$P = 0.017^{a}$	$P = 0.034^{a}$	$P = 0.027^{a}$	$P = 0.08^{a}$
		$P = 0.5^{b}$	$P = 0.98^{b}$	$P = 0.6^{b}$	$P = 0.25^{b}$	$P = 0.9^{b}$	$P = 0.9^{b}$	$P = 0.07^{b}$	$P = 0.9^{b}$	$P = 0.75^{b}$	$P = 0.9^{b}$	$P = 0.012^{b}$	$P = 0.9^{b}$
+	−	112 ± 37	39 ± 6.7	257 ± 77	209 ± 40	102 ± 34	43 ± 5.4	212 ± 44	193 ± 38	134 ± 28	37.3 ± 6.4	244 ± 79	237 ± 40
		$P = 0.042^{a}$	$P < 0.001^{a}$	$P < 0.001^{a}$	$P = 0.1^{a}$	$P = 0.36^{a}$	$P = 0.021^{a}$	$P = 0.07^{a}$	$P = 0.9^{a}$	$P < 0.001^{a}$	$P < 0.001^{a}$	$P = 0.006^{a}$	$P < 0.001^{a}$
		$P < 0.001^{b}$	$P < 0.001^{b}$	$P < 0.001^{b}$	$P = 0.001^{b}$	$P = 0.003^{b}$	$P = 0.001^{b}$	$P = 0.004^{b}$	$P = 0.13^{b}$	$P < 0.001^{b}$	$P < 0.001^{b}$	$P < 0.001^{b}$	$P = 0.9^{b}$
+	−	130 ± 34	36.7 ± 7.5	272 ± 81	229 ± 34	97 ± 20	43 ± 11	199 ± 31	189 ± 21	141 ± 27	35.8 ± 7.2	265 ± 80	248 ± 27
		$P = 0.025^{a}$	$P = 0.008^{a}$	$P = 0.04^{a}$	$P = 0.018^{a}$	$P = 0.048^{a}$	$P = 0.047^{a}$	$P = 0.2^{a}$	$P = 0.5^{a}$	$P = 0.007^{a}$	$P = 0.001^{a}$	$P = 0.069^{a}$	$P = 0.003^{a}$
		$P < 0.001^{b}$	$P < 0.001^{b}$	$P < 0.001^{b}$	$P < 0.001^{b}$	$P = 0.24^{b}$	$P = 0.7^{b}$	$P = 0.043^{b}$	$P = 0.7^{b}$	$P < 0.001^{b}$	$P < 0.001^{b}$	$P = 0.001^{b}$	$P = 0.9^{b}$
		$P < 0.001^{c}$	$P < 0.001^{c}$	$P < 0.001^{c}$	$P < 0.001^{c}$	$P = 0.002^{c}$	$P = 0.001^{c}$	$P = 0.001^{c}$	$P = 0.14^{c}$	$P < 0.001^{c}$	$P < 0.001^{c}$	$P < 0.001^{c}$	$P < 0.001^{c}$

APOE and BCHE alleles		Total CAD patients				Control subjects			
		LDL-C	HDL-C	TG	TC	LDL-C	HDL-C	TG	TC
APOE-ε4 BCHE-K									
−	−	83 ± 17	47.3 ± 6	181 ± 43	176 ± 23	79.6 ± 18.7	49.1 ± 4.9	173 ± 31	172 ± 24
		$P = 0.09$	$P = 0.013$	$P = 0.09$	$P = 0.14$				
−	+	88 ± 23	47 ± 7.5	198 ± 60	182 ± 31	82.5 ± 14	50.2 ± 4.3	183 ± 29	178 ± 22
		$P = 0.19^{a}$	$P = 0.023^{a}$	$P = 0.13^{a}$	$P = 0.56^{a}$				
		$P = 0.75^{b}$	$P = 0.9^{b}$	$P = 0.012^{b}$	$P = 0.58^{b}$	$P = 0.8^{b}$	$P = 0.6^{b}$	$P = 0.3^{b}$	$P = 0.4^{b}$
+	−	107 ± 36	41 ± 6.4	238 ± 68	202 ± 40	94 ± 15	47.2 ± 6.3	193 ± 21	194 ± 18
		$P = 0.037^{a}$	$P < 0.001^{a}$	$P = 0.004^{a}$	$P = 0.35^{a}$				
		$P < 0.001^{b}$	$P < 0.001^{b}$	$P = 0.001^{b}$	$P < 0.001^{b}$	$P = 0.002^{b}$	$P = 0.4^{b}$	$P = 0.017^{b}$	$P < 0.001^{b}$
+	−	116 ± 33	41 ± 10.3	241 ± 73	212 ± 40	86.5 ± 2.7	48.5 ± 1.7.	178 ± 3.	182 ± 9
		$P = 0.04^{a}$	$P = 0.036^{a}$	$P = 0.1^{a}$	$P = 0.11^{a}$				
		$P < 0.001^{b}$	$P < 0.001^{b}$	$P = 0.007^{b}$	$P < 0.001^{b}$	$P = 0.8^{b}$	$P = 0.98^{b}$	$P = 0.9^{b}$	$P = 0.7^{b}$
		$P < 0.001^{c}$	$P < 0.001^{c}$	$P < 0.001^{c}$	$P < 0.001^{c}$	$P = 0.005^{c}$	$P = 0.19^{c}$	$P = 0.019^{c}$	$P < 0.001^{c}$

[a] Lipid parameter levels compared between corresponding alleles in control group with CAD/T2DM, CAD/ND, T2DM and TCAD

[b] Comparison of levels of LDL-C, HDL-C, TG and TC within each group aspect APOE-ε4, BCHE-K (−, −) with APOE-ε4, BCHE-K (−, +), APOE-ε4, BCHE-K (+, −) and APOE-ε4, BCHE-K (+,+) were used by one-way ANOVA test

[c] Compared among APOE-ε4, BCHE-K (−, −), APOE-ε4, BCHE-K (−, +), APOE-ε4, BCHE-K (+, −) and APOE-ε4, BCHE-K (+, +) within group related to lipid parameters levels

levels of LDL-C are all classic risk factors for CAD and diabetes [1, 6, 8–11, 28–33].

The mechanism whereby the BCHE-K variant and APOE-ε4 allele may act as a risk factor for CAD in Type 2 diabetic patients is unknown [1, 6–12, 18, 24–26]. However, it has been proposed that the presence of BCHE-K and APOE-ε4 in the vascular lesions and the presence of amyloid plaques in CAD and in types I and II diabetes may be an indicative of a possible interaction between BCHE-K and APOE-ε4 protein and β amyeloid [3, 32, 34–36]. Amyloidal fibrils cause excessive production of superoxide radicals leading to vascular endothelial damage. In addition, lipid peroxidation by superoxide radicals and nitric oxide inactivation may contribute to atherogenesis and beta cell pancreatic apoptosis [3–32, 34–36]. It has also been shown that BCHE interacts with lipoproteins [37], thus altering (modified) lipoprotein metabolism, which may further contribute to the development and progression of atherosclerosis [20, 21]. We have previously shown that the role of the APOE-ε4 allele as a risk factor for CAD is not only due to its association with a high level of LDL-C, but also by its association with a low level of the antiatherogenic HDL-C [9, 10].

In addition, we recently reported a negative correlation between BuCHE activities with total cholesterol level, also the frequency of BuChE phenotypes with low activity is high in stroke patients, who have high levels of cholesterol, may have increased susceptibility to stroke [38]. These observations emphasize the significance of lipid peroxidation, APOE and BCHE proteins in the development of CAD, however further studies are needed to shed light on contribution of BCHE-K and APOE and their mechanism of action in the development of CAD and diabetes in different populations.

In conclusion, we have found that BCHE-K variant and APOE-ε4 alleles are significant risk factors for CAD. We have demonstrated that BCHE-K synergistically increases the risk of CAD in individuals carrying APOE-ε4 allele. The presence of these alleles increases the risk of CAD especially in diabetic patients in the western population of Iran. In addition, individuals carrying both APOE-ε4 allele and BCHE-K variant have distinct plasma lipids profile and carrier of these alleles with low levels of HDL-C and with high levels of LDL-C are more susceptible to CAD and myocardial infarction.

References

1. Kalix S, Meynet MCB, Garin MCB, James RW (2001) The apolipoprotein e2 allele and the severity of coronary artery disease in Type 2 diabetic patients. Diabet Med 18:445–450
2. Mahfouz RAR, Sabbagh AS, Zahed LF, Mahfoud ZR, Kalmoni RF, Otrock ZK, Taher AT, Zaatari GS (2006) Apolipoprotein E gene polymorphism and allele frequencies in the Lebanese population. Mol Bio Rep 33:145–149
3. Jaikaran ETAS, Clark A (2001) Islet amyloid and Type 2 diabetes: from molecular misfolding to islet pathophysiology. Biochim Biophys Acta 1537:179–203
4. Zimmet PZ (1999) Diabetes epidemiology as a tool to trigger diabetes research and care. Diabetologia 42:499–518
5. Hirashiki A, Yamada Y, Murase Y, Suzuki Y, Kataoka H, Morimoto Y, Tajika T, Murohara T, Yokota M (2004) Association of gene polymorphisms with coronary artery disease in low- or high-risk subjects defined by conventional risk factors. J Am Coll Cardiol 44(1):209–210
6. Nassar BA, Rockwood K, Kirkland SA, Ransom TP, Darvesh S, Macpherson K, Johnstone DE, O'Neill BJ, Bata IR, Andreou P, Jeffery JS, Cox JL, Title LM (2006) Improved prediction of early-onset coronary artery disease using APOE epsilon4, BChE-K, PPARgamma2 Pro12 and ENOS T-786C in a polygenic model. Clin Biochem 39(2):109–114
7. Nassar BA, Darvesh S, Bevin LD, Rockwood K, Kirkland SA, O'neill BJ, Bata IR, Johnstone DE, Title LM (2002) Relation between butyrylcholinesterase K variant, paraoxonase 1 (PON1) Q and R and apolipoprotein E epsilon 4 genes in early-onset coronary artery disease. Clin Biochem 35(3):205–209
8. Hashim Y, Shepherd D, Wiltshire S, Holman RR, Levy JC, Clark A, Cull CA (2001) Butyrylcholinesterase K variant on chromosome 3 q is associated with Type II diabetes in white caucasian subjects. Diabetologia 44:2227–2230
9. Kharrazi H, Vaisi Raygani A, Sabokroh AR, Pourmotabbed T (2006) Association between apolipoprotein E polymorphism and coronary artery disease in the Kermanshah population in Iran. Clin Biochem 39:613–616
10. Vaisi Raygani A, Rahimi Z, Nomani H, Tavilani H, Pourmotabbed T (2006) The presence of apolipoprotein å4 and å2 alleles augments the risk of coronary artery disease in type 2 diabetic patients. Clin Biochem 40:1150–1156
11. Vaisi Raygani A, Rahimi Z, Entezami H, Kharrazi H, Bahrhemand F, Tavilani H, Rezaei M, Kiani A, Nomanpour B, Tavilani H, Pourmotabbed T (2008) Butyrylcholinesterase K variants increase the risk of coronary artery disease in the population of western Iran. Scandin J Clin and Lab Invest 68(2): 123–129
12. Lepienski LM, Alcantara VM, Souza RL, Rea RR, Chautard-Freire-Maia EA (1993) Variant K of butyrylcholinesterase and types 1 and 2 of diabetes mellitus. Clin Sci Lond 85(1):77–81
13. Raygani AV, Zahrai M, Solt Anzadeh A, Doosti M, Javadi E, Pourmotabbed T (2004) Analysis of association between butyrylcholinesterase K variant and apolipoprotein E genotypes in alzheimer's disease. Neurosci Lett 371(2–3):142–146
14. WHO (1999) Study Group Report of a WHO consultation: part 1: diagnosis and classification of diabetes mellitus. World Health Organization, Geneva
15. Johan SWM, Weitzner G, Rozen R (1991) A rapid procedure for extracting genomic DNA from leukocytes. Nucleic Acids Res 19:408
16. Jenese FS, Nielsen LR, Schwartz M (1996) Detection of the plasma cholinesterase K variant by PCR using amplification–created restriction site. Hum Heard 46:26–31
17. Wenham PR, Price WH, Blandell G (1991) Apolipoprotein E genotyping by onstage PCR. Lancet 337:1158–1159
18. Humphries SE, Ridker PM, Talmud PJ (2004) Genetic testing for cardiovascular disease susceptibility: a useful clinical management tool or possible misinformation. Arterioscler Thromb Vasc Biol 24:628–636
19. Saito M, Eto M, Nitta H, Kanda Y, Shigeto M, Nakayama K, Tawaramoto K, Kawasaki F, Kamei S, Kohara K, Matsuda M, Matsuki M, Kaku K (2004) Effect of apolipoprotein E4 allele on

plasma LDL cholesterol response to diet therapy in type 2 diabetic patients. Diabetes Care 27(6):1276–1280
20. Lehmann DJ, Nagy Z, Litchfield S, Cortina Borja M, Smith DA (2000) Association of butyrylcholinesterase K variant with cholinesterasepositive neuritic plaques in the temporal cortex in late-onset alzheimer's disease. Hum Genet 106:447–452
21. Abbott CA, Mackness MI, Kumar S, Olukoga AO, Gordon C, Arrol S, Bhatnagar D, Boulton AJ, Durrington PN (1993) Relationship between butyrylcholiesterase activity, hypertriglyceridemia and insulin sensitivity in diabetes mellitus. Clin Sci 85:77–81
22. Duman BS, Ozturk M, Yilmazer S, Hatemi H (2004) Apolipoprotein E polymorphism in Turkish subjects with Type 2 diabetes mellitus: allele frequency and relation to serum lipid concentrations. Diabetes Nutr Metab 7(5):267–274
23. Isbir T, Yilmaz H, Agachan B, Karaali ZE (2003) Cholesterol ester transfer protein, apolipoprotein E and lipoprotein lipase genotypes in patients with coronary artery disease in the Turkish population. Clin Genet 64(3):228–234
24. Lepienski LM, Souza RL, Chautard-Freire-Maia EA, Alcantara VM, Rea RR (2006) Variant K of butyrylcholinesterase and risk of early-onset type 1 diabetes mellitus in Euro-Brazilians. Diabetes Obes Metab 8(6):709–711
25. Corbo RM, Vilardo T, Ruggeri M, Gemma AT, Scacchi R (1999) Apolipoprotein E genotype and plasma levels in coronary artery disease. a case control study in the Italian population. Clin Biochem 32(3):217–222
26. Guang-da X, Xiang-Jiu Y, Lin-Shuang Z, Zhi-Song C, Yu-Sheng H (2005) Apolipoprotein e4 allele and the risk of CAD death in type 2 diabetes mellitus with ischaemia electrocardiographic change. Diabetes Res Clin Pract 68(3):223–229
27. Johansen A, Nielsen EM, Andersen G, Hamid YH, Jensen DP, Glumer C, Drivsholm T, Borch-Johnsen K, Jorgensen T, Hansen T, Pedersen O (2004) Large-scale studies of the functional K variant of the butyrylcholinesterase gene in relation to Type 2 diabetes and insulin secretion. Diabetologia 47(8):1437–1441
28. Wilson PWF, Schaefer EJ, Larson MG, Ordovas JM (1996) Apolipoprotein E alleles and risk of coronary disease: a meta-analysis. Arterioscler Thromb Vasc Biol 16:1250–1255
29. Mero N, Malmstrom R, Steiner G, Taskinen MR, Syvanne M (2000) Postprandial metabolism of apolipoprotein B-48- and B-100-containing particles in type 2 diabetes mellitus: relations to angiographically verified severity of coronary artery disease. Atherosclerosis 150:67–177
30. Ou T, Yamakawa-Kobayashi K, Arimani T et al (1998) Methylenetetrahydrofolate reductase and apolipoprotein E polymorphisms are independent risk factors for coronary heart disease in Japanese: a case–control study. Atherosclerosis 137:23–28
31. Gensini GF, Comeglio M, Colella A (1998) Classical risk factors and emerging elements in the risk profile for coronary artery disease. Eur Heart J 19(A):53–61
32. Singh PP, Naz I, Gilmour A, Singh M, Mastana S (2006) Association of APOE (Hha1) and ACE (I/D) gene polymorphisms with type 2 diabetes mellitus in north west India. Diabetes Res Clin Pract 74:95–102
33. Powell DS, Maksoud H, Charge SB, Moffitt JH, Desai M, Da Silva Fihlo RL et al (2003) Apolipoprotein E genotype, islet amyloid deposition and severity of Type 2 diabetes. Diab Res Clin Pract 60:105–110
34. Souza DR, Nakachima L, Biagioni RB, Nakazone MA, Pinhel MA, Trindade DM, Mafra VT, Tacito LH, Martin JF, Pinheiro Junior S, Brandao AC (2007) Relevance of apolipoprotein E4 for the lipid profile of Brazilian patients with coronary artery disease. Braz J Med Biol Res 40(2):189–197
35. Altamirano CV, Bartels CF, Lockridge O (2000) The butyrylcholinesterase K-variant shows cellular protein turnover and quaternary interaction to the wild-type enzyme. J Neurochem 74:869–877
36. Thomas T, Thomas G, Mclendon C, Sutton T, Mullan M (1996) β-amyloid mediated vasoactivity, and vascular endothelial damage. Nature 380:168–171
37. Randell EW, Mathews MS, Zhang H, Seraj JS, Sun G (2005) Relationship between serum butyrylcholinesterase and the metabolic syndrome. Clin Biochem 38:799–805
38. Vaisi Raygani A, Tavilani H, Rahimi Z, Zahrai M, Sheikh N, Aminian M, Pourmotabbed T (2009) Serum butyrylcholinesterase activity and phenotype associations with lipid profile in stroke patients. Clin Biochem 42:210–214