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The interaction between a HSP-70 gene variant with dietary calories in determining serum markers of inflammation and cardiovascular risk

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Abstract:

Background: The high prevalence of cardiovascular disease (CVD) globally is attributable to an interaction between environmental and genetic factors. Gene × diet interaction studies aim to explore how a modifiable factor interacts with genetic predispositions. Here we have explored the interaction of a heat shock protein (*HSP70*) gene polymorphism (*+1267A>G*) with dietary intake and their possible association with serum C-reactive protein (CRP), an inflammatory marker, that is a major component of CVD risk.

Methods: *HSP70* genotype was determined using a TaqMan real time PCR based method. Genetic variation of the *HSP70* gene *+1267A>G* locus. Dietary intake was assessed using a dietary questionnaire. Serum high sensitivity (Hs) CRP and other cardiovascular risk factors were assessed by routine methods. This included coronary angioplasty to determine the presence of coronary artery stenosis.

***Results*:** There were significant differences between serum lipid profile and Hs-CRP across the genotypes for Hsp70. The carriers of G allele had higher serum hs-CRP concentrations, compared with the AA homozygotes, with the wild genotype. Interaction analysis showed the association was modulated by total energy intake; the interaction of high energy intake with GG genotype: RERI= 0.77, AP= 0.26, S=1.6.

Conclusion: We have found a significant association between the *+1267A>G* variant of the *HSP70* gene with cardiovascular risk factors and serum hs-CRP concentrations. It is possible that a low energy diet could ameliorate the unfavorable effects of G allele of *HSP70*.

Key words: Chronic disease, Cardiovascular disease, Inflammation, HSP70, Gene/diet interaction

Introduction

The heat shock proteins (HSPs) are a family of molecules that are released by cells in response to cell stress, that include: free radicals, sheer stress and toxins (1). Hsp70 has been shown to be highly expressed in different physiological and environmental stress, and protects cell and tissues (2). There are, three human genes encoding members of HSP70 class including HSP70-1/2 and HSP70-hom (3) and this locus appears to be involved in determining CAD risk(4-6).

We have previously reported an association between the *HSP70-2* gene *+1267A>G* polymorphism with cardiovascular disease (7) and also with obesity as an important risk factor for cardiovascular disease (8). There have been other reports of a relationship between the 1267A>G Hsp70 variant with CVD risk factors (9, 10). These studies have evaluated the role of HSP70 gene variants on serum inflammatory biomarkers such as Hs-CRP (5, 10). Serum CRP, is an well established inflammatory marker that is related with an increased CVD risk (11). It is also possible that genetic predisposition and dietary factors interact to play an important role in determining CVD (12).

We have therefore evaluated the association of a genetic variant, *HSP70-2* gene *+1267A>G* with the presence of CAD , comparing CAD patients with 740 healthy individuals recruited from the Mashhad Stroke and Heart Atherosclerotic Disorders (MASHAD) cohort. Moreover, to test our hypothesis that this *HSP70-2 +1267A>G* may be associated with CAD and CRP, we examined the interaction of this genetic variant with dietary calories on serum hsCRP.

Materials and methods

Study population

740 CAD patients undergoing coronary angiography with obstructive coronary artery disease (43% male, aged 49±8 years) were recruited from Ghaem Hospital. Written consent was obtained from all the participants. This research was approved by the Ethics Committee of the MUMS.

Anthropometric and biochemical determination

Anthropometric determinants, including height, body weight, waist and hip circumference were assessed (13). Biochemical parameters, including C-reactive protein (CRP), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG), fasting blood glucose (FBG) were evaluated as described previously (14).

Assessment of dietary intake

A 24-h recall questionnaire was used to assess daily intake, and this was analyzed as described previously (15) using Dietplan6 software (Forest field Software Ltd., UK).

Genotyping

The DNA was extracted using QIAamp- DNA Mini-Kit (Qiagen, San Diego, CA) based on the manufacturer's instructions. To assess the concentration and purity of DNA, NanoDrop-1000-Detector (NanoDrop-Technologies, Wilmington, USA) was used. Genotyping was carried out using Taqman-probes-based method with ~20 ng of DNA in TaqMan-Universal Master Mix with specific primers and probes (Applied Biosystems Foster City, CA). The ABIPRISM-7500 device equipped with the SDS software (version-2.0) was applied for determination of the allelic content of the samples (16, 17).

Statistical analyses

Data analysis was undertaken using SPSS 22 software (SPSS Inc, Chicago, IL). The Kolmogorov–Smirnov test was utilized for evaluation of the normality of the variables within groups. Categorical data were assessed using a χ^2 test. The statistical difference for genotype distribution and allele frequencies among groups was assessed by the χ^2 analysis and Hardy-Weinberg equilibrium using a

Pearson chi-square test. Differences between groups were investigated by ANOVA and t tests, or Kruskal–Wallis and Mann–Wilcoxon U tests. Residual models were used to adjust dietary intake variables for energy intake (18).We also examined multiplicative and additive interactions between the SNP and environmental factors (i.e. dietary intake) on the risk of high serum Hs-CRP concentrations. Multiplicative interaction was analyzed using the multiplicative term in a multiple logistic regression model. To examine the modifying influence of the studied variant on dietary intake in association with Hs-CRP concentration, we used multivariate logistic regression models. Potential confounders were adjusted for; these included: age, sex, physical activity, smocking, energy intake, body mass index and inflammatory markers, and white blood cell count (WBCC). The main indices of biological interaction: AP, the attributable proportion due to interaction; RERI, the relative excess risk due to interaction; and S, the synergy index (19) were computed and calculated using the method of Andersson et al.(20). All the analyses were done using SPSS 20 (SPSS Inc., IL, USA) and a two-sided statistical significance was set at P value ≤ 0.05.

Results

Association of HSP70-2 gene +1267A>G genetic variant with general characteristics of population

The association of the HSP70-2 variant with demographic characteristics, fasted lipid profile, blood pressure, Inflammatory biomarkers and dietary Intake are shown in Table 1. The genotype distribution of the polymorphism was in HWE (*P>0.05*). The frequency of the risk-associated G allele was 47.53 %, and the frequencies of AA, AG, and GG genotypes were respectively 18.7%, 67.6% and 13.7%, in the total sample. There was no significant differences between different genotypes with respect to: weight, waist circumference and physical activity level (p value>0.05). However the sex distribution and age were significantly different. There were no significant differences between serum TG in different genotypes in different models (*p value>0.05*). However serum cholesterol [*p value (in recessive model) = 0.05*), LDL [*p value(in codominant model) =*

<0.001; p value (in recessive model) <0.001)], HDL [*p value (in codominant model) = <0.001; p value (in recessive model) <0.001*)] and FBS [*p value (in additive model) = 0.05*) were statistically different in subjects with different genotypes. Subjects who were homozygous for the G variant (GG genotype) also had higher DBP than CC genotypes however there was no significant difference between genotypes and SBP. Individuals who carried the GG genotype had higher serum HSP70 [*p value (in recessive model) = 0.05*] with higher serum Hs-CRP [*p value (in recessive model) = 0.04*], however there was no association with WBCC across different genotypes.

Association of HSP70-2 gene +1267A>G genetic variant with serum Hs-CRP

As shown in Table 2, after adjustment for the potential confounders, the HSP70-2 gene +1267A/G variant was associated with an increased likelihood of a high serum Hs-CRP concentration. We found a gene-disease association with an OR of 1.24 with an accuracy of >80% under the dominant genetic model with CRP. Therefore, subjects with GG genotype had a higher likelihood of a high CRP level (in adjusted dominant model, OR= 1.1, 95%CI (0.7-1.8), than those with the A allele.

Interaction of life style with HSP70-2 gene +1267A> on and energy intake with serum Hs- CRP under dominant genetic model

We also studied the nutrient intake across this genetic variant to determine the modulatory influence of diet on the outcome. We observed no statistically significant difference in dietary habit between groups in relation to macronutrients and energy consumption (Table 1). Interaction between gene × diet intakes was conducted on multiplicative and biological interaction analysis (Table 3 and figure 1). There was no statistically significant multiplicative interaction (*p value=0.5*). However, results suggested an additive interaction between this variant with energy intake. These data showed that subjects with a GG genotype and high energy intake had an increased likelihood of a high serum Hs-CRP (*OR=3, 95%CI 1.2-7, p=0.01*) compared with the reference group, defined as subjects with low risk; low energy intake and carrying A protective allele. The influence of both exposures together

exceeds the effect of the two exposures separately and there was a positive and significant additive interaction. The parameters of additive interaction were also reported: *RERI= 0.77, 95%CI: (-1.2-2.8); AP=0.26, 95%CI: (-0.4-0.9)* and *SI=1.6, 95%CI: (0.3-8.6).* A super additive interaction or positive interaction is said to exist when; RERI >0, AP >0, or S >1 (21).

Discussion

We have demonstrated that CAD patients with GG genotype and a high energy intake had an increased likelihood of a high serum Hs-CRP (*OR=3, 95%CI 1.2-7, p=0.01*), compared to the reference group that was defined based on subjects with less risk; low energy intake and carrying A protective allele. Moreover, we found that this effect was more pronounced when both factors were present, and there was a positive and significant additive interaction. This is consistent with previous observations on the role of this genetic marker with CAD, although our data is the first study showing a novel role of this genetic variant in interaction with life style as a susceptible predisposition marker in predicting the risk of CVD. Moreover, our data showed that subjects who carried a G allele had higher serum cholesterol, LDL, TG/HDL. Although atherogenesis is a complex disorder, it is suggested that abnormalities in lipoprotein metabolism are one of the central factors. Lipid concentrations are important measures of cardiovascular disease risk, however several lipoprotein ratios or "atherogenic indices" have been defined to improve the predictive capability of the lipid profile. Dobiasova et al. found that Log (TG/HDL-C) had an association with the LDL-C particle diameter , and it has been proposed as an atherogenic index of plasma (AIP) which is indirect measure of the diameter of LDL-C particle(22). The current study also showed that Hsp70-2 polymorphism may affect hsCRP levels as a marker of inflammation. This supports our previous findings in which we demonstrated a higher prevalence of CAD and obesity as chronic inflammatory disease in subjects with G allele of HSP70-2 gene +1267ANG variant. Similarly, Hrira et.al have

reported a positive correlation between P2–Hsp70-2 homozygous, higher level of hsCRP, LDL cholesterol, and the presence of CVD (5). The study of Nakhjavani et.al in diabetic patients showed a direct correlation between asymmetric dimethylarginine (ADMA) and serum HSP70 with high serum hs-CRP in type 2 diabetes suggested that both ADMA and HSP70 play an inhibitory role on nitric oxide synthase (NOS) in inflammatory conditions (23). Consistent with these observations, Giacconi et.al, showed the association of Hsp70 1267 A/G SNP with pro-inflammatory cytokine production in healthy elderly and proposed this biomarker as a possible determinant of individual susceptibility to chronic diseases (24). Emerging evidence has shown that hsCRP and Hsp70 are biomarkers of increased risk of several chronic diseases; however, little is known about the function of these two biomarkers in combination and with the other inflammatory markers.(25-27).

(9).

We did not observe a relationship between the HSP-70 polymorphism and serum HSP-70 level in our study. This is consistent with the study of Contreras-Sesvold et.al, who also reported no significant differences for HSP70 concentrations across genotypes (28). Similarly, another study also examined the association of heat shock protein with different related polymorphisms (*HSPA1B* (2074G/C and 1267A/G). They showed no differences in the serum HSP70 related to HSP70 gene polymorphism for HSPA1B gene locus 1267A/G (29). However, Gombos et.al has reported an interaction between the HspA1B +1267 allele G and Hsp70 concentrations (9). These contradictory data can be explained at least in part by sample size, different method of genotyping or expression level analysis, ethnicity, etc.

Inflammation is an important risk factor of CVD (30), and the onset and progress of an atherosclerotic lesion. The inflammatory response may be modulated by changes in dietary intake through both pro- and anti inflammatory pathways (31) . It has been shown that genetic alterations can influence the

modulatory effect of diet on inflammation status. Therefore, we have investigated whether the magnitude of association between this variant was modulated by diet intake. Our results show that GG carriers for the HSP70-2 gene +1267ANG polymorphism with a high consumption of energy have a higher serum Hs-CRP concentration compared with the control subjects. Adiponectin is a well-known anti-inflammatory biomarker due to its suppression of TNF-alpha and adhesion molecules. Martinez et.al, reported in men with C/C homozygous of -11377 C > G at *adipoQ* were less insulin resistant by following a diet rich of MUFA and carbohydrate in compared with the diet high in SFA(32-33).Another study by Song et.al demonstrated that the IL6R Asp358Ala (T/G) would interact with energy intake and obesity in Japanese population(34).

In conclusion, we found that a genetic variant at the HSP70-2 gene locus (the +1267ANG) appears to be a factor in the inter-individual differences in the inflammation response that is modulated by a high energy diets in CAD patients. Our results suggest that a energy dense diet may exaserbate the inflammation status in CAD patient who carries risk genotype. Further studies are required to identify individuals who may benefit from a more personalized approach to diet modification.

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Table 1. General characteristics of study population

Variable	Genotype			P-value in genetic models[#]	
				Additive	Recessive
	AA	AG	GG		
Demographic characteristics					
Age (y)	51.7±8.1	48.3±8	48.9±7.9	<0.001	<0.001
Weight (kg)	71.8±11	73.8±13	73.5±10.8	NS	NS
Height (m)	1.6±0.09	1.61±0.09	1.57±0.09	0.04	NS
WC (cm)	92.8±11	93.8±13	94.7±13.4	NS	NS
PAL	1.66±0.3	1.67±0.3	1.68±0.3	NS	NS
Lipid Profile/ Serum Glucose					
TG (mg/dl)	145±76	136±74	153±105	NS	NS
LDL (mg/dl)	115.3±38	96.7±35	101.5±41	<0.001	<0.001
Cholesterol (mg/dl)	195.4±41	191.5±37.8	200±38	NS	0.05
HDL (mg/dl)	43.7±10	49.6±11.6	49.4±11	<0.001	<0.001
Cholesterol/LDL	4.6±1	4±1	4.3±1.3	<0.001	0.001
TG/HDL	3.6±2.3	3±1.9	3.4±3	0.005	NS
FBS (mg/dl)	96±37.6	92.4±30	100.7±41	NS	NS
Blood pressure					
DBP (mmHg)	83±12	79.9±10	81.4±10	0.052	NS
SBP (mmHg)	127.8±21	123.9±20	126±20	NS	NS
Inflammatory biomarkers					
HSP70 (ng/ml)	4.3±6	5±8.6	4.2±7	NS	0.07
HS-CRP (mg/l)	5.3±11	6.6±12	7.3±11	0.05	0.04
WBC ($\times 10^9$/L)	6±1.6	6±1.5	5.8±1.3	NS	NS
Dietary Intake					
Energy (kcal)	1803±650	1731±624	1681±593	NS	NS
Fat (g)	73.6±23	75±17	75.7±17	NS	NS
Carbohydrate (g)	237±58	225.2±48	230.9±52	NS	NS
Protein (g)	66±18.6	72.7±27	65.5±18.5	NS	NS

[#] Additive genetic model (AA genotype vs. GG genotype); Recessive genetic model (AA genotype vs. GG/AG genotype).
Abbreviation: NS: Not Significant, PAL, physical activity level; WC, Waist circumference; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; LDL, Low density lipoprotein; HDL, high density lipoprotein; HSP, Heat shock protein; Hscrp, high sensitive CRP; WBC, white blood cell.

Table 2. Association of HSP70-2 gene +1267A>G variant with serum Hs-CRP in Iranian under different genetic models.

Risk allele	Genetic Model	Genotype	N (%)		Crude OR* (95% CI)	Adjusted OR* (95% CI)
			Case (n=342)	Control (n=343)		
G	**Co-dominant**	AA	52	65	1	
		AG	216	208	1.26 (0.8-1.9)	1.24 (0.8-1.9)
		GG	46	40	1.3 (0.7-2.3)	1.26 (0.7-2.2)
	Recessive				1.27 (0.8-1.9)	1.24 (0.86-1.9)
	Dominant				1.1 (0.7-1.7)	1.1 (0.7-1.8)
	Additive				1.1 (0.7-2.1)	1.4 (0.8-2.4)

*Adjusted for age, sex, BMI, smoking, WBC.

P value for dominant model=0.08

Abbreviations: Hs-CRP; high sensitive CRP.

Table 3. Interaction of HSP70-2 gene +1267A>G gene and energy intake with serum Hs- CRP under dominant genetic model (n=463).

	Genotype distribution				Interaction parameters			
	AA/AG		GG		Multiplicative interaction	Additive interaction measures		
Energy intake	OR (95%CI)	p value	OR (95%CI)	p value	P value	RERI	AP	SI
low	1		1.06 (0.4-2.5)	0.8	0.5	0.77(-1.2-2.8)	0.26(-0.4-0.9)	1.6(0.3-8.6)
high	2.1 (0.78-5.8)	0.1	3 (1.2-7)	0.01				

Adjusted for age, sex, BMI, smoking, WBC

Dietary energy intake: high and low were above and below the median in the control group.

Measures of biological interaction: RERI, the relative excess risk due to interaction; AP, the attributable proportion due to interaction; and S, the synergy index

Statistically significant with the 95% CI of RERI > 0, the 95% CI of AP > 0, or the 95% CI of S > 1, indicating positive additive interaction.