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The association between annexin A5 (*ANXA5*) gene polymorphism and left ventricular hypertrophy (LVH) in Chinese endogenous hypertension patients

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Abstract

Left ventricular hypertrophy (LVH) is common in endogenous hypertension (EH). We evaluated annexin A5 (*ANXA5*) promoter polymorphism in a cross-sectional study with a total of 850 EH patients, including 337 EH patients with LVH.

Genotyping of *ANXA5* promoter single nucleotide polymorphisms (SNPs) was conducted by SNaPshot assays and statistical analyses were performed to quantify its association with LVH.

Of all potential SNPs, rs1050606 showed significant association with LVH ($P = .008$ in dominant and $P = .006$ in codominant models, respectively). During further analysis of SNPs on *ANXA5* promoter region, rs1050606 had the most prominent effect. Furthermore, haplotypes M2 had higher risk of inducing LVH in EH patients compared with M1 ($P = .032$, OR = 1.42, 95%CI = 1.03–1.94). Patients with *ANXA5* promoter haplotype GATGTC were also more susceptible to LVH ($P = .022$, OR = 1.35, 95%CI = 1.04–1.74). In the luciferase experiment, ANXA5 rs1050606 had the most promoter activity in myocardial cells ($P < .001$).

These results showed that *ANXA5* rs1050606 was significantly associated with LVH in Chinese EH patients, likely via influencing *ANXA5* expression in serum and in myocardial cells.

Abbreviations: ANXA5 = annexin A5, EH = endogenous hypertension, FBG = fasting blood glucose, LVH = left ventricular hypertrophy, LVMI = left ventricular myocardial mass index, TG = triglyceride, WHO = World Health Organization.

Keywords: ANXA5, endogenous hypertension, left ventricular hypertrophy, polymorphism, susceptibility

1. Introduction

Heart failure is a fatal clinical syndrome worldwide, which usually appears as progressive left ventricular systolic or diastolic dysfunction.[1] It is usually caused by untreated cardiac hypertrophy, including left ventricular hypertrophy (LVH) that is characterized by increasing cardiomyocyte size and fibrosis. Cardiac remodeling is the culprit to the cardiac hypertrophy, which could be triggered by hypertension, cardiomyopathy, valvular dysfunction, and myocardial infarction.[2] Hypertension is an increasing multifactorial global health problem: genetic and environmental factors as well as their interactions all contribute to its pathogenesis.[3] As an intermediate cardiac event and phenotype of hypertensive heart diseases, LVH has been focused for heart disease studies.[4]

Annexin A5 (*ANXA5*) is a 35 kDa protein and usually exists in plasma. A family of calcium-dependent phospholipid binding proteins, ANXA5 is one of the most abundant annexins in rat and human myocardium,[5,6] which has a high affinity for phosphatidylserine. In a recent study, Susana et al has reported that reduction in plasma ANXA5 is associated with cardiac resynchronization therapy induced left ventricular reverse remodeling.[7]

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BZ and XZ contributed equally to this work.

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Single nucleotide polymorphism (SNP) is able to influence susceptibility, occurrence, and development of many diseases, including LVH.[8,9] There are numerous reported SNPs in *ANXA5*, which have been investigated in antiphospholipid syndrome,[10,11] ischemic stroke,[12] and myocardial infarction.[13,14] SNPs in *ANXA5* promoter is generally acknowledged to influence ANXA5 expression and bind with different transcriptional factors.[15]

In this study, we conducted a hospital-based case–control study to identify and quantify the association between potential tag polymorphisms of ANXA5 (rs6830321, rs1050606, rs35317464, rs2306416, rs117677079, and rs41278075) and susceptibility to LVH in hypertension patients. Furthermore, we investigated 6 additional SNPs (rs112782763, rs28717001, rs28651243, rs113588187, rs1050606, and rs11538099, which have been well studied as functional SNPs in *ANXA5* activity in the previous study)[15] and evaluated their impact of LVH in endogenous hypertension (EH) patients.

2. Methods and materials

2.1. Study participants

The study was in compliance with the declaration of Helsinki and approved by the Institutional Ethics Committee of the Hospital Affiliated to Guizhou Medical University (approval no. 201667, Feb.20, 2016). All methods described in this study were carried out in accordance with approved guidelines. All participants were informed the content of this research and signed a written informed consent before donating blood samples.

A total of 337 patients diagnosed with EH and LVH were recruited as the EH with LVH group, while 513 age-matched and gender-matched EH patients without LVH were recruited as the EH group. All patients were recruited by the hospital affiliated to the Guizhou Medical University between April 2013 and August 2016. Hypertension symptom was defined according to the World Health Organization criteria: mean clinic systolic blood pressure $\geq$140 mm Hg and/or mean clinic diastolic blood pressure $\geq$90 mm Hg on average of 2 measurements or by current antihypertensive treatment.

2.2. DNA extraction and genotyping

From each participant, 5 mL peripheral blood sample was collected and then fixed in EDTA for DNA isolation and genotyping. Genomic DNA from blood samples was extracted by QIAcube HT Plasticware and QIAamp 96 DNA QIAcube HT Kit (Qiagen, Dusseldorf, Germany) following manufacturer's protocol, and then stored at -80 °C.

Genotyping of SNPs was performed by SNaPshot assays. 2 μL PCR product was purified with 0.3 μL shrimp alkaline phosphatase (Thermo Fisher Scientific, MA) following manufacturer's protocol. After purification, production of SNaPshot extension reaction was mixed with 2 μL SNaPshot ready reaction mix (Applied Biosystems, CA) for further amplification. Hi-Di form amide and GeneScan-120 LIZ size standard (Applied Biosystems) were mixed with purified mini-sequencing products. Raw genomic data were collected using 3730 Genetic Analyzer Data Collection Software version 3.0 and analyzed with GeneMapper Software Version 4.1 (Applied Biosystems).

2.3. Construction of ANXA5 promoter–reporter plasmid and variant plasmids on SNPs

The promoter region of ANXA5 was confirmed using methods from previous study.[15] ANXA5 promoter sequences were synthesized and constructed into pGL3-BASIC vector (Promega, Madison, WI) by Biolight Tec. Company (Nanjing, China). Plasmid variants were adopted by single point mutation. All plasmids were further confirmed by DNA sequencing.

2.4. Cell culture and transfection

Primary cardiac myocyte was adopted from mice and cultured in Dulbecco modified eagle medium glucose (Invitrogen) culture medium, supplemented with 10% fetal bovine serum (GIBCO, Burlington, Canada). The corresponding cultural condition was 37°C in a humidified atmosphere with 5% CO_2.

For further plasmid transfection, 1×10^6 cells were seeded in each of the 24-well culture plate. Cells were transfected with the help of Lipofectamine LTX plus reagent with 0.5 μg above-mentioned luciferase reporter plasmids. pRL-SV40 was transiently cotransfected into cells as internal control.

2.5. Luciferase assay

Twenty-four hours after transfection, luciferase activity was monitored by the dual luciferase report assay system (Promega) following manufacturer's protocol.

2.6. Statistical analysis

Statistical analyses were performed in SAS 10.0.3 software. χ^2 test was conducted to determine the Hardy–Weinberg equilibrium of SNPs in *ANXA5* promoter region. Categorical variables and continuous variables were described as percentages and mean ± standard deviation (mean ± SD), respectively. Unconditional logistic regression was carried out to calculate the odds ratios (ORs) and 95% CI for genotypes and evaluate the association between *ANXA5* polymorphism and LVH in EH patients. P value $< .05$ (2-sided) was considered as statistically significant.

3. Results

3.1. Participants' demographic characteristics and clinical features

Demographic and clinical characteristics of the participants were demonstrated in Table 1. There were no significant differences in age, gender, family history, body mass index, fasting blood glucose, triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, systolic blood pressure, and diastolic blood pressure between the 2 groups. Left ventricular end-diastolic dimension, left ventricular posterior wall, interventricular septum, relative wall thickness, left ventricular myocardial mass index (LVMI), pulmonary artery pressure, and isovolumetric relacsation time were all significantly higher in EH patients with LVH compared with EH patients without LVH.

3.2. The influence of ANXA5 tag SNPs on LVH in EH patients

Linkage disequilibria of SNPs on ANXA5 gene were demonstrated in Fig. 1. With the help of analysis by Haploview software, 6 tagSNPs were selected for genotyping: rs6830321, rs1050606, rs35317464, rs2306416, rs117677079, and rs41278075. As shown in Table 2, rs1050606 had significant influence on the risk of LVH in EH patients (TG and TG + GG for

Table 1

The clinical biochemical characteristics of EH and EH with LVH.

Variables	EH (n = 513)	EH with LVH (n = 337)	*P*
Age	59.2 ± 8.9	58.9 ± 9.4	.660
Sex			.837*
Male	264	171	
Female	249	166	
Family history, %			.079*
Yes	166	90	
No	347	247	
BMI, kg/m^2	24.1 ± 3.0	24.4 ± 2.7	.149
FBG, mmol/L	9.2 ± 2.7	9.0 ± 2.4	.287
TG, mmol/L	2.6 ± 1.3	2.7 ± 1.2	.368
TC, mmol/L	6.0 ± 2.0	5.9 ± 1.9	.514
HDL-C, mmol/L	1.6 ± 0.6	1.7 ± 0.9	.473
LDL-C, mmol/L	4.2 ± 1.4	4.3 ± 1.3	.102
SBP, mm Hg	156 ± 20	154 ± 17	.153
DBP, mm Hg	98 ± 8	97 ± 10	.195
LVEDD, (mm	41.9 ± 4.6	42.9 ± 4.3	**.002**
LVPW, mm	9.4 ± 1.0	12.8 ± 1.3	**<.001**
IVS, mm	9.6 ± 1.1	13.1 ± 0.9	**<.001**
LA, mm	29.6 ± 4.1	35.3 ± 5.8	**<.001**
RWT	0.49 ± 0.08	0.64 ± 0.10	**<.001**
LVMI, (mm	91863 ± 22728	135231 ± 32390	**<.001**

BMI = body mass index, DBP = diastolic blood pressure, EH = endogenous hypertension, FBG = fasting blood glucose, HDL-C = high-density lipoprotein cholesterol, IVRT = isovolumetric relacsation time, IVS = interventricular septum, LDL-C = low-density lipoprotein cholesterol, LVEDD = left ventricular end-diastolic dimension, LVH = left ventricular hypertrophy, LVMI = left ventricular myocardial mass index, LVPW = left ventricular posterior wall, PAP = pulmonary artery pressure, RWT = relative wall thickness, SBP = systolic blood pressure, TC = total cholesterol, TG = triglyceride.
* *P* value of chi-square test for both sex and family history.

P = .008, OR = 1.52, 95% CI = 1.12–2.08, and P = *.006, OR = 1.50, 95% CI = 1.12–2.01, respectively), while rs6830321, rs35317464, rs2306416, rs117677079, and rs41278075 did not have statistically significant influence on LVH in EH patients.*

3.3. Association between ANXA5 promoter polymorphisms and risk of LVH in EH patients

rs1050606 was one of the 6 linked functional SNPs on ANXA5 promoter region with different affinity to different transcription factors.[15] We further evaluated these SNPs (Table 3). Besides rs1050606, GA + AA genotype in rs112782763, AC + CC in rs28717001, TC + CC in rs28651243, GA + AA in rs113588187, and TG + GG in rs1050606 were also significantly different in EH patients with LVH than those without LVH (*P* = .032, .037, .046, and .029, respectively). Nevertheless, there was no statistically significance of CT + TT genotype in rs11538099.

Considering the substantial influence of rs1050606 on LVH, we further investigated its impact in a successive stratification analysis. LVMI was adopted as a comprehensive index considering influence from left ventricular end-diastolic dimension, left ventricular posterior wall, interventricular septum, LA, and relative wall thickness. We focused on the association between rs1050606 polymorphism and LVMI.

3.4. Association of ANXA5 haplotype with LVH in EH patients

Two forms of common haplotypes in *ANXA5* gene promoter (GATG as *M1* and ACCA as *M2* in Bogdanova research)[16] were analyzed in EH patients with and without LVH. As described in Table 4, M2 haplotype was associated with a significantly higher risk of LVH than M1 haplotype in EH patients (*P* = .032, OR = 1.42, 95% CI = 1.03–1.94). The global *P* for M2 compared with M1 was less than .001. Also, EH patients with GATGGC haplotype were at a higher risk of LVH onset, compared to those with GATGTC as reference (*P* = .022, OR = 1.35, 95% CI = 1.04–1.74) (Table 4).

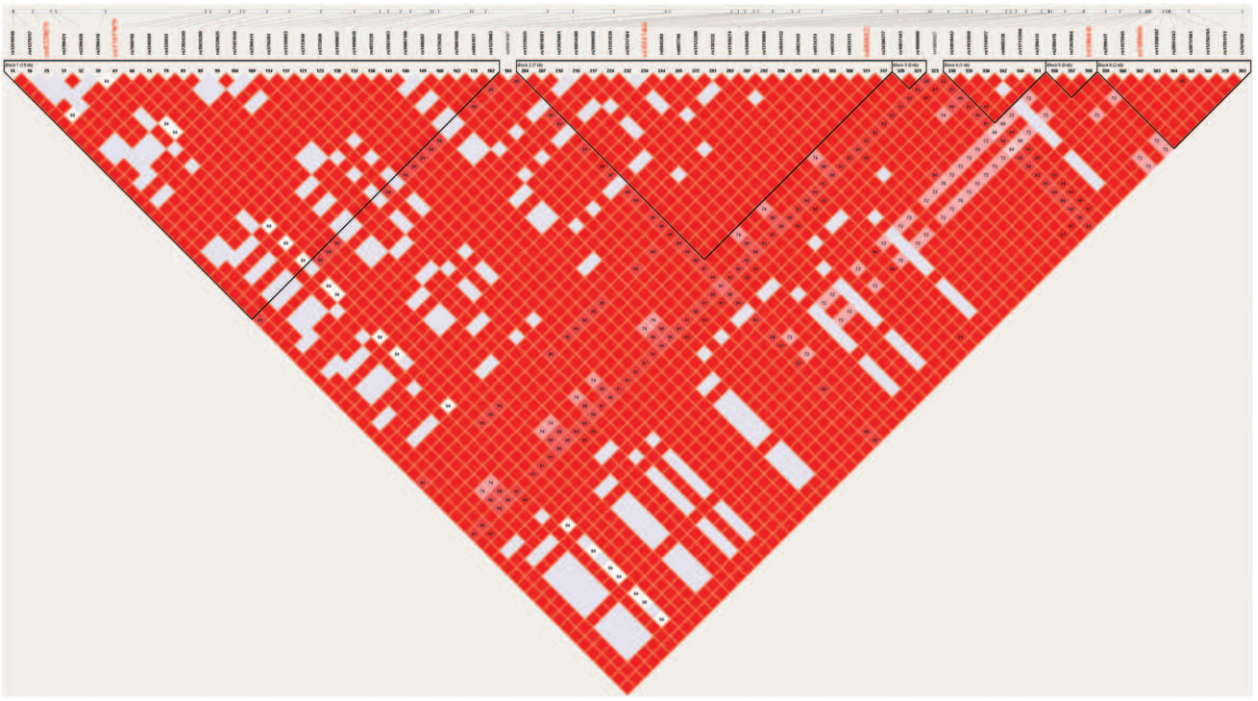

Figure 1. Six SNPs generated by Haploview. Shading represents the magnitude and significance of pair wise LD, with a red-to white gradient reflecting higher to lower LD values. LD = linkage disequilibrium, SNP = single nucleotide polymorphism.

Table 2

Association between ANXA5 tag-SNPs and risk of LVH in EH patients.

		EH (n = 513)		EH with LVH (n = 337)			
SNP	**Genotypes**	**N**	**%**	**N**	**%**	***P****	**Adjusted OR (95%CI)***
rs6830321	TT	248	48.3	173	51.3		1.00 (Ref)
	TC	181	35.3	109	32.3	.376	0.85 (0.61–1.15)
	CC	84	16.4	55	16.3	.633	0.89 (0.59–1.30)
	TC + CC	265	51.7	164	48.7	.265	0.83 (0.62–1.09)
rs1050606	TT	348	67.8	197	58.5		1.00 (Ref)
	TG	133	25.9	115	34.1	**.008**	**1.52 (1.12–2.08)**
	GG	32	6.3	25	7.4	.227	1.41 (0.81–2.47)
	TG + GG	165	32.2	140	41.5	**.006**	**1.50 (1.12–2.01)**
rs35317464	CC	348	67.8	222	65.9		1.00 (Ref)
	CT	154	30.0	108	32.0	.575	1.10 (0.84–1.50)
	TT	11	2.1	7	2.1	.802	1.03 (0.41–2.62)
	CT + TT	165	32.2	115	34.1	.603	1.08 (0.82–1.47)
rs2306416	TT	331	64.5	203	60.2		1.00 (Ref)
	TC	169	32.9	124	36.8	.258	1.23 (0.90–1.63)
	CC	13	2.5	10	3.0	.737	1.27 (0.56–2.98)
	TC + CC	182	35.5	134	39.8	.235	1.20 (0.88–1.59)
rs117677079	TT	324	64.5	203	60.2		1.00 (Ref)
	TC	175	32.9	124	36.8	.258	1.23 (0.90–1.63)
	CC	14	2.5	10	3.0	.737	1.27 (0.56–2.98)
	TC + CC	182	35.5	134	39.8	.235	1.20 (0.88–1.59)
rs41278075	GG	306	59.6	212	62.9		1.00 (Ref)
	GA	188	36.6	116	34.4	.474	0.88 (0.66–1.19)
	AA	19	3.7	9	2.7	.465	0.65 (0.31–1.52)
	GA + AA	207	40.4	125	37.1	.378	0.87 (0.69–1.18)

ANXA5 = annexin A5, BMI = body mass index, CI = confidence interval, DBP = diastolic blood pressure, EH = endogenous hypertension, FBG = fasting blood glucose, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, LVH = left ventricular hypertrophy, OR = odds ratio, SBP = systolic blood pressure, SNP = single nucleotide polymorphism, TC = total cholesterol, TG = triglyceride.
* Adjusted for age, sex, family history, BMI, FBG, TG, TC, HDL-C, LDL-C, SBP, and DBP in logistic regression model.

3.5. Impact of SNPs on ANXA5 promoter activity

The potential influence of each promoter SNP on ANXA5 transcription was analyzed by luciferase assay in Fig. 2. After transient transfections, variants of rs113588187 and rs1050606 increased significantly in luciferase ($P = .013$ for rs113588187 and $P < .001$ for rs1050606, respectively). Compared with rs113588187, increasing promoter activity of rs1050606 was more substantial ($P = .021$). For the remaining polymorphisms, no significant luciferase activity was detected comparing to the wild type.

4. Discussion

In this study, we analyzed, quantified, and contrasted SNPs in *ANXA5* gene in EH patients with and without LVH. *ANXA5* SNP rs1050606 had the strongest association with increased risk of LVH in the sampled Chinese EH patients. Also, EH patients carrying M2 and GATGGC haplotype had a higher risk of LVH. These results suggested that *ANXA5* promoter SNP (rs1050606, rs112782763, rs28717001, rs28651243, and rs113588187) and their corresponding haplotypes (including M2 and GATGGC) could be potential biomarkers for LVH in EH patients.

ANXA5 has been generally acknowledged as the most abundant annexins in rat and human myocardium.[5,6] Several studies have reported that ANXA5 level increased after myocardial infarction[17,18] and unstable angina.[18] Its dysregulation was also illuminated in heart failure patients, especially in cardiomyocyte.[5] In Ravassa's study, overexpression of ANXA5 influenced phosphorylation of Akt and p38 MAPK, aberrant expression of which could further inhibiting the antiapoptotic activity of cells.[7] It was reported that myocardial infarction and transverse aortic constriction could induce p38 and p42/44 MAPK pathways by early transient and sustained effects, respectively.[19] After myocardial infarction, the left ventricular and cardiomyocyte hypertrophy were triggered.[19] Besides, ANXA5 could influence the LVH via the p38 pathway.

Polymorphisms of *ANXA5* promoter have been reported in recurrent pregnancy loss,[16,20] and our study is the first time to evaluate their effects on LVH. In previous research, functions of these SNPs were investigated via molecular biology experiments in human placental cells.[15] rs62319820 and rs112782763 were considered to have significant impact on ANXA5 promoter activity. However, variants of rs1050606 did not alter luciferase activity in BeWocell line. As the result, rs1050606 mutation might influence potential affinities to transcription factors (eg, FOXP3, RARβ, RXRα, and ETF).[15] Our study demonstrates that rs1050606 is associated with significantly higher risk of LVH in EH patients. There are some differences of tissue specificity between placental trophoblastic cells in Tiscia et al report and left ventricular cells in our study. We suggest such difference is reasonable and could be attributed to the minimal impact on expressional trails of gene in a single allele.[21] In our following functional analysis, rs1050606 variant also demonstrated strongest promoter activity, compared with other SNPs in primary cardiomyocyte cells, which was far from the conclusion of its functional characterization in human placenta membrane cancer cell.

Nevertheless, there are still some limitations in this study. First, all participants were recruited from hospital, hence selection and information bias was inevitable. Second, a total of 337 LVH patients with EH were included in this study, and future studies with larger sample size would be desirable to increase statistical

Table 3

Association between ANXA5 promoter SNPs and risk of LVH in EH patients.

SNP	Genotypes	EH (n = 513)		EH with LVH (n = 337)		P*	Adjusted OR (95%CI)*
		n	%	n	%		
Promoter							
SNP1							
rs112782763	GG	426	83.0	259	76.8		1.00 (Ref)
-467G>A	GA	75	14.6	71	21.1	**.022**	**1.53 (1.06–2.21)**
	AA	12	2.4	7	2.1	.980	1.01 (0.39–2.63)
	GA + AA	87	17.0	78	23.2	**.032**	**1.46 (1.03–2.08)**
SNP2							
rs28717001	AA	427	83.2	260	77.2		1.00 (Ref.)
-448A>C	AC	73	14.3	69	20.5	**.026**	**1.53 (1.06–2.21)**
	CC	13	2.5	8	2.4	.935	1.04 (0.42–2.56)
	AC + CC	86	16.8	77	22.9	**.037**	**1.46 (1.03–2.07)**
SNP3							
rs28651243	TT	427	83.2	261	77.4		1.00 (Ref.)
-442T>C	TC	73	14.3	67	19.9	**.040**	**1.48 (1.02–2.14)**
	TT	13	2.5	9	2.7	.740	1.16 (0.48–2.78)
	TC + CC	86	16.8	76	22.6	**.046**	**1.43 (1.01–2.03)**
SNP4							
rs113588187	GG	428	83.4	260	77.1		1.00 (Ref.)
-373G>A	GA	71	13.8	69	20.5	**.017**	**1.57 (1.08–2.28)**
	AA	14	2.7	8	2.4	.956	0.98 (0.40–2.38)
	GA + AA	85	16.6	77	22.9	**.029**	**1.48 (1.04–2.10)**
SNP5							
rs1050606	TT	348	67.8	197	58.5		1.00 (Ref.)
-302T>G	TG	133	25.9	115	34.1	**.008**	**1.52 (1.12–2.08)**
	GG	32	6.3	25	7.4	.227	1.41 (0.81–2.47)
	TG + GG	165	32.2	140	41.5	**.006**	**1.50 (1.12–2.01)**
SNP6							
rs11538099	CC	426	83.0	261	77.4		1.00 (Ref.)
-1C>T	CT	73	14.3	69	20.5	**.026**	**1.52 (1.05–2.20)**
	TT	14	2.7	7	2.1	.743	0.86 (0.34–2.17)
	CT + TT	87	17.0	76	22.6	.051	1.42 (1.00–2.01)

ANXA5 = annexin A5, BMI = body mass index, CI = confidence interval, DBP = diastolic blood pressure, EH = endogenous hypertension, FBG = fasting blood glucose, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, LVH = left ventricular hypertrophy, OR = odds ratio, SBP = systolic blood pressure, SNP = single nucleotide polymorphism, TC = total cholesterol, TG = triglyceride.
* Adjusted for age, sex, family history, BMI, FBG, TG, TC, HDL-C, LDL-C, SBP, and DBP in logistic regression model.

power. Third, because all the participants in our study are Chinese population, it largely restricts the clinical applications of our study to a specific Asian population. In addition, more molecular biology experiment should be performed for further investigation of the specific function of rs1050606 and its relative signal pathway in LVH.

5. Conclusion

In conclusion, we have identified the significant association between *ANXA5* promoter SNPs and LVH risk in EH patients. Our study would contribute to the potential biomarkers for LVH susceptibility in EH.

Table 4

Association of ANXA5 SNP haplotypes with risk of LVH in EH patients.

Haplotypes*	EH (n = 513)		EH with LVH (n = 337)		P-value†	OR (95%CI)†	Global P‡
	n	%	n	%			
SNP1–6§							**<0.001**
GATGTC	758	73.9	448	66.5		1.00 (Ref.)	
GATGGC	167	16.3	133	19.7	**0.022**	**1.35 (1.04–1.74)**	
ACCATT	66	6.4	51	7.6	0.171	1.31 (0.8901.92)	
ACCAGT	28	2.7	28	4.2	0.055	1.69 (0.99–2.89)	
SNP1–4§							**<0.001**
GATG (M1)	925	90.2	583	86.5		1.00 (Ref.)	
ACCA (M2)	94	9.2	83	12.3	**0.032**	**1.42 (1.03–1.94)**	

* The alleles of haplotypes were arrayed as the location of the SNPs in ANXA5 from5' to3'.
† Adjusted for age, sex, famil history, BMI, FBG, TG, TC, HDL-C, LDL-C, SBP and DBP.
‡ Generated by permutation test with 1000 times of simulation.
§ Haplotypes with a frequency <0.01 were pooled into the mixed group.

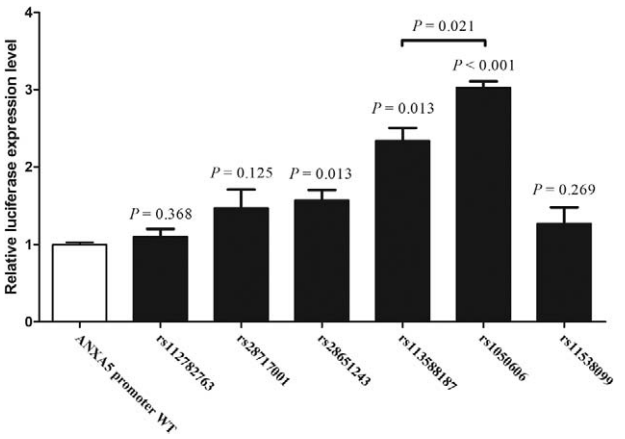

Figure 2. Luciferase activity of functional SNPs on *ANXA5* promoter region. ANXA5 = annexin A5, SNP = single nucleotide polymorphism.

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