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RESEARCH LETTER

Atrial natriuretic peptide (ANP) gene promoter variant and increased susceptibility to early development of hypertension in humans

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Previous evidence supports a role of atrial natriuretic peptide (ANP) as a candidate gene for hypertension. We characterized an ANP gene promoter variant, which has been associated with lower peptide levels, in a sample of young male subjects from Southern Italy ($n=395$, mean age $=35.2\pm2$ years) followed up for 28 years. In this cohort, the ANP gene variant was associated with early blood pressure increase and predisposition to develop hypertension.

Primary hypertension represents a complex cardiovascular trait determined by both environmental and genetic factors.[1,2] The gene encoding ANP, a cardiac hormone with natriuretic diuretic and vasodilatory properties,[3] has been shown to favour development of high BP levels in genetically manipulated animal models.[4,5] Similar results were obtained with the manipulation of NPRA (natriuretic peptide receptor type A) gene.[6] Furthermore, the efficacy of a regulatable ANP gene therapy for hypertension has been recently demonstrated.[7] In humans, the contributory role of ANP gene into predisposition to develop hypertension has been so far explored through a few case–control association studies with variable results.[8,9]

In this study, we tested the hypothesis that an ANP gene promoter polymorphism, which has been associated to lower NT-proANP plasma levels,[10] could represent a valuable tool to investigate the contributory role of ANP on susceptibility to develop hypertension. We used a cohort of young male subjects ($n=395$) followed up for 28 years from the Olivetti Heart Study, a survey of cardiovascular risk factors carried out in Southern Italy.[11] At baseline (1975) and subsequent follow-up visits (1987, 1994–1995, and 2002–2004), each participant underwent anthropometry and BP measurements, and a venous blood sample was drawn. Personal and familiar medical history was collected in the interview. Hypertension was diagnosed when BP was $\geqslant140$ and/or 90 mm Hg or in subjects under regular antihypertensive therapy.

Genotyping for the ANP gene was performed on genomic DNA throughout the analysis of a –C664G promoter polymorphism by an *Rsa*I restriction fragment length polymorphism (RFLP) assay, as previously reported.[8,10,12] The Hardy–Weinberg equilibrium (HWE) was tested by χ^2 goodness-of-fit test. Differences in quantitative variables according to the genotype were tested by Student's *t*-test for unpaired data and by analysis of covariance to account for possible confounders. The frequencies of outcomes (hypertension, antihypertensive therapy) by exposure factor (genotype) were compared by χ^2 statistics. Results were expressed as mean $\pm$ s.d. Statistical significance was accepted at $P<0.05$. Statistical analysis was performed by SPSS 10.0.

The less common ANP promoter allele was present in 17 individuals (4.3% of the study population), a proportion similar to that reported for other Caucasian populations.[10,12] The genotype distribution followed the HWE ($\chi^2=0.191$; $P=0.66$). At the time of enrolment (1975), when the mean age of the subjects was 35 ± 2 years, no between-genotype difference was observed with regard to prevalence of obesity, levels of metabolic parameters and smoking habits. In contrast, systolic blood pressure (BP) was significantly higher in the heterozygous (131.8 ± 23.5 mm Hg) as compared to the wild-type genotype (123.6 ± 14.2 mm Hg), $P=0.02$. Diastolic BP tended also to be higher in heterozygous than in wild type (86 ± 15.7 vs 81.5 ± 9.9 mm Hg, $P=0.07$), also accounting for age and body mass index. At the last follow-up visit (2002–2004) no significant differences between heterozygous and wild-type participants were detected.

As shown in Figure 1 (upper panel), prevalence of hypertension was significantly higher among heterozygous at baseline ($\chi^2=4.376$, $P<0.05$), while the difference was no longer significant at subsequent follow-up visits. The use of antihypertensive treatment was significantly higher among heterozygous at the earlier 1987 examination ($\chi^2=7.902$, $P=0.02$) but not at the following examinations (Figure 1, bottom panel). At the 2002–2004 visit (28-year follow-up), the heterozygous group reported a higher occurrence of transitory cerebrovascular ischemic events (5.9 vs 0.5% in wild type, $P<0.05$), and, among their first-degree relatives, a greater number

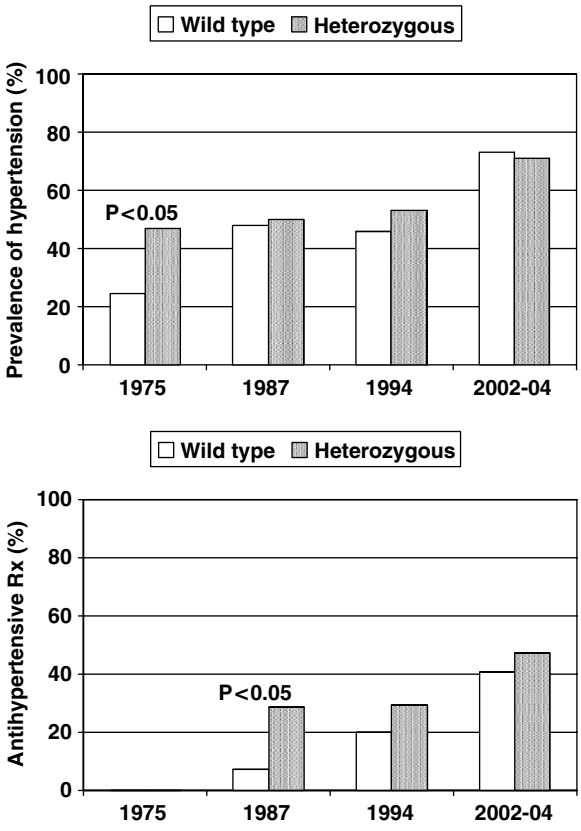

Figure 1 Over time prevalence of hypertension (upper panel) and of antihypertensive therapy (bottom panel) by –C664G ANP gene promoter polymorphism. The prevalence of hypertension at the 1975 examination was significantly higher in the heterozygous individuals than in the wild-type carriers ($\chi^2 = 4.376$, $P < 0.05$) as well as the use of antihypertensive treatment at the 1987 examination ($\chi^2 = 7.902$, $P = 0.02$).

of strokes along with a trend for a higher prevalence of hypertension.

We show that subjects harbouring the ANP promoter gene allelic variant are more susceptible to develop hypertension at a younger age, and show in an early phase of life higher BP levels as compared to subjects carrying the wild-type ANP gene variant. The same individuals required more often and at an earlier age the prescription of an antihypertensive treatment. The influence of ANP gene appeared to be independent of age, obesity, smoking and diabetes status. This result provides the first evidence of a direct contributory role of ANP gene to hypertension development in humans.

The ANP promoter polymorphism investigated in the present analysis has been associated with lower plasma NT-proANP levels.[10] Therefore, it seems reasonable that lower ANP levels determine higher BP levels and favour development of sustained hypertension at a younger age.

It is well known that genetic studies performed in young subjects are expected to be more powerful on identifying genes causing diseases. In fact, the effects of exposure to risk factors related to environment and lifestyles have less of an impact in young individuals. In addition, the 28 years longitudinal observation has allowed us to recognize the precocious need for antihypertensive therapy among carriers of the promoter allelic variant. Of interest, a greater incidence of transient ischemic cerebrovascular events and a certain degree of familial aggregation for cardiovascular diseases were observed among heterozygous compared with wild-type homozygous individuals for the ANP gene variant.

What is known about this topic:

- Hypertension represents a complex cardiovascular trait due to both genetic and environmental factors.[1]
- The atrial natriuretic peptide (ANP) plays a pivotal role in the maintenance of blood pressure homeostasis through its diuretic, natriuretic and vasodilatory effects.[3]
- The gene encoding ANP is a plausible contributor to increased susceptibility to develop hypertension. In fact, ablation of ANP gene leads to a form of salt-sensitive hypertension,[4] whereas overexpression of ANP causes hypotension.[5]

What this study adds:

- A molecular variant of ANP gene, which falls within the promoter region and has been previously associated to reduced NT-proANP plasma levels,[10] is associated with significantly higher blood pressure levels and with an increased prevalence of hypertension in young male subjects.
- Carriers of the ANP gene promoter allelic variant show an increased predisposition to develop hypertension along with an increased occurrence of transitory cerebrovascular ischemic events over a 28-year follow-up period.
- This study provides the first epidemiological evidence in humans of the effect of an ANP gene variant on susceptibility to early development of hypertension, thanks to a combined cross-sectional and longitudinal approach.

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