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Association of heat shock protein70-2 (*HSP70-2*) gene polymorphism with coronary artery disease in an Iranian population

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ABSTRACT

Background: Coronary artery disease (CAD) is an inflammatory process and a major cause of mortality and morbidity. The (heat shock protein70-2) *HSP70-2* gene is reported to be associated with coronary artery disease possibly by affecting the regulation of pro-inflammatory cytokines such as TNF-α. The association between CAD and the *HSP70-2* gene +*1267A>G* polymorphism has been studied in some populations but there are no data about this association in the Iranian population.
Aim: We have investigated the association between the *HSP70-2* gene +*1267A>G* polymorphism and angiographically defined CAD within an Iranian population.
Methods: We determined the presence of the *HSP70-2* gene +*1267A>G* polymorphism in 628 patients with CAD and 307 healthy individuals using PCR-RFLP. Of the patients, 433 (68%) had >50% stenosis (CAD+) and the remaining 195 patients had <50% stenosis (CAD−), based on coronary angiography. Angiogram positive patients were subdivided into three groups: those with single (n = 113), double (n = 134), and triple vessels (n = 186) disease.
Results: A significant higher frequency of AG + GG genotypes (G allele carriers) was observed in angiogram positive and angiogram negative groups compared to controls in a dominant analysis model of the *HSP70-2* gene +*1267A>G* position (51.2 vs. 43.2, P = 0.002, OR = 1.37) (51.0 vs. 43.2, P = 0.01, OR = 1.37). The allele frequency of the *HSP70-2 G* was also significantly higher in angiogram positive and angiogram negative groups compared to the control group (51.2 vs. 43.2, P = 0.002, OR = 1.37) (51.0 vs. 43.2, P = 0.01, OR = 1.37).
Conclusion: These results suggest that *HSP70-2* +*1267* polymorphism may influence the risk of CAD in Iranian population, however further studies are needed to clarify the role of other *HSP70-2* gene polymorphisms in the pathogenesis of the CAD.

1. Introduction

Coronary artery disease (CAD) is the most common form of cardiovascular disease and one of the major causes of mortality and morbidity (Go et al., 2013; Lozano, 2013). CAD it is likely to remain as the most important cause of mortality in the world until 2020 (Hatmi et al., 2007; Murray and Lopez, 1997). The prevalence of CAD and risk factors for CAD is high in Iran (Ebrahimi et al., 2011). Therefore identifying those individuals at high risk for coronary diseases is important. The risk of CAD can be partially evaluated through the ascertainment of the risk factor profile, that includes family history, hyperlipidemia, excessive weight, hypertension and smoking (Ebrahimi et al., 2011). Among

Abbreviations: CAD, coronary artery disease; *HSP70-2*, heat shock protein70-2; TNF-α, tumor necrosis factor-α; RFLP, restriction fragment length polymorphism; OR, odd ratio; P, p value; HSP, heat shock proteins; MHC, major histocompatibility class; BMI, body mass index; FBG, fasting blood glucose; dNTPs, deoxynucleotide triphosphate; SPSS, statistical package for social science; WC, waist circumference; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HC, hip circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; MI, myocardial infarction; MUMS, Mashhad University of Medical Sciences; SVD, single vascular disease; 2VD, two vascular disease; 3VD, three vascular disease; CI, confidence interval; MetS, metabolic syndrome.

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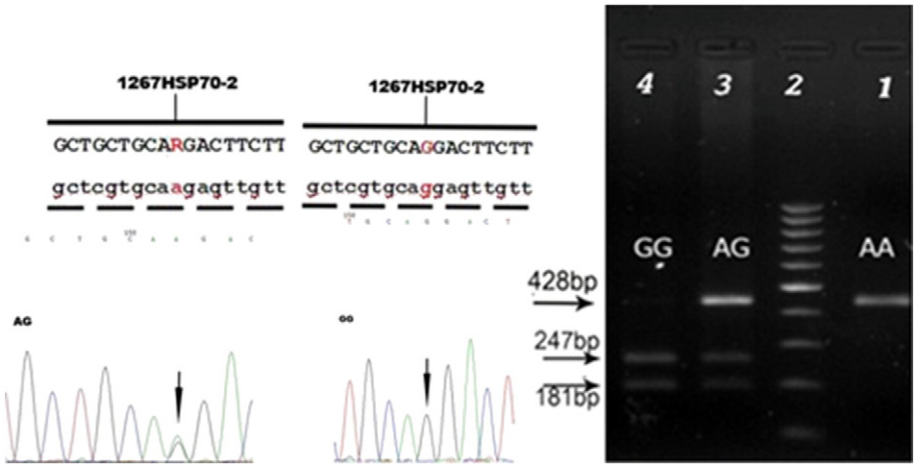

Fig. 1. PCR-RFLP and sequence of the amplified segment in *HSP70-2* gene +*1267A>G*. The genotype was labeled on corresponding sequences, and the sites which were marked with black arrows were the SNP of *HSP70-2* gene electrophoresed on 1.5% agarose, stained with ethidium bromide. Lane 2, 100 bp DNA ladder. Lane 3, heterozygote, Lane 4 homozygote GG and Lane 1 homozygote AA for *HSP70-2* genotype.

these, family history is an important factor and genetics may play a substantial role in the development of CAD. Study of genetic variants in human and animal models has so far shown the role of more than 100 genes involved in the development of atherosclerotic plaques (Lusis et al., 2004). *HSP70-2* is one of the strong candidate genes for the evaluation of coronary artery disease risk.

HSP70-2 gene is a member of multigene *HSP70* family. Heat shock proteins (HSPs) are expressed in almost all cells and organisms from bacteria to humans, in response to a variety of different stress stimuli including heat, cold, heavy metals, inflammatory cytokines, oxidized LDL and hypoxia. The expression of these proteins might be constitutive or inducible (Kiang and Tsokos, 1998; Whitley et al., 1999). In humans, three genes encode members of the *HSP70* class including *HSP70-1* (*HSPA1A*), *HSP70-2* (*HSPA1B*) and *HSP70-hom* (*HSPA1L*) (Milner and Campbell, 1990; Sargent et al., 1989). All three heat shock protein70 (*HSP70*) genes are located within the MHC class III region (6p21.3) (Milner and Campbell, 1992). Antibody titers related to HSP70 has been shown to be associated with coronary risk factors, increased risk and severity of cardiovascular disease (Ghayour-Mobarhan et al., 2008). HSP70 expression in human endothelial and smooth muscle cells has been documented in response to the oxidized LDL in vitro (Zhu et al., 1994, 1995). Overexpression of HSP70 in advanced atherosclerotic lesions occurs in several cell types including macrophages, dendritic cells and smooth muscle cells, which leads to expression of pro-inflammatory cytokines from macrophages (Bobryshev and Lord, 2002). Expression of cytokines such as TNF-α from atheromas may stimulate the innate immune response. Induction of HSP70 prevents NF- B activation and leads to reduction of inflammatory cytokine activity. This pathway partly reflects the anti-inflammatory activity of HSP70 (Shimizu et al., 2002). High levels of HSP70 expression lead to cardiac cell protection from stressful damage by binding to denatured or inappropriately folded proteins (Snoeckx et al., 2001; Xu, 2002). Increased serum levels of HSP70 are associated with reduced atherosclerotic intima thickness and risk of coronary artery disease (Neschis et al., 1998; Zhu et al., 2003).

Previous studies suggested that the *HSPA1B +1267* allele G was associated with coronary artery disease (Giacconi et al., 2006). Another study suggests that *HSP70-2* gene +*1267A>G* polymorphism is associated with diabetic nephropathy (Buraczynska et al., 2009).

Table 1
Characteristics of Angio+, Angio− and control subjects.

Characteristics	Control (n = 307)	Angio+ (n = 433)	Angio− (n = 195)	Comparison between the groups		
				P1	P2	P3
Age, year	52.2 ± 8.6	58.7 ± 10	53.2 ± 12	<0.001	0.50	<0.001
Gender, No. (%) Male	232 (77.9)	271 (63.0)	66 (34.2)	<0.001	<0.001	<0.001
BMI (kg/m^2)	28.8 ± 4.6	26.6 ± 4.7	26.7 ± 5.5	<0.001	<0.001	0.90
Weight (kg)	73.8 ± 12.1	69.7 ± 14.4	67.9 ± 14.5	<0.001	<0.001	0.20
WC (cm)	97.9 ± 11.8	92.2 ± 13.2	90.4 ± 13.9	<0.001	<0.001	0.20
Height (cm)	160 (14)	162 (15)	158 (12)	0.08	0.50	0.01
TC (mg/dl)	193 (64)	165 (85)	159 (61)	<0.001	<0.001	0.30
TG (mg/dl)	122 (71)	127 (92)	114 (75)	0.05	0.20	0.006
HDL (mg/dl)	43 (11)	39 (15)	43 (17)	0.002	0.70	0.006
LDL (mg/dl)	119 (38)	96 (50)	86 (40)	<0.001	<0.001	0.10
FBG (mg/dl)	82 (20)	103 (53)	96 (24)	<0.001	<0.001	<0.001
HC (cm)	104 (12)	95 (12)	97 (15)	<0.001	<0.001	0.20
Waist/hip ratio	0.9 (0.1)	0.9 (0.09)	0.9 (0.1)	<0.001	0.40	<0.001
SBP (mm Hg)	122 (28)	140 (40)	130 (40)	<0.001	<0.001	0.20
DBP (mm Hg)	80 (9)	80 (17)	70 (10)	0.001	<0.001	0.03
MI, No. (%)	–	121 (28.9)	18 (9.5)	–	–	–
Hypertension, No. (%)	52 (19.0)	208 (49.3)	80 (41.7)	0.001	<0.001	0.07
Diabetes, No. (%)	20 (9.2)	125 (29.6)	33 (17.2)	<0.001	0.01	<0.001

Values are expressed as mean ± SD, median and interquartile range for normally and non-normally distributed variables, respectively. Comparisons were performed by one-way ANOVA and Kruskal–Wallis test. Also the post hoc test and Mann–Whitney *U* test were used for comparison between groups. X^2 of test results for categorical data.
BMI: body mass index; WC: waist circumference, TC: total cholesterol; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; FBG: fasting blood glucose; HC: hip circumference, SBP: systolic blood pressure; DBP: diastolic blood pressure. MI: myocardial infarction.
P1: comparison between groups of Angio+ and control, P2: comparison between groups of Angio− and control, P3: comparison between groups of Angio+ and Angio−.