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THE ANP GENETIC VARIANT rs5068 AND CIRCULATING LEVELS OF NATRIURETIC PEPTIDES IN PATIENTS WITH CHRONIC HEART FAILURE

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Keywords

rs5068; genetic variant; atrial natriuretic peptide; B-type natriuretic peptide; natriuretic peptides; heart failure

Atrial (ANP) and B-type natriuretic peptides (BNP) are cardiac hormones released by the heart in response to myocardial stretch and volume overload (1). ANP and BNP induce vasodilation, natriuresis and suppress the renin-angiotensin-aldosterone system. In heart failure (HF), ANP and BNP are highly produced and secreted from the atria and ventricles in the attempt to compensate the increased cardiac filling pressures. Natriuretic peptides exert also a metabolic action, indeed, ANP and BNP induce lipolysis in vitro and vivo (2). In consideration of the metabolic properties of natriuretic peptides, it is still debated whether these hormones play a role in weight loss observed in cardiac cachexia. Previous studies conducted in general populations from Massachusetts, Sweden, Finland, Minnesota and Italy reported that the ANP genetic variant rs5068 is associated with higher circulating levels of ANP, BNP and lower blood pressure values, body mass index (BMI), waist circumference, prevalence of hypertension, obesity and metabolic syndrome (3-5). To date no studies have

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[1]This author takes responsability for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

described the phenotype related to rs5068 in the clinical context of chronic heart failure. Hence, the aim of our study was to characterize in-depth the cardiovascular and metabolic phenotype associated with rs5068 in a cohort of heart failure patients.

We genotyped a subset of 1173 HF patients (with available blood samples) from the GISSI Heart Failure (GISSI-HF) study cohort (6). The study has been approved by the Ethic Committees of the hospitals participating in the GISSI-HF study and conforms to the principles outlined in the Declaration of Helsinki. All subjects gave written informed consent to participate in the study. Continuous variables were compared by rs5068 genotype (AG vs AA) by analysis of variance (ANOVA) or Wilcoxon rank test as appropriate, as well as categorical variables were compared by logistic regression. Skewed variables were transformed to approximate normality with the logarithmic transformation. The association of the genotype with the study outcomes was assessed by multivariable Cox proportional hazards model.

Genotype frequencies of rs5068 were 94% (n= 1098) for AA and 6% (n= 75) for AG corresponding to a minor allele frequency of 3% (table 1). All comparisons are AG vs AA. In a multivariable analysis adjusted for age, sex, BMI, serum creatinine and New York Heart Association (NYHA) class, the G allele was associated with higher plasma concentrations of mid-regional pro-ANP (MR-proANP) (median 235 vs 206 pmol/L, p= 0.048) and BNP (180 vs 141 pg/ml, p= 0.027) (figure 1). Carriers of the minor allele tended to have higher NT-proBNP plasma levels (1161 vs 837 pg/ml, p= 0.13). At the age and sex adjusted analysis the two groups did not differ in terms of systolic (126.8 vs 125.2 mmHg, p= 0.53), diastolic (76.2 vs 76.5 mmHg, p= 0.85) blood pressures, prevalence of hypertension (61.3% vs 55.3%, p = 0.35) and BMI (26.5 vs 27.0 kg/m^2, p= 0.43) (tables 1). The prevalence of weight loss ≥ 5% in one year was similar between groups in the regression model adjusted for NYHA class, atrial fibrillation, age, heart rate, total cholesterol, glomerular filtration rate, central venous pressure, previous hospitalization, ascites, hepatomegaly, pulmonary congestion, peripheral edema (OR: 0.73 [95% CI 0.36-1.52], p = 0.40). In the 4-year follow-up, incidence of all-cause mortality (30.7% vs 26.7%, p= 0.37), cardiovascular mortality (25.3% vs 19.5%, p = 0.18) and hospitalization for cardiovascular reasons (60% vs 56.4%, p= 0.76) were not different between genotypes. In the multivariable analysis adjusted for age, sex, NYHA class, atrial fibrillation, diabetes mellitus, chronic obstructive pulmonary disease, diastolic blood pressure, LV ejection fraction, serum levels of total cholesterol, creatinine and triglycerides, third heart sound, uricemia and therapy with diuretics or beta-blockers, the G allele was not associated with risk of all-cause mortality (HR: 0.94 [95% CI 0.6 – 1.46], p=0.78), cardiovascular mortality (HR: 1.03 [95% CI 0.6 – 1.69], p=0.89) or hospitalization for cardiovascular reasons (HR: 1.07 [95% CI 0.79 – 1.46], p=0.65) during a 4-year follow up.

Consistent with previous studies conducted in general populations (3-5), HF patients who are carriers of rs5068 minor allele have significantly higher circulating levels of MR-proANP, which is a reliable estimator of ANP secretion, and BNP. Levels of NT-proBNP tend to be higher in the AG genotype. Regarding the mechanism that might cause the increase in circulating ANP levels in the carriers of rs5068 minor allele, an elegant recent study showed that the presence of the G allele counteracts the regulatory action of

microRNA miR-425, which inhibits *NPPA* expression (7). By preventing binding of miR-425 to *NPPA* gene, rs5068 G allele results in increased expression of the ANP gene and higher circulating levels of ANP. Whether the interaction between miR-425 and rs5068 might also cause higher circulating levels of BNP and NT-proBNP is still unknown but more likely, rs5068 could be in linkage disequilibrium with another single nucleotide polymorphism that is associated with higher BNP and NT-proBNP plasma values.

In our study, systolic and diastolic blood pressure, BMI and prevalence of hypertension did not differ according to rs5068 genotypes. In consideration of the metabolic properties of NPs we also analyzed whether rs5068 could be associated with a decrease in body weight ≥ 5% in one year, which is one of the main features for the diagnosis of cachexia (8), and no relation was found. A possible explanation could be related to the altered molecular forms of natriuretic peptides (NPs) that are produced in HF and might have impaired cardiovascular as well as metabolic properties (9,10). Further, in the metabolic dysfunction of heart failure the role played by inflammatory cytokines, hormones and neurotransmitters might minimize the possible metabolic effect exerted by NPs in general and more specifically by the increase in circulating NPs associated with rs5068.

In our 4 year follow-up analysis, the genetic variant rs5068 is not associated with all-cause mortality, cardiovascular mortality and hospitalization for cardiovascular reason. The biological effect of the increased circulating levels of NPs associated with rs5068 minor allele are likely overcome by major components such as therapy, compliance to therapy and comorbidities that have a stronger clinical impact in the final outcome of chronic heart failure.

Further investigations in larger cohorts may be required to evaluate whether rs5068 has an influence on clinical outcomes in HF patients.

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Abbreviations

ANP	Atrial natriuretic peptide
ANOVA	Analysis of variance
BMI	Body mass index
BNP	B-type natriuretic peptide
GISSI-HF	GISSI Heart Failure
HF	Heart failure
MR-proANP	Mid-regional pro-atrial natriuretic peptide
NPs	Natriuretic peptides
NT-proBNP	N-terminal-proBNP
NYHA	New York Heart Association

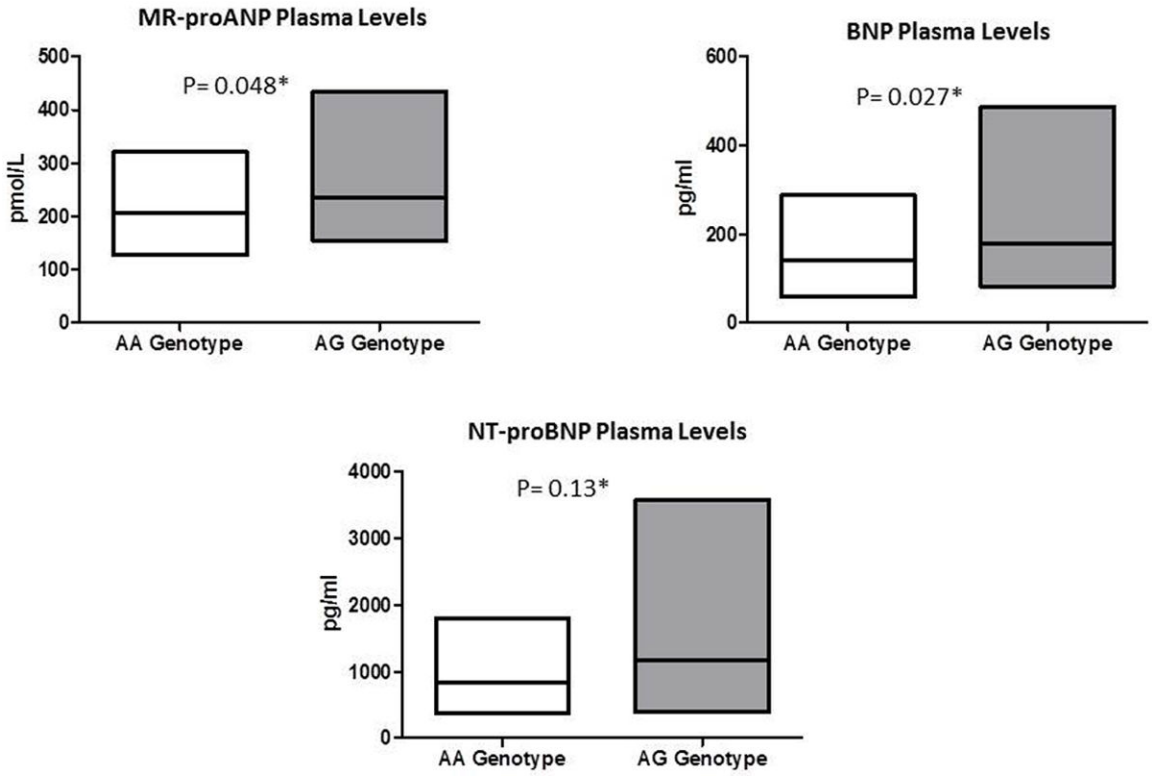

Figure 1. Natriuretic Peptides Plasma Levels according to rs5068 Genotypes

* P values obtained from analysis of variance on log transformed data, adjusted for age, sex, New York heart association class, body mass index and creatinine. Horizontal lines in the graphs represent first quartile, median and third quartile. Mid-regional pro-atrial natriuretic peptide (MR-proANP), B-type natriuretic peptide (BNP), N-terminal-proBNP (NT-proBNP)

Table 1

Characteristics of the study population according to rs5068 genotypes

Characteristics	Overall (n= 1173)	AA (n=1098)	AG (n=75)	p-value	Adjusted p-value
Male, %	80.5	80.4	81.3	0.85	
Age, y	67.0 (10.8)	66.9 (10.9)	67.9 (9.5)	0.44	
Systolic blood pressure, mmHg	125.3 (18.9)	125.2 (18.7)	126.8 (21.4)	0.47	0.53#
Diastolic blood pressure, mmHg	76.5 (10.5)	76.5 (10.5)	76.2 (11.3)	0.83	0.85#
BMI, kg/m^2	26.9 (4.5)	27 (4.6)	26.5 (3.9)	0.36	0.43#
Obesity (BMI≥ 30 kg/m^2),%	21.6	21.7	18.7	0.56	
LV ejection fraction, %	33.4 (9.4)	33.4 (9.3)	33.5 (11.4)	0.92	0.96#
NYHA III-IV, %	26.4	25.9	34.7	0.09	0.11#
Creatinine, mg/dl	1.1 (0.9-1.36)	1.1 (0.9-1.36)	1.1 (0.97-1.37)	0.70*	0.79#
Total Cholesterol, mg/dl	189.3 (41.7)	189.6 (41.8)	184.5 (41.4)	0.31	0.35#
HDL Cholesterol, mg/dl	48.1 (13.8)	48.1 (13.9)	47.9 (12.3)	0.89	0.89#
LDL Cholesterol, mg/dl	114.2 (35.6)	114.5 (36.0)	109.7 (29.3)	0.20	0.35#
Triglycerides, mg/dl	123 (91-177)	124 (91-177)	109 (80-151)	0.07*	0.10#
Glycaemia (serum glucose), mg/dl	118.8 (46.7)	118.9 (47.3)	117.2 (37.0)	0.71	0.78#
Weight Loss ≥ 5% in 1 year, n (%)	171 (14.6)	161 (14.7)	10 (13.3)	0.75	0.40**
Hypertension, %	55.7	55.3	61.3	0.31	0.35†
Diabetes Mellitus, %	26.1	26.2	24.0	0.67	0.67†
Diuretics, %	92.7	92.7	92.0	0.82	
ACE-inhibitors, %	81.4	81.2	85.3	0.37	
Beta-blockers, %	67.7	68.0	62.7	0.33	
Angiotensin Receptor Blockers, %	17.6	17.8	14.7	0.46	
Calcium Antagonist, %	8.3	8.1	10.7	0.60	
Spironolactone, %	42.4	41.8	50.7	0.13	

*Wilcoxon Two-Sample Test

#Analysis of Variance (ANOVA) adjusted for age and sex

† Logistic regression model adjusted for age and sex

** Logistic regression model adjusted for New York Heart Association class, atrial fibrillation, age, heart rate, total cholesterol, glomerular filtration rate, central venous pressure, previous hospitalization, ascites, hepatomegaly, pulmonary congestion, peripheral edema

Continuous variables are expressed as mean (standard deviation) or median (first quartile – third quartile).

Angiotensin-converting enzyme (ACE), body mass index (BMI), high-density lipoprotein (HDL), left ventricular (LV), low-density lipoprotein (LDL), New York Heart Association (NYHA)