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The relationship between HSP60 gene polymorphisms and susceptibility to atherosclerosis

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Abstract. – OBJECTIVE: To explore the potential correlation between heat shock protein 60 (HSP60) gene polymorphisms and susceptibility to atherosclerosis.

PATIENTS AND METHODS: A total of 160 atherosclerosis patients treated in our hospital from February 2017 to February 2019 were randomly enrolled as case group, and 200 healthy adults receiving physical examination were selected as control group at the same period. Venous blood was drawn from all subjects to extract deoxyribonucleic acid (DNA). TaqMan probe technology was employed to genotype two loci rs2340690 and rs788016 of HSP60 gene in all 260 subjects. The correlations between HSP60 gene polymorphisms and the incidence rate and pathological grade of atherosclerosis were analyzed.

RESULTS: There were three genotypes (AA, AG, and GG) in HSP60 rs2340690 and three (GG, AG, and AA) in HSP60 rs788016. No significant differences in the frequency of each genotype were found between the two groups (p>0.05). HSP60 rs2340690 and HSP60 rs788016 had no significant associations with the incidence rate of atherosclerosis in the dominant, recessive, and additive genetic models. In the case of pathological grade IV, the proportion of atherosclerosis patients carrying GG genotype of HSP60 rs2340690 was higher than those carrying AA genotype and AG genotype of HSP60 rs2340690 (p<0.05). The probability in atherosclerosis patients carrying rs788016 A was higher than those carrying rs2340690 G (p<0.05). When atherosclerosis patients carried both genotype G of HSP60 rs2340690 and genotype A of HSP60 rs788016, the odds ratio (OR) was 1.721 (p=0.049).

CONCLUSIONS: The HSP60 gene polymorphisms are certainly correlated with the pathological grade and incidence rate of atherosclerosis.

Key Words:
Atherosclerosis, HSP60 gene, Gene polymorphism, Correlation.

Introduction

Atherosclerosis, a common cardiovascular disease, manifested as blood vessel wall sclerosis and millet congee-like necrosis due to thickened inner wall of blood vessels and narrowed lumen of blood vessels. The accumulation of lipids and complex carbohydrates on the inner wall of the artery, as well as increase of collagen fibers and smooth muscle cells, are the typical features of atherosclerosis[1,2]. Atherosclerosis has a great influence on important organs such as the heart and brain, resulting in myocardial infarction and cerebral infarction. Besides, it is reported that atherosclerosis in the kidneys can also lead to infarction[3,4]. Currently, there is no authoritative consensus since various influencing factors and complicated mechanisms are involved, including living habits and external environment ,as well as personal genetics[5,6]. Among them, the physiological imbalance and hormone parasecretion due to genetic changes are basic causes of the disease[7]. Atherosclerosis involves many genes[8,9], including MTHFD2, SLC2A1, ApoE, and KLF14. It is reported that heat shock protein 60 (HSP60) participates in the whole process of atherosclerosis, with relatively conservative and stable molecular structure, which is a potential marker for the early diagnosis of this disease[10]. However, reports on the effects of HSP60 gene polymorphisms on atherosclerosis are rare. Given this, this research

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Table I. General clinical data.

Parameter	Case group (n=160)	Control group (n=200)	χ^2/t	*p*
Gender				
Male	95 (59.4 %)	112 (56%)	0.237	0.626
Female	65 (40.6%)	88 (44%)		
Age (y)	49.21±14.32	49.57±15.73	0.225	0.823
Weight (kg)	73.86±20.21	73.72±20.44	0.065	0.948
Smoking addiction	55 (34.4)	76 (38)	0.281	0.596
Fondness of liquor	68 (42.5)	90 (45)	0.127	0.722

investigated the role of HSP60 gene polymorphisms in atherosclerosis on the basis of its important role.

Patients and Methods

General Data

A total of 160 atherosclerosis patients admitted to our hospital from February 2017 to February 2019 were enrolled as case group, including 87 patients with atherosclerosis in the heart, 26 in the brain, 35 in the neck, and 12 in the kidneys based on disease location. In terms of pathological grade[11], there were 45 cases of grade I atherosclerosis, 45 cases of grade II, 48 cases of grade III, and 22 cases of grade IV. Meanwhile, 200 healthy adults undergoing physical examination were selected as control group. All subjects and their families were informed of this study that was approved by the Ethics Committee and signed the informed consent. General data, including gender, weight, age, and living habits were comparable between the two groups (p>0.05; Table I).

Extraction of Genomic Deoxyribonucleic Acid (DNA)

Venous blood (5 mL) was drawn from all subjects to extract the genomic DNA therefrom using a genomic DNA extraction kit (Beijing Tiangen Biotechnology Co., Ltd., Beijing, China). After that, the concentration and purity of the DNA were detected using a spectrophotometer (NanoDrop), and the DNA was stored at –20°C.

Single Nucleotide Polymorphism (SNP) Genotyping Via Polymerase Chain Reaction (PCR)

Oligo 6.0 was employed to design the primer sequences of SNP loci and their TaqMan probe sequences (Table II), and the primers were synthesized by Thermo Fisher Scientific (Waltham, MA, USA). The pre-prepared 17.8 μL of TransStart Probe quantitative PCR (qPCR) SuperMix (Beijing TransGen Biotech Co., Ltd., Beijing, China) was added with 1 μL of DNA solution and 1.2 μL of prepared primer solution (containing 0.4 μL of forward primers, 0.4 μL of reverse primers and 0.4 μL of probe primers). After gentle shaking, the mixture was placed in a CFX96 fluorescence qPCR instrument (Bio-Rad, Hercules, CA, USA), with the reaction condition as follows: 94°C for 3 min, 94°C for 15 s and 60°C for 30 s, 40 cycles. For each sample, 3 duplicate wells were set, with diethyl pyrocarbonate (DEPC)-treated water as the negative control and the positive plasmid containing the sequences (Shanghai Sangon Biotech Co., Ltd., Shanghai, China) as positive control.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 19.0 (IBM, Armonk, NY, USA) was utilized for statistical analysis. The Chi-square test was employed to analyze the difference in the distribution of genotypes between case group and control group. The relationship between each genotype and risk of atherosclerosis was analyzed by Logistic regression analysis. p<0.05 indicated that the difference was statistically significant.

Table II. Primer sequences.

SNP	Primer sequence	Probe sequence
rs2340690	Forward: 5'-AGCTAGCACTATTCCCAATTG-3' Reverse: 5'-ACTTCTCATGTCCATTAGAT-3'	HEX: 5'-TGTTAGATCATAATACGTACT-3' FAM: 5'-TGTTAGGTCATAATACGTACT-3'
rs788016	Forward: 5'-TTGTCACTGCCTGCACTCCAG-3' Reverse: 5'-TGTGCCCATCAACAATTCTC-3'	HEX: 5'-GGAAAGTATTCTTACTGCCAGGC-3' FAM: 5'-GGAAAGTATTCTTACTGCCAGGC-3'

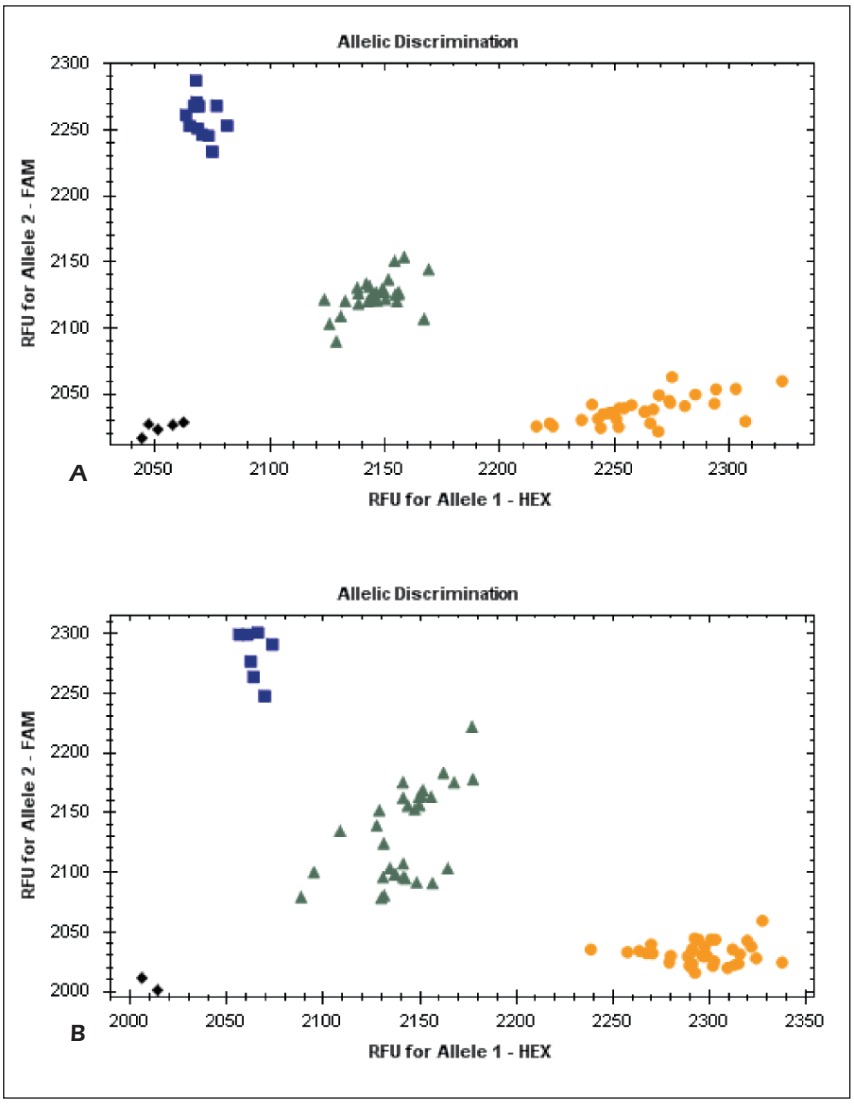

Figure 1. Genotyping results of rs2340690 and rs788016. A, FAM: GG, HEX: AA, and middle green: AG. B, FAM: AA, HEX: GG, middle green: AG.

Results

Genotypes of Rs2340690 and Rs788016 Polymorphism Loci and Their Distribution Frequency

Genotyping results on HSP60 of all subjects (Figure 1) were obtained. In all subjects, there were 3 genotypes in HSP60 rs2340690 (GG, AG, and AA) and HSP60 rs788016 (AA, AG, and GG), respectively. No significant differences were found in the genotype frequency between case group and control group ($p>0.05$; Table III), and the distribution met the Hardy-Weinberg equilibrium law ($p_{rs2340690}=0.45$, $p_{rs788016}=0.32$).

Risk of Atherosclerosis Analyzed Through Different Genetic Models of Rs2340690

The odds ratio (OR) was 1.285 ($p=0.381$) in dominant model (AG+GG/AA), 0.985 ($p=0.975$) in recessive model (GG/AA+AG), and 1.098 ($p=0.847$) in additive model (GG/AA), respectively (Table IV).

Risk of Atherosclerosis Analyzed Via Different Models of Rs788016

The OR was 1.128 ($p=0.671$), 2.284 ($p=0.072$), and 2.222 ($p=0.098$) in recessive model (AA/GG+AG), dominant model (AG+AA/ GG), and additive model (GG/AA), respectively, (Table V).

Table III. Frequency distribution of genotypes

Genotype	Control group		Case group		χ^2	*p*
	n	%	n	%		
rs2340690						
AA	121	60.5	87	54.38	0.766	0.381
AG	60	30	58	36.25	0.882	0.348
GG	19	9.5	15	9.37	0.001	0.975
A	302	75.5	232	72.5	0.234	0.629
G	98	24.5	88	27.5		
rs788016						
GG	96	48	72	45	0.181	0.671
AG	89	44.5	63	39.37	0.54	0.462
AA	15	7.5	25	15.63	3.231	0.072
G	281	70.25	207	64.69	0.704	0.401
A	119	29.75	113	35.31		

Relationship Between Rs2340690 and Atherosclerosis Grade

Among the 3 genotypes of rs2340690, the proportion of grade IV atherosclerosis patients carrying GG genotype was higher than those carrying AA genotype and AG genotype ($p<0.05$; Table VI).

Association Between Rs788016 and Atherosclerosis Grade

Among the three genotypes of rs788016, the number of grade IV atherosclerosis patients carrying AA genotype and AG genotype was greater than those carrying GG genotype ($p<0.05$; Table VII).

Correlation Between Combined Action of Rs2340690 and Rs788016 and Atherosclerosis Grade

For atherosclerosis patients carried both G genotype of rs2340690 and A genotype of rs788016, the OR was 1.721 ($p=0.049$; Table VIII).

Discussion

As a common cardiovascular disease, symptoms, and signs of atherosclerosis in the early stage are atypical and easily neglected. However, when the disease progresses to the middle and advanced stages, it will cause fatal cardiovascular and cere-

Table IV. Analysis on risk of atherosclerosis via different models of rs2340690.

Model	Genotype	Control group (n=200)	Case group (n=160)	OR [95% confidence interval (CI)]	*p*
Dominant model	AA	121 (60.5)	87 (54.38)	1	0.381
	AG+GG	79 (39.5)	73 (45.62)	1.285 (1.012-1.481)	
Recessive model	AA+AG	181 (90.5)	145 (90.6)	1	0.975
	GG	19 (9.5)	15 (9.4)	0.985 (0.814-1.412)	
Additive model	AA	121 (60.5)	87 (54.38)	1	0.847
	GG	19 (9.5)	15 (9.4)	1.098 (0.927-1.227)	

Table V. Risk of atherosclerosis in different models of rs788016.

Model	Genotype	Control group (n=200)	Case group (n=160)	OR (95% CI)	*p*
Dominant model	GG	96 (48)	72 (45)	1	0.671
	AG+AA	104 (52)	88 (55)	1.128 (0.932-1.414)	
Recessive model	GG+AG	185 (92.5)	135 (84.37)	1	0.072
	AA	15 (7.5)	25 (15.63)	2.284 (1.912-2.571)	
Additive model	GG	96 (48)	72 (45)	1	0.098
	AA	15 (7.5)	25 (15.63)	2.222 (1.912-2.685)	

Table VI. Relationship between rs2340690 and atherosclerosis grade.

Grade	n (160)	rs2340690				
		AA (n=87)	AG (n=58)	GG (n=15)	A (n=232)	G (n=88)
I	45	26 (29.89)	16 (27.59)	3 (20)	68 (29.31)	22 (25)
II	45	24 (27.59)	18 (31.03)	3 (20)	66 (28.45)	24 (27.27)
III	48	26 (29.89)	18 (31.03)	4 (26.67)	70 (30.17)	26 (29.55)
IV	22	11 (12.63)	6 (10.34)	5 (33.3)*	28 (12.07)	16 (18.18)

*p<0.05 *vs.* AA.

Table VII. Association between rs788016 and atherosclerosis grade.

Grade	n (160)	rs3828336				
		GG (n=72)	AG (n=63)	AA (n=25)	G (n=207)	A (n=113)
I	45	22 (30.56)	17 (26.98)	6 (24)	61 (29.47)	29 (25.66)
II	45	22 (30.56)	18 (28.57)	5 (20)	62 (29.95)	28 (24.78)
III	48	23 (31.94)	17 (26.98)	8 (32)	63 (30.43)	33 (29.20)
IV	22	5 (6.94)	11 (17.46)*	6 (24)*	21 (10.14)	23 (20.35)#

*p<0.05 *vs.* CC, #p<0.05 *vs.* G

brovascular diseases, including myocardial infarction and cerebral infarction. At that time, cross-sectional area of the vascular cavity is reduced or even blocked, and cell necrosis is pronounced because of occluded blood flow[12]. Currently, the pathogenesis of atherosclerosis remains unclear. The progression of the disease is mainly judged by measuring cross-section of the artery, but it is an invasive, expensive, and non-real-time examination[13,14]. Hence, the recognition of personal genetic physique and early prevention are also research focuses at present in addition to the usual living habit and regular diet. Recently, many studies have explored the incidence rate of the disease at the genetic level. Genetic genes greatly correlated with the development of atherosclerosis need to be developed. Sangiorgi et al[15] showed that HSP60 is able to bind to the TLRs on the inner wall of blood vessels to eliminate debris in blood vessels and prevent atherosclerosis.

HSP60, a member of the HSP family, is widely distributed in the mitochondria of animal cells, which is responsible for the transfer of polypeptide molecules in the cell matrix, the folding of peptide chains and the assembly of proteins[16]. It is reported that the high expression of HSP60 is capable of raising the risk of coronary heart disease. The higher the serum concentration of HSP60 is, the higher the incidence rate of acute myocardial infarction will be[17]. In addition, He et al[18] reported that the chain effect of four mutations rs2340690, rs788016, rs2305560, and rs2565163 in HSP60 gene polymorphisms is related to the incidence rate of coronary heart disease in chinese han population. Among them, HSP60 rs2340690 and rs788016 have a relatively high mutation frequency (above 20%). Rs2340690 located in the intron region of HSPD1 gene has an important influence on

Table VIII. Frequency distribution and OR of the combined action genotypes of rs2340690 and rs788016.

Genotype		Control group		Case group		OR (95% CI)	*p*
		n	%	n	%		
I rs2340690	rs788016						
A	G	109	54.5	95	59.37	1	0.487
A	A	42	21	21	13.13	0.574 (0.322-0.631)	0.139
G	G	25	12.5	8	5	0.367 (0.273-0.401)	0.061
G	A	24	12	36	22.5	1.721 (1.463-1.928)	0.049

the binding of transcription factors and is associated with differentially expressed genes[19]. Rs788016 is located in the upstream intron region of HSPD1 gene, which has been proven in many studies to play a vital role in regulating the expression of downstream HSPD1 gene expression[20]. In this study, based on the high mutation rate of the two loci in han population, three genotypes AA, AG, and GG were found in HSP60 rs2340690, and three genotypes GG, AG, and AA were detected in HSP60 rs788016. There was no significant difference in the frequency of the 3 genotypes in the two loci between case group and control group. The dominant, recessive, and additive genetic models of the two loci showed that the two loci had no remarkable association with the incidence rate of atherosclerosis. However, for atherosclerosis patients carrying both genotype G of rs2340690 and genotype A of rs788016, the OR was 1.721 (p=0.049), suggesting that patients with double-mutations already have atherosclerosis, and the risk was nearly doubled. For patients with pathological grade IV atherosclerosis, the frequency of GG genotype of rs2340690 was higher than that of those carrying AA genotype and AG genotype of rs2340690. In addition, the probability in patients carrying rs788016 A was higher than that of those carrying rs788016 G, implying that atherosclerosis patients carrying mutated base of HSP60 had a worse aggravation.

There are several noteworthy limitations in this study. First, it was neglected that the reported SNP-associated parts might have rare synthesis deletion. Second, the sample size in case and control groups was relatively small. Additionally, we only did this investigation among Han Chinese patients. We also need to consider the impact of race on the study in future studies. Third, atherosclerosis is a complicated disease, and the interaction features between the genes and environment were not included in this study due to condition limits.

Conclusions

In summary, the polymorphism of HSP60 gene has a relation with the pathological grade and incidence rate of atherosclerosis.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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