The Atrial Natriuretic Peptide Genetic Variant rs5068 Is Associated With a Favorable Cardiometabolic Phenotype in a Mediterranean Population

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OBJECTIVE—We hypothesized that the minor allele of the atrial natriuretic peptide (ANP) genetic variant rs5068 is associated with a favorable cardiometabolic phenotype in a general Mediterranean population.

RESEARCH DESIGN AND METHODS—We genotyped a random sample of the residents of Ventimiglia di Sicilia, Sicily, for rs5068.

RESULTS—Genotype frequencies of rs5068 are AA, 93.5%; AG, 6.4%; and GG, 0.1%. All subsequent analyses are AA versus AG+GG. After adjusting for age and sex, the minor G allele is associated with lower BMI (estimate [SE]: -1.7 kg/m^2 [0.8], $P = 0.04$). In the AG+GG group, males with HDL cholesterol levels <40 mg/dL are less frequent ($P = 0.05$) and obesity tends to be less prevalent ($P = 0.07$). Importantly, the G allele is associated with a lower prevalence of metabolic syndrome ($P = 0.02$). After adjusting for BMI, the above associations were attenuated. Independently of age, sex, and BMI, the minor allele is also associated with lower systolic blood pressure (-6.0 mmHg [2.5], $P = 0.02$) and lower prevalence of hypertension (odds ratio 0.41 [95% CI 0.20–0.83], $P = 0.01$).

CONCLUSIONS—The association between the minor allele of rs5068 and a favorable cardiometabolic phenotype that we previously reported in a U.S. population is now replicated in a Mediterranean population in which the G allele of rs5068 is associated with lower blood pressure, BMI, and prevalence of hypertension and metabolic syndrome. These findings may lead to a diagnostic strategy to assess cardiometabolic risk and lay the foundation for the future development of an ANP or ANP-like therapy for metabolic syndrome.

The cardiac hormones atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) are secreted by the heart in response to myocardial stretch and play a key role in water and sodium homeostasis as well as in the regulation of blood pressure (1). Indeed, ANP and BNP possess cardiovascular and renal properties, inducing vasodilation, natriuresis, suppression of the renin-angiotensin-aldosterone system, and inhibition of cardiac hypertrophy and fibrosis. Both hormones activate natriuretic peptide receptor A (NPR-A), which is linked to particulate guanylyl cyclase generating the second messenger cGMP. The natriuretic peptide clearance receptor (NPR-C) and neutral endopeptidase 24.11 are responsible for the degradation of ANP and BNP and therefore, importantly, contribute to the regulation of circulating hormone concentrations.

During the last two decades, several studies have shown that beyond cardiovascular and renal properties, natriuretic peptides also exert metabolic actions. Specifically, the natriuretic peptides ANP and BNP induce lipolysis in human adipocytes in vitro and in vivo (2). In the study by Bordicchia et al. (3), activation of NPR-A through the infusion of BNP promoted energy expenditure in mice. Furthermore, intravenous infusion of ANP in healthy, normal-weight subjects leads to an ANP concentration–dependent increase in venous glycerol and nonesterified fatty acid values, stimulating lipid mobilization and oxidation (4); whereas a marked lipolysis occurred in the subcutaneous abdominal adipose tissue of heart failure patients after administration of BNP (5). The lipid-mobilizing effect of ANP is not related to the activation of the sympathetic nervous system and it is not affected by obesity as ANP remains a potent lipolytic agent even in obese young individuals (6). Further, the metabolic properties of ANP are not mediated by insulin (7) but by the activation of hormone-sensitive lipase and perilipin A through a cGMP-dependent pathway (8). An important interaction between natriuretic peptides and metabolism is also suggested by community-based analyses. Interestingly, plasma levels of natriuretic peptides are reduced in obese subjects (9) and in individuals affected by metabolic syndrome, suggesting that these two pathological states may represent natriuretic peptide deficiency states (10,11). Moreover, insulin resistance is associated with a lower natriuretic peptide plasma value in obese and nonobese subjects (12).

The natriuretic peptide precursor A gene (*NPPA*) encodes for the prohormone from which ANP and N-terminal-proatrial-natriuretic peptide (NT-proANP) are obtained in equimolar amounts (13) and lies in tandem with the BNP gene (*NPPB*) on chromosome 1 (14). Several studies have investigated the cardiovascular phenotype associated with the genetic

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variant rs5068, which is located in the 3′ untranslated region of *NPPA*. A large study on common variants of *NPPA* and *NPPB* performed by Newton-Cheh et al. (15) revealed that the minor G allele of rs5068 is associated with higher circulating levels of ANP and BNP, lower values of blood pressure, and reduced odds of hypertension. Further, Ellis et al. (16) confirmed the association between the G allele and increased plasma levels of ANP and NT-proANP and lower values of blood pressure, whereas Maimaitiming et al. (17) found the minor allele of this genetic variant associated with higher plasma levels of BNP and lower blood pressure values.

In consideration of the metabolic properties exerted by ANP and BNP, for the first time, a recent study conducted in our laboratory investigated not only the cardiovascular but also the metabolic phenotype associated with rs5068 genotypes in a random sample of the general adult population from Olmsted County, Minnesota (18). Increased levels of NT-proANP, which is a robust estimator of ANP secretion, lower systolic blood pressure values, and reduced prevalence of myocardial infarction were associated with the G allele of rs5068. Importantly, the minor allele was also associated with lower BMI, prevalence of obesity, and values of waist circumference. Carriers of the minor allele had higher values of HDL cholesterol, and the prevalence of metabolic syndrome was significantly lower in the group characterized by the presence of the G allele.

Replicating these newly discovered metabolic associations with rs5068 is of high importance. With this purpose in the current study, we genotyped a randomly selected subset of a Mediterranean population and analyzed the cardiovascular and metabolic phenotype associated with rs5068 genotypes. We hypothesized that in the general population of the small town of Ventimiglia di Sicilia, the G minor allele of rs5068 is associated with a favorable cardiometabolic phenotype characterized by lower blood pressure, BMI, prevalence of obesity, and metabolic syndrome.

RESEARCH DESIGN AND METHODS

Study population
In 1989, all residents of Ventimiglia di Sicilia, a small town of the Sicilian countryside, were invited to participate in the Ventimiglia di Sicilia Heart Study. A total number of 1,351 subjects (622 male and 729 female) out of 1,796 residents responded and were enrolled, with a participation rate of ~75% (19). The project design included a medical questionnaire, blood pressure, anthropometric and biochemical measurements, and electrocardiogram recording. The procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 1983, and were approved by the Department Council of Istituto di Medicina Interna e Geriatria, University of Palermo (20). At the beginning of the study, all subjects were interviewed about their family history, consumption of drugs, and personal clinical history. The participants underwent a medical examination and anthropometric measurements; a blood sample was drawn in order to measure several biochemical parameters (19). Blood pressure was measured twice by mercury sphygmometry, at the beginning and at the end of the visit, and the average systolic and diastolic pressures were recorded (20). Height and weight were also recorded; BMI was expressed as kg/m². Waist (midway between lower rib margin and the iliac crest in the horizontal plane) and hip (the maximum width over the greater trochanters) circumferences were also measured and the waist-to-hip ratio calculated (21). In all participants, C-reactive protein, serum creatinine, and lipid and glucose panel, including total cholesterol, HDL cholesterol, triglycerides, fasting glucose plasma, and insulin levels, were measured by standard procedures. LDL cholesterol was calculated by using the Friedewald formula (22). All cardio- and cerebrovascular events were collected with the help of the local practitioners, from their own files or hospital records registered. The diagnosis of myocardial infarction was supported by the presence of chest pain and/or typical electrocardiogram changes and/or cardiac enzyme (creatine kinase MB or troponin I) elevations (23). Stroke was identified as a neurological deficit observed by a physician and persisting for >24 h, without any other disease explaining the symptoms (24). Participants were considered as having *1*) normal weight if BMI was <24.99 kg/m², overweight if BMI was between 25.00 and 29.99 kg/m², and obese if BMI was ≥30 kg/m²; *2*) impaired fasting glucose if fasting glucose plasma levels were ≥110 mg/dL; *3*) diabetes if fasting glucose plasma concentrations were ≥126 mg/dL or if they were on pharmacological therapy with antidiabetic drugs or insulin (25); *4*) hypercholesterolemia if total cholesterol plasma levels were ≥200 mg/dL or they were on treatment with lipid-lowering medications (26); or *5*) hypertriglyceridemia if triglyceride plasma levels were ≥150 mg/dL or they were on lipid-lowering therapy. Metabolic syndrome was defined according to the criteria of the National Cholesterol Education Program Adult Treatment Panel III guidelines. Hypertension was diagnosed using Joint National Committee VI criteria (27).

Genotyping
Samples of DNA were available from a randomly selected subgroup (812 subjects) of the cohort in the analysis. Genotyping of rs5068 was carried out using TaqMan (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions, using 10–20 ng DNA. Primers and probes were Assay by Design (Applied Biosystems). After PCR amplification, end reactions were read on the ABI Prism 7900ht using 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 by using ABI's proprietary calling algorithm, which determines how well that sample fits into the cluster. Genotypes scoring <95% on the quality value percentage are located further from the clusters corresponding to the various calls and therefore have a lower reliability. An electronic data file was generated that contained genotypes and the quality value.

Statistical methods
Data are summarized and compared between groups based on observed genotypes. For continuous variables, mean and standard deviation or median and quartiles are presented and data comparisons between groups were made using two-sample Student *t* test or nonparametric Wilcoxon rank sum test. For categorical variables, comparisons were made using Pearson χ^2 tests, and data are presented as frequency and percentage. The effect of genotype on each continuous variable of interest was examined using a series of linear regression models with and without adjustment for additional covariates such as age, sex, and BMI. In cases where the data were not normally distributed, a log transform was taken

prior to the linear regression analysis. Linear regression models are summarized as β estimates and standard errors. Genotype effects for categorical variables were tested using logistic regression models, also with and without adjustment for additional covariates. Results of logistic regression models are presented as odds ratio (OR) and 95% CIs. SAS version 9.3 (Cary, NC) was used for all analyses, and $P \leq 0.05$ was considered to be statistically significant.

RESULTS

Prevalence of rs5068 genotypes and cardiovascular phenotype

A DNA sample was available from 812 subjects. A total number of 804 subjects were successfully genotyped for the genetic variant rs5068. Genotype frequencies are AA, 93.5% ($n = 752$); AG, 6.4% ($n = 51$); and GG, 0.1% ($n = 1$), and the minor allele frequency is 3.3%. The distribution is in Hardy-Weinberg equilibrium ($P = 0.89$). Recognizing the low frequency of the minor allele, all analyses were performed assuming a dominant model with AG and GG genotypes combined. In Table 1, the characteristics of the study population are reported. The two groups do not differ in terms of age and sex. Adjusted for age and sex, the minor G allele is associated with lower systolic (estimate [SE]: −7.6 mmHg [2.6], $P = 0.003$) and diastolic (−3.0 mmHg [1.4], $P = 0.03$) blood pressure. After additional adjustment including BMI and treatment for hypertension, the minor allele is still associated with lower values of systolic blood pressure (−5.2 mmHg [2.4], $P = 0.03$) (Fig. 1), but no significant association is detected with diastolic blood pressure (−1.8 mmHg [1.3], $P = 0.17$). In the analysis adjusted for age and sex, the G allele is associated with a lower prevalence of hypertension (OR 0.35 [95% CI 0.18–0.70], $P = 0.003$), and this association remains valid even after adjusting for age, sex, and BMI (0.41 [0.20–0.83], $P = 0.01$) (Fig. 2). The two groups do not differ in terms of prevalence of cardiovascular diseases, including myocardial infraction, stroke, and heart failure. No association is found between the G allele and creatinine, C-reactive protein, fibrinogen, and factor VII plasma levels.
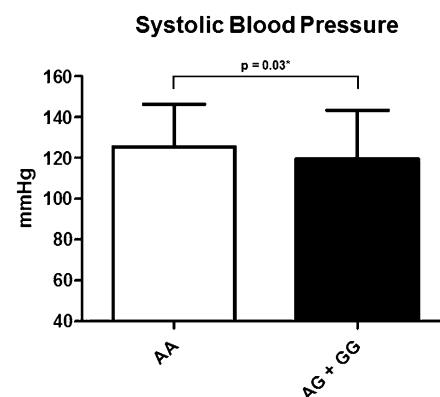

Figure 1—*Systolic blood pressure values in the study population according to rs5068 genotypes. *P value obtained from logistic regression model adjusting for age, sex, BMI, and treatment for hypertension. Error bars represent 1 SD above the mean.*

Metabolic phenotype

Carriers of the minor allele show significantly lower values of BMI (estimate [SE]: -1.7 kg/m^2 [0.8], $P = 0.04$) when compared with the homozygotes for the major allele after adjusting for age and sex (Table 1 and Fig. 3). Moreover, obesity tends to

Table 1—*Characteristics of the study population*

Characteristics	AA ($n = 752$)	AG+GG ($n = 52$)	Unadjusted P value	Adjusted P value*
Age, years	52.7 ± 19.7	55.6 ± 19.1	0.31	
Male sex, n (%)	333 (44)	23 (44)	0.99	
Systolic blood pressure, mmHg	125.6 ± 20.8	119.6 ± 23.8	0.046	0.003
Diastolic blood pressure, mmHg	76.2 ± 10.0	73.5 ± 10.5	0.06	0.030
Creatinine, mg/dL	0.82 ± 0.26	0.83 ± 0.31	0.73	0.94
C-reactive protein	0.38 ± 0.56	0.40 ± 0.42	0.79	0.87
Fibrinogen, mg/dL	274.8 ± 65.9	284.7 ± 50.8	0.29	0.38
Factor VII activity, %	95.5 ± 22.8	90.6 ± 26.3	0.18	0.13
BMI, kg/m^2	28.2 ± 5.7	26.7 ± 4.9	0.07	0.039
Obesity, n (%)	244 (33)	11 (21)	0.09	0.07
Waist circumference, cm	92.9 ± 14.4	90.6 ± 11.8	0.25	0.10
Waist-to-hip ratio	0.89 ± 0.10	0.88 ± 0.06	0.49	0.27
Total cholesterol, mg/dL	189.8 ± 39.1	187.8 ± 40.6	0.73	0.58
LDL cholesterol, mg/dL	125.7 ± 34.0	124.4 ± 34.9	0.79	0.68
HDL cholesterol, mg/dL	42.8 ± 11.5	44.6 ± 11.5	0.30	0.28
Females with HDL cholesterol <50 mg/dL, n (%)	279 (37)	20 (38)	0.84	0.79#
Males with HDL cholesterol <40 mg/dL, n (%)	203 (61)	9 (39)	0.039	0.053#
Hypercholesterolemia, n (%)	317 (42)	22 (42)	0.98	0.89
Triglycerides, mg/dL	105.3 ± 58.3	94.3 ± 51.6	0.18	0.12
Hypertriglyceridemia, n (%)	173 (23)	9 (17)	0.34	0.27
Serum glucose, mg/dL##	89.5 ± 22.4	86.9 ± 13.7	0.43	0.30
Insulin, μU/mL##	8.6 (6.0–12.7)	7.8 (6.2–11.3)	0.27	0.23
Impaired fasting glucose, n (%)	77 (10)	4 (8)	0.56	0.43
Diabetes, n (%)	73 (10)	4 (8)	0.63	0.50
Metabolic syndrome, n (%)	238 (32)	10 (19)	0.06	0.02

*P value obtained from regression model adjusting for age and sex. #P value obtained from regression model adjusting only for age. ##Analyzed on subgroup of subjects free of type 1 and 2 diabetes, presented as median (IQR) due to distribution.
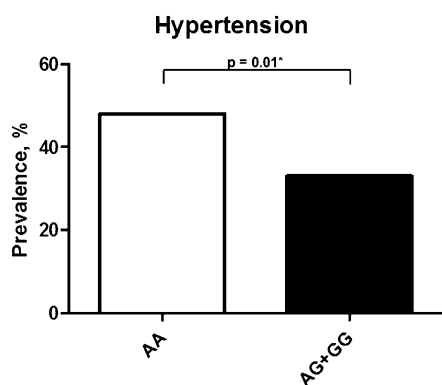
Hypertension

Figure 2—*Prevalence of hypertension in the study population according to rs5068 genotypes. *P value obtained from logistic regression model adjusting for age, sex, and BMI.*

be less prevalent in the group characterized by the presence of the G allele (OR 0.52 [95% CI 0.26–1.04], $P = 0.07$). Male subjects presenting HDL cholesterol plasma levels <40 mg/dL are less frequent in the AG+GG group (0.42 [0.18–1.01], $P = 0.053$), but the significance of this association is not maintained after adjusting for age and BMI ($P = 0.11$). Importantly, the G allele is significantly associated with a lower prevalence of metabolic syndrome (19 vs. 32%) with adjustment for age and sex (0.42 [0.20–0.89], $P = 0.02$). When BMI is included in the multivariate model, the association is attenuated (0.49 [0.22–1.10]; $P = 0.09$). The analysis of metabolic parameters as waist circumference and waist-to-hip ratio does not reveal any significant difference between the two groups. The minor allele is not associated with insulin, glucose,
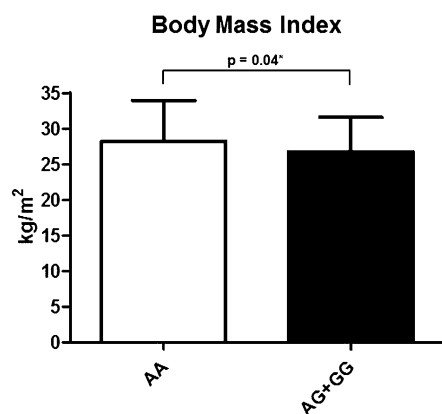
Body Mass Index

Figure 3—*BMI in the study population according to rs5068 genotypes. *P value obtained from logistic regression model adjusting for age and sex. Error bars represent 1 SD above the mean.*

total cholesterol, LDL cholesterol, and triglyceride plasma levels. Prevalence of diabetes, hypercholesterolemia, hypertriglyceridemia, and impaired fasting glucose are similar between the AA and GG+GG groups.

CONCLUSIONS—The minor G allele of the ANP genetic variant rs5068 has been reported to be associated with higher values of natriuretic peptides, lower blood pressure, and reduced odds of hypertension (15–17). A recent analysis conducted on a random sample of the adult residents in Olmsted County confirmed that the minor allele of rs5068 is associated with higher levels of NT-proANP and lower systolic blood pressure values, but it also revealed, for the first time, an association between the G allele of rs5068 and a metabolic phenotype characterized by lower BMI and waist circumference and higher values of HDL. Importantly, obesity and metabolic syndrome were less prevalent between the carriers of the G allele (18).

In the current study, we genotyped a random subset of the general population from Ventimiglia di Sicilia, which is a small town in the Sicilian countryside, and investigated the cardiovascular and metabolic phenotype associated with rs5068 genotypes. Importantly, the newly discovered association between the minor allele of rs5068 and a favorable metabolic phenotype was replicated in this Mediterranean population. Independently from age and sex, the G-allele carriers have significantly lower BMI when compared with the homozygotes for the major allele, and obesity tends to be less prevalent in the AG+GG group. Male carriers of at least one copy of the minor allele are less likely to present HDL <40 mg/dL. The carriers of the G allele also have significantly lower values of systolic and diastolic blood pressure, and this allele is associated with a lower prevalence of hypertension. Importantly, metabolic syndrome is also less prevalent in the group characterized by the presence of the G allele.

After including BMI in the multivariate model, the association between the G allele and lower prevalence of male subjects with HDL <40 mg/dL was attenuated, as was the association with lower prevalence of metabolic syndrome. Therefore, these data may suggest a possible key role played by the primary association between rs5068 minor allele and lower BMI in the metabolic phenotype

observed in the G-allele carriers. In contrast, the systolic blood pressure values are significantly lower in the AG+GG group in both the univariate and multivariate analysis including age, sex, BMI, and antihypertensive treatment as confounding factors. The G allele is also associated with a lower prevalence of hypertension.

The association between the rs5068 genotypes and blood pressure has been investigated in four recent studies (15–18) that consistently reported a phenotype characterized by higher plasma levels of natriuretic peptides and lower blood pressure values for the carriers of the minor allele of this ANP genetic variant. Such reports are supported by previous seminal genetic studies in mice that advanced the blood pressure–lowering properties of natriuretic peptides. Indeed, transgenic mice overexpressing the ANP gene had chronically elevated plasma levels of ANP with mean arterial pressure reduced by 24 mmHg when compared with their nontransgenic siblings (28); whereas mice with a disruption of *NPPA* presented no circulating levels of ANP and developed chronic hypertension (29).

Almost two decades ago, in vitro studies conducted by Sarzani et al. (30,31) elegantly revealed that natriuretic peptide receptors were expressed in adipocytes from both rats and humans. In 2000, Sengenès et al. (2) demonstrated that ANP and BNP induce lipolysis in isolated human fat cells through guanylyl cyclase activation and cGMP production, while also reporting that infusion of ANP through a microdialysis probe implanted in abdominal subcutaneous adipose tissue of healthy individuals resulted in a significant increase in extracellular glycerol concentration, thus confirming the lipolytic properties of ANP in vivo. Similar results were obtained after acute intravenous infusion of ANP in lean and obese subjects in which a 60-min administration of ANP resulted in a lipid-mobilizing effect consisting of a vasodilating action in adipose tissue and higher circulating levels of plasma glycerol and nonesterified fatty acids (6). Interestingly, systemic infusion of ANP stimulates not only lipid mobilization but also lipid oxidation in a dose-dependent manner in healthy individuals (4). A 7-day infusion of BNP into mice increased oxygen consumption and energy expenditure without a significant increase in food intake or physical activity compared with mice receiving vehicle (3). Studies on

animal models have also contributed to clarify the metabolic consequences of chronic exposure to increased levels of natriuretic peptides. Genetically engineered mice that are exposed throughout life to a plasma BNP concentration that is 100 times higher than the physiological concentration are protected from obesity and insulin resistance when fed with a high-fat diet (32). Moreover, mice overexpressing cGMP-dependent kinase, the activation of which constitutes a key point in the signal transduction of the natriuretic peptides, are leaner and more insulin sensitive even on standard diet when compared with wild-type mice. Both types of genetically engineered mice showed reduction in fat tissue and an increase in fat oxidation.

In humans, BMI is inversely associated with plasma natriuretic peptide levels, and both obese men and women have significantly lower values of BNP and NT-proANP when compared with subjects with normal BMI (9). In the complex interaction between natriuretic peptides and metabolic phenotype, a key role may be played by NPR-C. In the adipose tissue, NPR-C is highly expressed (33) and modulated by dietary intake, specifically decreased with fasting (34). Interestingly, when compared with obese normotensives, obese hypertensive subjects have even lower plasma ANP levels, and the NPR-A/NPR-C ratio of expression in adipose tissue is significantly decreased (33). Moreover, a genetic variant located in a promoter element of the NPR-C gene is associated with plasma levels of ANP and blood pressure values in obese, hypertensive patients (35); the same variant is also associated with prevalence of overweight, obesity, and abdominal adiposity in a random cohort of untreated male subjects from the south of Italy (36). These interesting data suggest that NPR-C and its expression in adipose tissue might exert an important effect on natriuretic peptide plasma levels. This network between natriuretic peptides and adipose tissue, in which both sides may play a mutual regulatory role, is definitely a field of extreme importance. Thus, further investigations are warranted to elucidate mechanisms mediating these complex interactions.

Consistent with physiological studies demonstrating the cardiovascular and metabolic properties of the natriuretic peptides in animal models and humans, our previous analysis on a random sample of the general population from Olmsted County (18) showed that the minor allele of rs5068, which is associated with higher values of NT-proANP, is also associated with lower systolic blood pressure, BMI, and waist circumference, higher values of HDL cholesterol, and lower prevalence of obesity and metabolic syndrome. Importantly, in the current study, we have been able to replicate the associations between the G allele of the ANP genetic variant rs5068 and a favorable cardiometabolic phenotype. Specifically, in a random subset of the general population of Ventimiglia di Sicilia, the G allele is associated with lower systolic and diastolic blood pressure and lower prevalence of hypertension. The carriers of the G allele have lower BMI and tend to be less likely to develop obesity, males with HDL cholesterol levels <40 mg/dL are less prevalent in the AG+GG group, and the minor allele is associated with a lower prevalence of metabolic syndrome.

As in our previous analysis on a sample of the Olmsted County residents (18), the study conducted on a random subset of the Sicilian population suggests that the primary association between the G allele and lower BMI probably mediates the relationship of the minor allele with lower prevalence of metabolic syndrome and males presenting HDL cholesterol levels <40 mg/dL. Indeed, these two associations are attenuated after including BMI in the multivariate analysis. Conversely, after adjusting for BMI, the associations between the minor allele and lower systolic blood pressure and prevalence of hypertension still remained significant.

The association between the minor allele of rs5068 and lower BMI represents one of the key results of our analyses conducted in the U.S. and Sicilian cohorts. Values of BMI (27.9 vs. 28.2 kg/m^2 for the AA genotypes and 26.7 vs. 26.7 kg/m^2 for the AG+GG genotypes in the U.S. and Sicilian cohort, respectively) and the immediately related metabolic parameters of prevalence of obesity (35 vs. 33% for the AA genotypes and 22 vs. 21% for the AG+GG genotypes in the U.S. and Sicilian cohort, respectively) and waist circumference (93 vs. 92.9 cm for the AA genotypes and 90 vs. 90.6 cm for the AG+GG genotypes in the U.S. and Sicilian cohort, respectively) are similar between the two cohorts. The two populations, however, seem to differ more in terms of lipid panel (total cholesterol, HDL, LDL, and triglyceride plasma levels), insulin, and glucose plasma values and prevalence of metabolic syndrome. Such variables were not significantly associated with rs5068 genotypes, or if an association was present, it was attenuated after including BMI in the multivariate-adjusted model. We hypothesize that different diets in culturally and naturally different environments such as Olmsted County and Sicily might contribute to differences in the metabolic parameters observed in the two cohorts. In addition, our results might have been influenced by the measurement variability, which may also have had an impact on which variables survive multivariable adjustment best. The similarity in terms of BMI findings between the two populations provides us confidence regarding the reliability and reproducibility of our data. Moreover, the consistency of our results in two different cohorts suggests a key role played by the association between the G allele of rs5068 and BMI in the metabolic phenotype observed, but certainly further investigations are needed to confirm this hypothesis.

A limitation of our study is the lack of natriuretic peptide plasma levels, which were not available from the subjects of the cohort studied. Nonetheless, several studies conducted in populations of different ethnicities showed an association between the minor allele of rs5068 and higher plasma values of the natriuretic peptides. Newton-Cheh et al. (15) identified an association between the G allele and higher circulating ANP and BNP concentrations in the Framingham cohort from Massachusetts, in the Malmö cohort from Sweden, and in the Finrisk97 cohort from Finland. Ellis et al. (16) confirmed the association between the minor allele and higher ANP and NT-proANP plasma levels in patients with coronary artery disease from New Zealand, whereas in a cohort randomly selected from the French middle-aged general population, Maimaitiming et al. (17) found a significant association between the rs5068 minor allele and increased values of BNP. Our analysis on a random sample of the general population from Olmsted County showed that the G-allele carriers have higher values of NT-proANP when compared with the homozygotes for the major allele (18). Such previous evidence, along with the well-established cardiovascular and metabolic properties of natriuretic peptides, supports our hypothesis that increased circulating levels of ANP and/or BNP with activation of the NPR-A receptor may play a key role in the phenotype observed in the Mediterranean population that we analyzed. Further investigations

have already been planned and are clearly needed to better characterize the Ventimiglia di Sicilia cohort in terms of circulating natriuretic peptide plasma levels.

There remains a huge, unmet need for novel drugs for metabolic syndrome, especially agents that also have cardio-protective properties. ANP and BNP are both approved as intravenously administered agents for acute heart failure in Japan and the U.S., respectively. Most importantly, a recent study documented the actions of chronically delivered BNP by twice daily subcutaneous administration in humans with stable heart failure (37); chronic BNP administration was safe and associated with reduced left ventricular mass and improved symptoms. Thus, it is tempting to advance the concept of natriuretic peptide–based therapy in patients with metabolic syndrome where both increased BMI and elevated blood pressure are present.

In conclusion, the current study confirms the association between the minor allele of rs5068 and a favorable cardiometabolic phenotype, which was identified for the first time by our previous analysis conducted on a random subset of the general community from Olmsted County and built on the seminal first report by Newton-Cheh et al. (15,18) in regard to the association of rs5068 with blood pressure values. Specifically, in a randomly selected subset of the Mediterranean population of Ventimiglia di Sicilia, the minor allele of the ANP genetic variant rs5068 is associated with lower values of systolic blood pressure and prevalence of hypertension. Importantly, the carriers of the G allele have a significantly lower BMI and tend to be less likely to develop obesity when compared with the homozygotes for the major allele. Males with HDL cholesterol levels <40 mg/dL are less frequent in the AG and GG genotypes. Metabolic syndrome is less prevalent between the carriers of the minor allele. The relationship between the G allele and the metabolic phenotype observed is probably mediated by the primary association between the minor allele of rs5068 and a lower BMI. Additional studies are warranted in order to evaluate the possible implications in terms of cardiovascular risk assessment and to better clarify the physiological mechanisms that led to the phenotype detected in the carriers of the rs5068 minor allele. Moreover, our findings along with our previous data obtained from the analysis of a cohort from Olmsted County (18) may support the hypothesis of future novel metabolic therapeutics based on administration of natriuretic peptides and/or stimulation of the NPR-A receptor.

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