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]
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, \(^{11,13}\) and myocardial infarction. \(^{13,14}\) SNPs in ANXA5 promoter is generally acknowledged to influence ANXA5 expression, and bind with different transcriptional factors. \(^{15}\) The promoter region of ANXA5 was confirmed by 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. 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 participants were 24 hours after transfection, luciferase activity was measured with 2.5 μL of luciferase reporter plasmid. 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. \(\chi^2\) 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. \(P \text{ value}<.05\) (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 relaxation 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 significantly higher in EH patients compared with EH patients without LVH.

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% CO₂.

For further plasmid transfection, 1 × 10⁴ 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.
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. 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) 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 GATGTC as reference (P=.022, OR=1.35, 95%CI=1.04–1.74) (Table 4).
Table 2

| SNP     | Genotypes | EH (n=513) | EH with LVH (n=337) | P  | Adjusted OR (95%CI)* |
|---------|-----------|------------|---------------------|----|---------------------|
| rs6830321 | TT        | 248 48.3   | 173 51.3            | .100 | (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            | .008 | 1.52 (1.12–2.08)    |
|         | TG        | 133 25.9   | 115 34.1            | .006 | 1.50 (1.12–2.01)    |
|         | 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            | .006 | 1.50 (1.12–2.01)    |
|         | 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            | .000 | 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            | .000 | 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            | .100 | 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 GATGCC haplotype had a higher risk of LVH. These results suggested that ANXA5 promoter SNP (rs1050606, rs112782763, rs28717001, rs2865124, and rs113588187) and their corresponding haplotypes (including M2 and GATGCC) could be potential biomarkers for LVH in EH patients.

ANXA5 has been generally acknowledged as the most abundant annexin in rat and human myocardium. Several studies have reported that ANXA5 level increased after myocardial infarction and unstable angina. Its dysregulation was also illuminated in heart failure patients, especially in cardiomyocyte. In Ravassa’s study, overexpression of ANXA5 influenced phosphorylation of Akt and p38 MAPK, aberrant expression of which could further inhibiting the antia apoptotic activity of cells. 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. After myocardial infarction, the left ventricular and cardiomyocyte hypertrophy were triggered. Besides, ANXA5 could influence the LVH via the p38 pathway. Polymorphisms of ANXA5 promoter have been reported in recurrent pregnancy loss 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 and variants 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
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 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) |
|--------------|-----------|------------|----|---------------------|----|-----------|---------------------|
| Promoter     |           |            |    |                      |    |           |                     |
| rs112782763  | GG        | 426        | 83.0 | 259                  | 76.8 | 1.00 (Ref) |                     |
| -467G>A      | GA        | 75         | 14.6 | 71                   | 21.1 | 0.022 | 1.53 (1.06–2.21)   |
|              | AA        | 12         | 2.4  | 7                    | 2.1  | 0.980 | 1.01 (0.39–2.63)   |
|              | GA+AA     | 87         | 17.0 | 78                   | 23.2 | 0.032 | 1.46 (1.03–2.08)   |
| SNP2         | AA        | 427        | 83.2 | 260                  | 77.2 | 1.00 (Ref) |                     |
| -448A>C      | AC        | 73         | 14.3 | 69                   | 20.5 | 0.026 | 1.53 (1.06–2.21)   |
|              | CC        | 13         | 2.5  | 8                    | 2.4  | 0.935 | 1.04 (0.42–2.56)   |
|              | AC+CC     | 86         | 16.8 | 77                   | 22.9 | 0.037 | 1.46 (1.03–2.07)   |
| SNP3         | TT        | 427        | 83.2 | 260                  | 77.2 | 1.00 (Ref) |                     |
| -442T>C      | TC        | 73         | 14.3 | 67                   | 19.9 | 0.040 | 1.16 (0.48–2.78)   |
|              | TT        | 13         | 2.5  | 9                    | 2.7  | 0.740 | 1.01 (0.39–2.63)   |
|              | TC+CC     | 86         | 16.8 | 76                   | 22.6 | 0.046 | 1.43 (1.01–2.03)   |
| SNP4         | GG        | 428        | 83.4 | 260                  | 77.1 | 1.00 (Ref) |                     |
| -373G>A      | GA        | 71         | 13.8 | 69                   | 20.5 | 0.017 | 1.57 (1.08–2.28)   |
|              | AA        | 14         | 2.7  | 8                    | 2.4  | 0.956 | 0.98 (0.40–2.38)   |
|              | GA+AA     | 85         | 16.6 | 77                   | 22.9 | 0.029 | 1.48 (1.04–2.10)   |
| SNP5         | TT        | 348        | 67.8 | 197                  | 58.5 | 1.00 (Ref) |                     |
| -302T>G      | TG        | 133        | 25.9 | 115                  | 34.1 | 0.008 | 1.52 (1.12–2.08)   |
|              | GG        | 32         | 6.3  | 25                   | 7.4  | 0.227 | 1.14 (0.81–1.57)   |
|              | TG+GG     | 165        | 32.2 | 140                  | 41.5 | 0.006 | 1.50 (1.12–2.01)   |
| SNP6         | CC        | 426        | 83.0 | 261                  | 77.4 | 1.00 (Ref) |                     |
| -1C>T       | CT        | 73         | 14.3 | 69                   | 20.5 | 0.026 | 1.52 (1.05–2.20)   |
|              | TT        | 14         | 2.7  | 7                    | 2.1  | 0.743 | 0.93 (0.34–2.47)   |
|              | CT+TT     | 87         | 17.0 | 76                   | 22.6 | 0.051 | 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.

### 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 |
|--------------|------------|----|---------------------|----|--------|------------|----------|
| SNP1        |            |    |                      |    |        |            |          |
| GATGTC       | 758        | 73.9 | 448                  | 66.5 | 1.00 (Ref) | <0.001   |
| 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 from 5’ to 3’.

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

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