Evaluating the association of common *UBE2Z* variants with coronary artery disease in an Iranian population

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Abstract

Coronary artery disease (CAD) is the leading cause of cardiovascular mortality worldwide. Genome-wide association studies have discovered several variants associated with CAD. Notably, a recent study has identified *UBE2Z* rs46522 at 17q21.32 as a CAD-susceptibility variant in Europeans. However, association of this locus with CAD in non-Europeans has not been investigated. Herein, we evaluated the contribution of rs46522 and a variant in high linkage disequilibrium in *UBE2Z* 3'-UTR (rs1057897) to the CAD susceptibility by performing association study in an Iranian population. This study recruited 300 angiographically-confirmed CAD patients and 300 asymptomatic controls. Genotypes were determined by TaqMan genotyping assay. Multivariate logistic regression analysis revealed that rs46522 was associated with the susceptibility to CAD assuming codominant [TT vs. CC: 2.68 (1.36-5.31), *P*: 1.1717e-2], dominant [CT+TT vs. CC: 1.74 (1.12-2.69), *P*: 1.2675e-2], recessive [TT vs. CC+CT: 2.12 (1.13-3.98), *P*: 1.9369e-2] and log-additive [1.61 (1.17-2.21), *P*: 2.967e-3] models. However, no association was observed for rs1057897 under any genetic models. In conclusion, we provide the first evidence for association of rs46522 with the susceptibility to CAD in an Iranian population and discussed about regulatory potential and functional role of the studied variants to provide clues for its association with CAD and promote further research.

Key words: Coronary artery disease, polymorphism, *UBE2Z*, Iranian population, association.

Introduction

Coronary artery disease (CAD), one of the leading causes of death and a major health concern, is a complex disease which is believed to be caused by genetic and environmental factors, and also interactions among these factors (1, 2). Despite some validated classical risk factors, it is estimated that about 40-60% of inter-individual variation in the CAD risk is attributable to genetic components (3, 4). The genetic contributors to CAD are not fully understood and the so-far identified CAD-associated variants can explain only a small portion of the disease heritability (5).

To date, genome wide association studies (GWASs) have successfully discovered a total of about 50 robust associations of single nucleotide polymorphisms (SNP) with CAD (6, 7). Notably, a recent study has performed large scale meta-analysis of 14 GWASs on CAD in individuals of European ancestry and identified a locus at 17q21.32 encompassing *UBE2Z*, *GIP*, *ATP5G1* and *SNF8* genes and confirmed the lead SNP as a novel variant conferring the CAD risk (8). The lead SNP at 17q21.32, rs46522, is located in the intronic region of *UBE2Z* (ubiquitin-conjugating enzyme E2Z). Disease-associated variants may function in an ethnicity-specific manner, and this merits the need for performing association studies in different populations to unequivocally confirm the results (9-11). However, to the best of our knowledge, no association study on a non-European population has focused on this variant thus far. Herein,

we selected rs46522 and also a variant in high linkage disequilibrium (LD) in *UBE2Z* 3'-UTR (rs1057897) and evaluated the contribution of these polymorphisms to the CAD susceptibility by performing an association study in an Iranian population. We also discussed about the regulatory potential and functional role of the studies variants to provide clues for its association with CAD and promote further research to elucidate the molecular mechanism underlying the disease susceptibility.

Materials and methods

The study population

The study population comprised 600 unrelated Iranian individuals including 300 CAD patients and 300 control subjects matched for age and sex (Table 1). CAD patients were recruited from individuals referred to hospital for symptoms of CAD and underwent coronary angiography. CAD was defined as angiographic evidence of more than 50% stenosis in at least one major epicardial coronary artery. Controls were asymptomatic individuals (≥ 45 years old) who referred for routine checkup with no personal or family history of CAD. Individuals with known congenital heart disease, cardiomyopathy, valvular disease, familial hypercholesterolemia, cancer, and renal, liver or thyroid disorders were excluded from both patients and controls. For each participant, demographic and clinical data including age, gender, hypertension, body mass index (BMI), fasting blood glucose (FBG) and lipid concentrations [i.e.

total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-c) and high density lipoprotein cholesterol (HDL-c)] were collected (Table 1). Two consecutive blood pressure readings were performed and the average values were considered as systolic/diastolic blood pressures. Hypertension was defined as systolic blood pressure of ≥140 mmHg or diastolic blood pressure of ≥90 mmHg and/or using anti-hypertensive drugs. Diabetes mellitus (DM) was diagnosed according to following criteria: FBG concentration of ≥7.0 mmol/L or consumption of hypoglycemic agents or insulin. Dyslipidemia was defined as TC level >6.2 mmol/L, TG level >1.7 mmol/L, HDL-c level <1.0 mmol/L for males or <1.3 mmol/L for females, LDL-c level ≥4.1 mg/dl or a combination of these abnormalities. Body mass index (BMI) was calculated as weight in kilograms divided by height in square meters and BMI categories were defined as BMI≥30 kg/m$_2$ or BMI<30 kg/m$_2$. Written informed consent was obtained from all participants. This study was approved by the ethical committee of Shahid Beheshti University of Medical Sciences.

DNA extraction and genotyping

High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany) was used to extract genomic DNA from peripheral whole blood. SNPs were genotyped using TaqMan Genotyping Assay (C____587742_10 for rs46522 and C___7454104_10 for rs1057897, Applied Biosystems, Foster City, CA, USA). A 10 µl of TaqMan assay reaction contained 5 µl of 2X TaqMan universal mastermix, 0.5 µl of 20X predesigned probe-primer mix, 2.5 µl nuclease-free water and 2µl genomic DNA. PCR reactions were performed according to manufacturer's instructions and all reactions, including no template control, were performed on a lightcycler 96 instrument (Roche, Germany). Genotypes were determined by allelic discrimination using lightcycler 96

software (version 1.1).

Statistical Analysis

Statistical Analysis were performed using R version 3.1.0 for windows (12). X^2 test or unpaired Student's t test was used to evaluate differences in demographic/clinical variables among cases and controls. SNPs were tested for significant departure from Hardy-Weinberg equilibrium among controls using a X^2 test. Association of the SNPs with CAD was evaluated using logistic regression analysis implemented in the association function of SNPassoc version 1.9-2 R package (13). Odds ratios (OR) and respective 95% confidence intervals (95% CI) were calculated considering codominant, dominant, recessive, overdominant and log-additive models. Multivariate logistic regression analysis was performed to adjust for risk factors including age, gender, hypertension, BMI category, DM status, and dyslipidemia. *P values* correspond to the likelihood ratio tests obtained by comparing the multivariate models with the null model. A *P value* <0.05 was considered statistically significant.

Results

Characteristics of the study population

Demographic and clinical characteristics of the study population were reported in Table 1. Patients and controls were age (60.53 vs. 61.00 years, P>0.05) and sex (56.33% male in both groups) matched. Patients had higher levels of systolic (126.80 vs. 121.51 mmHg, *P*: 1.377e-03) and diastolic (79.64 vs. 75.83 mmHg, *P*: 1.607e-04) blood pressures, FBG (7.11 vs. 5.31 mM, *P*: 1.296e-15), TG (1.67 vs. 1.33 mM, *P*: 1.084e-08), and LDL-c (3.03 vs. 2.71 mM, *P*: 3.349e-02) in comparison to controls. However, the proportion of individuals with hypertension was not significantly different between CADs and controls (35.00% vs. 27.33%, *P*: 5.249e-02).

Table 1. Characteristics of the study population.

Characteristics	CAD patients (n = 300)	Controls (n = 300)	*P* [3]
Age (years) [1]	60.53 ± 10.1 [2]	61.00 ± 8.1	5.297e-01
Gender, male (%)	56.33	56.33	1
Hypertension (%)	35.00	27.33	5.249e-02
SBP (mmHg)	126.80 ± 22.50	121.51 ± 17.44	**1.377e-03** [4]
DBP (mmHg)	79.64 ± 12.46	75.83 ± 12.07	**1.607e-04**
BMI (kg/m²)	26.94 ± 5.46	24.53 ± 4.03	**1.411e-09**
Diabetes (%)	33.00	7.66	**2.794e-14**
FBG (mM)	7.11 ± 3.36	5.31 ± 1.64	**1.296e-15**
Dyslipidemia (%)	62.33	18.33	**< 2.2e-16**
TC (mM)	4.89 ± 2.60	4.65 ± 0.71	0.1239
TG (mM)	1.67 ± 0.84	1.33 ± 0.53	**1.084e-08**
HDL-c (mM)	1.09 ± 0.35	1.32 ± 0.29	**< 2.2e-16**
LDL-c (mM)	3.03 ± 2.52	2.71 ± 0.63	**3.349e-02**

SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; FBG, Fasting blood Glucose; TC, Total Cholesterol; TG, Triglyceride; HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol;

[1] Age refers to age at diagnosis for the patients and age at the time of enrolment for the controls.

[2] Quantitative and qualitative variables are shown as mean +/- standard deviation and percent (%) respectively.

[3] *P values* from X^2 test or unpaired Student's t-test for comparison of, respectively, categorical or continuous variables between cases and controls.

[4] *P values* less than 0.05 are shown in bold face.

Table 2. Association of *UBE2Z* rs46522 and rs1057897 with the CAD risk under different genetic models.

Model	Genotype	CADs No. (%)	Controls No. (%)	OR[1] (95% CI)	*P[1]*
rs46522					
Codominant	CC	101 (33.7)	134 (44.7)	1.00	1.1717e-2
	CT	154 (51.3)	136 (45.3)	1.54 (0.97-2.45)	
	TT	45 (15.0)	30 (10.0)	2.68 (1.36-5.31)	
Dominant	CC	101 (33.7)	134 (44.7)	1.00	1.2675e-2
	CT+TT	199 (66.3)	166 (55.3)	1.74 (1.12 2.69)	
Recessive	CC+CT	255 (85.0)	270 (90.0)	1.00	1.9369e-2
	TT	45 (15.0)	30 (10.0)	2.12 (1.13 3.98)	
Overdominant	CC+TT	146 (48.7)	164 (54.7)	1.00	3.8241e-1
	CT	154 (51.3)	136 (45.3)	1.21 (0.79 1.85)	
log-Additive	-	-	-	1.61 (1.17-2.21)	2.967e-3
rs1057897					
Codominant	TT	74 (24.7)	80 (26.7)	1.00	9.294e-1
	GT	162 (54.0)	156 (52.0)	1.10 (0.65-1.87)	
	GG	64 (21.3)	64 (21.3)	1.10 (0.58-2.07)	
Dominant	TT	74 (24.7)	80 (26.7)	1.00	7.021e-1
	GT+GG	226 (75.3)	220 (73.3)	1.10 (0.67-1.82)	
Recessive	TT+GG	236 (78.7)	236 (78.7)	1.00	9.176e-1
	GG	64 (21.3)	64 (21.3)	1.03 (0.61-1.74)	
Overdominant	TT+GG	138 (46.0)	144 (48.0)	1.00	8.056e-1
	GT	162 (54.0)	156 (52.0)	1.06 (0.68-1.63)	
log-Additive	-	-	-	1.05 (0.77-1.44)	7.604e-1

[1] Odds ratios (ORs) and *P values (P)* were adjusted for age, gender, hypertension, BMI, DM and dyslipidemia

CAD patients were more diabetic (33.00% vs. 7.66%, *P*: 2.794e-14) and dyslipidemic (62.33% vs. 18.33%, *P*< 2.2e-16) than the controls. In addition, the mean of BMI was significantly higher in patients (26.94 vs. 24.53 kg/m$_2$, *P*: 1.411e-09). In contrast, the mean of HDL-c level was significantly higher in controls (1.32 vs. 1.09 mM, *P*< 2.2e-16). The mean of TC level was higher in patients than controls but the difference was not statistically significant (4.89 vs. 4.65 mM, *P*: 0.1239).

Association of *UBE2Z* rs46522 and rs1057897 with CAD

The genotype frequencies of rs46522 and rs1057897 were not significantly deviated from Hardy-Weinberg equilibrium among controls (rs46522 *P*: 0.7941, rs1057897 *P*: 0.4889). Using multivariate logistic regression analysis, we fitted codominant, dominant, recessive, overdominant and log-additive genetic models and calculated the effect of SNPs as ORs and corresponding 95% CIs adjusted for risk factors. The results revealed that rs46522 is associated with the increased susceptibility to CAD under different genetic models (Table 2). Fitting codominant model, we found that subjects carrying TT genotype have increased risk of CAD in comparison to subjects with CC genotype [adjusted OR (95% CI) of 2.68 (1.36-5.31) for TT vs. CC, *P* =1.1717e-2]. When CT and TT genotypes were grouped and compared to CC genotype, higher risk for CAD was observed under dominant model [adjusted OR (95% CI) of 1.74 (1.12-2.69) for CT+TT vs. CC, *P* = 1.2675e-2]. This SNP was also associated with CAD under recessive model indicating that individuals carrying TT genotype have increased risk of CAD in comparison to

the combined CC+CT carriers [adjusted OR (95% CI) of 2.12 (1.13-3.98) for TT vs. CC+CT, *P* = 1.9369e-2]. According to the lowest *P value*, the log-additive model best fitted to the data [adjusted OR (95% CI): 1.61 (1.17-2.21), *P* = 2.967e-3]. In addition, rs1057897 was not associated with CAD in any of the analyzed genetic models (Table 2).

Discussion

Understanding the genetic contributors to CAD is a preliminary step to obtain insight on the pathophysiology of the disease (14, 15). In recent years, GWASs have been successful in identifying a number of polymorphisms that influence the susceptibility to CAD (3, 11, 14, 16). However, many of these studies have been conducted on populations of European descent (7, 17) and there are no sufficient data on other populations including the studied population. Here, we evaluated the association of *UBE2Z* rs46522 and rs1057897 at 17q21.32 locus with the susceptibility to CAD in an Iranian population. We found a positive association between rs46522 and CAD in the same direction as the original study (8), suggesting that this locus may contribute to the CAD risk not only in Europeans but also in the studied population. To the best of our knowledge, no other association study has evaluated the association of this variant with CAD thus far. However, a study has found no association between *UBE2Z* rs46522 and coronary stenosis index and incidence of pathological myocardial infraction in a cohort of elderly Japanese patients with atherosclerosis of coronary arteries (18).

GWAS associations are enriched for regulatory va-

riants (19), and as such, much effort is currently being directed toward integrating disease associations with increasing knowledge on regulatory elements especially ENCODE projects data. Annotating rs46522 with ENCODE data using RegulomeDB (Version 1.1, available at http://www.regulomedb.org) (20) revealed that, although located in the intronic region, this SNP has RegulomeDB score of 1f, representing a measure of the regulatory potential of the locus and indicating that it is linked to gene expression and likely to affect binding of protein(s). It overlaps transcription factor ChIP-seqs of NFKB1, NFYB, RUNX3 and RELA in GM12878 cell lines (20). Moreover, *UBE2Z* rs46522 is resided in an active regulatory region characterized by H3K27Ac mark and enhancer chromatin state in many cells (20). It has been shown that the risk allele (T) is associated with the higher expression levels of *SNF8* in endothelial cells (21), and with lower expression levels of *UBE2Z* in blood (20). In monocytes, however, this SNP modulates expression of *UBE2Z* and *ATP5G1* and has no effect on *SNF8* (22). These tissue-specific effects of rs46522 on regional gene expression have shed some lights on the functional role of this SNP and also suggest that mechanism of CAD susceptibility may differ in endothelial cells and monocytes. The precise underlying molecular mechanism and the possible role of *UBE2Z*, *ATP5G1* or *SNF8* in CAD susceptibility is not yet clear. However, apoptosis may be a probable link between the function of *UBE2Z* and developing CAD. The gene encodes an enzyme ubiquitinating proteins involved in signaling pathways and apoptosis (Provided by RefSeq, Accession: NM_023079.4). During atherosclerosis development, apoptosis occurs in endothelial cells, macrophages and vascular smooth muscle cells (VSMCs) and seems to play a dual role in atherogenesis (23-25). Increasing apoptosis in endothelial cells may initiate atherosclerosis (26, 27). In VSMC and macrophages, apoptosis may delay the atherosclerotic process or cause plaque rupture and thrombosis depending on the stage of the plaque (25).

Since the GWAS identified variants are not necessarily disease causative and usually point to large LD blocks with many theoretical candidate variants (28), identifying casual variants is a major challenge. We noticed that rs46522, although seems to be a regulatory variant, is not the only potentially functional SNP at 17q21.32 locus and therefore may not necessarily be the causative variant. *UBE2Z* rs1057897 overlaps a miR-196a binding site sequence supported by CLASH (29) (sequence ID: A12-2097909_1) and is in high LD with rs46522 in European super-population of 1000 Genomes Project (r^2: 0.99) (30). However, no association was observed for rs1057897 implying that this SNP may not be the disease causative variant of 17q21.32 locus. There are numerous other functional variants in LD block of 17q21.32 that are theoretically candidate disease causative variants. Therefore, a positive association with CAD for the lead SNP of this locus in Iranian population does not necessarily point to disease-causative variant, but merits the need for more studies to fine-map the locus and functionally analyze the relevance of candidate variants to CAD.

In conclusion, we provide the first evidence for association of *UBE2Z* rs46522 with the susceptibility to CAD in an Iranian population. Future studies may benefit from analyzing more SNPs at this locus to pave the way for elucidating causality and shed more lights on the molecular mechanism of this association. Genotyping the locus in different ethnicities could substantially help in identifying causative variant. In addition, as Iranian population itself are consisted of different ethnicities, it would be interesting to investigate this locus in different ethnicities of the population and assess the effects of ethnic-specific allele or genotype frequencies on the susceptibility to CAD.

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