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Hypertension

Association of Atrial Natriuretic Peptide and Type A Natriuretic Peptide Receptor Gene Polymorphisms With Left Ventricular Mass in Human Essential Hypertension

Speranza Rubattu, MD,*† Giada Bigatti, MD,‡ Anna Evangelista, PhD,* Chiara Lanzani, MD,‡ Rosita Stanzione, PhD,* Laura Zagato, BBSC,‡ Paolo Manunta, MD,‡ Simona Marchitti, BS,* Vanessa Venturelli, MD,† Giuseppe Bianchi, MD,‡ Massimo Volpe, MD,*† Paola Stella, MD‡

Pozzilli, Rome, and Milan, Italy

OBJECTIVES The goal of our study was to investigate the relationships between atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and type A natriuretic peptide receptor (NPRA) gene polymorphisms and left ventricular structure in human essential hypertension.

BACKGROUND Experimental evidence supports a key role for natriuretic peptides in the modulation of cardiac mass. This relationship has not yet been described in human disease.

METHODS A total of 203 hypertensive patients were studied by mono-bidimensional echocardiography. Three markers of the ANP gene (−C664G, G1837A, and T2238C polymorphisms) and a microsatellite marker of both NPRA and BNP genes were characterized.

RESULTS Patients carrying the ANP gene promoter allelic variant had increased left ventricular mass index (117.4 ± 1.7 g vs. 95.7 ± 1.7 g, $p = 0.005$), left ventricular posterior wall thickness (1.14 ± 0.07 cm vs. 0.96 ± 0.01 cm, $p < 0.0001$), left ventricular septal thickness (1.12 ± 0.10 cm vs. 1.04 ± 0.01 cm, $p = 0.01$), and relative wall thickening (47.5 ± 4.1% vs. 39.4 ± 5.3%, $p = 0.001$) as compared with the wild-type genotype. These associations were independent from anthropometric factors and major clinical features and were confirmed in a large subgroup of never-treated hypertensive patients ($n = 148$). Carrier status of the ANP gene promoter allelic variant was associated with significantly lower plasma proANP levels: 1,395 ± 104 fmol/ml versus 3,110 ± 141 fmol/ml in hypertensive patients carrying the wild-type genotype ($p < 0.05$). A significant association for NPRA gene variants with left ventricular mass index and left ventricular septal thickness was found. The analysis of BNP did not reveal any effect on cardiac phenotypes.

CONCLUSIONS Our findings show that the ANP/NPRA system significantly contributes to ventricular remodeling in human essential hypertension. (J Am Coll Cardiol 2006;48:499–505)
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Left ventricular hypertrophy (LVH) is a common clinical feature in hypertension and represents a powerful independent risk factor for cardiovascular morbidity and mortality in this condition (1). It is influenced by a number of factors, including blood pressure (BP) levels, age, gender, obesity, dietary habits, and pharmacological treatments (2,3). Hypertension contributes only partially to the determination of cardiac mass, whereas the role of genetic factors responsible for cardiac mass variance has been estimated up to 60% both in humans (4,5) and in animal models (6). Identification of genes involved in the development of LVH, considered as an intermediate disease phenotype, can provide useful clues to the understanding of the complex etiopathogenesis of hypertension-induced end-organ damage. The approach to the understanding of the molecular genetic basis of LVH has been more successful, for obvious reasons, in animal models than in humans (7).

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Atrial natriuretic peptide (ANP) is a potent natriuretic and vasorelaxant hormone that is mainly secreted by cardiomyocytes and plays a significant contributory role in cardiovascular homeostasis (8). It has been shown recently that inactivation of the ANP gene (9) or of its receptor type A natriuretic peptide receptor (NPRA) (10) leads to a significant increase of left ventricular mass (LVM), independently of BP levels. In contrast, elevation of ANP levels in the heart seems to protect from hypertrophic stimuli, thus supporting a protective role of ANP toward left ventricular enlargement (11). In vitro data confirm the antihypertrophic effect of ANP on cardiomyocytes in

From the *IRCCS Neuromed, Polo Molisano University of Rome La Sapienza, Pozzilli, Italy; †U.O. Cardiologia, IInd School of Medicine, University of Rome La Sapienza, S. Andrea Hospital, Rome, Italy; and the ‡Division of Nephrology, Dialysis and Hypertension, University Vita e Salute, San Raffaele Hospital, Milan, Italy. This work was supported by a grant from the Italian Ministry of Health (IRCCS) to Drs. Rubattu and Volpe; a grant from MURST 2004 to Dr. Rubattu; by grants from Ministero Università e Ricerca Scientifica of Italy (FIRB Grant RBNE01724C_001 and PRIN Grant 2004069314_01) to Dr. Bigatti; and a grant from Italian Ministry of Health (ICS 110.4/RF02353) to Dr. Bigatti.

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Abbreviations and Acronyms

ANOVA = analysis of variance
ANP = atrial natriuretic peptide
BNP = brain natriuretic peptide
BP = blood pressure
LVH = left ventricular hypertrophy
LVM = left ventricular mass
NPRA = type A natriuretic peptide receptor
RFLP = restriction fragment length polymorphism
ST = septal thickness

culture (12). Experimental evidence also supports an involvement of brain natriuretic peptide (BNP) in the process of cardiac remodeling (13,14).

A functional deletion mutation of the 5′-flanking region of the human type A natriuretic peptide receptor gene, responsible of reduced gene transcriptional activity, was found to confer increased susceptibility to essential hypertension or LVH in a study performed in a Japanese population (15). However, the question of whether ANP and BNP can directly modulate LVM, independently of hypertension, remains unanswered for the human disease.

The present study was designed to investigate the relationships between natriuretic peptide gene molecular variants and left ventricular structure in a highly homogenous population of middle-age Caucasian patients with mild to moderate essential hypertension. To strengthen our findings and to rule out any long-term effects of antihypertensive therapy (16), the analysis was repeated in a subgroup of never-treated hypertensive patients.

A positive association between ANP gene molecular variants and left ventricular mass index in essential hypertension was found. This finding, although confirming the experimental evidence, represents the first demonstration of the antihypertrophic role played by ANP in the process of cardiac remodeling in human essential hypertension.

METHODS

Selection of patients. Consecutive patients were recruited at the outpatient clinic of the Division of Nephrology, Dialysis and Hypertension of the San Raffaele Hospital, Milan. To minimize the environmental variability, the inclusion criteria were very restrictive. Patients were required: 1) to be of the same race; 2) to have only essential hypertension without collateral diseases with cardiovascular implications (i.e., diabetes); 3) to have not undergone pharmacological treatment for hypertension for at least 3 months before the study and during the study; 4) to be not obese (body mass index ≤30 kg/m^2); and 5) to have normal sodium intake (24-h sodium excretion ≤300 mEq). This selection originated a population of 203 Caucasian patients (168 male, 35 female). All patients were Italians: 148 patients were never treated, and 55 patients had interrupted the pharmacological treatment 3 months before the study. All patients provided an informed written consent. The study protocol was approved by the local ethics committee.

BP measurement. Hypertension was diagnosed, as previously reported (17), on the basis of the presence of systolic BP >135 mm Hg and diastolic BP >85 mm Hg by daytime ambulatory BP monitoring (Spacelabs 90207, Redmond, Washington) and of an office systolic BP >140 mm Hg and diastolic BP >90 mm Hg (average of 3 repeated measurements made by the same doctor with a mercury sphygmomanometer).

Biochemistry. Serum and urine electrolytes and creatinine were measured by standard methods (ion-selective electrode and autoanalyzer), and plasma renin activity and plasma aldosterone with a radioimmunoassay (DiaSorin, Vercelli, Italy; and Medical System SpA, Genova, Italy). The aldosterone/plasma renin activity ratio was computed for each patient.

Measurement of LVH. Patients underwent monodimensional and bi-dimensional echocardiography using a Hewlett-Packard imaging system (Sonos 2500 model, Palo Alto, California). All measurements were performed by the same investigator, who was blinded to the patient's genotypes.

As previously reported (17), left ventricular internal diameters, septal thickness (ST), and posterior wall thickness were measured according to the guidelines of the American Society of Echocardiography (18). The LVM was calculated at end diastole by applying the Devereux correction to the American Society of Echocardiography cube LVM formula (19). The relative wall thickening was calculated as previously reported (20).

Only patients with normal left ventricular function were included. Patients with either wall motion or cardiac valve abnormalities were excluded.

Genetic analysis of ANP, NPRA, and BNP. Extraction of DNA from peripheral whole blood was done using a commercially available kit (Qiagen Italia SPA, Milan, Italy). Characterization of ANP polymorphisms was performed by previously reported procedures (21). Briefly, we analyzed a −C664G promoter polymorphism by an *RsaI* restriction fragment length polymorphism (RFLP) assay; an intronic G1837A polymorphism by an *HpaII* RFLP assay; and a coding T2238C polymorphism by a *ScaI* RFLP assay. These polymorphisms were previously shown to be in linkage disequilibrium (21,22). For NPRA, we used a microsatellite marker localized within the promoter region (23). For BNP, we used a microsatellite marker localized within the 3′ end of the gene (24).

All polymerase chain reactions were performed with a PTC-100 thermal cycler. Digestion with the corresponding enzyme was carried out for the ANP markers as recommended by the manufacturer (NEB, Boston, Massachusetts). The digested polymerase chain reaction products were resolved on agarose gels and visualized by ethidium bromide staining. Both NPRA and BNP microsatellites were amplified with ^{32}P-labeled forward primer and unla-

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beled reverse primer and visualized by autoradiography. The genotypes were read by 2 blinded independent investigators.

Measurement of plasma proANP levels. Fifteen hypertensive patients carrying the wild-type genotype for the ANP promoter polymorphism and 5 heterozygous hypertensive patients were randomly selected among the whole sample.

To obtain more definite information on the functional relevance of the ANP gene promoter variant, 12 normotensive patients carrying the wild-type allele and 10 heterozygous normotensive patients (from the same ethnic group as the hypertensive population) were also included in the analysis. Plasma proANP levels were measured by the use of a sensitive enzyme-linked immunosorbent assay kit (Biomedica Gruppe, Wien, Austria).

Statistical analysis. All data are given as the mean value ± SEM. Deviation from Hardy-Weinberg equilibrium was analyzed with the chi-square test. Differences between groups were analyzed either by *t* test or 1-way analysis of variance (ANOVA) if groups were more than 2. The post-hoc least significant difference test was performed to complete the analysis for multiple comparisons. A covaried 1-way ANOVA was performed considering each cardiac parameter as a dependent variable; considering age, gender, body mass index, BP, duration of hypertension, 24-h urinary Na^+ output, and aldosterone/plasma renin activity ratio as covariates; and considering the polymorphism showing significant differences in the analyses among groups as an independent value. Analysis was performed with SPSS version 10 (SPSS Inc., Chicago, Illinois) for the Mac OS statistical software on an e-mac personal computer.

Table 1. Descriptive Anthropometric and Cardiac Variables of the Whole Sample

Variables	Mean ± SEM
Age (yrs)	43.5 ± 0.6
Body mass index (kg/m^2)	26.0 ± 0.2
Estimated duration of hypertension (mo)	38.9 ± 3.7
SBP (mm Hg)	144.9 ± 1.0
DBP (mm Hg)	96.4 ± 0.6
HR (beats/min)	72.9 ± 0.6
Daytime SBP (mm Hg)	145.8 ± 0.9
Daytime DBP (mm Hg)	96.6 ± 0.7
Daytime HR (beats/min)	78.7 ± 0.6
sNa^+ (mmol/l)	141.2 ± 0.1
sK^+ (mmol/l)	4.1 ± 0.02
GFR (ml/min/BSA)	114.7 ± 2.8
uNa^+ (mmol/day)	136.8 ± 4.5
uK^+ (mmol/day)	54.0 ± 1.4
PRA (mg/ml · h)	1.664 ± 0.10
Aldosterone (pg/ml)	206.4 ± 7.5
ALDO/PRA ratio	180.8 ± 9.6
LA diameter (cm)	3.75 ± 0.6
LV septum (cm)	1.06 ± 0.01
LV PW (cm)	0.98 ± 0.01
LVIDD (cm)	4.96 ± 0.03
LVISD (cm)	2.94 ± 0.03
RWT (%)	39.9 ± 5.1
LVMI (g/m^2)	97.4 ± 1.5
EF (%)	65.6 ± 0.6

Aldosterone = plasma aldosterone; ALDO/PRA = aldosterone/plasma renin activity; ANP = atrial natriuretic peptide; DBP = diastolic blood pressure; EF = ejection fraction; GFR = glomerular filtration rate; HR = heart rate; LA = left atrium diameter; LV = left ventricular; LVIDD = left ventricular internal diastolic diameter; LVISD = left ventricular internal systolic diameter; LVMI = left ventricular mass index; RWT = relative wall thickness; SBP = systolic blood pressure; sNa^+ = serum sodium; sK^+ = serum potassium; uK^+ = 24-h urinary potassium output; uNa^+ = 24-h urinary sodium output.

RESULTS

Characteristics of the study sample. Main anthropometric, clinical, and biochemical variables are reported in Table 1.

Here we report allele frequencies and chi-square test results for Hardy-Weinberg equilibrium for each genotype:

1) ANP polymorphic markers:
   a) −C664G promoter polymorphism: 97.4% for the −644C allele and 2.6% for the −664G allele; chi-square test: p = 0.65
   b) G1837A intronic polymorphism: 97.5% for the 1837G allele and 2.5% for the 1837A allele; chi-square test: p = 0.11
   c) T2238C stop codon polymorphism: 2238T 83% for the 2238T allele and 17% for the 2238C allele; chi-square test: p = 0.45
2) NPRA polymorphism: Three allelic variants were identified for this microsatellite. Their frequency was 46.7% for allele 10, 44.6% for allele 11, and 4.4% for allele 12; chi-square test: p = 0.38.
3) BNP polymorphism: Five allelic variants were identified for this microsatellite. Their frequency was 96.3% for the wild-type allele and 3.7% for the combined mutant alleles; chi-square test: p = 0.83.

The frequency of the observed genotypes was not different from those expected from the allelic frequencies. The Hardy-Weinberg equilibrium was respected for all markers as shown by chi-square significances.

ANP gene polymorphisms and cardiovascular phenotypes. −C664G POLYMORPHISM. Among the whole sample, 192 patients were homozygous for the −664C allele, 11 patients were heterozygous, and none homozygous for the −664G allele was found. Among the subgroup of never-treated patients, 142 patients were homozygous for the −664C allele, 6 patients were heterozygous, and none homozygous for the −664G allele were found.

No significant differences between the 2 genotypes were found by *t* test with regard to gender distribution, body mass index, BP levels, age, duration of hypertension, and 24-h urinary sodium excretion (Table 2). In contrast, the analysis of possible associations with echocardiographic parameters showed that hypertensive patients who were carriers of the −664G allele had a significantly increased posterior wall thickness and ST and an increased LVMI, whereas no significant differences were observed with regard to end-systolic and end-diastolic diameters, left atrial diameter, or left ven-

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Table 2. Clinical Variables According to the −C664G ANP Polymorphism Genotypes

Variables	−664CC (n = 192)	−664CG (n = 11)	p Value*
Gender (M/F)	160/32	8/3	NS
Age (yrs)	43.5 ± 0.7	38.9 ± 2.8	NS
Body mass index (kg/m^2)	25.4 ± 0.2	26.0 ± 0.6	NS
Duration of hypertension (months)	38.8 ± 4.4	38.3 ± 11.9	NS
SBP (mm Hg)	144.6 ± 1.1	150.7 ± 6.4	NS
DBP (mm Hg)	96.2 ± 0.7	101.5 ± 3.9	0.09
HR (beats/min)	73.1 ± 0.7	70.5 ± 2.5	NS
uNa+ (mmol/day)	130.1 ± 4.1	117.3 ± 17.7	NS

*Analysis performed by *t* test for independent samples.
NS = not significant; other abbreviations as in Table 1.

tricular function. These significant associations were present both in the overall population and in the subgroup of never-treated hypertensive patients (Table 3).

To better separate out the genetic effect, a covariate ANOVA was performed for each cardiac variable as described in Methods section. The analysis results were significant both in the whole sample and in the never-treated subgroup, as shown in Table 4 (column titled "Total Model"). In the same table, the R^2 value enclosed within brackets in the column titled "ANP" indicates the amount of total variability explained by the effect of ANP polymorphism for each cardiac variable, independently of all covariates.

Measurement of circulating proANP levels in a subgroup of both wild-type and heterozygous hypertensive patients showed that the −664G allelic variant was associated with a significant decrease of proANP: 1,395 ± 104 fmol/l versus 3,110 ± 141 fmol/ml in wild-type genotype individuals ($p < 0.05$). In normotensive patients, plasma proANP levels were 1,165 ± 47 fmol/ml versus 2,319 ± 64 fmol/ml in mutant versus wild-type allele individuals ($p < 0.001$).

G1837A INTRONIC POLYMORPHISM. Among the whole sample, 192 patients were homozygous for the 1837G allele and 10 patients were heterozygous; 1 patient homozygous for the 1837A allele was joined to the heterozygous patients for the analysis. Among the subgroup of never-treated patients, 143 individuals were homozygous for the 1837G allele, 5 patients were heterozygous, and none homozygous for the 1837A allele were found. No differences between the 2 genotypes were found with regard to either clinical or cardiac variables by *t* test.

T2238C POLYMORPHISM. Among the whole sample, 141 patients were homozygous for the 2238T allele, 53 patients were heterozygous, and 8 patients were homozygous for the 2238C allele. Among the subgroup of never-treated patients, 101 patients were homozygous for the 2238T allele, 38 patients were heterozygous, and 7 patients were homozygous for the 2238C allele.

No differences between genotypes with regard to clinical variables were seen by 1-way ANOVA.

To perform the analysis of this polymorphism with regard to cardiac variables, patients carrying the variant allele were joined because of the low number of double mutant allele individuals. Interestingly, within the subgroup of never-treated hypertensive patients an increase of LVMI was detected in patients carrying the variant allele, although the significance was reached only for ST (1.02 ± 0.02 cm vs. 1.09 ± 0.03 cm in wild-type homozygotes vs. carriers of the mutant allele, respectively; $p = 0.03$).

NPRA receptor polymorphism. To perform the analysis, individuals were grouped, based on the most common genotypes, as follows: 10/10 (n = 49), 10/11 (n = 91), 11/11 (n = 45), 11/12 (n = 18) [whole sample] and 10/10 (n = 33), 10/11 (n = 67), 11/11 (n = 35), 11/12 (n = 13) [never-treated subgroup]. No differences between genotypes

Table 3. Echocardiographic Variables According to the −C664G ANP Polymorphism Genotypes

	All Patients			Never-Treated Patients		
Variables	−664CC (No. 192)	−664CG (No. 11)	p Value*	−664CC (No. 142)	−664CG (No. 6)	p Value*
LA (cm)	3.66 ± 0.07	4.05 ± 0.40	NS	3.73 ± 0.08	3.97 ± 0.55	NS
LV septum (cm)	1.04 ± 0.01	1.12 ± 0.10	0.01	1.04 ± 0.02	1.21 ± 1.00	0.03
LV PW (cm)	0.96 ± 0.01	1.14 ± 0.07	0.000	0.95 ± 0.01	1.15 ± 0.09	0.002
LVIDD (cm)	4.92 ± 0.04	4.95 ± 0.23	NS	4.92 ± 0.04	5.21 ± 0.38	NS
LVISD (cm)	2.91 ± 0.04	3.02 ± 0.22	NS	2.91 ± 0.04	3.01 ± 0.34	NS
RWT (%)	39.4 ± 5.3	47.5 ± 4.1	0.001	39.0 ± 5.9	46.3 ± 6.7	0.02
LVMI (g/m^2)	95.7 ± 1.7	117.4 ± 1.7	0.005	94.4 ± 1.9	126.3 ± 15.6	0.001
EF (%)	65.8 ± 0.6	63.8 ± 3.1	NS	65.7 ± 0.7	66.5 ± 3.4	NS

*Analysis performed by *t* test for independent samples.
PW = posterior wall; other abbreviations as in Table 1.

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Table 4. One-Way ANOVA Adjusted for the Following Covariates: Age, Gender, Body Mass Index, BP, Duration of Hypertension, 24-h Urinary Na^+ Output, ALDO/PRA Ratio, With the ANP −C664G Polymorphism as Independent Variable

Variables	All Patients: Total Model p Value; (R^2)	All Patients: ANP p Value; (R^2)	Never-Treated Patients: Total Model p Value; (R^2)	Never-Treated Patients: ANP p Value; (R^2)
LV septum (cm)	0.000 (31.7%)	0.000 (8.8%)	0.000 (29.8%)	0.007 (6.9%)
LV PW (cm)	0.000 (32.6%)	0.000 (13.1%)	0.000 (27.0%)	0.003 (8.4%)
RWT (%)	0.000 (19.2%)	0.000 (10.1%)	0.03 (14.8%)	0.02 (5.2%)
LVMI (g/m^2)	0.000 (29.7%)	0.001 (7.4%)	0.000 (28.2%)	0.004 (8.1%)

Each row represents a covaried 1-way analysis of variance of 1 individual cardiac parameter, considered as the dependent variable, both in the whole sample and in the subgroup of never-treated patients. The columns titled Total Model report the significance (p value) and amount of variability (R^2) explained by the complete analysis. The columns titled ANP report the significance (p value) and amount of variability (R^2) explained by the effect of the ANP gene promoter polymorphism independently of all covariates.
ANOVA = analysis of variance; other abbreviation as in Table 1.

with regard to clinical variables were seen by 1-way ANOVA (Table 5).

Carriers of the 11/12 genotype had significantly higher values of ST and LVMI versus all other genotypes in the whole sample, whereas the significance was not reached in the never-treated subgroup of patients despite the presence of a similar trend (Table 6). Moreover, the significant association between the NPRA 11/12 genotype and LVMI was confirmed by the covariate ANOVA, after the exclusion of the same confounding factors considered for the ANP promoter polymorphism analysis (whole model: $p = 0.000$, $R^2 = 28.6\%$; individual genotype effect: $p = 0.05$; $R^2 = 5.3\%$).

Finally, because of the limited number of hypertensive patients carrying both ANP and NPRA promoter gene variants, the analysis of possible epistatic interactions between the 2 genes on determination of LVMI was not performed.

BNP polymorphism. The frequency of all allelic variants (n = 5) for the BNP gene microsatellite marker was low. In fact, carriers of all allelic variants (13 patients) were grouped for the analysis. No effect of the BNP gene variants on the cardiovascular phenotypes considered in this study was detected.

DISCUSSION

Our study shows that an ANP gene promoter variant, responsible for a significant downregulation of ANP gene transcription, is associated with increased cardiac wall thicknesses and LVMI in essential hypertension independently from BP levels as well as from anthropometric and clinical factors. A role of NPRA gene molecular variants on the determination of LVMI was also found.

To identify the "true" genotype/phenotype relationships, the study was performed in a hypertensive population selected on the basis of very restrictive inclusion criteria. In fact, we enrolled patients with similar race, age, body dimensions, duration and stage of hypertension, and dietary habits, and with no current pharmacological treatment. Remarkably, these findings were confirmed in a considerable subgroup of never-treated hypertensive patients, allowing us to completely rule out any pharmacological effect on cardiac phenotypes (16).

Although the clinical relevance of our results is limited by the low frequency of the ANP gene promoter variant, these findings, although confirming previous experimental evidence, represent the first demonstration in humans of the antihypertrophic role played by ANP in the process of cardiac remodeling in essential hypertension. Moreover, preliminary echocardiographic examinations performed in normotensive patients carrying the promoter polymorphism allelic variant have shown a trend for increased LVMI as compared with wild-type normotensive patients, thus suggesting an autocrine/paracrine role for ANP in the regulation of ventricular mass that seems to be further enhanced in the presence of hypertension.

Table 5. Clinical Variables According to the NPRA Genotypes

Variables	1 (n = 49) (10/10)	2 (n = 91) (10/11)	3 (n = 45) (11/11)	4 (n = 18) (11/12)	p Value*
Age (yrs)	43.4 ± 1.6	42.2 ± 1.0	43.8 ± 1.4	45.9 ± 1.7	NS
Body mass index (kg/m^2)	25.2 ± 0.4	25.5 ± 0.2	24.9 ± 0.3	26.0 ± 0.5	NS
Duration of hypertension (mo)	42.5 ± 8.7	37.8 ± 5.9	31.9 ± 8.6	54.4 ± 19.8	NS
SBP (mm Hg)	139.7 ± 2.1	146.1 ± 1.8	146.3 ± 2.4	147.0 ± 3.8	NS
DBP (mm Hg)	94.0 ± 1.4	97.1 ± 1.1	96.3 ± 1.4	99.5 ± 2.6	NS
HR (beats/min)	74.1 ± 1.6	72.2 ± 0.9	73.5 ± 1.2	73.3 ± 2.7	NS
uNa^+ (mmol/day)	127.1 ± 7.8	124.1 ± 5.9	141.5 ± 9.1	131.2 ± 14.3	NS

*Analysis performed by 1-way ANOVA with least-squares difference post-hoc test for multiple comparisons.
Abbreviations as in Table 1.

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Table 6. Echocardiographic Variables According to the NPRA Genotypes

Variables	All Patients					Never-Treated Patients				
	1 (n = 49) (10/10)	2 (n = 91) (10/11)	3 (n = 45) (11/11)	4 (n = 18) (11/12)	p Value*	1 (n = 33) (10/10)	2 (n = 67) (10/11)	3 (n = 35) (11/11)	4 (n = 13) (11/12)	p Value*
LV septum (cm)	1.05 ± 0.03	1.03 ± 0.02	1.02 ± 0.03	1.19 ± 0.08†	0.01	1.04 ± 0.04	1.02 ± 0.02	1.04 ± 0.04	1.13 ± 0.07	NS
LV PW (cm)	0.95 ± 0.03	0.98 ± 0.01	0.99 ± 0.02	1.05 ± 0.05	NS	0.93 ± 0.03	0.94 ± 0.02	0.99 ± 0.03	1.02 ± 0.06	NS
LVIDD (cm)	4.88 ± 0.07	4.93 ± 0.06	4.92 ± 0.08	5.09 ± 0.12	NS	4.85 ± 0.09	5.00 ± 0.07	4.89 ± 0.10	5.07 ± 0.14	NS
LVISD (cm)	2.87 ± 0.06	2.93 ± 0.06	2.90 ± 0.09	3.02 ± 0.12	NS	2.88 ± 0.08	2.93 ± 0.06	2.90 ± 0.09	2.94 ± 0.13	NS
RWT (%)	38.9 ± 1.2	39.7 ± 0.7	41.0 ± 1.3	41.7 ± 2.5	NS	38.4 ± 1.3	38.7 ± 0.8	41.0 ± 1.6	40.7 ± 2.5	NS
LVMI (g/m^2)	95.4 ± 4.1	94.7 ± 2.3	97.0 ± 3.4	115.0 ± 8.4†	0.02	92.3 ± 4.9	93.8 ± 2.6	97.6 ± 3.7	109.3 ± 9.5	NS
LA (cm)	3.53 ± 0.01	3.64 ± 0.01	3.99 ± 0.02	3.80 ± 0.02	NS	3.60 ± 0.01	3.65 ± 0.01	4.11 ± 0.02	3.75 ± 0.01	NS
EF (%)	66.0 ± 0.9	65.3 ± 1.0	66.1 ± 1.2	65.6 ± 1.8	NS	65.8 ± 1.2	65.5 ± 1.1	65.9 ± 1.4	67.3 ± 1.9	NS

*Analysis performed by 1-way ANOVA with least-squares difference post-hoc test for multiple comparisons. †Versus all other genotypes.
Abbreviations as in Table 1.

Our data support previous evidence of a role of NPRA on LVM in human hypertension (15).

The natriuretic peptides are mainly produced within the heart and are involved in the regulation of BP and blood volume through their direct effects on kidney and systemic vasculature (24). An important contributory role of natriuretic peptides in the regulation of cellular growth, cellular proliferation, and cardiac hypertrophy has been recently documented. All effects are primarily mediated through the NPRA receptor (25). In particular, a strong relationship between ANP/NPRA and LVM has been so far convincingly shown only in genetically manipulated animal models (9,10,11,26,27). Based on these findings, the ANP-NPRA system seems to behave as a relevant regulatory mechanism antagonizing hypertrophic growth responses within the heart. Thus, the increase of ANP that generally accompanies cardiac hypertrophy should be interpreted as the response of an intrinsic "friendly" mechanism that protects myocardiocytes against hypertrophic stimuli (28,29).

To further strengthen the interpretation of the results from association studies, it is important to establish a plausible biological explanation for observed statistical relationships between gene polymorphisms and pathological phenotypes. Our data provided good evidence for a biological link for the association between ANP and cardiac variables. In fact, we found that carrier status of the promoter allelic variant was associated with significantly lower levels of circulating proANP. The latter represents the most reliable marker of the total amount of secreted ANP because of its longer half-life and higher stability as compared with alpha-ANP, for which a parallel trend for lower levels in mutant allele patients was also observed (data not shown). Our findings parallel those obtained in ANP knockout mice. The −C664G polymorphism is located within a portion of the 5′ flanking sequence of the human gene containing a series of *cis*-acting regulatory elements able to modulate ANP gene expression (30–32). Our results cannot exclude the possibility that another polymorphism in linkage disequilibrium with the −C664G locus actually causes the phenotype.

A stop codon polymorphism was associated with a certain degree of cardiac mass remodeling. The stop codon variant adds 2 extra arginine residues to alpha-ANP, and its functional consequences are still unknown. We previously reported its significant association with an increased risk of ischemic stroke (21), whereas other studies have shown its contributory role to hypertensive renal damage (33) and to ischemic cardiac disease (34).

The NPRA gene molecular variant used in this study is located within the 5′ flanking region, and its effect on the transcriptional activity is unknown (23). The results of our study, showing a significant effect on LVMI in patients carrying the mutant alleles, suggest that the functional relevance of the promoter gene variants may be related to a reduced NPRA activity.

Finally, the BNP gene was unrelated to all cardiovascular phenotypes considered in this study, and particularly to LVMI. This negative result, which does not support previ-

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ous observations (13,14), may be partly explained by the low frequency of allelic variants detected in our population (although it was slightly higher than that observed for the ANP promoter and NPRA gene variants) and most probably by the location of this marker within the 3′ end of the gene. It is likely that the characterization of a more functionally significant gene marker may have led to different results. Moreover, the lack of measurement of BNP levels can be considered as a limitation of our study.

Conclusions. The present study shows an association between ANP/NPRA gene polymorphisms and left ventricular structure in human essential hypertension. A reduced proANP level mediates the observed increase in LVMI in carriers of the ANP gene promoter variant, whereas a reduced NPRA activity is most likely responsible for the same phenotype. Our data, although confirming the antihypertrophic role of ANP/NPRA toward the hypertrophic growth response previously obtained in experimental models, provide the first evidence that the ANP/NPRA system regulates LVM in human hypertension.

Reprint requests and correspondence: Dr. Speranza Rubattu, IRCCS Neuromed, Località Camerelle, 86077 Pozzilli (Isernia), Italy. E-mail: rubattu.speranza@neuromed.it.

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Association of Atrial Natriuretic Peptide and Type A Natriuretic Peptide Receptor Gene Polymorphisms With Left Ventricular Mass in Human Essential Hypertension

Speranza Rubattu, Giada Bigatti, Anna Evangelista, Chiara Lanzani, Rosita Stanzione, Laura Zagato, Paolo Manunta, Simona Marchitti, Vanessa Venturelli, Giuseppe Bianchi, Massimo Volpe, and Paola Stella

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