Association of heat shock protein polymorphisms with patient susceptibility to coronary artery disease comorbid depression and anxiety in a Chinese population

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Background. Coronary artery disease (CAD) is one of the severe diseases that threaten human health worldwide. In addition, the associated rate of comorbidity with depression and anxiety is extremely high. Heat shock proteins (HSPs) are a group of proteins that possesses cardiovascular and psychological protection properties. The objective of this study is to determine the association of the two most widely studied HSPs, namely, HSP70 and HSP90, with CAD comorbid depression and anxiety in a Chinese population. **Methods.** A case-control study involving 271 CAD patients and 113 healthy individuals was conducted. The 271 CAD patients include individuals with (123) and without depression (148) and individuals with (57) and without anxiety (214). Ten single nucleotide polymorphisms (SNPs) for HSP70 and seven SNPs for HSP90 were selected and genotyped. **Results.** Results revealed that the HSP70 rs10892958 C allele and HSP70 rs2236658 T allele were associated with a decreased risk of CAD (P< 0.05), whereas the G allele of the rs11218941 polymorphism was associated with an increased risk of CAD. The haplotype analysis results indicated that the haplotype TGGGC of the HSPA8 gene (coded the HSP70 family, rs4936770/rs4802/rs10892958/rs11218941/rs2236658) significantly increased the risk of CAD (P = 0.008). Among the patients with CAD, the carriers of the CC genotype for the HSP90 rs1042665 showed higher risks of anxiety than the carriers of another genotypes. However, no significant relationships were found among the CAD with depression and CAD without depression groups for the selected SNPs. These findings suggested that the genetic polymorphisms in the HSP gene, especially the HSPA8 of HSP70, contribute to CAD susceptibility and rs1042665 genetic polymorphisms might have an effect on the anxiety incidence among CAD patients.

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Abstract:

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Methods. A case-control study involving 271 CAD patients and 113 healthy individuals was conducted. The 271 CAD patients include individuals with (123) and without depression (148) and individuals with (57) and without anxiety (214). Ten single nucleotide polymorphisms (SNPs) for HSP70 and seven SNPs for HSP90 were selected and genotyped.

Results. Results revealed that the HSP70 rs10892958 C allele and HSP70 rs2236658 T allele were associated with a decreased risk of CAD (P< 0.05), whereas the G allele of the rs11218941 polymorphism was associated with an increased risk of CAD. The haplotype analysis results indicated that the haplotype TGGGC of the HSPA8 gene (coded the HSP70 family, rs4936770/rs4802/rs10892958/rs11218941/rs2236658) significantly increased the risk of CAD (P = 0.008). Among the patients with CAD, the carriers of the CC genotype for the HSP90 rs1042665 showed higher risks of anxiety than the carriers of another genotypes. However, no significant relationships were found among the CAD with depression and CAD without depression groups for the selected SNPs. These findings suggested that the genetic polymorphisms in the HSP gene, especially the HSPA8 of HSP70, contribute to CAD susceptibility and rs1042665 genetic polymorphisms might have an effect on the anxiety incidence among CAD patients.

Keywords: Heat shock proteins, Coronary artery disease, anxiety, depression, HSP90, HSP70

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Introduction

81 Cardiovascular diseases (CVDs) are the number one cause of mortality in the world. These
82 diseases account for one-third of the total deaths and approximately 17.9 million lives each year.
83 Coronary artery disease (CAD) is an atherosclerotic CVD manifested by stable or unstable
84 angina, myocardial infarction (MI), or sudden cardiac death. CAD is the leading cause of death
85 in developed and developing countries (Malakar et al. 2019; Roth et al. 2017). More than
86 900,000 individuals in the United States suffered from MI or died of CAD in 2017(Benjamin et
87 al. 2018). CAD-driven death ranked first in six provinces in China. Patients with CAD
88 experience high levels of mental disorder, which might further influence the course of illness and
89   the recovery of the physical and psychological states. Anxiety and depression are highly
90   prevalent among CAD patients and cause adverse outcomes, which affect over 20% and 30%
91 of patients with CAD, respectively.(Gu et al. 2016; Rudisch & Nemeroff 2003) Wang reported
92 that anxiety is independently associated with the severity of coronary atherosclerosis and
93 predicted the worse outcome in CAD patients with Han Chinese ethnicity (Wang et al. 2013).
94 Depressive disorders also increase the mortality in CAD patients and the risk of developing the
95 disease. Therefore, increasing attentions have been devoted to the importance of comorbid
96 anxiety and depression among CAD patients.

97 The relationship between psychiatric disorders and CAD is multidimensional. Several potential
98 mechanisms were posited. Stapelberg et al. conducted a thorough review(Stapelberg et al. 2011)
99 and proposed six domains of causal mechanism for the potentially linked depression and cardiac
100 disease, namely, behavioral mechanisms, genetic mechanisms, inflammatory mechanisms,
101 endothelial dysfunction and platelet activation, polyunsaturated omega-3 free fatty acid
102 deficiency and hypothalamic–pituitary–adrenal axis, and autonomic mechanisms. Numerous
103 studies have focused on the genetic mechanisms of the relationship between psychiatric diseases
104 and CAD(Amare et al. 2019; Liu et al. 2014; Meyer et al. 2020).

105 Heat shock proteins (HSPs) are a group of highly conserved stress proteins whose expression is
106 stimulated by heat shock, inflammation, infection, ischemia hypoxia, physiological stress, and
107 carcinogens. Many HSPs have a molecular chaperone activity involved in various aspects of
108 protein biogenesis, including folding, oligomeric assembling, transporting to a particular
109 subcellular compartment, or controlled switching among active/inactive conformations. In
110 addition, HSPs are governed tightly by cellular regulatory mechanism and get expressed under
111 varying metabolic stress, including CAD, anxiety, depression and other psychiatric diseases,
112 protecting cells and tissue from misfolding of denatured proteins by regulating both transcription
113 and translation (Dhama et al., 2019). HSPs are classified into six families in accordance with

114   their molecular weight, which ranges from 17 kDa to more than 100 kDa. These families include
115   small HSP, HSP40, HSP60, HSP70, HSP90, and HSP100 families. Out of these six, HSP70 and
116   HSP90 are the most well-known families.

117   Various studies have reported the cardiovascular and psychological regulatory effects of HSP70
118   and HSP90. The phosphorylated HSP70 provides an on/off switch for the regulation of Ca
119   signaling, which leads to therapeutic benefits in human diseases, such as atherosclerosis,
120   cardiomyopathy, congestive heart failure and ischemia (Lakshmikuttyamma et al. 2006).
121   Song(Song et al. 2019) highlighted that HSP70 is a promising therapeutic target for myocardial
122   ischemia–reperfusion injury. Fibronectin levels and collagen production would be decreased by
123   inhibiting the extracellular HSP90, implicating for the fibrosis-related pathology of chronic heart
124   conditions (García et al. 2016). Moreover, extracellular HSP70 and HSP90 are considered as
125   potential diagnostic markers of chronic heart disease (Edkins et al. 2018).

126   Additionally, the abnormal functioning of HSP70 is hypothesized as an underlying target for
127   altered stress response in major depression(Cheng et al. 2018). The genetic variants of the
128   HSP70 family proteins may affect the action of antidepressants (Pae et al. 2007). HSP90, a
129   molecular chaperone of FK-506 binding protein 5 (FKBP5), coordinates with FKBP5 to regulate
130   glucocorticoid receptor (GR) activity; both are factors of several affective disorders, including
131   anxiety, depression, and post-traumatic stress disorder (PTSD) (Criado-Marrero et al. 2018).

132   Numerous studies focused on the relationship between HSP70 gene polymorphism and CAD.
133   It stated that several HSP70 SNPs are associated with the risk of developing CAD. Several works
134   reported that rs1061581 (+1267), which is the most studied polymorphism, is associated with
135   CAD. This inference is supported by studies from Iran, Tunisia, Italy, and China(Giacconi et al.
136   2006; Hrira et al. 2012; Mardan-Nik et al. 2016; Wei et al. 2014).He et al. suggested that the
137   genetic variants of HSP70 gene, especially the promoter single nucleotide polymorphism (SNP)
138   rs2236659, contribute to coronary heart disease (CHD) susceptibility by affecting the HSP
139   protein expression levels (He et al. 2010).

140   HPS70 and HSP90 show neuroprotective effects in many studies. HSP70 could suppress the
141   progression of Alzheimer's disease with in vitro and in vivo experiments and presented
142   protective effects in neurodegenerative shocks(Campanella & Pace 2018; Pratt et al. 2015).
143   Shimizu et al.(Shimizu et al. 1996) found abnormal HSP70 mRNA with a 162-base deletion in
144   patients with major depression, whereas another group reported that the 162-base deletion in the
145   5'-flanking region of the HSP70 gene mRNA is not associated with major depression(Takimoto
146   et al. 2003). The result of a study from South Korea reported that the genetic variants within the
147   gene coding for the HSP70 family proteins may affect the action and therapeutic efficacy of
148   antidepressants(Pae et al. 2007). Vlachos et al. (Vlachos et al. 2014) found the correlation among
149   depression, anxiety, and polymorphonuclear cell resilience in ulcerative colitis, which was
150   mediated by HSP70. Moreover, studies from Taiwan suggested that HSP70 polymorphisms
151   might play protective role against Alzheimer's disease but are unlikely to play a major role in
152   mitigating the risk of developing Parkinson's disease(Chen et al. 2008; Hsu et al. 2008). A

153 previous review(Criado-Marrero et al. 2018) stated that HSP90 served as complex regulators of
154 various psychiatric diseases, such as depression, PTSD, and anxiety.

155 GR is a crucial part of the stress hormone axis and dysregulation for GR is consistently proven
156 its importance for stress-related diseases, such as depression and anxiety (Holsboer F, 2000). It
157 has been demonstrated that Hsp90 is required for GR maturation and the related regulation
158 function of HSP90 is primarily accomplished through interactions with its co-chaperones,
159 FKBP5 (FKBP51 and FKBP52). A large number of studies focused their attention on the
160 association between FKBP5 and depression or antidepressant response and Fries's group
161 provided a summary (Fries, et al., 2017). Another study overviewed the association of the
162 FKBP5 SNPs with mental health disorders and proposed four FKBP5 SNPs (rs9470080,
163 rs1360780, rs9296158, rs3800373) are significantly associated with at least five diseases,
164 including anxiety and depression (Criado-Marrero et al. 2018). One of our recent study also
165 reported the significant association between FKBP5 gene variations and the risk of CAD
166 comorbid depression in a north Chinese population(Wang et al. 2020). Given the close molecular
167 chaperone relation between FKBP5 and HSP90, the assumption that the HSP90 gene
168 polymorphisms may affect the susceptibility to CAD comorbid depression/anxiety is reasonable.

169 The objective of the present study is to address the lack of study on the association of HSP
170 polymorphisms with patient susceptibility to CAD comorbid depression and anxiety. After
171 searching information for related studies (Lei et al. 2017; Sun et al. 2018; Zou et al. 2016), we
172 selected 17 SNPs from HSP70 (rs2763979 (C>T), rs2075800(C>T), rs1380154(C>T),
173 rs391957(C>T), rs17840761(A>G), rs11218941(A>G), rs10892958(G>C), rs2236658(C>T),
174 rs4802(A>G), rs4936770(T>C)) and HSP90 (rs1042665(C>T), rs10873531(A>G),
175 rs8005905(A>T), rs1165678(A>G), rs1165681(C>T), rs1882019 (G>A), and rs3794241(C>T)),
176 which were extensively studied in different diseases and some have been selected as tagSNPs for
177 gene-disease associations, to identify the importance of genetic polymorphisms in the
178 susceptibility to CAD comorbid depression and anxiety .

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180 ## Materials & Methods

181 ### Subjects

182 The participants in this study were recruited from the First Peoples' Hospital of Jining between
183 May 2016 and July 2019. A total of 271 CAD patients and 113 healthy individuals (controls)
184 were involved in the study. All participants were unrelated North Chinese Han individuals.

185 The software of Power and Sample Size Calculation (Version 3.1.2, Department of Biostatistics,
186 Vanderbilt University, Nashville, TN, United States) was employed to calculate the sample size.
187 It was calculated that the minimum sample size for experimental subjects and control subjects
188 was 138 and 111, respectively. The calculation was made according to the following parameters:
189 an independent case–control study; a type I error (α) of 0.05; a statistical power of 0.8; the

190 probability of exposure in cases (p0) of 0.15; the probability of exposure in the control (p1) of
191 0.3; and the control to case ratio (m) of 0.8. Therefore, the total of 271 CAD patients and 113
192 healthy controls basically meet the study requirements.

193 This study was approved by the ethics committee of the First Peoples' Hospital of Jining
194 (approval number: JY2016062) and adhered to the principles set by the Declaration of Helsinki.
195 Written informed consent was provided by every participant prior to the study.

196 The CAD cases were diagnosed by experienced cardiologists in accordance with the following
197 diagnostic criteria: (1) at least one of the major segments of the coronary arteries has 50% or
198 higher stenosis based on coronary angiography, (2) history of percutaneous coronary intervention,
199 and (3) history of coronary artery bypass graft surgery. Patients were excluded if they have other
200 serious diseases, such as congenital heart disease, severe autoimmune disease, renal failure,
201 immune system disorders, and malignancies.

202 The symptoms of depression and anxiety of CAD patients were assessed by two experienced
203 psychiatrists in accordance to the 5th Edition of the Diagnostic and Statistical Manual of Mental
204 Disorders. The Patient Health Questionnaire-9 (PHQ-9) and the seven-item Generalized Anxiety
205 Disorder 7 (GAD-7) were used to assess such symptoms, respectively. A score of 5 or higher in
206 the PHQ-9 scale was selected as the cutoff score for identifying the symptoms of depression,
207 whereas a threshold of >5 points in the GAD-7 scale was set to indicate the possibility of anxiety.

208 The controls who matched the sex and age of the patients were selected from the medical clinical
209 examination program, which included clinical physical examination, electrocardiogram analysis,
210 radiographic chest examination, and medical history evaluation. After screening, 113 healthy
211 volunteers who met the criteria were included in this study.

212 **SNP selection and Genotyping**

213 Candidate SNPs in this study were selected as follows: (1) SNPs from public databases and
214 literatures; (2) SNPs that previously were studied in gene-disease association outcomes (for
215 instance, noise-induced hearing loss, cancer risk, bipolar disorder, Alzheimer's disease and
216 diabetes), and (3) SNPs with a minor allele frequency not less than 5%. Finally, 17 SNPs from
217 HSP70 (rs2763979, rs2075800, rs1380154, rs391957, rs17840761, rs11218941, rs10892958,
218 rs2236658, rs4802, rs4936770) and HSP90 (rs1042665, rs10873531, rs8005905, rs1165678,
219 rs1165681, rs1882019, and rs3794241) were selected and genotyped.

220 First, 4 ml of peripheral blood were collected from each subject, and the genomic DNA was
221 extracted using a TIANamp Blood DNA Kit (TIANGEN, China) in accordance with the
222 manufacturer's instructions. NanoDrop-1000 (NanoDrop, United States of America [USA]) was
223 used to detect the concentration and purity of the DNA samples for the subsequent experiments.
224 Second, all DNA samples were genotyped through polymerase chain reaction (PCR)–ligase
225 detection reaction. Table 1 presents the primers for amplifying the PCR of the target SNPs for

226 each participant. After treating with shrimp alkaline phosphatase, the samples were extended and
227 purified using iPLEX extension reagents (Agena Bioscience, USA) and Nanodispenser RS1000.
228 Finally, the primer extension products were detected through matrix-assisted laser
229 desorption/ionization time-of-flight mass spectrometry, and the genotyping data were
230 automatically analyzed by the SpectroTyper. At least 10% of the samples were randomly
231 selected and retested to verify the reliability of the MassARRAY results.

232 **Statistical Analysis**

233 SPSS Statistics version 19.0 (SPSS, Inc., Chicago, IL, USA) was used for the statistical analysis.
234 The continuous and categorical variables were presented as mean ± standard deviation and
235 numbers (%), respectively. The chi-square (χ2) and t-test statistics were used to compare the
236 categorical and continuous variables, respectively. The PHQ-9 and GAD-7 scores that fit a
237 nonparametric distribution were tested for significance by using the two-tailed Mann–Whitney U
238 test. The χ2 test was also adopted to assess the Hardy–Weinberg equilibrium (HWE) for
239 polymorphisms, the genotype distributions, and the allele frequencies. Pairwise linkage
240 disequilibrium (LD) analyses were conducted using SHEsisPlus (http://shesisplus.bio-
241 x.cn/SHEsis.html). The differences in the risks of CAD and psychiatric diseases among different
242 groups were calculated using a binary logistic regression model. Odds ratios and 95% confidence
243 intervals were also calculated when needed. A two-sided p value of P< 0.05 was considered
244 statistically significant.

Results

246 The χ2 test results demonstrate that the 17 observed genotype frequencies in the CAD and
247 control groups are in accordance with the HWE (P ≥0.111), which means that the participants
248 represent the target population.

249 No significant differences (P >0.05) are found in terms of age, gender, smoking habit, or
250 drinking habit between the CAD patients and controls or among the different subgroups divided
251 by comorbid depression (D) or anxiety (A).

252 **Characteristics of the Study Participants**

253 The demographic and clinical characteristics of the participants in this study are presented in
254 Table 2. No significant differences (P >0.05) were found in term of age, gender or smoking,
255 drinking, no matter between the CAD patients and the health control or between the different
256 subgroups divided by whether comorbid depression (D) or anxiety (A). The clinical
257 characteristics of CAD patients from these subgroups were also presented in Table 2. The
258 patients with comorbid depression or anxiety had a decreased rate of hypertension relative to
259 those without these psychiatric disorders (53.7% vs. 97.3%, and 57.9% vs. 82.7%, respectively;
260 P < 0.05). We also found that CAD patients who had depression or anxiety had markedly higher
261 rates of comorbid insomnia than did those without depression or anxiety (47.2% vs. 25.0% and
262 50.9% vs. 30.8%, respectively; P < 0.005).

Association between Gene Polymorphisms and CAD Risk

The genotypic distribution and allele frequencies of the 17 genetic polymorphisms between the CAD and control groups were compared. The results are summarized in Tables 3 and 4, respectively. No statistically significant difference is observed between the CAD patients and controls for the genotypic and allelic distributions of rs2763979 (C>T), rs2075800(C>T), rs1380154(C>T), rs391957(C>T), rs17840761(A>G), rs4936770(T>C), HSP90 (rs1042665(C>T), rs10873531(A>G), rs8005905(A>T), rs1165678(A>G), rs1165681(C>T), rs1882019 (G>A), and rs3794241(C>T) polymorphisms.

For rs10892958, the CC (OR = 0.394, 95% CI [0.197–0.786], and p = 0.008) and GC+CC genotypes (OR = 0.477, 95% CI [0.255–0.893], and p = 0.021) are associated with significantly decreased risks of CAD when the GG genotype is used as the reference. The C allele occurs less frequently in the CAD group (52.4%) than in the control group(62.8%, p = 0.021), thereby indicating that it might be a protective factor against the development of CAD. Similarly, the TT and CT+TT genotypes and T allele frequency of rs2236658 are significantly lower in the CAD cases than in the controls (P = 0.018, 0.030, 0.023, respectively). The GG genotype and G allele of the rs11218941 polymorphism are associated with an increased risk of CAD (P = 0.019 and P = 0.021, respectively). No statistically significant difference is observed in terms of the rs4802 genotype frequency, whereas a statistically significant difference is found between the G and A allele frequencies (OR = 1.372, 95% CI [1.005–1.875], and p = 0.047).

Haplotype Analysis

Haplotype analysis was conducted to investigate the linkage among the studied SNPs. Figure 1 shows that in the LD block of the HSPA8 gene, which codes the HSP70 family, rs4936770/ rs4802/rs10892958/rs11218941/rs2236658 demonstrate a strong LD (rs4936770/rs4802: D′ = 0.99, r^2 =0.72;rs4936770/rs10892958: D′ = 0.99, r^2 =0.57; rs4936770/rs11218941: D′ = 0.97, r^2 =0.55; rs4936770/rs2236658: D′ = 0.96, r^2 =0.41; rs10892958/rs11218941: D′ = 1, r^2 =0.98; rs10892958/rs2236658: D′ = 0.98, r^2 =0.73; rs11218941/rs2236658: D′ = 0.98, r^2 =0.72). The haplotype frequencies are presented in Table 5. The results show that haplotype TGGGC significantly increases the risk of CAD (P = 0.008, OR = 1.609, 95% CI = 1.124–2.302).

Influence of Gene Polymorphisms on the Risk of Depression/Anxiety in CAD Patients

The results of the association analysis between the studied polymorphisms and the risk of depression and anxiety among CAD patients are summarized in Tables 6 and 7, respectively. No significant relationships are found among the CAD with depression (CAD+D) and CAD without depression (CAD−D) groups.

Among the CAD patients sub-grouped by comorbid anxiety, the presence of the AG (OR = 3.378, 95% CI [1.258–9.072], and p = 0.016) and GG genotypes (OR = 3.236, 95% CI [1.081–9.684], p = 0.036) of rs17840761 is associated with the increased risk of developing CAD comorbid anxiety compared with those without anxiety. Among the patients with CAD, the carriers of the CT genotype of rs1042665 have lower risk of anxiety than the carriers of the CC genotype (OR =

301   0.355, 95% CI [0.173–0.726], and p = 0.005), whereas the carriers of the CT genotype of
302   rs1165681 demonstrate higher risk of anxiety than the carriers of the CC genotype (OR = 2.530,
303   95% CI [1.178–5.434], and p = 0.017). The risk of anxiety is significantly lower among CAD
304   patients carrying the CT+TT genotypes of rs1042665 (OR = 0.334, 95% CI [0.167–0.667], and p
305   = 0.002) and markedly higher (OR = 2.142, 95% CI [1.021–4.495], and p = 0.044) than those
306   carrying the CC genotype of rs1165681.

307   We further examine the association of rs17840761, rs1042665, and rs1165681 with the severity
308   of anxiety symptoms. The results are illustrated in Figure 2. No significant difference is observed
309   among the different genotypes of rs17840761. In addition, the GAD-7 scores of patients with the
310   CC genotype of rs1042665 are markedly higher those of patients with the CT genotypes (P <
311   0.016). The rs1165681 polymorphism is also associated with the severity of anxiety symptoms
312   due to the lower GAD-7 scores of CC genotype carriers than CT and TT genotype carriers (p =
313   0.001).

314   **Discussion**

315   Genetics study is an effective tool for understanding the underlying mechanism of CAD
316   comorbid depression and anxiety. The present study investigates the relationship between
317   HSP70/HSP90 polymorphisms and CAD comorbid depression and anxiety in a Chinese
318   population.

319   We investigated the association of 11 HSP70 SNP polymorphisms with CAD susceptibility, and
320   the results revealed that the rs4802, rs10892958, rs11218941, and rs2236658 gene variants are
321   associated with CAD in the studied population. Further analysis suggested that these SNPs
322   belonged to a component of HSPA8, which is an important member of the HSP70 family.
323   HSPA8 is also referred to as HSC70, HSC71, HSP71, or HSP73 and represents a constitutively
324   expressed cognate protein of the HSP70 family. HSPA8 is an essential protein in the
325   housekeeping of the HSP70 family and plays a major role in the protein quality control, with its
326   chaperone in the folding protein process. He et al. (He et al. 2010) identified four tag SNPs
327   (rs2236659, rs2276077, rs10892958, and rs1461496) of HSPA8 and evaluated their association
328   with the susceptibility to CHD. The findings showed that the promoter SNP rs2236659 was
329   associated with the susceptibility to CHD, and the carriers of the C allele of rs2236659 possessed
330   a decreased CHD risk. By contrast, rs10892958 did not exhibit any association with the
331   susceptibility to CHD, which contradicted our conclusion that the TT and CT+TT genotypes and
332   T allele frequency of rs2236658 were significantly lower in the CAD cases than in the controls.

333   Further haplotype analysis showed that the HSPA8 SNPs in this study displayed strong LD,
334   whereas the other SNPs did not. These results were also inconsistent with the findings of a study
335   on a Chinese population, which indicated that a haplotype of HSPA8 SNPs contributes to a lower
336   CHD risk compared with the common haplotype (He et al. 2010). Studies showed that HSPA8
337   acted as an accessory protein of a hyperpolarization-activated chloride channel from rat
338   pulmonary vein cardiomyocytes(Okamoto & Nagasawa 2019). In addition, the HSPA8

339 chaperone system played a major role in regulating the cardiac myosin binding protein C(Glazier
340 et al. 2018). This inference could partly explain the obtained results. A genome-wide association
341 study reported that HSPA8 is associated with the new onset of atrial fibrillation, MI, acute
342 kidney injury, and stroke after cardiac surgery, thereby indicating its potential role in cardiac
343 protection (Westphal et al. 2019). Furthermore, He et al. suggested that HSPA8 might take part
344 in the development of CHD in two ways. First, HSPA8 could protect against reactive oxygen
345 species with other HSPs, which were involved in the etiology of CHD. Second, HSPA8 could
346 protect against hypoxia-induced apoptosis in hypoxia-induced apoptosis-resistant macrophages.

347 HSP90 played a myocardial protective role in the crosslinking with actin filaments in a Ca^{2+}- and
348 ATP-dependent manner, thereby helping the modulation of the cytoskeletal dynamics and its
349 positive signal in the cytoplasm of myocardial cells after heat stress (Islam et al. 2014). Our
350 previous study revealed a remarkable association between FKBP5 gene variations and CAD risk.
351 The most perceived molecular function of FKBP5 was its role as a co-chaperone of HSP90
352 heterocomplexes. Therefore, we investigated the relationship between HSP90 polymorphisms
353 and CAD. However, the results showed no significant difference in the HSP90 SNP between the
354 CAD patients and healthy individuals in this study. The limited sample size and single species
355 restricted the scope of the present study. Hence, further studies with larger samples from
356 different races should be conducted to provide more evidence.

357 In this study, we focused on the influence of HSP70/HSP90 gene polymorphisms on the risk of
358 depression/anxiety in CAD patients. Our data suggested that genetic polymorphisms of HSPs
359 have no significant impact on comorbid depression. This result was consistent with a report on a
360 Chinese population, which highlighted that no significant association exists between HSP70 gene
361 (rs2075799) SNPs and the comorbidity of CAD with depression (Min et al. 2017). However, the
362 different genotypes of rs17840761, rs1042665, and rs1165681 presented anxiety risk among the
363 CAD patients. The presence of the AG and GG genotypes of rs17840761, CT and CT+TT
364 genotype of rs1165681 were associated with the increased risk of developing CAD comorbid
365 with anxiety compared with those without anxiety. The carriers of the CT genotype of rs1042665
366 had lower risk of anxiety than the carriers of the CC genotype. These paradoxical results might
367 be explained by the relatively limited sample size, which was the major limitation of the present
368 study. Moreover, the regional and racial biases, as well as the lack of correction for potential
369 population stratification were also the potential limitations for this study.

370 Conclusions

371 The results suggest the association between HSPA8 gene polymorphism and CAD in a Chinese
372 population. The rs17840761 of HSP70 and rs1042665/rs1165681 of HSP90 gene polymorphisms
373 may influence the comorbidity of anxiety among CAD patients. However, the limited sample
374 size, especially the healthy subjects, may urge us to draw conservative conclusions for the
375 conclusion for the association between HSP70/HSP90 gene polymorphisms and CAD risks.
376 Therefore, further investigations through replication studies with large samples are necessary to
377 confirm these conclusions and clarify the role of HSP polymorphisms in the pathogenesis of the

378   comorbidity of anxiety/depression and CAD.

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Table1

Primers of HSP Genes Used in the PCR

1   Table1 Primers of HSP Genes Used in the PCR

SNP	Ancestor allele	Primer sequence	Product size
rs2763979	C	F: 5'- ACGTTGGATGTCTTACTCGGGACTGTGAGG-3' R: 5'- ACGTTGGATGCACCTCCTTCCTACTTTCTC-3'	99
rs2075800	C	F: 5'- ACGTTGGATGAGGTCAATCAACTGGCAGAG-3' R: 5'- ACGTTGGATGCTCCTTGGTAGAGTTTTGTG-3'	116
rs1380154	T	F: 5'- ACGTTGGATGCAAACCATGTGTTTACTTAC-3' R: 5'- ACGTTGGATGTTGATATGGGACATTCTGCC-3'	102
rs391957	T	F: 5'- ACGTTGGATGATGGAGGAAGGGAGAACAAG-3' R: 5'- ACGTTGGATGAGTAGGTCCAGCAGGAGTGA -3'	102
rs17840761	G	F: 5'- ACGTTGGATGACAAGTCCCGCCTTCACTC-3' R: 5'- ACGTTGGATGAAGTTTCAGATCCCACAGCC-3'	105
rs11218941	A	F: 5'- ACGTTGGATGCAGCGTTTTCTTTCACCCAG -3' R: 5'- ACGTTGGATGATGTACCCCCATACTGGAAG-3'	112
rs10892958	C	F: 5'- ACGTTGGATGGAGGTGATGGGCACTATTAC -3' R: 5'- ACGTTGGATGCCCTCATCCCTTAACAGAAC-3'	96
rs2236658	T	F: 5'- ACGTTGGATGGGTAACTGAGGACTCCCGC -3' R: 5'- ACGTTGGATGGCGTTCTGGAACTTTCAAGC -3'	119
rs4802	A	F: 5'- ACGTTGGATGGGGTTGCAAACTTTCTCCAG -3' R: 5'- ACGTTGGATGATTCCTTTTTCTCTTCCTC -3'	100
rs4936770	C	F: 5'- ACGTTGGATGGGTAAACCAAGCTTGAGCTG-3' R: 5'- ACGTTGGATGTGAATTCTGGTGGAAACCGC-3'	103
rs1042665	T	F: 5'- ACGTTGGATGATTTTCCGTGTCTCACCTGC-3' R: 5'- ACGTTGGATGACAACATGGCACTTCAGAGG-3'	107
rs10873531	G	F: 5'- ACGTTGGATGACTCTGTCTCTGGAAACAGC -3' R: 5'- ACGTTGGATGCACCTTGGCTCTGTCTGAAG -3'	100
rs8005905	T	F: 5'- ACGTTGGATGTCCAGAGACAGAGTAGAGTG-3' R: 5'- ACGTTGGATGGTACCAAGAAAAGGCCCAAG-3'	94
rs1165678	G	F: 5'- ACGTTGGATGCATCTGCAGAAACGTCTACC -3' R: 5'- ACGTTGGATGTGGAGAGAACTGAGACAGAG -3'	92
rs1165681	T	F: 5'- ACGTTGGATGAGGTAAAGCCAAGACAGAAC -3' R: 5'- ACGTTGGATGTTAGAAGTGGTGACTGCCTC -3'	108
rs1882019	G	F: 5'- ACGTTGGATGAAGATTGGGTAAGGGGCAAC -3' R: 5'- ACGTTGGATGCATGAAAGCACAAGCGTACC -3'	117

| rs3794241 | C | F: 5'- ACGTTGGATGTAACTCTTAGTGATGCCTCC-3' | 99 |
| | | R: 5'- ACGTTGGATGGTTCCCAATTTACCTTCCCC -3' | |

Table 2

Demographic and Clinical Characteristics of the Participants

3   Table 2 Demographic and Clinical Characteristics of the Participants

Variables	CAD (n= 271)	Controls (n=113)	P-value[a]	CAD+D (n= 123)	CAD-D (n= 148)	P-value[b]	CAD+A (n= 57)	CAD-A (n= 214)	P-value[c]
Age (yrs)	58.4±10.1	54.1±10.3	0.126	58.9±10.9	57.9±9.5	0.147	58.1±9.4	58.4±10.2	0.376
Gender (M/F, n)	128/143	62/51	0.173	53/70	75/72	0.213	25/32	103/111	0.566
Smoking (n, %)	90, 33.2	-	-	38, 30.9	52, 35.1	0.461	17,29.8	73,34.1	0.541
Drinking (n, %)	97, 35.8	-	-	37, 30.1	60, 40.5	0.074	18,31.6	79,36.9	0.455
Hypertension (n, %)	210, 77.5	-	-	66, 53.7	144, 97.3	0.000*	33, 57.9	177, 82.7	0.000*
Diabetes mellitus (n, %)	53, 19.6	-	-	23, 18.7	30, 20.3	0.657	9, 15.8	44, 20.6	0.420
Stroke (n, %)	52, 19.3	-	-	22, 17.9	30, 20.3	0.539	7, 12.3	45, 21.0	0.136
Insomnia (n, %)	95, 35.1	-	-	58, 47.2	37, 25.0	0.000*	29, 50.9	66, 30.8	0.005*

5   [a]CAD versus Controls, [b]CAD+D versus CAD-D,. [c]CAD+A versus CAD-A.

6   CAD: coronary heart disease, CAD+D: CHD with depression, CAD-D: CAD without depression, CAD+A: CAD with anxiety, CAD-

7   A: CAD without anxiety

Table 3.

Genotypic Distribution of 17 HSP Gene Between All CAD Patients (n=271) and Controls (n =113).

1 Table 3. Genotypic Distribution of 17 HSP Gene Between All CAD Patients (n=271) and

2 Controls (n =113).

SNP	Genotype	Case,(%)	Control,(%)	P value[a] (χ^2)	P value[b]	OR (95% CI)
rs2763979	CC	104(38.4)	37(32.7)	0.352(2.088)	Referent	1.00
	CT	122(45.0)	60(53.1)		0.192	0.723(0.445-1.176)
	TT	45(16.6)	16(14.2)		0.999	1.001(0.506-1.981)
	CT+TT	167(61.6)	76(67.3)	0.297(1.089)	0.297	0.782(0.492-1.242)
rs2075800	CC	112(41.3)	52(46.0)	0.466(1.526)	Referent	1.00
	CT	127(46.9)	52(46.0)		0.547	1.134(0.715-1.798)
	TT	32(11.8)	9(8.0)		0.293	1.651(0.735-3.708)
	CT+TT	159(58.7)	61(54.0)	0.397(0.717)	0.398	1.210(0.778-1.883)
rs1380154	CC	78(28.8)	30(26.5)	0.905(0.199)	Referent	1.00
	CT	147(54.2)	63(55.8)		0.680	0.897(0.537-1.501)
	TT	46(17.0)	20(17.7)		0.721	0.885(0.451-1.734)
	CT+TT	193(71.2)	83(73.5)	0.657(0.197)	0.657	0.894(0.546-1.465)
rs391957	CC	121(44.6)	57(50.4)	0.451(1.593)	Referent	1.00
	CT	128(47.2)	50(44.2)		0.419	1.206(0.766-1.899)
	TT	22(8.1)	6(5.3)		0.263	1.727(0.664-4.493)
	CT+TT	150(55.4)	56(49.6)	0.300(1.076)	0.300	1.262(0.813-1.959)
rs17840761	AA	57(21.0)	22(19.5)	0.942(0.12)	Referent	1.00
	AG	155(57.2)	66(58.4)		0.736	0.906(0.513-1.603)
	GG	59(21.8)	25(22.1)		0.788	0.911(0.462-1.796)
	AG+GG	214(79.0)	91(80.5)	0.730(0.119)	0.730	0.908(0.524-1.573)
rs11218941	AA	75(27.7)	41(36.3)	0.060(5.64)	Referent	1.00
	AG	134(49.4)	57(50.4)		0.317	1.285(0.787-2.100)
	GG	62(22.9)	15(13.3)		0.019*	2.260(1.144-4.462)
	AG+GG	196(72.3)	72(63.7)	0.094(2.803)	0.095	1.488(0.933-2.373)
rs10892958	GG	62(22.9)	14(12.4)	0.027(7.228)*	Referent	1.00
	GC	134(49.4)	56(49.6)		0.067	0.540(0.280-1.044)
	CC	75(27.7)	43(38.1)		0.008*	0.394(0.197-0.786)
	GC+CC	209(77.1)	99(87.6)	0.019(5.527)*	0.021*	0.477(0.255-0.893)
rs2236658	CC	45(16.6)	9(8.0)	0.053(5.867)	Referent	1.00
	CT	130(48.0)	54(47.8)		0.067	0.481(0.220-1.053)
	TT	96(35.4)	50(44.2)		0.018*	0.384(0.174-0.849)
	CT+TT	226(83.4)	104(92.0)	0.026(4.927)*	0.030*	0.435(0.205-0.922)
rs4802	AA	83(30.6)	45(39.8)	0.201(3.211)	Referent	1.00
	AG	91(33.6)	35(31.0)		0.206	1.410(0.828-2.401)
	GG	97(35.8)	33(29.2)		0.089	1.594(0.932-2.725)
	AG+GG	188(69.4)	68(60.2)	0.082(3.035)	0.082	1.499(0.949-2.367)
rs4936770	TT	98(36.2)	33(29.2)	0.261(2.69)	Referent	1.00

	TC	129(47.6)	55(48.7)		0.360	0.790(0.477-1.309)
	CC	44(16.2)	25(22.1)		0.103	0.593(0.316-1.112)
	TC+CC	173(63.8)	80(70.8)	0.190(1.718)	0.191	0.728(0.453-1.171)
rs1042665	CC	164(60.5)	71(62.8)	0.417(1.748)	Referent	1.00
	CT	93(34.3)	33(29.2)		0.422	1.220(0.751-1.982)
	TT	14(5.2)	9(8.0)		0.380	0.673(0.279-1.628)
	CT+TT	107(39.5)	42(37.2)	0.671(0.180)	0.671	1.103(0.701-1.734)
rs10873531	AA	165(60.9)	66(58.4)	0.074(5.22)	Referent	1.00
	AG	97(35.8)	37(32.7)		0.844	1.049(0.653-1.685)
	GG	9(3.3)	10(8.8)		0.034	0.360(0.140-0.926)
	AG+GG	106(39.1)	47(41.6)	0.651(0.204)	0.651	0.902(0.577-1.410)
rs8005905	AA	186(68.6)	77(68.1)	0.306(2.368)	Referent	1.00
	AT	77(28.4)	29(25.7)		0.712	1.099(0.665-1.817)
	TT	8(3.0)	7(6.2)		0.162	0.473(0.166-1.350)
	AT+TT	85(31.4)	36(31.9)	0.924(0.009)	0.924	0.977(0.610-1.566)
rs1165678	AA	191(70.5)	72(63.7)	0.378(1.945)	Referent	1.00
	AG	72(26.6)	38(33.6)		0.167	0.714(0.443-1.151)
	GG	8(3.0)	3(2.7)		0.994	1.005(0.259-3.894)
	AG+GG	80(29.5)	41(36.3)	0.194(1.690)	0.194	0.736(0.463-1.170)
rs1165681	CC	77(28.4)	26(23.0)	0.491(1.424)	Referent	1.00
	CT	135(49.8)	63(55.8)		0.237	0.724(0.423-1.236)
	TT	59(21.8)	24(21.2)		0.575	0.830(0.433-1.590)
	CT+TT	194(71.6)	87(77.0)	0.276(1.187)	0.277	0.753(0.451-1.256)
rs1882019	GG	131(48.3)	60(53.1)	0.479(1.471)	Referent	1.00
	GA	114(42.1)	46(40.7)		0.588	1.135(0.717-1.796)
	AA	26(9.6)	7(6.2)		0.241	1.701(0.699-4.137)
	GA+AA	140(51.7)	53(46.9)	0.395(0.722)	0.396	1.210(0.779-1.878)
rs3794241	CC	166(61.3)	67(59.3)	0.758(0.555)	Referent	1.00
	CT	93(34.3)	39(34.5)		0.873	0.962(0.602-1.539)
	TT	12(4.4)	7(6.4)		0.459	0.692(0.261-1.833)
	CT+TT	105(38.7)	46(40.7)	0.720(0.129)	0.720	0.921(0.589-1.442)

5 Abbreviations: CI, confidence interval; OR, odds ratio.

6 [a]Pvalue for allele frequencies in cases and controls using 2-sidedχ^2 test.

7 [b]Pvalues adjusted by age and gender using logistic regression.

8 * $P<0.05$

Table 4.

Allelic Distribution of 17 HSP Gene Between All CAD Patients (2n=542) and Controls (2n =226).

1 Table 4. Allelic Distribution of 17 HSP Gene Between All CAD Patients (2n=542) and Controls

2 (2n =226).

SNP	Allele	Case,(%)	Control,(%)	P value^a (χ^2)	P value^b(χ^2)	OR (95% CI)
rs2763979	C	330(60.9)	134(59.3)	0.681(0.169)	Referent	1.00
	T	212(39.1)	92(40.7)		0.681	0.936(0.682-1.284)
rs2075800	C	351(64.7)	156(69.0)	0.255(1.294)	Referent	1.00
	T	191(35.3)	70(31.0)		0.256	1.213(0.870-1.691)
rs1380154	C	303(55.9)	123(54.4)	0.707(0.141)	Referent	1.00
	T	239(44.1)	103(45.6)		0.707	0.942(0.690-1.287)
rs391957	C	370(68.3)	164(72.6)	0.238(1.392)	Referent	1.00
	T	172(31.7)	62(27.4)		0.238	1.230(0.872-1.734)
rs17840761	A	269(49.6)	110(48.7)	0.809(0.059)	Referent	1.00
	G	273(50.4)	116(51.3)		0.809	0.962(0.706-1.313)
rs11218941	A	284(52.4)	139(61.5)	0.021(5.345)*	Referent	1.00
	G	258(47.6)	87(38.5)		0.021*	1.451(1.058-1.992)
rs10892958	G	258(47.6)	84(37.2)	0.008(7.029)*	Referent	1.00
	C	284(52.4)	142(62.8)		0.008*	0.651(0.474-0.895)
rs2236658	C	220(40.6)	72(31.9)	0.023(5.161)*	Referent	1.00
	T	322(59.4)	154(68.1)		0.023*	0.684(0.493-0.950)
rs4802	A	257(47.4)	125(55.3)	0.046(3.974)*	Referent	1.00
	G	285(52.6)	101(44.7)		0.047*	1.372(1.005-1.875)
rs4936770	T	325(60.0)	121(53.5)	0.100(2.703)	Referent	1.00
	C	217(40.0)	105(46.5)		0.101	0.769(0.563-1.052)
rs1042665	C	421(77.7)	175(77.4)	0.942(0.005)	Referent	1.00
	T	121(22.3)	51(22.6)		0.942	0.986(0.680-1.430)
rs10873531	A	427(78.8)	169(74.8)	0.225(1.471)	Referent	1.00
	G	115(21.2)	57(25.2)		0.226	0.799(0.555-1.149)
rs8005905	A	449(82.8)	183(81.0)	0.537(0.382)	Referent	1.00
	T	93(17.2)	43(19.0)		0.537	0.881(0.591-1.315)
rs1165678	A	454(83.8)	182(80.5)	0.279(1.171)	Referent	1.00
	G	88(16.2)	44(19.5)		0.280	0.802(0.537-1.197)
rs1165681	C	289(53.3)	115(50.9)	0.538(0.380)	Referent	1.00
	T	253(46.7)	111(49.1)		0.538	0.907(0.665-1.237)
rs1882019	G	376(69.4)	166(73.5)	0.258(1.278)	Referent	1.00
	A	166(30.6)	60(26.5)		0.259	1.221(0.863-1.728)
rs3794241	C	425(78.4)	173(76.5)	0.571(0.322)	Referent	1.00
	T	117(21.6)	53(23.5)		0.571	1.359(0.954-1.937)

3 Abbreviations: CI, confidence interval; OR, odds ratio.

4 ^{a}Pvalue for allele frequencies in cases and controls using 2-sidedχ^2 test.

5   [b]P values adjusted by age and gender using logistic regression.

6   * $P<0.05$

Table 5.

Haplotype frequencies for *HSP70* polymorphisms in CAD and control group.

2 Table 5. Haplotype frequencies for *HSP70* polymorphisms in CAD and control group.

Haplotype (rs4936770/ rs4802/ rs10892958/ rs11218941/ rs2236658)	CAD 2n=542, (%)	Controls 2n=226 (%)	OR (95% CI)	*P* value
TGGGC	176(32.4)	52(23.0)	1.609 [1.124~2.302]	0.008*
CACAT	213(39.2)	102(45.1)	0.787 [0.575~1.076]	0.134
TGCAT	68(12.5)	37(16.3)	0.732 [0.474~1.131]	0.159
TAGGC	40(7.3)	20(8.8)	0.82 [0.468~1.437]	0.489
TGGGT	41(7.5)	12(5.3)	1.459 [0.752~2.831]	0.261

6 CAD, coronary artery disease; CI, confidence interval; OR, odds ratio. Haplotypes were omitted

7 if the estimated haplotype frequency was <3%.

8 * *P*<0.05

Table 6 Genotypic Distribution of 17 HSP polymorphisms among the CAD with depression group (n=123) and CAD without depression group (n=148)

CAD+D: CAD with depression, CAD-D: CAD without depression [a]*P*value for genotype frequencies in CAD+D and CAD-D using 2-sidedχ^2 test. [b]*P*values adjusted by smoking and drinking habit, hypertension, diabetes mellitus, insomnia and stroke history using binary logistic regression.

1 Table 6 Genotypic Distribution of 17 HSP polymorphisms among the CAD with depression
2 group (n=123) and CAD without depression group (n=148)

SNP	Genotype	CAD+D,(%)	CAD-D,(%)	P value[a] (χ^2)	P value[b]	OR (95% CI)
rs2763979	CC	53(43.1)	51(34.5)	0.347(2.118)	Referent	
	CT	51(41.5)	71(48.0)		0.169	0.691(0.408-1.170)
	TT	19(15.4)	26(17.6)		0.328	0.703(0.347-1.424)
	CT+TT	70(56.9)	97(65.5)	0.146(2.115)	0.146	0.694(0.424-1.136)
rs2075800	CC	54(43.9)	58(39.2)	0.093(4.742)	Referent	1.00
	CT	50(40.7)	77(52.0)		0.169	0.697(0.417-1.166)
	TT	19(15.4)	13(8.8)		0.267	1.570(0.708-3.483)
	CT+TT	69(56.1)	90(60.8)	0.433(0.615)	0.433	0.823(0.507-1.338)
rs1380154	CC	41(33.3)	37(25.0)	0.102(4.562)	Referent	1.00
	CT	58(47.2)	89(60.1)		0.060	0.588(0.338-1.023)
	TT	24(19.5)	22(14.9)		0.966	0.984(0.475-2.042)
	CT+TT	82(66.7)	111(75.0)	0.131(2.276)	0.132	0.667(0.393-1.131)
rs391957	CC	49(39.8)	72(48.6)	0.241(2.848)	Referent	1.00
	CT	65(52.8)	63(42.6)		0.104	1.516(0.918-2.504)
	TT	9(7.3)	13(8.8)		0.971	1.017(0.404-2.563)
	CT+TT	74(60.2)	76(51.4)	0.146(2.110)	0.147	1.431(0.882-2.321)
rs17840761	AA	21(17.1)	36(24.3)	0.333(2.199)	Referent	1.00
	AG	73(59.3)	82(55.4)		0.184	1.526(0.818-2.848)
	GG	29(23.6)	30(20.3)		0.182	1.657(0.789-3.479)
	AG+GG	102(82.9)	112(75.7)	0.145(2.126)	0.147	1.561(0.856-2.849)
rs11218941	AA	36(29.3)	39(26.4)	0.328(2.230)	Referent	1.00
	AG	64(52.0)	70(47.3)		0.974	0.990(0.563-1.744)
	GG	23(18.7)	39(26.4)		0.201	0.639(0.322-1.269)
	AG+GG	87(70.7)	109(73.6)	0.593(0.286)	0.593	0.865(0.507-1.474)
rs10892958	GG	23(18.7)	39(26.4)	0.328(2.230)	Referent	1.00
	GC	64(52.0)	70(47.3)		0.164	1.550(0.837-2.873)
	CC	36(29.3)	39(26.4)		0.201	1.565(0.788-3.108)
	GC+CC	100(81.3)	109(73.6)	0.135(2.229)	0.137	1.556(0.869-2.785)
rs2236658	CC	17(13.8)	28(18.9)	0.381(1.930)	Referent	1.00
	CT	64(52.0)	66(44.6)		0.186	1.597(0.798-3.196)
	TT	42(34.1)	54(36.5)		0.503	1.281(0.620-2.645)
	CT+TT	106(86.2)	120(81.1)	0.262(1.261)	0.263	1.455(0.754-2.806)
rs4802	AA	43(35.0)	40(27.0)	0.326(2.245)	Referent	1.00
	AG	37(30.1)	54(36.5)		0.141	0.637(0.350-1.162)
	GG	43(35.0)	54(36.5)		0.317	0.741(0.411-1.334)
	AG+GG	80(65.0)	108(73.0)	0.158(1.989)	0.159	0.689(0.410-1.157)
rs4936770	TT	43(35.0)	55(37.2)	0.410(1.782)	Referent	1.00
	TC	56(45.5)	73(49.3)		0.944	0.981(0.578-1.666)

	CC	24(19.5)	20(13.5)		0.240	1.535(0.751-3.138)
	TC+CC	80(65.0)	93(62.8)	0.707(0.141)	0.707	1.100(0.668-1.811)
rs1042665	CC	80(65.0)	84(56.8)	0.246(2.806)	Referent	1.00
	CT	39(31.7)	54(36.5)		0.291	0.758(0.454-1.267)
	TT	4(3.3)	10(6.8)		0.156	0.420(0.127-1.393)
	CT+TT	43(35.0)	64(43.2)	0.165(1.929)	0.165	0.705(0.431-1.155)
rs10873531	AA	74(60.2)	91(61.5)	0.702(0.709)	Referent	1.00
	AG	46(37.4)	51(34.5)		0.686	1.109(0.671-1.834)
	GG	3(2.4)	6(4.1)		0.502	0.615(0.149-2.542)
	AG+GG	49(39.8)	57(38.5)	0.824(0.049)	0.824	1.057(0.648-1.725)
rs8005905	AA	84(68.3)	102(68.9)	0.877(0.263)	Referent	1.00
	AT	36(29.3)	41(27.7)		0.814	1.066(0.626-1.816)
	TT	3(2.4)	5(3.4)		0.671	0.729(0.169-3.138)
	AT+TT	39(31.7)	46(31.1)	0.912(0.012)	0.912	1.030(0.615-1.723)
rs1165678	AA	83(67.5)	108(73.0)	0.614(0.974)	Referent	1.00
	AG	36(29.3)	36(24.3)		0.342	1.301(0.756-2.240)
	GG	4(3.3)	4(2.7)		0.715	1.301(0.316-5.357)
	AG+GG	40(32.5)	40(27.0)	0.324(0.974)	0.324	1.301(0.771-2.196)
rs1165681	CC	31(25.2)	46(31.1)	0.466(1.526)	Referent	1.00
	CT	66(53.7)	69(46.6)		0.226	1.419(0.805-2.502)
	TT	26(21.1)	33(22.3)		0.656	1.169(0.588-2.323)
	CT+TT	92(74.8)	102(68.9)	0.285(1.141)	0.286	1.338(0.783-2.287)
rs1882019	GG	60(48.8)	71(48.0)	0.753(0.568)	Referent	1.00
	GA	53(43.1)	61(41.2)		0.914	1.028(0.621-1.701)
	AA	10(8.1)	16(10.8)		0.493	0.740(0.312-1.751)
	GA+AA	63(51.2)	77(52.0)	0.895(0.018)	0.895	0.968(0.600-1.562)
rs3794241	CC	78(63.4)	88(59.5)	0.697(0.722)	Referent	1.00
	CT	39(31.7)	54(36.5)		0.433	0.815(0.488-1.360)
	TT	6(4.9)	6(4.1)		0.840	1.128(0.349-3.642)
	CT+TT	45(36.6)	60(40.5)	0.506(0.443)	0.506	0.846(0.517-1.384)

5 CAD+D: CAD with depression, CAD-D: CAD without depression

6 [a]Pvalue for genotype frequencies in CAD+D and CAD-D using 2-sidedχ^2 test.

7 [b]Pvalues adjusted by smoking and drinking habit, hypertension, diabetes mellitus, insomnia and

8 stroke history using binary logistic regression.

Table 7 Genotypic Distribution of 17 HSP polymorphisms among the CAD with anxiety group (n=57) and CAD without anxiety group (n=214)

CAD+A: CAD with anxiety, CAD-A: CAD without anxiety [a]Pvalue for genotype frequencies in

CAD+A and CAD-A using 2-sidedχ^2 test. [b]Pvalues adjusted by smoking and drinking habit,

hypertension, diabetes mellitus, insomnia and stroke history using binary logistic regression.

* Significant difference (P˂0.05)

1   Table 7 Genotypic Distribution of 17 HSP polymorphisms among the CAD with anxiety group
2   (n=57) and CAD without anxiety group (n=214)

SNP	Genotype	CAD+A,(%)	CAD-A,(%)	P value^a (χ^2)	P value^b	OR (95% CI)
rs2763979	CC	25(43.9)	79(36.9)	0.190(3.321)	Referent	1.00
	CT	27(47.4)	95(44.4)		0.734	0.898(0.483-1.670)
	TT	5(8.8)	40(18.7)		0.078	0.395(0.141-1.109)
	CT+TT	32(56.1)	135(63.1)	0.338(0.918)	0.339	0.749(0.414-1.354)
rs2075800	CC	20(35.1)	92(43.0)	0.438(1.652)	Referent	1.00
	CT	31(54.4)	96(44.9)		0.219	1.485(0.791-2.791)
	TT	6(10.5)	26(12.1)		0.908	1.062(0.386-2.917)
	CT+TT	37(64.9)	122(57.0)	0.282(1.159)	0.283	1.395(0.760-2.561)
rs1380154	CC	17(29.8)	61(28.5)	0.963(0.076)	Referent	1.00
	CT	30(52.6)	117(54.7)		0.808	0.920(0.470-1.799)
	TT	10(17.5)	36(16.8)		0.994	0.997(0.412-2.410)
	CT+TT	40(70.2)	153(71.5)	0.845(0.038)	0.845	0.938(0.494-1.780)
rs391957	CC	26(45.6)	95(44.4)	0.955(0.093)	Referent	1.00
	CT	26(45.6)	102(47.7)		0.820	0.931(0.505-1.716)
	TT	5(8.8)	17(7.9)		0.897	1.075(0.362-3.188)
	CT+TT	31(54.4)	119(55.6)	0.869(0.027)	0.869	0.952(0.529-1.712)
rs17840761	AA	5(8.8)	52(24.3)	0.038(6.550)*	Referent	1.00
	AG	38(66.7)	117(54.7)		0.016*	3.378(1.258-9.072)
	GG	14(24.6)	45(21.0)		0.036*	3.236(1.081-9.684)
	AG+GG	52(91.2)	162(75.7)	0.011(6.534)*	0.015*	3.338(1.266-8.801)
rs11218941	AA	15(26.3)	60(28.0)	0.449(1.600)	Referent	1.00
	AG	32(56.1)	102(47.7)		0.520	1.255(0.629-2.505)
	GG	10(17.5)	52(24.3)		0.560	0.769(0.318-1.858)
	AG+GG	42(73.7)	154(72.0)	0.796(0.067)	0.796	1.091(0.563-2.112)
rs10892958	GG	10(17.5)	52(24.3)	0.449(1.600)	Referent	1.00
	GC	32(56.1)	102(47.7)		0.222	1.631(0.744-3.575)
	CC	15(26.3)	60(28.0)		0.560	1.300(0.538-3.141)
	GC+CC	47(82.5)	162(75.7)	0.281(1.164)	0.283	1.509(0.712-3.196)
rs2236658	CC	8(14.0)	37(17.3)	0.704(0.703)	Referent	1.00
	CT	30(52.6)	100(46.7)		0.459	1.387(0.583-3.300)
	TT	19(33.3)	77(36.0)		0.777	1.141(0.457-2.848)
	CT+TT	49(86.0)	177(82.7)	0.557(0.344)	0.558	1.280(0.560-2.928)
rs4802	AA	16(28.1)	67(31.3)	0.296(2.435)	Referent	1.00
	AG	24(42.1)	67(31.3)		0.268	1.500(0.732-3.074)
	GG	17(29.8)	80(37.4)		0.762	0.890(0.418-1.895)
	AG+GG	41(71.9)	147(68.7)	0.637(0.222)	0.638	1.168(0.612-2.228)
rs4936770	TT	17(29.8)	81(37.9)	0.346(2.123)	Referent	1.00
	TC	32(56.1)	97(45.3)		0.178	1.572(0.814-3.035)

		CAD+A	CAD-A	P^a value(χ^2)	P^b value	OR(95%CI)
	CC	8(14.0)	36(16.8)		0.904	1.059(0.419-2.677)
	TC+CC	40(70.2)	133(62.1)	0.262(1.256)	0.264	1.433(0.762-2.694)
rs1042665	CC	45(78.9)	119(55.6)	0.005(10.423)*	Referent	1.00
	CT	11(19.3)	82(38.3)		0.005*	0.355(0.173-0.726)
	TT	1(1.8)	13(6.1)		0.130	0.203(0.026-1.600)
	CT+TT	12(21.1)	95(44.4)	0.001(10.262)*	0.002*	0.334(0.167-0.667)
rs10873531	AA	41(71.9)	124(57.9)	0.150(3.791)	Referent	1.00
	AG	15(26.3)	82(38.3)		0.076	0.553(0.288-1.064)
	GG	1(1.8)	8(3.7)		0.366	0.378(0.046-3.114)
	AG+GG	16(28.1)	90(42.1)	0.055(3.697)	0.057	0.538(0.284-1.018)
rs8005905	AA	42(73.7)	144(67.3)	0.608(0.996)	Referent	1.00
	AT	14(24.6)	63(29.4)		0.429	0.762(0.389-1.494)
	TT	1(1.8)	7(3.3)		0.510	0.490(0.059-4.094)
	AT+TT	15(26.3)	70(32.7)	0.355(0.855)	0.356	0.735(0.382-1.414)
rs1165678	AA	39(68.4)	152(71.0)	0.914(0.180)	Referent	1.00
	AG	16(28.1)	56(26.2)		0.749	1.114(0.577-2.149)
	GG	2(3.5)	6(2.8)		0.754	1.299(0.252-6.687)
	AG+GG	18(31.6)	62(29.0)	0.701(0.147)	0.701	1.132(0.602-2.128)
rs1165681	CC	10(17.5)	67(31.3)	0.032(6.896)*	Referent	1.00
	CT	37(64.9)	98(45.8)		0.017*	2.530(1.178-5.434)
	TT	10(17.5)	49(22.9)		0.519	1.367(0.528-3.538)
	CT+TT	47(82.5)	147(68.7)	0.041(4.193)*	0.044*	2.142(1.021-4.495)
rs1882019	GG	25(43.9)	106(49.5)	0.748(0.581)	Referent	1.00
	GA	26(45.6)	88(41.1)		0.474	1.253(0.676-2.323)
	AA	6(10.5)	20(9.3)		0.641	1.272(0.463-3.496)
	GA+AA	32(56.1)	108(50.5)	0.446(0.580)	0.447	1.256(0.698-2.261)
rs3794241	CC	37(64.9)	129(60.3)	0.797(0.453)	Referent	1.00
	CT	18(31.6)	75(35.0)		0.580	0.837(0.445-1.573)
	TT	2(3.5)	10(4.7)		0.651	0.697(0.146-3.324)
	CT+TT	20(35.1)	85(39.7)	0.524(0.407)	0.524	0.820(0.446-1.508)

4 CAD+A: CAD with anxiety, CAD-A: CAD without anxiety

5 [a]Pvalue for genotype frequencies in CAD+A and CAD-A using 2-sidedχ^2 test.

6 [b]Pvalues adjusted by smoking and drinking habit, hypertension, diabetes mellitus, insomnia and

7 stroke history using binary logistic regression.

8 * Significant difference (P<0.05)

Figure 1

Linkage disequilibrium pattern between five SNPs, rs4936770, rs4802, rs10892958, rs11218941 and rs2236658, in CAD patients and healthy controls.
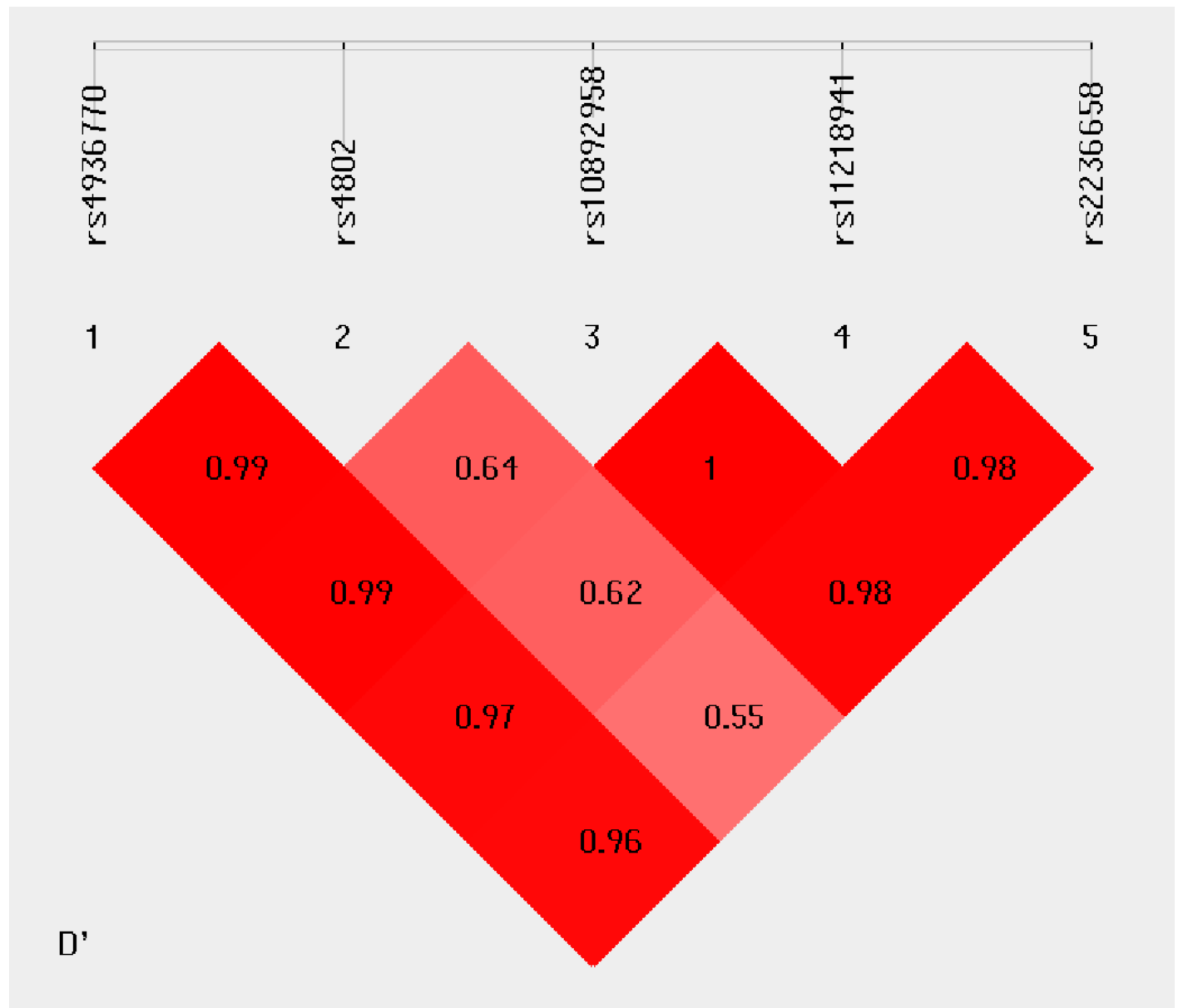
Figure 2

Association of HSP polymorphisms and GAD-7 scores in CAD patients with comorbid anxiety.

(A) rs178040761 (B) rs1042665 and (C) rs1165681 . GAD-7, Generalized Anxiety Disorder -7.