Journal of the American College of Cardiology
© 2011 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 58, No. 6, 2011
ISSN 0735-1097/$36.00
doi:10.1016/j.jacc.2011.05.011

A Genetic Variant of the Atrial Natriuretic Peptide Gene Is Associated With Cardiometabolic Protection in the General Community

Valentina Cannone, MD, PHD,*† Guido Boerrigter, MD,* Alessandro Cataliotti, MD, PHD,* Lisa C. Costello-Boerrigter, MD, PHD,* Timothy M. Olson, MD,‡ Paul M. McKie, MD,* Denise M. Heublein, CLT,* Brian D. Lahr, MS,§ Kent R. Bailey, PHD,§ Maurizio Averna, MD, PHD† Margaret M. Redfield, MD,*‖ Richard J. Rodeheffer, MD,‖ John C. Burnett, JR, MD*

Rochester, Minnesota; and Palermo, Italy

Objectives We sought to define the cardiometabolic phenotype associated with rs5068, a genetic variant of the atrial natriuretic peptide (ANP) gene.

Background The ANP and B-type natriuretic peptide play an important role in cardiorenal homeostasis but also exert metabolic actions.

Methods We genotyped 1,608 randomly selected residents from Olmsted County, Minnesota. Subjects were well-characterized.

Results Genotype frequencies were: AA 89.9%, AG 9.7%, and GG 0.4%; all subsequent analyses were AA versus AG+GG. The G allele was associated with increased plasma levels of N-terminal pro-atrial natriuretic peptide ($p = 0.002$), after adjustment for age and sex. The minor allele was also associated with lower body mass index (BMI) ($p = 0.006$), prevalence of obesity ($p = 0.002$), waist circumference ($p = 0.021$), lower levels of C-reactive protein ($p = 0.027$), and higher values of high-density lipoprotein cholesterol ($p = 0.019$). The AG+GG group had a lower systolic blood pressure ($p = 0.011$) and lower prevalence of myocardial infarction ($p = 0.042$). The minor allele was associated with a lower prevalence of metabolic syndrome ($p = 0.025$). The associations between the G allele and high-density lipoprotein cholesterol, C-reactive protein values, myocardial infarction, and metabolic syndrome were not significant, after adjusting for BMI; the associations with systolic blood pressure, BMI, obesity, and waist circumference remained significant even after adjusting for N-terminal pro-atrial natriuretic peptide.

Conclusions In a random sample of the general U.S. population, the minor allele of rs5068 is associated with a favorable cardiometabolic profile. These findings suggest that rs5068 or genetic loci in linkage disequilibrium might affect susceptibility for cardiometabolic diseases and support the possible protective role of natriuretic peptides by their favorable effects on metabolic function. Replication studies are needed to confirm our findings. (J Am Coll Cardiol 2011;58:629–36) © 2011 by the American College of Cardiology Foundation

Since the discovery of atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) over 2 decades ago, the endocrine role of the heart in the control of salt and water balance and cardiac structure has been clearly established (1–3). Both ANP and BNP are secreted by the heart and are endogenous ligands for the particulate guanylyl cyclase-A receptor, mediating their biological actions via the second messenger 3′,5′ cyclic guanosine monophosphate (cGMP) and consequent activation of cGMP-dependent protein kinase, phosphodiesterases, and ion channels. Their actions include vasodilation, natriuresis, suppression of the renin-angiotensin-aldosterone system, and inhibition of both cardiomyocyte hypertrophy and cardiac fibroblast activation (4).

The natriuretic peptide precursor A gene (*NPPA*) lies in tandem with the BNP gene on chromosome 1 and encodes for the prohormone from which ANP and N-terminal

From the *Cardiorenal Research Laboratory, Division of Cardiovascular Diseases, Mayo Clinic and Mayo Clinic College of Medicine, Rochester, Minnesota; †Department of Clinical Medicine and Emerging Diseases, University of Palermo, Palermo, Italy; ‡Cardiovascular Genetics Research Laboratory, Divisions of Cardiovascular Diseases and Pediatric Cardiology, Mayo Clinic and Mayo Clinic College of Medicine, Rochester, Minnesota; §Division of Biostatistics, Mayo Clinic and Mayo Clinic College of Medicine, Rochester, Minnesota; and the ‖Division of Cardiovascular Diseases, Mayo Clinic and Mayo Clinic College of Medicine, Rochester, Minnesota. This work was supported by grants from the National Institutes of Health (RO1 HL55502, RO1 HL36634, and PO1 HL76611). All other authors have reported that they have no relationships to disclose.

Manuscript received December 15, 2010; revised manuscript received April 14, 2011, accepted May 15, 2011.

JACC Vol. 58, No. 6, 2011
August 2, 2011:629–36

Abbreviations and Acronyms

ANP = atrial natriuretic peptide
BMI = body mass index
BNP = B-type natriuretic peptide
BP = blood pressure
cGMP = 3′,5′ cyclic guanosine monophosphate
CRP = C-reactive protein
DNA = deoxyribonucleic acid
HDL = high-density lipoprotein
LV = left ventricular
NPPA = natriuretic peptide precursor A gene
NT-proANP = N-terminal pro-atrial natriuretic peptide
NT-proBNP = N-terminal pro–B-type natriuretic peptide
SBP = systolic blood pressure
SNP = single nucleotide polymorphism

pro-atrial natriuretic peptide (NT-proANP) are derived in equimolar amounts (5). Most recently, Newton-Cheh et al. (6) reported in a seminal study that 2 common genetic variants in the 3′ untranslated region and 2 kilobase downstream of *NPPA*—the single nucleotide polymorphisms (SNPs) rs5068 and rs198358, respectively—are associated with increased circulating levels of NT-proANP, N-terminal pro–B-type natriuretic peptide (NT-proBNP), lower blood pressure (BP), and reduced prevalence of hypertension. This recent study underscores the importance of ANP and BNP in BP homeostasis as well as the impact of common genetic variations on cardiovascular function as predicted from the early studies in mouse models in which ANP gene disruption resulted in increased BP (4,7).

Increasing evidence supports the view that both ANP and BNP might also play a fundamental role in metabolic regulation. Indeed, low levels of ANP infusion in healthy volunteers increase free fatty acids mobilization and lipid oxidation (8). In humans with heart failure, ANP infusion results in increased circulating levels of the adipokine adiponectin (9), which is an important regulator of glucose and lipid metabolism and possesses anti-hypertrophic properties in cardiomyocytes (10–12). In mice, activation of the guanylyl cyclase-A receptor has been shown to slow gastric emptying (13). Most recently, Miyashita et al. (14) demonstrated in a mouse model that BNP overexpression prevented the development of obesity in response to a high fat diet. Moreover, in humans, rs198389—an SNP in the promoter region of the BNP gene that increases circulating BNP—is associated with reduced risk for type 2 diabetes mellitus (15,16).

The impact of rs5068 on the metabolic phenotype in the general population is unknown. In the current study we hypothesized that rs5068, which has previously been associated with higher levels of ANP and BNP and lower BP, is also associated with a protective cardiometabolic phenotype in the general population. To test this hypothesis, we used a subset of a large, well-characterized sample of the general population 45 years and older from Olmsted County, Minnesota both to reconfirm the elegant studies of Newton-Cheh et al. (6) on BP and to extend our studies to define the cardiometabolic phenotype of rs5068 in the general community (17).

Methods

This study was approved by the Mayo Clinic Institutional Review Board.

Study population. We analyzed a subset of a clinically well-characterized population-based sample of the general population 45 years or older living in Olmsted County, Minnesota from 1997 to 2000. This population was first characterized as part of the National Institutes of Health-funded "Prevalence of Left Ventricular Dysfunction Study" and "Cardiac Peptides in Cardiorenal Regulation" (RO1 HL55502 and HL36634). The design and selection criteria of the aforementioned study as well as the characteristics of the Olmsted County population have been previously described (17,18). This population was characterized clinically and biochemically and by echocardiography. From the 2,027 subjects in this study from whom there were collected deoxyribonucleic acid (DNA) samples, a total of 1,608 subjects were successfully genotyped and were included in this study.

Body mass index (BMI) was measured as kilograms/meter squared. Obesity was defined as BMI $\geq$30 kg/m^2. Waist circumference was measured in centimeters at the top of the umbilicus. In accordance with the National Cholesterol Education Program Adult Treatment Panel III criteria, metabolic syndrome was defined by the presence of 3 or more of the following criteria: 1) central obesity defined as a waist circumference >102 cm in men and >88 cm in women; 2) triglyceride level >150 mg/dl (to convert to mmol/l, multiply by 0.0113); 3) high-density lipoprotein (HDL) cholesterol level <40 mg/dl (to convert to mmol/l, multiply by 0.0259) in men and <50 mg/dl in women; 4) blood pressure of 130/85 mm Hg or higher; and 5) fasting glucose level of 110 mg/dl (to convert to mmol/l, multiply by 0.0555) or higher. Hypertension was diagnosed with Joint National Committee VI criteria (19).

Genotyping. Genotyping of rs5068 was carried out on 1,608 subjects with TaqMan (Applied Biosystems, Foster City, California) according to the manufacturer's instructions, with 10 to 20 ng DNA. Primers and probes were Assay-by Design (Applied Biosystems). After polymerase chain reaction amplification, end reactions were read on the ABI Prism 7900ht with Sequence Detection Software (Applied Biosystems). The quality value percentage is a quality metric that indicates the reliability of called genotypes generated by the SDS software. The quality value was calculated with the ABI proprietary calling algorithm determining how well that sample fits into the cluster. Genotypes <95% are located further from their clusters and have a lower reliability. An electronic data file was generated that contains genotypes and the quality value.

Natriuretic peptide assays. Plasma NT-proANP levels were available in a subgroup of 1,485 subjects with a radioimmunoassay (Phoenix Pharmaceuticals, Belmont, California) (20). The NT-proBNP values were measured in

a subgroup of 1,566 subjects with an electrochemiluminescence immunoassay (Roche Diagnostics, Indianapolis, Indiana) (21).

Doppler echocardiography. All echocardiograms were performed with the same echocardiographic instrument (HP-2500, Hewlett Packard, Palo Alto, California) and were interpreted by a single echocardiologist blinded to clinical data. Two-dimensional and color Doppler imaging were performed to screen for valvular stenosis and regurgitation. In each subject, ejection fraction was measured, and diastolic function was classified as mild, moderate, and severe as previously described (17). Left ventricular (LV) dimension and mass and left atrial volume were calculated from M-mode and 2-dimensional measurements, respectively, and were indexed to body surface area (22–24). The LV mass was calculated according to the Devereux formula. Presence of LV hypertrophy was defined based on LV mass index >130 g/m^2 for men and >100 g/m^2 for women (25). Presence of left atrial enlargement was defined as left atrial volume index >33 ml/m^2 in men and >30 ml/m^2 in women (26).

Statistical analysis. Data pertaining to patient demographic information and clinical characteristics were summarized with descriptive statistics. These included counts and percentages for categorical and ordinal variables or medians and interquartile ranges for continuous variables. To test for an association with the rs5068 genotype, specifically whether or not a subject had at least 1 copy of the minor G allele, each clinical factor was modeled as the dependent variable via linear regression (or logistic regression if the factor was binary) with rs5068 genotype as the explanatory variable. All modeling was performed unadjusted and adjusted for potential confounding variables such as age and sex. Further adjustments were done for BMI and NT-proANP. Due to highly skewed distributions of the NT-proANP and NT-proBNP biomarkers, a probit transformation was applied to the ranked values of each, thus producing normal distributional properties. Similarly, other skewed variables, including C-reactive protein (CRP), serum glucose, insulin, and triglycerides levels, were transformed to approximate normality with the logarithmic transform. Furthermore, because age and sex are highly correlated with these biomarkers and with several other clinical factors, both were treated as adjusting covariates in the logistic models to control for their potential confounding. Although multiple hypothesis tests were carried out, a nominal 2-sided significance level of 0.05 was used with no formal adjustment for multiple testing. Given that approximately 30 to 40 variables were evaluated, the expected number of approximately 1 or 2 nominally significant results by chance alone should be considered in the interpretation of our findings. All analyses were carried out with the SAS statistical software package (version 8.2, SAS Institute, Inc., Cary, North Carolina).

Results

Prevalence of rs5068 and circulating natriuretic peptides. From collected DNA samples on 2,027 subjects, a total of 1,608 subjects were successfully genotyped and included in this study. Genotype frequencies of rs5068 were AA: 89.9% (n = 1,445), AG: 9.7% (n = 157), and GG: 0.4% (n = 6), corresponding to a minor allele frequency of 5.3%. The distribution was in Hardy-Weinberg equilibrium (p = 0.435). Due to the low frequency of homozygotes for the G allele, all analyses were performed assuming a dominant model with AG and GG genotypes combined. The characteristics of the study population are summarized in Table 1. Neither age nor sex were significantly associated with the genotype, although there was a trend toward a higher prevalence of women among those who had at least 1 minor allele compared with those with none (58% vs. 52%, age-adjusted p = 0.122). Importantly, the presence of at least 1 copy of the minor allele was associated with increased plasma levels of NT-proANP (median 2,584 vs. 2,188 pg/ml), both unadjusted (p < 0.001), and after adjustment for age, sex, and BMI (p = 0.006). In contrast, circulating levels of NT-proBNP were not significantly different between genotypes.

Cardiovascular phenotype. The G allele was significantly associated, controlling for age and sex, with lower systolic blood pressure (SBP) (delta = −4.28 mm Hg, p = 0.011) but not with diastolic BP (delta = −1.24 mm Hg, p = 0.132) (Table 1). The effect on SBP remained marginally significant after adjusting for BMI (p = 0.051) or further adjusting for NT-proANP (p = 0.054). The analysis of left atrial volume and LV structure and function as determined by echocardiography (LV ejection fraction, LV dimensions, LV mass, and LV volume index) did not reveal any significant associations with the rs5068 genotype.

With regard to cardiovascular diseases in a model adjusted for age and sex, the regression analysis showed that fewer minor allele carriers of rs5068 had a history of myocardial infarction (adjusted odds ratio: 0.29, p = 0.042), whereas in a model adjusted also for BMI, the result was slightly attenuated (odds ratio: 0.32, p = 0.061) (Table 2). There was no significant association between genotype and hypertension, coronary artery disease, congestive heart failure, atrial fibrillation, or cerebrovascular accident.

Metabolic phenotype. In a regression model adjusted for age and sex, the presence of at least 1 copy of the G allele was associated with lower BMI (delta = −1.22 kg/m^2, p = 0.006) (Table 3) and waist circumference (delta = −2.45 cm, p = 0.021) as well as reduced rate of obesity (odds ratio: 0.54, p = 0.002) (Fig. 1A). In addition, the minor allele was associated with higher levels of HDL cholesterol (delta = 2.46 mg/dl, p = 0.019), lower plasma values of CRP (0.17 vs. 0.20 mg/dl, p = 0.027) (Table 3), and—although not significant—with lower levels of insulin in subjects free of diabetes mellitus type 1 and 2 (4.7 μU/ml vs. 5.2 μU/ml, p = 0.069) (Table 3). Moreover, having at least 1 minor allele was associated with lower prevalence of metabolic syndrome

Table 1 Characteristics of the Study Population Across the rs5068 Genotype

Characteristic	AA (n = 1,445)	AG + GG (n = 163)	Unadjusted p Value*	Adjusted p Value†	Adjusted p Value‡	Adjusted p Value§
Women	746 (52%)	95 (58%)	0.108	0.122	0.173	0.396
Age, yrs	61.5 (53.2, 70.4)	62.5 (54.6, 71.6)	0.182	0.208	0.300	0.998
Age categories, yrs			0.183	0.211	0.295	0.935
45–54	436 (30%)	41 (25%)				
55–64	433 (30%)	53 (33%)				
65–74	367 (25%)	39 (24%)				
75+	209 (14%)	30 (18%)				
Systolic blood pressure, mm Hg	132 (116, 147)	129 (115, 144)	0.063	0.011	0.051	0.054
Diastolic blood pressure, mm Hg	73 (67, 80)	72 (66, 77)	0.063	0.132	0.247	0.262
Creatinine, mg/dl	1.0 (0.9, 1.2)	1.0 (0.9, 1.1)	0.283	0.437	0.479	0.450
NT-proBNP, pg/ml‖	67.4 (27.8, 146.9)	80.2 (37.8, 175.1)	0.096	0.413	0.497	0.565
NT-proANP, pg/ml‖	2,188 (1,374, 3,238)	2,584 (1,693, 3,842)	< 0.001	0.002	0.006	—
Ejection fraction, %	65 (60, 68)	65 (60, 65)	0.701	0.916	0.897	0.642
Ejection fraction <40%	28 (2%)	1 (1%)	0.254	0.247	0.284	0.207
Ejection fraction <50%	83 (6%)	6 (4%)	0.279	0.267	0.323	0.229
Moderate-to-severe diastolic dysfunction	106 (7%)	11 (7%)	0.627	0.458	0.432	0.173
LV dimension index, cm/m^2	2.6 (2.4, 2.8)	2.6 (2.4, 2.8)	0.236	0.321	0.910	0.689
LV mass index, g/m^2	94 (82, 109)	93 (82, 107)	0.297	0.315	0.565	0.337
LA volume index, ml/m^2	23.3 (19.5, 28.4)	23.9 (19.2, 27.9)	0.377	0.211	0.314	0.085

Values are counts (%) or median (25th and 75th percentile). *p value obtained from univariate regression model. †p value obtained from regression model adjusting for age and sex. ‡p value obtained from regression model adjusting for age, sex, and body mass index (BMI). §p value obtained from regression model adjusting for age, sex, BMI, and N-terminal pro-atrial natriuretic peptide (NT-proANP). ‖p values reflect probit transformation applied to rank-ordered NT-proANP and N-terminal pro–B-type natriuretic peptide (NT-proBNP) values.
LA = left atrial; LV = left ventricular.

(odds ratio: 0.58, p = 0.025) (Fig. 1B). The association between the rs5068 minor allele HDL cholesterol and CRP values and metabolic syndrome failed to remain significant (Table 3), after including BMI in the regression model. However, the association of the G allele with BMI, obesity, and waist circumference remained significant after adjusting for NT-proANP. Genotypes did not differ with regard to plasma concentration of total and low-density lipoprotein cholesterol, triglycerides, and fasting glucose (Table 3). Of note, the proportion of subjects with anti-lipemic treatment was similar between the 2 genotype groups (17% in both), as was the prevalence of diabetes mellitus type 2 (Table 2).

Discussion

Although ANP and BNP have been known to play a fundamental role in cardiorenal homeostasis, significant metabolic actions of natriuretic peptides have only recently emerged. Here we report for the first time that an ANP genetic variant associated with higher NT-proANP levels is associated with a favorable metabolic profile, primarily via its association with BMI, and that it is also associated with a favorable cardiovascular profile. Specifically, according to a regression analysis adjusted for age and sex, the minor allele of the *NPPA* SNP rs5068 correlates not only with reduced SBP and lower prevalence of myocardial infarction but also with lower BMI, prevalence of obesity, waist circumference, higher levels of HDL cholesterol, as well as lower values of CRP. Carriers of the minor allele are also characterized by a lower risk of metabolic syndrome.

In our study of residents in Olmsted County, Minnesota, the genotype frequencies for rs5068 were similar to the recently reported Framingham Heart Study and Malmö Diet and Finrisk97 cohorts (6). We observed that the G allele was associated with increased levels of NT-proANP,

Table 2 Prevalence of Cardiovascular Diseases and Diabetes Mellitus Type 2 Across the rs5068 Genotype

Characteristic	AA (n = 1,445)	AG + GG (n = 163)	Odds Ratio (95% CI) [p Value]*	Odds Ratio (95% CI) [p Value]†	Odds Ratio (95% CI) [p Value]‡
Verified hypertension	425 (29%)	47 (29%)	0.97 (0.68–1.39) [0.879]	0.91 (0.63–1.31) [0.600]	1.01 (0.69–1.48) [0.950]
Coronary artery disease	185 (13%)	14 (9%)	0.64 (0.36–1.13) [0.124]	0.57 (0.31–1.05) [0.071]	0.61 (0.33–1.12) [0.109]
Myocardial infarction	80 (6%)	3 (2%)	0.32 (0.10–1.02) [0.055]	0.29 (0.09–0.96) [0.042]	0.32 (0.10–1.06) [0.061]
Congestive heart failure	31 (2%)	5 (3%)	1.44 (0.55–3.77) [0.453]	1.40 (0.52–3.74) [0.504]	1.57 (0.58–4.23) [0.373]
Atrial fibrillation	68 (5%)	10 (6%)	1.32 (0.67–2.63) [0.421]	1.29 (0.63–2.64) [0.487]	1.35 (0.66–2.77) [0.417]
Cerebrovascular accident	20 (1%)	4 (2%)	1.79 (0.61–5.31) [0.292]	1.76 (0.59–5.26) [0.311]	1.79 (0.60–5.37) [0.300]
Diabetes mellitus type 2	108 (7%)	13 (8%)	1.07 (0.59–1.95) [0.818]	1.05 (0.57–1.93) [0.875]	1.26 (0.68–2.35) [0.459]

*Odds ratio, 95% confidence interval (CI), and p value obtained from logistic regression univariate model. †Odds ratio, 95% CI, and p value obtained from logistic regression model adjusting for age and sex. ‡Odds ratio, 95% CI, and p value obtained from logistic regression model adjusting for age, sex, and body mass index.

Table 3 Metabolic Parameters in the Study Population According to the rs5068 Genotype

Characteristic	AA (n = 1,445)	AG + GG (n = 163)	Unadjusted p Value*	Adjusted p Value†	Adjusted p Value‡	Adjusted p Value§
BMI, kg/m^2	27.9 (25, 31.6)	26.7 (24.3, 29.4)	0.003	0.006	—	0.006‖
Obesity (BMI ≥30 kg/m^2)	503 (35%)	36 (22%)	0.001	0.002	—	0.001‖
Waist circumference, cm	93 (82, 101)	90 (80, 96)	0.006	0.021	—	0.025‖
Total cholesterol, mg/dl	200 (178, 222)	207 (183, 231)	0.190	0.274	0.301	0.336
HDL cholesterol, mg/dl	42 (35, 54)	47 (39, 58)	0.004	0.019	0.115	0.205
LDL cholesterol, mg/dl	125 (105, 146)	133 (108, 154)	0.296	0.275	0.280	0.197
Triglycerides, mg/dl¶	129 (95, 182)	123 (88, 169)	0.102	0.096	0.295	0.234
Serum glucose, mg/dl¶	94 (89, 101)	93.0 (89, 101)	0.573	0.535	0.960	0.486
Insulin, μU/ml¶#	5.2 (3.6, 7.8)	4.7 (3.5, 7.2)	0.059	0.069	0.446	0.469
C-reactive protein, mg/dl¶	0.20 (0.09, 0.44)	0.17 (0.07, 0.40)	0.083	0.027	0.220	0.120
Metabolic syndrome	296 (20%)	21 (13%)	0.024	0.025	0.286	0.133

Values are counts (%) or median (25th and 75th percentile). *p value obtained from univariate regression model. †p value obtained from regression model adjusting for age and sex. ‡p value obtained from regression model adjusting for age, sex and BMI. §p value obtained from regression model adjusting for age, sex, BMI, and NT-proANP. ‖BMI not adjusted for in the model; adjusting factors were age, sex, and NT-proANP only. ¶p values based on logarithmic transformed variable. #Analyzed on subgroup of subjects free of diabetes mellitus type 1 and 2.
HDL = high-density lipoprotein; LDL = low density lipoprotein; other abbreviations as in Table 1.

similar to this recent report. This increase in NT-proANP is notable, because Itoh et al. (27) reported that it is produced in equimolar amounts with ANP and represents a robust estimator of ANP secretion from the heart (28).

Although a major stimulus for ANP release is increased atrial stretch, there is no indication that this would explain the higher levels associated with the minor allele in our study, because there were no differences in terms of congestive heart failure, atrial fibrillation or left atrial enlargement across genotypes. Moreover, BP levels were lower in the group characterized by the presence of the minor allele. Newton-Cheh et al. (6) speculated that rs5068, which is located in the 3' untranslated region, could affect transcript stability and result in higher ANP production. These investigators pointed out that this mechanism would not explain the higher BNP levels seen in their study, the levels of which would be expected to decrease in a compensatory manner if the primary mechanism was an increase in ANP production. They propose an alternative explanation that rs5068 resides in a shared enhancer element that coordinately regulates expression of the adjacent *NPPA* and *NPPB* genes. However, unlike in the study by Newton-Cheh et al. (6), levels of NT-proBNP were not different between genotypes in our cohort. This discrepancy could be a consequence of the smaller size of the sample analyzed in our study as well as the different assay performed.

Given the known metabolic effects of natriuretic peptides and the increased levels of NT-proANP and NT-proBNP associated with the rs5068 minor allele, it was our main objective to define the metabolic phenotype associated with the rs5068 genotype. Indeed, minor allele carriers not only showed a favorable cardiovascular profile characterized by lower SBP and lower prevalence of myocardial infarction, but they also had a favorable metabolic phenotype characterized by lower BMI, waist circumference, and prevalence of obesity. Furthermore, they had higher levels of HDL cholesterol and lower levels of CRP. The differences in HDL and CRP cannot be explained by anti-lipemic treatment, because both genotypes were similar in this regard. Consistent with all these findings, the prevalence of the metabolic syndrome in our study population was lower in the minor allele carriers.

These associations support, once more, the view of a significant, clinically relevant metabolic action of natriuretic peptides, more specifically ANP. Interestingly, Miyashita et al. (14) provided recent important findings with regard to the critical role of natriuretic peptides and guanylyl cyclase-A receptor stimulation in lipid catabolism and glucose intolerance. Transgenic mice overexpressing BNP were protected against obesity and insulin resistance induced by high-fat diet, whereas transgenic mice overexpressing cGMP-dependent protein kinase revealed a significant reduction in body weight and higher insulin sensitivity compared with wild-type mice, even on standard diet. Both models showed augmented muscle mitochondrial biogenesis and fat oxidation. Of note, acute infusion of ANP in humans has been reported to promote insulin secretion and inhibit glucagon secretion (29,30). However, in our study this direct insulin-increasing effect of ANP is likely to be offset by the favorable metabolic status of rs5068 minor allele carriers, who would be expected to have improved insulin sensitivity and thus generally lower insulin levels.

Although our study is an association study that cannot establish a causal relationship between increased NT-proANP and cardiovascular-metabolic phenotype, our findings support the emerging metabolic role of guanylyl cyclase-A activation. The capacity of ANP and BNP to promote lipid mobilization and oxidation, slow gastric emptying, decrease blood pressure and body weight, and increase insulin sensitivity provides strong arguments in favor of the capacity of the natriuretic peptide/guanylyl cyclase-A/cGMP signaling system to enhance metabolic function and phenotype (6,8,13,14).

Moreover, our findings support the hypothesis that the presence of at least 1 copy of the minor allele of rs5068 associated with higher circulating level of NT-proANP

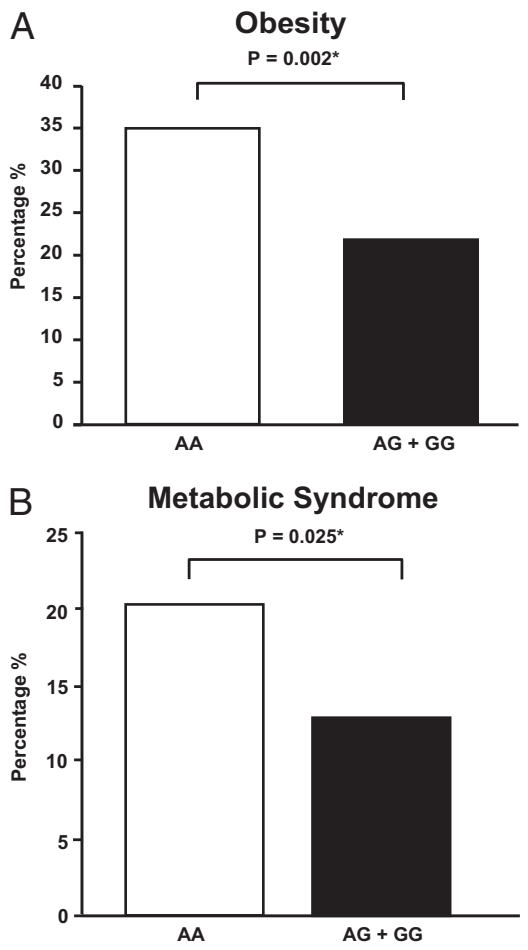

Figure 1 **Prevalence of Obesity and Metabolic Syndrome in the Study Population According to Genotype**

(A) Prevalence of obesity in the study population according to genotype. Obesity was defined as body mass index ≥30 kg/m^2. **(B)** Prevalence of metabolic syndrome in the study population according to genotype. *p value obtained from logistic regression model adjusting for age and sex.

might identify individuals with lower risk for cardiovascular and metabolic diseases.

Despite no formal adjustment for multiple comparisons, the consistency of the main significant findings with previous results as well as the number of significant findings tend to support the association between the minor allele of rs5068 and a favorable cardiometabolic phenotype. We acknowledge that a potential criticism of the study is that significant associations with multiple comparisons occurred by chance. On the basis of the null distribution of p values from the approximately 40 unique associations tested, the expected number of type I errors for this study is 2 (i.e., on average, 2 associations detected as nominally significant at the 0.05 level would have occurred by chance alone). Because there were a total of 6 such associations detected (counting the significant results for BMI, obesity, and waist circumference as only 1)—more than the expected chance finding—and because several of these associations with the rs5068 genotype reflected a consistent protective effect on cardiometabolic parameters, it is unlikely that chance alone explains the associations. The results might not meet a strict threshold for multiple testing, but given the a priori hypothesis and experimental data, the findings seem plausible.

We also note that the associations between the G allele and values of HDL cholesterol, CRP, metabolic syndrome, and myocardial infarction were attenuated after fitting a regression model adjusted for age, sex, and BMI. These data could reveal how these metabolic associations might be mediated by the primary important correlation between the *NPPA* SNP and lower BMI. We further performed a regression analysis including NT-proANP in our model. The associations between the minor allele of rs5068 and BMI, prevalence of obesity, and values of waist circumference still remained significant, whereas the associations between genotype and SBP and myocardial infarction remained marginally significant. These findings should be interpreted cautiously. Indeed, our data might indicate that rs5068 or genetic loci in linkage disequilibrium with it might affect susceptibility for cardiometabolic diseases. There is also the possibility that rs5068 alters lifelong levels of ANP, and this effect is not completely accounted for by the measurement of 1 value of NT-proANP in each individual. Moreover, it is important to remark that a lack of attenuation after assuming a regression model adjusted for NT-proANP does not exclude a possible biological role for ANP in determining the observed favorable metabolic phenotype. With respect to detection of a relationship between plasma ANP levels and metabolic phenotype, we were probably limited by the size of our sample, our ability to directly measure ANP due to the short half-life of ANP, and instability of the peptide under laboratory conditions. Although we tested for an association, we did not detect any difference between groups with respect to plasma ANP levels.

Our model considered, as a confounding variable, not ANP but NT-proANP, which was significantly higher in the group characterized by the presence of the minor allele. Indeed, NT-proANP is secreted in equimolar amounts with ANP (27). Due to its longer half-life, greater laboratory stability, and less variability in plasma concentration, NT-proANP is considered a reliable biomarker to estimate ANP secretion from the heart (27,28), but there is no evidence in published reports regarding a biological action of NT-proANP. On the contrary, several findings support the view of ANP as an important metabolic regulator. Infusion of ANP at pharmacological doses into healthy lean men and head-down bed rest position, both of which increase plasma ANP levels, promote lipid mobilization and use (8,31). This lipolytic effect of ANP is independent of insulin or sympathetic nervous system activation and is mediated by a cGMP-dependent pathway that induces the phosphorylation of hormone-sensitive lipase and perilipin A via the activation of cGMP-dependent protein kinase I (32,33). Furthermore, in a key Framingham study, NT-proANP and BNP correlated inversely with the metabolic

syndrome and its individual components, even after adjustment for BMI (34).

A replication study is clearly needed to confirm the validity of our data, and the lack of replication in the current study is a limitation. An additional limitation is the low genotyping call rate. To support the validity of our findings from a statistical point of view, we can assume that the failures occurred in a random fashion unrelated to any phenotype traits. Indeed, the genotype frequencies of rs5068 in our population are similar to the frequencies reported in the HapMap Project with regard to a population from Utah, in the Framingham Heart Study, Malmö Diet and Finrisk cohort.

Our investigation together with previous experimental data provide a rationale for the development of ANP or guanylyl cyclase-A agonist/ANP/BNP-like drugs as potential cardiometabolic therapeutics that are able to target the complex metabolic syndrome on several different levels. Indeed, our recent report of the feasibility of orally delivered BNP in an animal model of experimental hypertension supports such a therapeutic direction (35,36). Further studies are required to confirm our findings and to clarify the physiological mechanisms underlying the effects exerted by natriuretic peptides on metabolism.

Conclusions

Our findings in a general community population demonstrate that the minor allele of rs5068 is associated with a favorable cardiometabolic profile characterized by higher levels of NT-proANP and HDL cholesterol, lower SBP, prevalence of myocardial infarction, BMI, waist circumference, CRP levels, and prevalence of metabolic syndrome. Our results, which both confirm previous findings regarding a link to blood pressure and extend the relationship of rs5068 to cardiometabolic homeostasis, suggest that rs5068 or genetic loci in linkage disequilibrium might affect susceptibility for cardiometabolic diseases. Replication studies are needed to confirm our results. These findings support the possible protective role of natriuretic peptides by their favorable effects on metabolic function, including body weight and lipid metabolism with clinical implications in disease prevention and innovative therapeutics.

Reprint requests and correspondence: Dr. Valentina Cannone, Cardiorenal Research Laboratory, Guggenheim 915, Mayo Clinic and Foundation, 200 First Street Southwest, Rochester, Minnesota 55905. E-mail: cannone.valentina@mayo.edu.

REFERENCES

1. de Bold AJ, Borenstein HB, Veress AT, Sonnenberg H. A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. Life Sci 1981;28:89–94.
2. Kangawa K, Matsuo H. Purification and complete amino acid sequence of alpha-human atrial natriuretic polypeptide (alpha-hANP). Biochem Biophys Res Commun 1984;118:131–9.
3. Holtwick R, Gotthardt M, Skryabin B, et al. Smooth muscle-selective deletion of guanylyl cyclase-A prevents the acute but not chronic effects of ANP on blood pressure. Proc Natl Acad Sci U S A 2002;99:7142–7.
4. Garbers DL, Chrisman TD, Wiegn P, et al. Membrane guanylyl cyclase receptors: an update. Trends Endocrinol Metab 2006;17:251–8.
5. Nemer M, Chamberland M, Sirois D, et al. Gene structure of human cardiac hormone precursor, pronatriodilatin. Nature 1984;312:654–6.
6. Newton-Cheh C, Larson MG, Vasan RS, et al. Association of common variants in NPPA and NPPB with circulating natriuretic peptides and blood pressure. Nat Genet 2009;41:348–53.
7. Smithies O, Kim HS, Takahashi N, Edgell MH. Importance of quantitative genetic variations in the etiology of hypertension. Kidney Int 2000;58:2265–80.
8. Birkenfeld AL, Boschmann M, Moro C, et al. Lipid mobilization with physiological atrial natriuretic peptide concentrations in humans. J Clin Endocrinol Metab 2005;90:3622–8.
9. Tsukamoto O, Fujita M, Kato M, et al. Natriuretic peptides enhance the production of adiponectin in human adipocytes and in patients with chronic heart failure. J Am Coll Cardiol 2009;53:2070–7.
10. Maeda N, Shimomura I, Kishida K, et al. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nat Med 2002;8:731–7.
11. Shibata R, Ouchi N, Ito M, et al. Adiponectin-mediated modulation of hypertrophic signals in the heart. Nat Med 2004;10:1384–9.
12. Costello-Boerrigter LC, Burnett JC Jr. A new role for the natriuretic peptides: metabolic regulators of the adipocyte. J Am Coll Cardiol 2009;53:2078–9.
13. Addisu A, Gower WR Jr., Landon CS, Dietz JR. B-type natriuretic peptide decreases gastric emptying and absorption. Exp Biol Med (Maywood) 2008;233:475–82.
14. Miyashita K, Itoh H, Tsujimoto H, et al. Natriuretic peptides/cGMP/cGMP-dependent protein kinase cascades promote muscle mitochondrial biogenesis and prevent obesity. Diabetes 2009;58:2880–92.
15. Meirhaeghe A, Sandhu MS, McCarthy MI, et al. Association between the T-381C polymorphism of the brain natriuretic peptide gene and risk of type 2 diabetes in human populations. Hum Mol Genet 2007;16:1343–50.
16. Choquet H, Cavalcanti-Proenca C, Lecoeur C, et al. The T-381C SNP in BNP gene may be modestly associated with type 2 diabetes: an updated meta-analysis in 49 279 subjects. Hum Mol Genet 2009;18:2495–501.
17. Redfield MM, Jacobsen SJ, Burnett JC Jr., Mahoney DW, Bailey KR, Rodeheffer RJ. Burden of systolic and diastolic ventricular dysfunction in the community: appreciating the scope of the heart failure epidemic. JAMA 2003;289:194–202.
18. McKie PM, Cataliotti A, Lahr BD, et al. The prognostic value of N-terminal pro–B-type natriuretic peptide for death and cardiovascular events in healthy normal and stage A/B heart failure subjects. J Am Coll Cardiol 2010;55:2140–7.
19. The sixth report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure. Arch Intern Med 1997;157:2413–46.
20. Burnett JC Jr., Kao PC, Hu DC, et al. Atrial natriuretic peptide elevation in congestive heart failure in the human. Science 1986;231:1145–7.
21. Costello-Boerrigter LC, Boerrigter G, Redfield MM, et al. Amino-terminal pro-B-type natriuretic peptide and B-type natriuretic peptide in the general community: determinants and detection of left ventricular dysfunction. J Am Coll Cardiol 2006;47:345–53.
22. Shub C, Klein AL, Zachariah PK, Bailey KR, Tajik AJ. Determination of left ventricular mass by echocardiography in a normal population: effect of age and sex in addition to body size. Mayo Clin Proc 1994;69:205–11.
23. Murray JA, Kennedy JW, Figley MM. Quantitative angiocardiography. II. The normal left atrial volume in man. Circulation 1968;37:800–4.
24. Redfield MM, Rodeheffer RJ, Jacobsen SJ, Mahoney DW, Bailey KR, Burnett JC Jr. Plasma brain natriuretic peptide concentration: impact of age and gender. J Am Coll Cardiol 2002;40:976–82.
25. Levy D, Savage DD, Garrison RJ, Anderson KM, Kannel WB, Castelli WP. Echocardiographic criteria for left ventricular hypertrophy: the Framingham Heart Study. Am J Cardiol 1987;59:956–60.

26. Pritchett AM, Jacobsen SJ, Mahoney DW, Rodeheffer RJ, Bailey KR, Redfield MM. Left atrial volume as an index of left atrial size: a population-based study. J Am Coll Cardiol 2003;41:1036–43.
27. Itoh H, Nakao K, Sugawara A, et al. Gamma-atrial natriuretic polypeptide (gamma ANP)-derived peptides in human plasma: cosecretion of N-terminal gamma ANP fragment and alpha ANP. J Clin Endocrinol Metab 1988;67:429–37.
28. Thibault G, Murthy KK, Gutkowska J, et al. NH2-terminal fragment of rat pro-atrial natriuretic factor in the circulation: identification, radioimmunoassay and half-life. Peptides 1988;9:47–53.
29. Uehlinger DE, Weidmann P, Gnadinger MP, et al. Increase in circulating insulin induced by atrial natriuretic peptide in normal humans. J Cardiovasc Pharmacol 1986;8:1122–9.
30. Verspohl EJ, Bernemann IK. Atrial natriuretic peptide (ANP)-induced inhibition of glucagon secretion: mechanism of action in isolated rat pancreatic islets. Peptides 1996;17:1023–9.
31. Moro C, Pillard F, de Glisezinski I, et al. Atrial natriuretic peptide contribution to lipid mobilization and utilization during head-down bed rest in humans. Am J Physiol Regul Integr Comp Physiol 2007;293:R612–7.
32. Galitzky J, Sengenes C, Thalamas C, et al. The lipid-mobilizing effect of atrial natriuretic peptide is unrelated to sympathetic nervous system activation or obesity in young men. J Lipid Res 2001;42:536–44.
33. Sengenes C, Bouloumie A, Hauner H, et al. Involvement of a cGMP-dependent pathway in the natriuretic peptide-mediated hormone-sensitive lipase phosphorylation in human adipocytes. J Biol Chem 2003;278:48617–26.
34. Wang TJ, Larson MG, Keyes MJ, Levy D, Benjamin EJ, Vasan RS. Association of plasma natriuretic peptide levels with metabolic risk factors in ambulatory individuals. Circulation 2007;115:1345–53.
35. Cataliotti A, Chen HH, Schirger JA, et al. Chronic actions of a novel oral B-type natriuretic peptide conjugate in normal dogs and acute actions in angiotensin II-mediated hypertension. Circulation 2008; 118:1729–36.
36. Cataliotti A, Schirger JA, Martin FL, et al. Oral human brain natriuretic peptide activates cyclic guanosine 3′,5′-monophosphate and decreases mean arterial pressure. Circulation 2005;112:836–40.

Key Words: atrial natriuretic peptide ■ cardiometabolic disease ■ lipid metabolism ■ metabolic syndrome ■ natriuretic peptides.