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Original Article

Lack of association between rs3807989 in cav1 and atrial fibrillation

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Abstract: Background: A novel gene *Caveolin-1*(*CAV1*) was identified to be susceptibility to PR interval and also associated with atrial fibrillation (AF) in two Genome wide associations studies (GWAS) studies in European ancestry. The purpose of this study was to determine the association of the SNPs in CAV1 gene of rs3807989 with AF in Chinese Han patients. Methods and results: We attempted a replication in a cohort of 839 Chinese AF patients and 1215 healthy controls using melting temperature shift allele-specific genotyping analysis. One SNP in *CAV1* (rs3807989) was genotyped. The final study cohort consisted of 839 AF patients and 1215 healthy controls. No significant association was detected between rs3807989 and AF in a Chinese Han population (allelic *P-adj* = 0.828 with OR = 1.02; genotypic *P*-adj = 0.815, 0.405, 0.760 with a dominant model, recessive model, and additive model). After logistic regression with multiple covariates, the association remained non-significant with adjusted *P* value 0.828. When the AF cases were further divided into lone AF (31.5%) and other types of AF (68.5%), no significant association was found between rs3807989 and lone AF (*P-adj* = 0.929 with OR = 0.990) and other types of AF (*P-adj* = 0.597 with OR = 1.060). Conclusion: The SNP rs3807989 in CAV1 gene is not associated with AF or lone AF in our studies, which suggests that the SNP rs3807989 in CAV1 may not be a risk factor for AF in Chinese Han population.

Keywords: *CAV1*, atrial fibrillation, Rs3807989

Introduction

Atrial fibrillation (AF) is one of the most common cardiac arrhythmias, which affects 1-2% of the general population [1]. The prevalence of AF increases substantially with aging, from ~1% in young adults to ~10% in people older than 80 years [2]. AF has been shown to be an independent risk factor for ischemic stroke (IS), one of main causes of mortality and morbidity [3]. AF is mostly associated with cardiac risk factors, including hypertension, congenital heart disease, coronary artery disease and valvular heart disease [4]. Nearly 30% of AF cases, which have no predisposing factors, are therefore classified as lone AF [5].

Accumulating studies have indicated that genetic factors play an important role in the etiology of AF, in particular lone AF. In the last decade, a number of AF associated genes and loci, such as *KCNQ1, KCNH2, SCN5A, KCNE2, KCNJ2, NUP155, NPPA, SCN3B, 4q25*, *ZFHX3* and *KCNN3*, have been identified with genetic linkage and genome-wide association studies (GWAS) [6-10]. Caveolin-1 gene (*CAV1*), encoding a caveolae protein, is expressed in atrial myocytes [11]. In two independent GWAS studies, *CAV1* has been reported to be associated with AF [12, 13]. Mice deficient in Cav1 develop dilated cardiomyopathy and pulmonary hypertension [14]. More recently, a SNP in *CAV1* gene, rs3807989, has been associated with early-onset lone AF before the age of 40 years [15]. However, these study results need to be independently confirmed in other ethnic populations [16]. In the present case-control association study, we investigated the association of rs3807989, a tag SNP in *CAV1*, with AF in a Chinese Han population.

Materials and methods

Study subjects

The study subjects were enrolled from multiple hospitals in Dalian, Wuhan, Suizhou, Xiangfan

Table 1. Clinical characteristics of study population

Characteristic	AF patients (n = 839)	Controls (n = 1215)	*P*
Age[a]	53 ± 15	52 ± 15	0.479
Male/female	473/366	803/402	< 0.001
Lone AF (%)	264 (31.5%)	N/A	*
Other AF (%)	575 (80.5%)	N/A	*
paroxysmal AF (%)	495 (59.0%)	N/A	*
persistent AF (%)	298 (35.5%)	N/A	*
permanent AF (%)	46 (5.5%)	N/A	*
coronary artery disease (%)	309 (36.8%)	N/A	*
Hypertension (%)	265 (31.6%)	240 (19.8%)	< 0.001
Ischemic stroke (%)	93 (11.1%)	N/A	*

[a]Age for the case group refers to as the age at diagnosis of disease; age for the control group refers to as the age at which the subject was enrolled into the study. *untested *P* values.

and Shiyan cities and all were Chinese Han people. This study was approved by institutional review boards on human subject research, and was conformed to the guidelines of the Declaration of Helsinki. Informed consents were obtained from the participants or guardians.

Diagnosis criteria

AF was diagnosed by a panel of cardiologists, according to standard diagnostic criteria, including electrocardiograms (ECG) and/or Holter ECG [17]. Patients with other types of cardiac arrhythmias were excluded from this study. Patients with cardiomyopathies and valvular heart disease were also excluded by echocardiography (Echo). We recruited AF patients of younger than 80 years old with AF. Lone AF was defined as patients without history of hypertension, coronary artery disease (CAD), congenital heart disease, congestive heart failure, ischemic stroke or diabetes. All controls had no arrhythmias, coronary artery disease (CAD), congenital heart disease, cardiomyopathies or valvular heart disease by ECG, Echo, magnetic resonance imaging (MRI) and computed tomography (CT).

Genotyping

Peripheral venous blood samples were drawn from all participants and leukocytes were isolated. Genomic DNA was extracted from leucocytes following standard protocols of the Wizard Genomic DNA Purification Kit (Promega Corporation, Madison, WI, USA). The SNP rs3807989 was genotyped on a Rotor-Gene™ 6000 High Resolution Melt (HRM) system (QIAGEN, Hilden, Germany), with a reaction of 25 μL polymerase chain reaction (PCR), containing 1 μL of LC green dye, 5 pmol of each primer, 25 ng of genomic DNA, 2.5 μL of 10 × PCR buffer with 1.5 mmol/L $MgCl_2$, 5 mmol deoxynucleotide triphosphates, and 1 U of *Taq* polymerase. Two positive controls for each genotype (A/A, A/G, and G/G) were included in each run. A total of 48 cases and controls were randomly selected for verification of genotyping results using direct DNA sequence analysis. DNA sequencing was performed in both-directions using the BigDye Terminator v3.1 Cycle Sequencing Kits on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Power analysis was carried out by using PS software v3.0 (Dupont WD, Plummer WD: "Power and Sample Size Calculations: A Review and Computer Program", *Controlled Clinical Trials* 1990). SNP rs3807989 genotypes were tested for Hardy-Weinberg equilibrium in controls using PLINK v1.05. Allelic and genotypic association of rs3807989 with AF was assessed using Pearson's 2 × 2 and 2 × 3 contingency table χ^2 test (PLINK). Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using the χ^2 test (PLINK). Multivariate regression analysis was performed by incorporating age, sex, hypertension, CAD (for AF dataset) and ischemic stroke as covariates using multivariate logistic regression (PLINK). Empirical *P* values were determined using the PLINK v1.05 program with 100,000 Monte-Carlo simulations.

Table 2. Analysis of allelic association of rs3807989 with AF and CAD

Phenotype (case/control)	Allele A frequency (case/control)	*P-obs*	OR (95% CI)	*P-adj*	*P-emp*
Total AF (839/1215)	0.260/0.260	0.990	1.02 (0.85-1.22)	0.828	0.905
Lone AF (264/1215)	0.268/0.260	0.738	0.99 (0.75-1.23)	0.929	0.995
Other AF (575/1215)	0.266/0.260	0.688	1.06 (0.86-1.23)	0.597	0.758
AF with CAD (309/1215)	0.297/0.260	0.306	1.30 (0.90-1.89)	0.162	0.463
AF w/o CAD (530/1215)	0.262/0.260	0.970	0.97 (0.80-1.17)	0.720	0.842
AF with HT (265/1215)	0.252/0.260	0.744	0.83 (0.61-1.11)	0.206	0.627
AF w/o HT (574/1215)	0.274/0.260	0.379	1.06 (0.87-1.28)	0.573	0.775

AF, atrial fibrillation; CAD, coronary artery disease; HT, hypertension; IS, ischemic stroke. *P*-obs: unadjusted *P* values; *P*-adj: obtained using multivariate logistic regression analysis. *P*-emp: obtained by performing 100,000 Monte-Carlo simulations.

Table 3. Analysis of genotypic association of rs3807989 with AF

Phenotype	Numbers case/control	Model	*P*-obs	*P*-adj	*P*-emp
AF	839/1215	Dominant	0.788	0.815	0.789
		Recessive	0.588	0.405	0.628
		Additive	0.787	0.760	0.786

P-obs: unadjusted *P* values; *P*-adj: obtained using multivariate logistic regression analysis. *P*-emp: obtained by performing 100,000 Monte-Carlo simulations.

Results

Clinical characteristics of study subjects

The clinical characteristics of the study population were summarized in **Table 1**, consisting of 839 AF patients and 1215 controls. In AF patient group, the ratio of males to females was significantly higher than that in control group ($P < 0.001$). Prevalence of hypertension in AF patients was significantly higher than that in control group ($P < 0.001$). There is no age difference between the two groups. In the AF patient group, there were 495 paroxysmal AF (69.0%), 298 persistent AF (35.5%), and 46 permanent AF (5.5%). In addition, there were 264 lone AF.

For power analysis, we set the population parameters of an OR of 1.3 and rs3807989 allele A frequency of 0.260. At a type I error rate of 0.05, our study with 839 AF cases versus 1215 controls provided statistical power of more than 94.4% respectively. Therefore, the data suggested that our sample size provided sufficient power to identify the association between SNP rs3807989 and AF.

SNP rs3807989 was not associated with AF

The call rate was 100% for rs3807989 in all samples. This quality control of SNP in HRM genotyping was 100% according to direct sequencing verification of 48 random selected DNA samples. No deviation of rs3807989 genotype distribution from Hardy-Weinberg Equilibrium was found among 1,215 controls ($P > 0.05$, data not shown). As shown in **Table 2**, the association of the SNP rs3807989 with AF was not significant in our study population with a *P* value of 0.990. After logistic regression with multiple covariates, the association remained non-significant with adjusted *P* value 0.828 (**Table 2**). Furthermore, no significant association of the SNP rs3807989 with lone AF or other AF group was observed (**Table 2**). In addition, genotypic association of the SNP rs3807989 with AF was also not significant in three inheritance models, dominant, recessive and additive models (*P-obs* > 0.05 and *P-adj* > 0.05) (**Table 3**).

Discussion

In the present study, we carried out a large-scale case-control association study in a Chinese cohort consisting of 839 AF patients and 1215 controls. The SNP rs3807989 in the intron of *CAV1* gene was assessed. Although the SNP rs3807989 has been associated with AF in GWAS studies, this association has not been replicated in Chinese population. The results showed that the SNP rs3807989 in *CAV1* gene was not significantly associated with entire AF group, lone AF group or other AF group.

In two independent GWAS with Caucasian people, the SNP rs3807989 has been associated with risk of AF with relative low OR values [12, 13]. In a study with Canadian people, signifi-

cant association of rs3807989 with AF has been reported (P = 0.015 and OR = 1.35). However, the association becomes insignificant when correcting for multiple testing [15]. In this study, we did observe significant association of the SNP rs3807989 with AF or lone AF. Therefore, the SNP rs3807989 in *CAV1* gene may be a weak risk factor for AF.

Caveolin-1 (*CAV1*) is one of the major isoforms of caveolae. Caveolae are 50-100 nm cell surface plasma membrane wevaginations, which is rich in cholesterol [11]. The *CAV1* gene is highly expressed in endothelial cells. Most biological functions of these endothelial cells are directly or indirectly mediated by *CAV1*. *CAV1* has also been suggested to play an important role in the regulation of plasma lipoprotein metabolism [18]. In *Cav1*-deficient mice, higher plasma triglyceride (TG) levels are observed [19]. Recent studies have suggested that *CAV1* is involved in endothelial function and ischemia [20]. The association of the SNP rs3807989 in *CAV1* gene by GWAS studies has not been validated and reported. Recently, a GWAS study in African-American population, a SNP rs1177-3845 in *CAV1* gene has been associated with PR interval [21]. Therefore, the roles of the *CAV1* gene in AF need further investigation.

In summary, we carried out case-control study with large cohorts of AF patients and controls. The SNP rs3807989 in CAV1 gene was not associated with AF or lone AF. Our data suggested that the SNP rs3807989 in CAV1 may not be a risk factor for AF in Chinese Han population.

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Disclosure of conflict of interest

None.

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References

[1] Lloyd-Jones DM. Cardiovascular risk prediction: basic concepts, current status, and future directions. Circulation 2010; 121: 1768-1777.

[2] Kannel WB, Wolf PA, Benjamin EJ and Levy D. Prevalence, incidence, prognosis, and predisposing conditions for AF: population-based estimates. Am J Cardiol 1998; 82: 2N-9N.

[3] Wolf PA, Abbott RD and Kannel WB. Atrial fibrillation as an independent risk factor for stroke: the Framingham Study. Stroke 1991; 22: 983-988.

[4] Benjamin EJ, Wolf PA, D'Agostino RB, Silbershatz H, Kannel WB and Levy D. Impact of atrial fibrillation on the risk of death: the Framingham Heart Study. Circulation 1998; 98: 946-952.

[5] Fuster V, Rydén LE, Cannom DS, Crijns HJ, Curtis AB, Ellenbogen KA, Halperin JL, Kay GN, Le Huezey JY, Lowe JE, Olsson SB, Prystowsky EN, Tamargo JL and Wann LS. 2011 ACCF/AHA/HRS focused updates incorporated into the ACC/AHA/ESC 2006 Guidelines for the management of patients with atrial fibrillation: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines developed in partnership with the European Society of Cardiology and in collaboration with the European Heart Rhythm Association and the Heart Rhythm Society. J Am Coll Cardiol 2011; 57: e101-e198.

[6] Chen YH, Xu SJ, Bendahhou S, Wang XL, Wang Y, Xu WY, Jin HW, Sun H, Su XY, Zhuang QN, Yang YQ, Li YB, Liu Y, Xu HJ, Li XF, Ma N, Mou CP, Chen Z, Barhanin J and Huang W. KCNQ1 gain-of-function mutation in familial atrial fibrillation. Science 2003; 299: 251-254.

[7] Makiyama T, Akao M, Shizuta S, Doi T, Nishiyama K, Oka Y, Ohno S, Nishio Y, Tsuji K, Itoh H, Kimura T, Kita T and Horie M. A novel SCN5A gain-of-function mutation M1875T associated with familial atrial fibrillation. J Am Coll Cardiol 2008; 52: 1326-1334.

[8] Sinner MF, Pfeufer A, Akyol M, Beckmann BM, Hinterseer M, Wacker A, Perz S, Sauter W, Illig T, Näbauer M, Schmitt C, Wichmann HE, Schömig A, Steinbeck G, Meitinger T and Kääb S. The non-synonymous coding IKr-channel variant KCNH2-K897T is associated with atrial fibrillation: results from a systematic candidate gene-based analysis of KCNH2 (HERG). Eur Heart J 2008; 29: 907-914.

[9] Ren X, Xu C, Zhan C, Yang Y, Shi L, Wang F, Wang C, Xia Y, Yang B, Wu G, Wang P, Li X, Wang D, Xiong X, Liu J, Liu Y, Liu M, Liu J ,Tu X and Wang QK. Identification of NPPA variants associated with atrial fibrillation in a Chinese

GeneID population. Clin Chim Acta 2010; 411: 481-485.
[10] Zhang X, Chen S, Yoo S, Chakrabarti S, Zhang T, Ke T, Oberti C, Yong SL, Fang F, Li L, de la Fuente R, Wang L, Chen Q and Wang QK. Mutation in nuclear pore component NUP155 leads to atrial fibrillation and early sudden cardiac death. Cell 2008; 135: 1017-1027.
[11] Engelman JA, Zhang XL, Galbiati F and Lisanti MP. Chromosomal localization, genomic organization, and developmental expression of the murine caveolin gene family (Cav-1, -2, and -3). Cav-1 and Cav-2 genes map to a known tumor suppressor locus (6-A2/7q31). FEBS Lett 1998; 429: 330-336.
[12] Pfeufer A, van Noord C, Marciante KD, Arking DE, Larson MG, Smith AV, Tarasov KV, Müller M, Sotoodehnia N, Sinner MF, Verwoert GC, Li M, Kao WH, Köttgen A, Coresh J, Bis JC, Psaty BM, Rice K, Rotter JI, Rivadeneira F, Hofman A, Kors JA, Stricker BH, Uitterlinden AG, van Duijn CM, Beckmann BM, Sauter W, Gieger C, Lubitz SA, Newton-Cheh C, Wang TJ, Magnani JW, Schnabel RB, Chung MK, Barnard J, Smith JD, Van Wagoner DR, Vasan RS, Aspelund T, Eiriksdottir G, Harris TB, Launer LJ, Najjar SS, Lakatta E, Schlessinger D, Uda M, Abecasis GR, Müller-Myhsok B, Ehret GB, Boerwinkle E, Chakravarti A, Soliman EZ, Lunetta KL, Perz S, Wichmann HE, Meitinger T, Levy D, Gudnason V, Ellinor PT, Sanna S, Kääb S, Witteman JC, Alonso A, Benjamin EJ and Heckbert SR. Genome-wide association study of PR interval. Nat Genet 2010; 42: 153-159.
[13] Holm H, Gudbjartsson DF, Arnar DO, Thorleifsson G, Thorgeirsson G, Stefansdottir H, Gudjonsson SA, Jonasdottir A, Mathiesen EB, Njølstad I, Nyrnes A, Wilsgaard T, Hald EM, Hveem K, Stoltenberg C, Løchen ML, Kong A, Thorsteinsdottir U and Stefansson K. Several common variants modulate heart rate, PR interval and QRS duration. Nat Genet 2010; 42: 117-122.
[14] Zhao YY, Liu Y, Stan RV, Fan L, Gu Y, Dalton N, Chu PH, Peterson K, Ross J Jr and Chien KR. Defects in caveolin-1 cause dilated cardiomyopathy and pulmonary hypertension in knockout mice. Proc Natl Acad Sci U S A 2002; 99: 11375-11380.
[15] Olesen MS, Holst AG, Jabbari J, Nielsen JB, Christophersen IE, Sajadieh A, Haunsø S and Svendsen JH. Genetic loci on chromosomes 4q25, 7p31, and 12p12 are associated with onset of lone atrial fibrillation before the age of 40 years. Can J Cardiol 2012; 28: 191-195.
[16] NCI-NHGRI Working Group on Replication in Association Studies, Chanock SJ, Manolio T, Boehnke M, Boerwinkle E, Hunter DJ, Thomas G, Hirschhorn JN, Abecasis G, Altshuler D, Bailey-Wilson JE, Brooks LD, Cardon LR, Daly M, Donnelly P, Fraumeni JF Jr, Freimer NB, Gerhard DS, Gunter C, Guttmacher AE, Guyer MS, Harris EL, Hoh J, Hoover R, Kong CA, Merikangas KR, Morton CC, Palmer LJ, Phimister EG, Rice JP, Roberts J, Rotimi C, Tucker MA, Vogan KJ, Wacholder S, Wijsman EM, Winn DM and Collins FS. Replicating genotype-phenotype associations. Nature 2007; 447: 655-660.
[17] Fuster V, Rydén LE, Cannom DS, Crijns HJ, Curtis AB, Ellenbogen KA, Halperin JL, Le Heuzey JY, Kay GN, Lowe JE, Olsson SB, Prystowsky EN, Tamargo JL, Wann S, Smith SC Jr, Jacobs AK, Adams CD, Anderson JL, Antman EM, Halperin JL, Hunt SA, Nishimura R, Ornato JP, Page RL, Riegel B, Priori SG, Blanc JJ, Budaj A, Camm AJ, Dean V, Deckers JW, Despres C, Dickstein K, Lekakis J, McGregor K, Metra M, Morais J, Osterspey A, Tamargo JL, Zamorano JL; American College of Cardiology/American Heart Association Task Force on Practice Guidelines; European Society of Cardiology Committee for Practice Guidelines; European Heart Rhythm Association and Heart Rhythm Society. ACC/AHA/ESC 2006 Guidelines for the Management of Patients with Atrial Fibrillation: a report of the American College of Cardiology American Heart Association Task Force on Practice Guidelines and the European Society of Cardiology Committee for Practice Guidelines (Writing Committee to Revise the 2001 Guidelines for the Management of Patients With Atrial Fibrillation). Circulation 2006; 114: e257-e354.
[18] Frank PG, Pavlides S and Lisanti MP. Caveolae and transcytosis in endothelial cells: role in atherosclerosis. Cell Tissue Res 2009; 335: 41-47.
[19] Razani B, Engelman JA, Wang XB, Schubert W, Zhang XL, Marks CB, Macaluso F, Russell RG, Li M, Pestell RG, Di Vizio D, Hou H Jr, Kneitz B, Lagaud G, Christ GJ, Edelmann W and Lisanti MP. Caveolin-1 null mice are viable but show evidence of hyperproliferative and vascular abnormalities. J Biol Chem 2001; 276: 38121-38138.
[20] Sonveaux P, Martinive P, DeWever J, Batova Z, Daneau G, Pelat M, Ghisdal P, Grégoire V, Dessy C, Balligand JL and Feron O. Caveolin-1 expression is critical for vascular endothelial growth factor-induced ischemic hindlimb collateralization and nitric oxide-mediated angiogenesis. Circ Res 2004; 95: 154-161.
[21] Butler AM, Yin X, Evans DS, Nalls MA, Smith EN, Tanaka T, Li G, Buxbaum SG, Whitsel EA, Alonso A, Arking DE, Benjamin EJ, Berenson GS, Bis JC, Chen W, Deo R, Ellinor PT, Heckbert SR, Heiss G, Hsueh WC, Keating BJ, Kerr KF, Li

Y, Limacher MC, Liu Y, Lubitz SA, Marciante KD, Mehra R, Meng YA, Newman AB, Newton-Cheh C, North KE, Palmer CD, Psaty BM, Quibrera PM, Redline S, Reiner AP, Rotter JI, Schnabel RB, Schork NJ, Singleton AB, Smith JG, Soliman EZ, Srinivasan SR, Zhang ZM, Zonderman AB, Ferrucci L, Murray SS, Evans MK, Sotoodehnia N, Magnani JW and Avery CL. Novel loci associated with PR interval in a genome-wide association study of 10 African American cohorts. Circ Cardiovasc Genet 2012; 5: 639-646.