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Atrial Natriuretic Peptide Single Nucleotide Polymorphisms in Patients with Nonfamilial Structural Atrial Fibrillation

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Abstract

Background: Atrial natriuretic peptide (ANP) has antihypertrophic and antifibrotic properties that are relevant to AF substrates. The –G664C and rs5065 ANP single nucleotide polymorphisms (SNP) have been described in association with clinical phenotypes, including hypertension and left ventricular hypertrophy. A recent study assessed the association of early AF and rs5065 SNPs in low-risk subjects. In a Caucasian population with moderate-to-high cardiovascular risk profile and structural AF, we conducted a case-control study to assess whether the ANP –G664C and rs5065 SNP associate with nonfamilial structural AF.

Methods: 168 patients with nonfamilial structural AF and 168 age- and sex-matched controls were recruited. The rs5065 and –G664C ANP SNPs were genotyped.

Results: The study population had a moderate-to-high cardiovascular risk profile with 86% having hypertension, 23% diabetes, 26% previous myocardial infarction, and 23% left ventricular systolic dysfunction. Patients with AF had greater left atrial diameter (44 ± 7 vs. 39 ± 5 mm; $P < 0.001$) and higher plasma NTproANP levels (6240 ± 5317 vs. 3649 ± 2946 pmol/mL; $P < 0.01$). Odds ratios (ORs) for rs5065 and –G664C gene variants were 1.1 (95% confidence interval [CI], 0.7–1.8; $P = 0.71$) and 1.2 (95% CI, 0.3–3.2; $P = 0.79$), respectively, indicating no association with AF. There were no differences in baseline clinical characteristics among carriers and noncarriers of the –664C and rs5065 minor allele variants.

Conclusions: We report lack of association between the rs5065 and –G664C ANP gene SNPs and AF in a Caucasian population of patients with structural AF. Further studies will clarify whether these or other ANP gene variants affect the risk of different subphenotypes of AF driven by distinct pathophysiological mechanisms.

Keywords: atrial fibrillation, atrial natriuretic peptide, gene variants

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Introduction

Atrial fibrillation (AF) is the most common arrhythmia in clinical practice, as well as an important source of morbidity and mortality.¹ Although commonly associated with comorbidities,² AF also occurs in subjects without structural heart disease or left atrial remodeling.³ The Framingham Heart Study showed that the relative risk of AF is increased by 85% in individuals with parental history of AF,⁴ thus supporting a genetic predisposition. According to the pattern of heredity, AF is classified as familial or nonfamilial. The latter is the most common form of AF, and it occurs in association with cardiovascular diseases such as hypertension, myocardial infarction, heart failure, and valvular heart disease.⁵

Atrial natriuretic peptide (ANP) is a circulating hormone synthesized and secreted by atrial cardiomyocytes with natriuretic, vasorelaxant, antihypertrophic, and antifibrotic properties that are relevant to AF substrates.⁶ Single-nucleotide polymorphisms (SNPs) of the human *ANP* gene are known,⁷ and they have been associated to specific cardiovascular phenotypes and diseases.^{7–12} With regard to AF, a G664A variant within exon 1 (rs5063) was associated with lone AF¹² in a Chinese population but not in a larger American population of patients with early onset AF and low cardiovascular risk profile.¹³ In this latter report,¹³ a stop codon T2238C mutation within exon 3 (rs5065)¹⁴ was also explored with evidence of negative association. The –G664C variant, which is located within the *ANP* promoter region,¹⁵ has been described in association with early hypertension¹⁶ and left ventricular hypertrophy.⁹ However, it is presently unknown whether the –G664C variant is associated with structural AF. Moreover, as so far reported association studies rely on patients with lone AF or little comorbidities, it is also unclear whether carriers of these *ANP* gene variants may be more prone to develop AF when exposed to conventional risk factors.

We aimed to assess the potential contributory role of rs5065 and –G664C *ANP* gene variants to nonfamilial structural AF in a Caucasian population with a moderate-to-high cardiovascular risk profile.

Methods

Study population

Case (AF) and control (no-AF) patients were recruited among those admitted to the adult cardiology ward

or the hypertension unit for outpatients at St. Andrea Hospital in Rome. Case patients had at least 2 episodes of AF documented with baseline ECG or Holter ECG. Exclusion criteria were age <18 or >85 years, hyperthyroidism, electrolyte imbalance, severe chronic kidney dysfunction (eGFR < 30 mL/minute by the Cockcroft-Gault formula), left ventricular ejection fraction (EF) < 40%, and severe valvular heart disease. Patients with lone AF, familial AF, and first episode of AF were also excluded. For every AF patient, a matched control without history of AF was selected from the same geographical area. Case and control patients were matched with regard to their gender and age (difference <5 years).

Demographic, anthropometric, clinical, and laboratory data were recorded at study entry. AF was classified as paroxysmal, persistent, or permanent according to available guidelines.^{17,18} Frequency of AF episodes and symptoms were also recorded.

Hypercholesterolemia was defined as total cholesterol blood levels >200 mg/dL, LDL > 130 mg/dL or current treatment with statins.¹⁹ Hypertension was defined as systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg or current use of antihypertensive medications.²⁰ Diabetes was defined as baseline blood glucose >126 mg/dL or active treatment with insulin or with oral antidiabetic agents.²¹

All participants gave written informed consent before entering the study. The local Ethical Committee approved the research protocol.

Echocardiography

All participants underwent Doppler echocardiographic examination using an Acuson Sequoia C512 (Siemens Medical Solution, CA, USA) with a multifrequency transducer (2.5–4 MHz); images were performed using standardized acquisition methods.²² LV mass was calculated and normalized by (height^{2.7}).²³ Left ventricular hypertrophy was defined according to standard criteria.²⁴

Analysis of the ANP polymorphic markers

DNA was extracted from peripheral whole blood by the use of a commercially available kit (Qiagen, Venlo, The Netherlands). Characterization of *ANP* polymorphic markers was performed by previously reported procedures. We analysed a T2238C coding mutation (known



as rs5065) by a ScaI RFLP assay¹⁴ and a –G664C promoter mutation by an RsaI restriction fragment length polymorphism (RFLP) assay.²⁵ All polymerase chain reactions (PCRs) were performed with a PTC-100 thermal cycler. Digestion with the corresponding enzyme was carried out as recommended by the manufacturer (NEB). The PCR products were resolved on agarose gels and visualized by ethidium bromide staining. The genotypes were read by 2 blinded independent investigators, and any unclear result was resolved by new PCR.

NT-proANP plasma levels

A commercially available enzyme-linked immunosorbent assay (ELISA) kit (Gruppe Biomedica, Vienna, Austria) was used to assess plasma NT-proANP in a subgroup of patients in which plasma samples were collected at study entry (n = 247, 74% of the study population).

Assays were performed in duplicates.

Statistical analysis

Baseline characteristics of patients are presented as percentage for dichotomous variables and mean \pm SD of the mean for continuous variables. Normal distribution of data was assessed using histograms and Kolmogorov-Smirnov test. NT-proANP plasma con-

centrations showed a logarithmic normal distribution and were, therefore, logarithmically transformed. Differences between continuous variables were assessed using Student *t* test. Categorical variables were compared among groups by the Chi-square test. Hardy-Weinberg equilibrium for the 2 SNPs and allele frequencies in subjects with and without AF were tested using the chi-square test. The correlation between genotype and AF was examined with additive, dominant, and recessive models by logistic regression. Odds ratio and 95% CIs were calculated.

All tests were 2-sided, and a *P* value of less than 0.05 was considered statistically significant. All data analyses were performed with SPSS software package (version 19.0, SPSS, Inc., Chicago, Illinois). The power of our case-control sample was calculated by QUANTO statistical software. We estimated that we would have at least 80% power to detect relative risks of 1.65 for alleles with frequencies of 15% (rs5065) and 2.65 for risk alleles with frequencies of 2.5% (–G664C), assuming a dominant genetic model and a 2-sided significance level of 0.05.

Results

A total of 168 patients with nonfamilial structural AF and 168 age- and sex-matched controls were recruited. Clinical and echocardiographic characteristics of

Table 1. Baseline characteristics.

Variable	AF (n = 168)	Controls (n = 168)	<i>P</i> value
Demographic			
Male sex, n (%)	108 (64)	108 (64)	1
Age (years)	69 \pm 10	68 \pm 8	0.30
Comorbidities			
Hypertension, n (%)	147 (88)	143 (85)	0.63
SBP (mmHg)	126 \pm 15	126 \pm 16	0.69
DBP (mmHg)	75 \pm 10	75 \pm 29	0.75
Diabetes, n (%)	41 (24)	36 (21)	0.60
Hypercholesterolemia, n (%)	92 (55)	92 (55)	0.27
History of MI, n (%)	35 (21)	52 (31)	0.46
30 < CrCl < 60 mL/min, n (%)	44 (28)	52 (33)	0.27
Laboratory			
NT-proANP (pmol/mL)	6240 \pm 5317	3649 \pm 2946	<0.001
Echo			
Left atrial diameter (mm)	44 \pm 7	39 \pm 5	<0.001
LV mass (g/height ^{2.7})	50 \pm 15	48 \pm 14	0.23
40 < EF < 50%, n (%)	43 (26)	25 (22)	0.36

Note: Creatinine clearance was estimated according to the Cockcroft-Gault equation.

Abbreviations: AF, atrial fibrillation; SBP, systolic blood pressure; DBP, diastolic blood pressure; MI, myocardial infarction; CrCl, creatinine clearance; LV, left ventricular; EF, ejection fraction.



patients with and without AF are reported in Table 1. The study population had a moderate-to-high cardiovascular risk profile with 86% having hypertension, 55% hypercholesterolemia, and 23% diabetes. Of note, 26% of patients had previous myocardial infarction, and 23%, LV systolic dysfunction (Table 1).

As expected, patients with AF had greater left atrial diameter (44 ± 7 vs. 39 ± 5 mm; $P < 0.001$) and higher plasma NTproANP (6240 ± 5317 vs. 3649 ± 2946 pmol/mL; $P < 0.01$).

In univariate analysis, all variables listed in Table 1 were tested for association with AF. Only plasma log-NT-proANP (Hazard ratio [HR], 19.1; 95% CI, 6.5–55.6; $P < 0.0001$) and left atrial diameter (HR, 1.15; 95% CI, 1.10–1.20; $P < 0.001$) showed association with AF.

Genotype and allele frequencies of the 2 ANP gene variants are shown in Table 2. There was no deviation from Hardy–Weinberg equilibrium. Neither the rs5065 nor the –G664C variant displayed different distribution among patients with and without AF. Indeed, prevalence of the allelic variants for rs5065 and –G664C SNPs was 14.8% vs. 14.0% and 2.4% vs. 2.1% in patients with and without AF, respectively (Table 2). Moreover, 1.8% of patients with AF and 1.2% of controls were mutant homozygotes for the allelic variant of rs5065 ($P = 0.65$). No –G664C mutant homozygotes were found among subjects

with or without AF (Table 2). The calculated ORs for rs5065 and –G664C were 1.1 (95% CI, 0.7–1.8; $P = 0.71$) and 1.2 (95% CI, 0.3–3.2; $P = 0.79$), respectively, indicating no association with AF.

Carriers of the rs5065 and –G664C variants were similarly distributed among AF patterns (rs5065: paroxysmal 30%, persistent 27%, permanent 29%; $P = 0.92$ and –G664C: paroxysmal 8%, persistent 3%, permanent 6%; $P = 0.42$).

When analyzed by allele status, there were no significant differences in clinical characteristics among carriers and noncarriers of the rs5065 and –664C minor allele variants. In particular, plasma NT-proANP levels, left atrial diameter, and left ventricular mass were comparable (Table 3).

Discussion

Analysis of genetic determinants predisposing to AF could help in guiding research into causes, prevention, and treatment. Although evidence of a genetic basis of AF in selected patients has been reported,²⁶ identification of specific genetic determinants directly contributing to structural AF in at-risk populations is still under investigation. Noteworthy, identification of genetic elements contributing to or protecting from AF in the setting of multiple risk factors would strengthen the role of genetic predisposition.

Table 2. Association between genotypes, allele frequencies, and AF.

Gene variant	Genotype			Minor allele frequency and Hardy-Weinberg eq.	<i>P</i> value	
	WW	WM	MM			
rs5065 (n = 336)	244 (72.6%)	87 (25.9%)	5 (1.5%)	14.4%	HWE: 0.37	0.74
AF (n = 168)	121 (72.0%)	44 (26.2%)	3 (1.8%)	14.8%		
Controls (n = 168)	123 (73.2%)	43 (25.6%)	2 (1.2%)	13.9%		
–G664C (n = 336)	321 (95.5%)	15 (4.5%)	0	2.2%	HWE: 0.71	0.79
AF (n = 168)	160 (95.2%)	8 (4.8%)	0	2.4%		
Controls (n = 168)	161 (95.8%)	7 (4.2%)	0	2.1%		
Risk of AF and ANP SNPs						
Gene variant and model of inheritance	OR (95% IC)*			<i>P</i> value		
rs5065						
Recessive	1.5 (0.2–9.1)			0.65		
Additive	1.1 (0.7–1.7)			0.73		
Dominant	1.1 (0.7–1.8)			0.71		
–G664C						
Recessive	1			1		
Additive	1.2 (0.3–3.2)			0.79		
Dominant	1.2 (0.3–3.2)			0.79		

Abbreviations: WW, wild type; WM, heterozygous for allele gene variant; MM, homozygous for allele gene variant; HWE, Hardy-Weinberg equilibrium.

**Table 3.** Clinical characteristics according to rs5065/MA and –664C allele carrier status.

Variable	rs5065/MA		P value	–664C		P value
	Carriers (n = 91)	Non-carriers (n = 245)		Carriers (n = 15)	Non-carriers (n = 321)	
Atrial fibrillation, n (%)	47 (52%)	121 (49%)	0.71	8 (53%)	160 (50%)	0.79
Hypertension, n (%)	78 (86%)	212 (87%)	0.85	13 (87%)	277 (86%)	0.96
History of MI*, n (%)	24 (26%)	63 (26%)	0.90	6 (40%)	81 (25%)	0.20
Diabetes, n (%)	24 (26%)	53 (22%)	0.36	3 (20%)	74 (23%)	0.78
30 < CrCl < 60 mL/min, n (%)	29 (32%)	67 (27%)	0.43	6 (40%)	90 (28%)	0.41
NT-proANP (pmol/mL)	5576 ± 4171	4956 ± 4730	0.48	7299 ± 4786	5914 ± 4590	0.45
Left atrial diameter (mm)	42 ± 7	41 ± 7	0.34	40 ± 8	41 ± 7	0.24
LV mass _{2,7} (g/height ^{2.7})	49 ± 15	48 ± 14	0.86	48 ± 15	49 ± 14	0.76
40 < EF < 50%, n (%)	17 (19%)	61 (25%)	0.25	4 (27%)	74 (23%)	0.31

Abbreviations: MI, myocardial infarction; CrCl, creatinine clearance; LV, left ventricular; EF, ejection fraction; rs5065/MA, minor allele of rs5065 gene variant.

In the present study, we explored the potential contributory role of *ANP* genetic variants to AF in the context of predisposing factors. Accordingly, we recruited a study population with moderate-to-high cardiovascular risk profile and structural AF. Indeed, hypertension, diabetes, coronary artery disease, and left ventricular dysfunction may interplay in a setting of genetic predisposition that enhances or decreases the risk of AF. Our study was unable to detect any associations between nonfamilial structural AF and 2 *ANP* gene variants with known biological function and clinical role.^{8–11,27,28}

As expected, plasma NT-proANP levels were higher in AF patients as compared with control subjects. However, carriers and noncarriers of either –664C or rs5065 minor allele variants had similar plasma NT-proANP levels.

Development of nonfamilial structural AF may be genetically controlled to some extent, and the contributory role of the *ANP* gene has been investigated.^{12,13,27} In a family with 11 clinically affected members, Hodgson-Zingman et al²⁷ mapped an AF locus to chromosome 1p36-p35 and identified a heterozygous frameshift mutation in the gene encoding *ANP*. The rs5063 NT-proANP variant has been assessed in distinct populations with different conclusions. Indeed, it was associated with lone AF in a Chinese population¹² but not in a larger North American population of patients with early onset AF.¹³ In this latter report, the rs5065 variant was also explored without any evidence of association with AF.¹³ The rs5065 *ANP* variant is a relatively frequent polymorphism with known functional relevance,²⁷ and it has been previously

found to be associated with several cardiovascular phenotypes.^{7–11} Indeed, a post hoc analysis of more than 38,000 hypertensive patients from the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT)²⁹ showed that patients carrying the NT-ProANP rs5065 minor allele variant displayed tighter blood pressure control and more favorable cardiovascular outcomes when receiving a diuretic instead of a calcium channel blocker. Our results confirm lack of association between AF and *ANP* rs5065 minor allele variant and extend this finding to a population with structural AF.

We also aimed to determine whether the –G664C *ANP* variant associates with AF. Our results suggest lack of association. The –G664C polymorphism is located within a portion of the 5' flanking sequence of the human *ANP* gene and modulates *ANP* gene expression.⁹ We previously reported that the –G664C polymorphism is associated with increased left ventricular mass in essential hypertension,⁹ prompting us to explore whether an association exists with structural AF. In the present study, –664C minor allele carriers had similar left ventricular mass and were equally prone to develop AF as compared with noncarriers. Risk profile and comorbidities may account for the observed discrepancies. Indeed, the –G664C variant was shown to be associated with LV hypertrophy in hypertensives without cardiovascular comorbidities.⁹ On the contrary, the present study population includes many patients with comorbidities relevant to LV remodeling and AF substrates. Therefore, it cannot be ruled out at present that the –G664C SNP prompts AF independently from major predisposing factors.



Study limitations

Given the small number of rs5065 homozygous and –G664C homo/heterozygous minor alleles carriers, our study is globally underpowered to detect small contributions of gene variants to the risk of AF. A larger study population will help to confirm our results. Moreover, matching included age and sex but not other potential confounders. Risk factors relevant to structural AF were common in this study population. As a consequence, hypertensive patients constituted the large majority of both AF and control groups. It remains to be determined whether hypertension affects the risk of AF in rs5065 and –664C minor allele carriers.

Conclusions

Herein we report lack of association between the –G664C ANP promoter gene variant and AF. Moreover, we confirm lack of association of the rs5065 variant and AF by extending previous findings to a Caucasian population of patients with moderate-to-high cardiovascular risk and structural AF. Further studies are needed to understand whether these or other ANP gene SNPs may affect the risk of different subphenotypes of AF driven by distinct pathophysiological mechanisms.

Author Contributions

Conceived and designed the experiments: PF, AR, SR, MV. Analyzed the data: PF, AR, AF, RS, AM, FM, CA, IS, MC, CB. Wrote the first draft of the manuscript: PF, AR, SR. Contributed to the writing of the manuscript: PF, AR, CA, CB, SR, MV. Agree with manuscript results and conclusions: PF, AR, RS, AM, FM, CA, IS, MC, CB, SR, MV. Jointly developed the structure and arguments for the paper: PF, AR, SR, MV. Made critical revisions and approved final version: PF, AR, SR, MV. All authors reviewed and approved of the final manuscript.

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Competing Interests

Author(s) disclose no potential conflicts of interest.

Disclosures and Ethics

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

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