Considerable impacts of AGXT2 V140I polymorphism on chronic heart failure in the Chinese population

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Abstract

Background and aims: Alanine-glyoxylate aminotransferase 2 (*AGXT2*) polymorphisms have been extensively studied to be associated with many cardiovascular diseases, with the exception of chronic heart failure (CHF). The aim of this study was to determine whether the *AGXT2* rs37369 (V140I) polymorphism is associated with risk for and prognosis of CHF in Chinese patients.

Methods: 1000 CHF patients and 1200 healthy controls were recruited and polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP) was used to determine the genotypes of rs37369 polymorphism. Tube formation assay and transwell migration assay were performed to assess the effects of asymmetric dimethylarginine (ADMA) and to explore the significance of rs37369 polymorphism in the pathogenesis of CHF. 140 CHF patients underwent a median follow-up of 38.7 months by telephone.

Results: The rs37369 GG genotype was significantly over-represented in CHF patients compared to controls (18.9% vs 14.7%, $p=0.009$) and was significantly associated with increased risk of CHF ($p=0.030$), especially in patients with hypertension ($p=0.021$). Besides, the rs37369 GG genotype marginally increased the risk for CHF in smokers. ADMA stimulated migration and inhibited tube formation of cultured human umbilical vein endothelial cells (HUVECs). Overexpression of *AGXT2* with pc*AGXT2*-rs37369-A or G plasmid reversed ADMA-induced HUVECs migration and tube formation. *AGXT2* rs37369-A showed increased ADMA degradation activity and marginally prolonged the lifetime of CHF patients.

Conclusions: ADMA might accelerate the progression of CHF possibly by inhibiting angiogenesis and promoting migration of HUVECs. *AGXT2* rs37369 polymorphism is associated with increased risk for CHF, which may due to distinct disparities of alleles in ADMA degradation.

Keywords

Alanine-glyoxylate aminotransferase 2 (*AGXT2*); Chronic heart failure (CHF); Asymmetric dimethylarginine (ADMA); Polymorphism; Prognosis.

1. Introduction

Heart failure (HF) is becoming a global public health problem, which seriously threatens human health. The incidence of heart failure approaches 10 per 1000 subjects after 65 years of age and the prevalence of heart failure is supposed to increase of 46% from 2012 to 2030, which will cause an enormous social and economic burden [1, 2]. Chronic heart failure (CHF) is a relatively stable state of HF defined as abnormality in cardiac structure or function leading to failure of the heart to deliver oxygen at a rate commensurated with the requirements of the metabolizing tissues and it is characterized by ventricular remodeling and myocardial hypertrophy, impairment of active dilatation and contraction of the ventricle, as well as likely reduced cardiac ejection fraction [3-6].

Cardiac function is angiogenesis dependent. Disruption of angiogenesis and coordinated tissue growth in the heart contribute to the progression from adaptive cardiac hypertrophy to CHF [7]. It is well established that vascular endothelial growth factor (VEGF) is one of the most important angiogenic factors [8-10]. Nitric oxide (NO) is critically involved in VEGF mediated angiogenesis [11, 12] participates in the control of contractility and heart rate, limits the deleterious effects of cardiac remodeling after myocardial infarction and contributes to the protective effect of cardiac function [13-15]. Asymmetric dimethylarginine (ADMA), an endogenous nitric oxide synthase (NOS) inhibitor, which can competitively obstruct NO synthesis, has been regarded as an independent cardiovascular risk factor [16-19]. Plasma levels of ADMA are elevated in patients with CHF and positively associated with disease

severity [20, 21]. ADMA can also enhance cardiovascular risk prediction in CHF patients [22] and compromise therapeutic effect of anti-CHF drugs [23]. Besides, cardiac remodeling is one of the main manifestations of CHF and the cardiac fibroblasts exhibit increased migration in the remodeling heart [24]. A recent clinical study showed a positive correlation between plasma ADMA and right ventricular mass, pericardial fluid ADMA level and end-diastolic and systolic diameter of left ventricle, indicating an important role of ADMA in cardiac remodeling [25]. Considering the above, we speculate that ADMA may contribute to the development of CHF via inhibition of angiogenesis and promotion of migration.

Data from recent researches indicate that genetic factors are closely associated with the development and prognosis of CHF. According to results of case-control studies of candidate genes, multiple genetic polymorphisms in genes involved in nervous and humoral regulation, such as *ADRB1* (β 1-adrenergic receptor), *GRK5* (G-protein receptor kinase 5), *ACE* (angiotensin-converting enzyme), are associated with the risk for or prognosis of CHF [26-28]. Besides, a GWAS study showed that two single nucleotide polymorphisms (SNPs) loci at chromosomal position 15q22 and 12q14, respectively, were associated with the risk of CHF in European ancestry and African ancestry [29]. Therefore, it is of important and practical significance in the early screening of high-risk individuals and to clarify the pathogenesis of CHF, to further identify the potential genes predictive of CHF risk or prognosis.

Owing to the critical role of ADMA in the progression and prognosis of CHF and the important role of genetic factors in CHF, we speculated that genetic variants in

ADMA metabolic enzyme genes may affect the susceptibility and prognosis of CHF by influencing ADMA levels. Alanine-glyoxylate aminotransferase 2 (AGXT2), a mitochondrial aminotransferase primarily expressed in the kidney, was observed to play important role in ADMA metabolism [30-32]. Data from the International Consortium for BP Genome-Wide Studies indicated that *AGXT2* Val140Ile (rs37369) polymorphism was associated with diastolic blood pressure [31]. Rs37369 polymorphism also showed a strong association with urine BAIB level in a genome-wide association study (GWAS) for metabolic traits [33]. Furthermore, a recent GWAS revealed that rs37369 polymorphism was associated with heart rate variability, a risk factor of CHF [34, 35]. Coincidentally, we have previously reported that rs37369 polymorphism was associated with increased risk for coronary heart disease (CHD) in the Chinese population [36]. The polymorphism might indicate a potential significance in cardiovascular system and we aimed to explore the association between rs37369 polymorphism and CHF in the present study.

2. Materials and methods

2.1 Study population

We recruited 1000 consecutive Chinese Han patients with CHF who were admitted to the Xiangya Hospital (Changsha, China) between December 2011 and March 2015. All patients had New York Heart Association (NYHA) functional class (II-IV) and a history of CHF for at least 3 months. Exclusion criteria: 1) age less than 18 years or presence of congenital heart disease; 2) patients with advanced renal failure and severe liver disease; 3) any other diagnosis of any life-threatening disease. 1200 gender- and age-matched subjects, who attended routine health screenings in the outpatient clinics of the same hospital, were enrolled as the healthy controls and all the controls were free from histories of hypertension (systolic blood pressure above 140 mmHg and/or diastolic blood pressure above 90 mmHg, or history of anti-hypertension therapy), CHD (luminal stenosis $\geq 50\%$ in at least one major coronary artery branch or myocardial infarction, or history of anti-angina therapy), diabetes mellitus [DM, fasting plasma glucose (FPG) ≥ 7.0 mmol/L or history of DM therapy] and any other cardiovascular diseases (CVDs). Case report forms (CRF) were used to collect information including age, gender, cigarette smoking, and alcohol drinking. A similar questionnaire was also obtained from the controls. Smokers were defined as cigarette smoking ≥ 540 cigarettes/year. The investigation conforms with the principles outlined in the Declaration of Helsinki and was approved by the Ethics Committee of the School of Pharmaceutical Sciences, Central South University, Changsha and had registered on <http://www.chictr.org.cn/> (registration number:

ChiCTR-RCC-12002817). Written consents have been assigned by each of the patients or their relatives.

2.2 Genotyping of AGXT2 polymorphisms

Venous blood samples were drawn from all participants and hemocytes were separated by centrifugation (4°C, 3000 rpm, and 10 minutes) for DNA extraction. Genomic DNA was isolated using phenol-chloroform extraction method. Including rs37369 (Val140Ile) polymorphism, in total, three exonic SNPs at the *AGXT2* gene locus were selected and genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay, as previously described [36]. The other two SNPs were rs180749 (Ile212Thr) and rs466067 (Gly435Gly) polymorphisms, which were predicted to be of potential significance [30].

2.3 Constructions of human AGXT2 rs37369 overexpression plasmid

Human *AGXT2* cDNA was cloned from the kidney cDNA which was reversed transcribed from human renal tissue total RNA (Takara, Dalian, China). The *AGXT2* cDNA sequence was identical to the GenBank sequence (NM_031900) with the exception of SNP rs37369. The amplified fragment was inserted in the direct orientation into the pcDNA3.1(-) vector *via* PCR Cloning Kit (Promega, United States), according to the manufacturer's instructions. QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent Technologies, United States) was used to construct the mutant *AGXT2* rs37369 plasmid and *AGXT2* rs37369 A and G plasmids were verified by sequencing (Biosune, Changsha, China).

The empty vector pcDNA3.1(-) and two cloned plasmids pcAGXT2-rs37369-A

and pcAGXT2-rs37369-G were transferred into HUVEC-C cells using Lipofectamine® 2000 Transfection Reagent (Thermo Fisher Scientific, United States) according to the manufacturer's instructions. The cells were harvested to extract total RNA and total protein using Trizol (Takara, Dalian, China) and RIPA buffer (Beyotime, China) with 1 mM PMSF (Beyotime, China), respectively. Real-time PCR (Takara, Dalian, China) and immunoblotting were carried out to analyze the expression of AGXT2 mRNA and protein, respectively. Rabbit polyclonal anti-human AGXT2 antibody was purchased from Sigma-Aldrich (HPA037382) with a dilution of 1:1000.

Primers used in this study were shown in Supplemental Table 1.

2.4 Determination of ADMA metabolic activity in HUVECs cell lysate

HUVEC-C cell lysate was used to measure ADMA metabolic activity of AGXT2 after overexpression with AGXT2-rs37369-A (140Ile) and AGXT2-rs37369-G (140Val) plasmid. A reaction mixture containing 1 mM ADMA, 10 mM sodium glyoxylate, 10 μ M pyridoxal phosphate and cell lysate containing 60 μ g total protein was incubated at 37 °C for 120 min. Samples were boiled to stop the reaction. Concentration of ADMA in the mixture was measured by HPLC-tandem mass spectrometry (LCMS-2010 quadrupole mass spectrometer [Shimadzu, Kyoto, Japan]). The mobile phase containing 20 mM acetic ammonia (pH3.9) -methanol (95:5, v/v) was used at a flow rate of 0.2 mL/min. For the quantification of ADMA, the positive protonated molecule ions of ADMA at $m/z = 203$ $[M+H]^+$.

2.5 Tube formation assay

24 well plates were coated with precooled Matrigel (BD Biosciences, 100 μ l/well) on ice and incubated at 37°C in humidified atmosphere with 5% CO₂ for 30 minutes to allow gelation. HUVECs were added to the surface of the gel at a density of 15,000 cells/well in the presence or absence of ADMA (30 μ M). The control wells included the same amount of solvation vehicle (steriled ddH₂O) as the experimental condition. Cells were incubated for 6 hours and pictures were captured with a Nikon inverted microscope (Olympus, Japan). Images from 4 randomly selected fields were analyzed and total tubule length was calculated by using the Image J Software.

2.6 Transwell migration assay

BD BioCoat transwell chambers (BD Biosciences, Bedford, MA, USA) with 8- μ M pore size PET membrane were inserted in 24-well plates according to the manufacturer instructions. HUVECs were allowed to grow near to full confluence and then starved with cell medium containing 1% FBS overnight. 40,000 cells in serum free medium (DMEM) were plated in the upper well of the transwell chambers, whereas medium supplemented with 10% FBS was added to the lower chamber as the chemoattractant. Following incubation at 37°C with 5% CO₂ for 24 hours, cells on the upper side of the inserts were removed using a cotton swab. The inserts were fixed in 75% alcohol and stained with 0.1% crystal violet (Beyotime, China). The migrated cells in the lower surface were imaged with a Nikon inverted microscope. The stained crystal violet was eluted with 33% acetic acid and the OD value of eluent was measured at 570 nm to indicate degree of cell migration.

2.7 Statistical analysis

Statistical analysis was carried out by the software SPSS version 18.0 (IBM corporation, Armonk, NY, USA). Hardy–Weinberg equilibrium and differences in proportions were tested using the chi-square test. Linkage disequilibrium (LD) among three SNPs (rs37369, rs180749 and rs466067 polymorphisms) and haplotype association test under permutation test were analyzed by Haploview software. Differences in the baseline characteristics between CHF cases and controls were compared by t-test or ANOVA for continuous variables and by the Chi-square test for categorical variables. Association of the genotypes with risk for disease susceptibility and prognosis of CHF was analyzed by logistic regression and Kaplan-Meier survival analysis, respectively. A two-tailed $p < 0.05$ was considered significant.

3. Results

3.1 General characteristics of study population

The general characteristics of CHF patients and controls are summarized in Table 1. As compared with controls, CHF patients showed significantly higher systolic blood pressure (SBP), diastolic blood pressure (DBP), serum low-density lipoprotein levels, and prevalence of smoking. No significant difference in gender, age, serum total cholesterol and triglyceride, and history of alcohol consumption was observed between cases and controls.

3.2 AGXT2 rs37369 polymorphism associated with the risk of CHF

All SNPs investigated in both case and control samples followed Hardy–Weinberg equilibrium. Frequency of rs37369 GG genotype was over-represented in CHF patients (Table 2). Logistic regression analysis showed that the rs37369 GG genotype was associated with risk for CHF when adjusted for cardiovascular risk factors including gender, age, smoking status, history of alcohol drinking (Table 2). No difference in genotype distribution of rs180749 (Ile212Thr) and rs466067 (Gly435Gly) polymorphisms was observed between cases and controls (Supplemental Table 2). Results of linkage disequilibrium analysis showed low LD between AGXT2 rs37369 and rs180749/rs466067 polymorphisms ($r^2=0.056$ and 0.064 , respectively, Supplemental Figure 1). Besides, we observed strong LD between rs466067 and rs180749 polymorphisms ($r^2=0.843$, Supplemental Figure 1), but negative results were obtained from the haplotype association analysis (Supplemental Table 3).

In consideration that smoking was a risk factor for cardiovascular diseases while

CHD and hypertension were the two main risk factors for heart failure [37], a series of association analyses stratified by smoking status and status of accompanied disease (CHD or hypertension) were carried out. Carriers of the rs37369 GG genotype showed marginally increased risk for CHF in cases without CHD (corrected $p=0.054$) and smokers (corrected $p=0.108$, Table 2). While, in patients with hypertension, rs37369 GG genotype was associated with increased risk of CHF significantly (corrected $p=0.021$, Table 2).

3.3 Influence of rs37369 polymorphism on ADMA metabolism

To determine the function of AGXT2 rs37369 polymorphism, pcDNA3.1(-) plasmid carrying the AGXT2 rs37369-A and G alleles were constructed (Figure 1A). The plasmids were transferred into HUVEC-C cells, expression of human AGXT2 mRNA and protein was confirmed by real-time quantitative PCR and immunoblotting, respectively (Figure 1B, C). No difference in mRNA and protein expression between the plasmids was observed. Activity of AGXT2 was indicated by ADMA metabolic activity of the cell lysates transfected with pcAGXT2-rs37369-A or pcAGXT2-rs37369-G plasmid. Cell lysates overexpressing both AGXT2-rs37369-A (140Ile) and AGXT2-rs37369-G (140Val) significantly degraded ADMA ($p<0.001$, respectively, compared with control), while AGXT2-rs37369-A showed significantly higher ADMA metabolic activity than AGXT2-rs37369-G ($p<0.01$, Figure 1D).

3.4 Influence of rs37369 polymorphism on HUVECs migration and angiogenesis

To determine whether ADMA is capable of influencing the migration and angiogenesis ability of HUVECs, assays of transwell migration and tube formation

were performed. As compared with the control group, the relative migratory ratio of HUVECs increased by approximately 200% after treatment with 30 μ M ADMA (Figure 2A, B), while the tube formation ability of HUVECs was reduced significantly by 30 μ M ADMA pre-treatment (Figure 2C, 2D).

AGXT2 rs37369-A and AGXT2 rs37369-G plasmids were also transfected into HUVEC-C cells to observe the effect of AGXT2 rs37369 polymorphism on cell migration and the ability of angiogenesis. Migration of HUVECs was reduced by 46.2% and 32.4%, respectively, following the transfection of AGXT2 rs37369-A and AGXT2 rs37369-G plasmids after ADMA treatment as compared with ADMA treatment alone group (Figure 2A, B). AGXT2-rs37369-A transfected cells displayed a more significant decrease in ADMA-induced HUVECs migration (Figure 2A, B). The ability of tube formation destroyed by ADMA treatment could be reversed significantly by transfection of both AGXT2-rs37369-A and the AGXT2-rs37369-G plasmid (Figure 2C, D). However, total tubule length was significantly lower in AGXT2-rs37369-G transfected cells as compared with the AGXT2-rs37369-A transfected cells.

3.5 Influence of rs37369 polymorphism on the prognosis of CHF

To determine the association between rs37369 polymorphism and the prognosis of CHF, 140 CHF patients undergoing a median follow-up of 38.7 months after diagnosis were enrolled. The patients were followed-up by telephone. The results showed that New York Heart Association (NYHA) functional classification was significantly associated with the prognosis of CHF ($p < 0.001$, Figure 3A), while carriers of the

rs37369 A allele showed marginally prolonged lifetime as compared with rs37369 GG genotype ($p=0.092$, Figure 3B).

4. Discussion

In this study, we investigated the association of *AGXT2* rs37369 polymorphism with risk for and prognosis of CHF and revealed the functional role of this polymorphism in ADMA metabolism and endothelial angiogenesis. Data from 1000 CHF patients and 1200 healthy controls in our study provides strong evidence that *AGXT2* V140I polymorphism (rs37369) is associated with increased risk for CHF. The frequency of rs37369 GG genotype was over-represented in CHF patients and individuals with rs37369 GG genotype showed 35% (95% CI: 8%~71%) increase in risk for CHF. We also observed that the rs37369 GG genotype was marginally associated with increased risk for CHF in smokers. Our previous study showed that smokers carrying rs37369 GG genotype had increased plasma ADMA levels compared with non-smokers of the same genotype [36]. Elevated plasma ADMA levels were positively associated with disease severity and cardiovascular risk of CHF, which suggested that smoking may increase the probabilities of suffering from CHF. Considering CHD and hypertension were the main causes of CHF, association analyses stratified by disease state of CHD/hypertension were performed. The results indicated that carriers of rs37369 GG genotype showed significantly increased risk of CHF in patients with hypertension, suggesting an undiscovered function of *AGXT2* in the regulation of blood pressure, especially in the progression from hypertension to CHF. Apart from the rs37369 polymorphism, we also investigated two other *AGXT2* SNPs (rs180749 and rs466067), but failed to obtain any positive findings. (Supplemental Table 2).

Our study also provides evidence that ADMA could inhibit HUVEC angiogenesis, which is consistent with the elevated plasma level of ADMA in CHF [24, 38, 39]. The increase in cell migration and decrease in tubule formation induced by ADMA on HUVECs could be partially reversed by AGXT2-rs37369-A or AGXT2-rs37369-G plasmids. In CHF patients, ADMA might contribute to the progression of CHF by promoting migration and inhibiting angiogenesis in myocardial tissue. Compared with AGXT2 140Ile (rs37369-A), AGXT2 140Val (rs37369-G) showed significantly decreased ADMA metabolic activity, which helped explain the clinical findings that the rs37369 GG genotype was associated with increased risk for CHF in our study. One of the most remarkable findings of this study is the AGXT2 rs37369 polymorphism–ADMA pathway in the regulation of cardiac remodeling and CHF (Figure 4).

Despite the advancement in drug therapies, aggregate 5-year mortality rates of CHF remain high at approximately 50%, which might keep up with or even exceed that of many cancers [40]. Therefore, identification of novel prognostic biomarkers for CHF is of great significance. Through a follow-up study, we found that NYHA functional classification was significantly associated with the prognosis of CHF, which indicated that the patients involved in our study were appropriate and the results credible. Elevated plasma ADMA levels might be associated with higher NYHA functional classes and appeared to be an independent predictor of adverse clinical outcomes. As AGXT2 rs37369 polymorphism affects ADMA metabolism, we hypothesized that the rs37369 polymorphism may influence the prognosis of CHF. Our results indicated

that carriers of rs37369 GG genotype showed a worse prognosis, though only marginal statistical difference ($p=0.092$) was observed due to limited sample size. CHF is a complex clinical syndrome that can result from any functional or structural cardiac disorder which impairs the ability of the ventricle to fill or eject blood. Heart bypass surgery, coronary interventional therapy, pharmacotherapy or even mental factors could make contributions to the prognosis of CHF patients more or less. Any single factor may not explain the outcomes of CHF patients very well. Herein, we provide a new indicator that might affect the prognosis of CHF from the genetics perspective by means of ADMA metabolism.

Besides, it is interesting that the major allele of rs37369 polymorphism showed ethnic difference. Data from 1000 Genomes Project (http://browser.1000genomes.org/Homo_sapiens/Variation/Population?db=core;r=5:35036615-35037615;v=rs37369;vdb=variation;vf=28008) shows that the major allele of rs37369 is G in Americans of African Ancestry (ASW), but is A in Han Chinese (CHB), which indicates that the polymorphism might contribute to ethnic differences in genetic susceptibility to a certain disease. This situation is very common and further efforts are needed to reveal the clinical relevance of *AGXT2* rs37369 polymorphism in populations with different ethnic background.

At the same time, we must acknowledge some limitations in our study. First, the method of PCR-RFLP is time-consuming and our results were obtained from one population with limited sample size, multicenter studies are needed to establish the clinical significance of *AGXT2* rs37369 polymorphism in CHF. Second, the sample

size and the time period of follow-up are limited in the follow-up analysis, further studies with larger sample size and longer period of follow-up are warranted to validate the clinical significance of the SNP.

In summary, we are the first to reveal the functional mechanism of ADMA in the pathogenesis of CHF from the perspective of angiogenesis and HUVECs migration and we observed the influence of *AGXT2* rs37369 polymorphism on CHF susceptibility and prognosis. ADMA may contribute to the development of CHF by inhibiting angiogenesis and promoting migration. *AGXT2* rs37369 polymorphism is significantly associated with the risk of CHF, which could be explained by decreased ADMA metabolic activity. Thus, we provide a new target for therapeutic and prevention strategies of CHF. However, further studies in different populations and a larger population size are needed to confirm the clinical significance.

Conflict of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Figure 1

Figure 2

Figure 3

Figure 4

Figure legends

Fig. 1. Overexpression and activity assay of pcAGXT2-rs37369-A and G vectors.

(A) Diagram of the constructed vectors pcAGXT2-rs37369-A and pcAGXT2-rs37369-G. (B) Overexpression of human *AGXT2* mRNA in HUVEC transfected with different plasmid constructs. (C) *AGXT2* protein expression in HUVEC transfected with different plasmid constructs. (D) Relative ADMA metabolizing activity of *AGXT2*-rs37369-A and *AGXT2*-rs37369-G in HUVECs. ** $p<0.01$, *** $p<0.001$.

Fig. 2. Migration and tube formation in HUVEC. (A) Influence of ADMA and rs37369 polymorphism on migration in HUVECs. (B) Relative migratory ratio, the migrated cells were stained with 0.1% crystal violet, which could be eluted with 33% acetic acid and the OD value of the eluent was measured at 570 nm, to indicate the number of migrated cells. (C) Influence of ADMA and rs37369 polymorphism on tube formation of cultured HUVECs. (D) Quantitation of total tubule length. * $p<0.05$ ** $p<0.01$ *** $p<0.001$.

Fig. 3. Survival analysis of the CHF patients. Impact of NYHA functional classification (A) and *AGXT2* rs37369 polymorphism (B) on the prognosis of CHF.

Fig. 4. A working mechanism indicating the *AGXT2* V140I polymorphism–ADMA pathway in the regulation of cardiac remodeling and heart failure.

Table 1. Baseline demographics and clinical characteristics of the study population.

Characteristics	CHF	Control	<i>p</i> value
N	1000	1200	
Gender			0.175
Male (%)	611 (61.1)	699 (58.3)	
Female (%)	389 (38.9)	501 (41.7)	
Age (years)	62±13	61±8	0.068
SBP (mmHg)	133±25	116±12	<0.001
DBP (mmHg)	80±14	74±8	<0.001
TC (mmol/L)	4.39±1.31	4.33±0.62	0.118
TG (mmol/L)	1.70±1.29	1.61±1.21	0.072
HDL (mmol/L)	1.29±0.74	1.67±0.68	<0.001
LDL (mmol/L)	2.41±0.97	2.10±0.61	<0.001
Positive history of smoking (%)	389 (38.9)	313 (26.1)	<0.001
Positive history of alcohol consumption (%)	275 (27.5)	315 (26.3)	0.617

Coronary heart disease (%)	706 (70.6)	0 (0)	<0.001
Hypertension (%)	614 (61.4)	0 (0)	<0.001
Diabetes mellitus (%)	224 (22.4)	0 (0)	<0.001

SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 2. Association between *AGXT2* rs37369 polymorphism and risk for CHF in Chinese population.

Models	Genotypes	CHF N=1000 (%)	Controls N=1200 (%)	Crude OR (95% CI)	Adjusted OR ^a (95% CI)	<i>p</i>	Adjusted <i>p</i> ^a	Corrected <i>p</i> ^b
Entire cohort								
Codominant	AA	330 (33.0)	420 (35.0)	1.00 (reference)	1.00 (reference)			
	AG	481 (48.1)	603 (50.3)	1.02 (0.84-1.23)	1.02 (0.84-1.23)	0.874	0.857	1.000
	GG	189 (18.9)	177 (14.7)	1.36 (1.06-1.75)	1.36 (1.05-1.75)	0.016	0.019	0.057
Dominant	AA	330 (33.0)	420 (35.0)	1.00 (reference)	1.00 (reference)			
	AG/GG	670 (67.0)	780 (65.0)	1.09 (0.92-1.31)	1.10 (0.91-1.32)	0.324	0.320	0.960
Recessive	AA/AG	811 (81.1)	1023 (85.3)	1.00 (reference)	1.00 (reference)			
	GG	189 (18.9)	177 (14.7)	1.35 (1.08-1.69)	1.35 (1.08-1.71)	0.009	0.010	0.030
Smokers								
Codominant	AA	125 (32.1)	120 (38.3)	1.00 (reference)	1.00 (reference)			
	AG	190 (48.8)	150 (47.9)	1.22 (0.88-1.69)	1.23 (0.87-1.73)	0.245	0.244	0.732
	GG	74 (19.0)	43 (13.7)	1.65 (1.05-2.60)	1.73 (1.09-2.73)	0.029	0.020	0.060

Dominant	AA	125 (32.1)	120 (38.3)	1.00 (reference)	1.00 (reference)			
	AG/GG	264 (67.9)	193 (61.7)	1.31 (0.96-1.79)	1.34 (0.97-1.85)	0.086	0.073	0.219
Recessive	AA/AG	315 (81.0)	270 (86.3)	1.00 (reference)	1.00 (reference)			
	GG	74 (19.0)	43 (13.7)	1.48 (0.98-2.22)	1.56 (1.03-2.37)	0.062	0.036	0.108
Non-smokers								
Codominant	AA	205 (33.6)	300 (33.8)	1.00 (reference)	1.00 (reference)			
	AG	291 (47.6)	453 (51.1)	0.94 (0.75-1.18)	0.94 (0.75-1.20)	0.600	0.616	1.000
	GG	115 (18.8)	134 (15.1)	1.26 (0.93-1.71)	1.25 (0.91-1.71)	0.144	0.162	0.486
Dominant	AA	205 (33.6)	300 (33.8)	1.00 (reference)	1.00 (reference)			
	AG/GG	406 (66.4)	587 (66.2)	1.01 (0.81-1.26)	1.01 (0.81-1.26)	0.913	0.916	1.000
Recessive	AA/AG	496 (81.2)	753 (84.9)	1.00 (reference)	1.00 (reference)			
	GG	115 (18.8)	134 (15.1)	1.30 (0.99-1.71)	1.30 (0.98-1.72)	0.058	0.065	0.195
		Cases with CHD	Controls					
Codominant	AA	227 (32.2)	420 (35.0)	1.00 (reference)	1.00 (reference)			
	AG	350 (49.6)	603 (50.3)	1.07 (0.87-1.32)	1.07 (0.86-1.34)	0.502	0.530	1.000
	GG	129 (18.3)	177 (14.7)	1.35 (1.02-1.78)	1.35 (1.00-1.81)	0.035	0.047	0.141

Dominant	AA	227 (32.2)	420 (35.0)	1.00 (reference)	1.00 (reference)			
	AG/GG	479 (67.8)	780 (65.0)	1.14 (0.93-1.38)	1.14 (0.92-1.40)	0.205	0.231	0.693
Recessive	AA/AG	577 (81.7)	1023 (85.3)	1.00 (reference)	1.00 (reference)			
	GG	129 (18.3)	177 (14.7)	1.29 (1.01-1.66)	1.30 (0.99-1.69)	0.043	0.058	0.174
		Cases without CHD	Controls					
Codominant	AA	103 (35.0)	420 (35.0)	1.00 (reference)	1.00 (reference)			
	AG	131 (44.6)	603 (50.3)	0.89 (0.67-1.18)	0.88 (0.66-1.18)	0.407	0.383	1.000
	GG	60 (20.4)	177 (14.7)	1.38 (0.96-1.99)	1.36 (0.94-1.97)	0.080	0.101	0.303
Dominant	AA	103 (35.0)	420 (35.0)	1.00 (reference)	1.00 (reference)			
	AG/GG	191 (65.0)	780 (65.0)	1.00 (0.76-1.31)	0.99 (0.75-1.30)	0.991	0.935	1.000
Recessive	AA/AG	234 (79.6)	1023 (85.3)	1.00 (reference)	1.00 (reference)			
	GG	60 (20.4)	177 (14.7)	1.48 (1.07-2.05)	1.49 (1.07-2.08)	0.017	0.018	0.054
		Cases with hypertension	Controls					
Codominant	AA	205 (33.4)	420 (35.0)	1.00 (reference)	1.00 (reference)			
	AG	288 (46.9)	603 (50.3)	0.98 (0.79-1.22)	0.97 (0.77-1.24)	0.845	0.828	1.000

	GG	121 (19.7)	177 (14.7)	1.40 (1.05-1.86)	1.43 (1.05-1.95)	0.020	0.023	0.069
Dominant	AA	205 (33.4)	420 (35.0)	1.00 (reference)	1.00 (reference)			
	AG/GG	409 (66.6)	780 (65.0)	1.07 (0.88-1.32)	1.08 (0.86-1.35)	0.494	0.500	1.000
Recessive	AA/AG	493 (80.3)	1023 (85.3)	1.00 (reference)	1.00 (reference)			
	GG	121 (19.7)	177 (14.7)	1.42 (1.10-1.83)	1.47 (1.11-1.95)	0.007	0.007	0.021
		Cases without hypertension	Controls					
Codominant	AA	125 (32.4)	420 (35.0)	1.00 (reference)	1.00 (reference)			
	AG	193 (50.0)	603 (50.3)	1.08 (0.83-1.39)	1.07 (0.83-1.39)	0.579	0.603	1.000
	GG	68 (17.6)	177 (14.7)	1.29 (0.92-1.82)	1.28 (0.90-1.81)	0.145	0.165	0.495
Dominant	AA	125 (32.4)	420 (35.0)	1.00 (reference)	1.00 (reference)			
	AG/GG	261 (67.6)	780 (65.0)	1.12 (0.88-1.44)	1.12 (0.87-1.44)	0.346	0.370	1.000
Recessive	AA/AG	318 (82.4)	1023 (85.3)	1.00 (reference)	1.00 (reference)			
	GG	68 (17.6)	177 (14.7)	1.24 (0.91-1.68)	1.22 (0.89-1.66)	0.175	0.219	0.657

^a Adjusted for sex, age, smoking status (yes/no), history of alcohol drinking (yes/no).

^b Corrected by Bonferroni multiple adjustment.