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Genetic Polymorphisms of the Natriuretic Peptide System in the Pathogenesis of Cardiovascular Disease: What Lies on the Horizon?

Cristina Vassalle[1]* and Maria Grazia Andreassi[1]

BACKGROUND: The natriuretic peptide hormone family includes various proteins characterized by similar chemical structure and shared biological functions, with important effects on the cardiovascular system. Accordingly, these molecules are widely recognized as key clinical biomarkers in the diagnosis and monitoring of heart failure, hypertension, and coronary heart disease.

CONTENT: Several single-nucleotide polymorphisms have been recently identified in genes associated with the natriuretic system. This review provides an overview of new insights into the functional role of these genetic variants, as well as their impact on cardiovascular physiopathology and drug response.

CONCLUSIONS: Noteworthy relationships between some specific polymorphisms and clinical correlates of cardiovascular disease have emerged. Nevertheless, future confirming studies are needed to substantiate the clinical relevance of such variants.

Remarkable advances have occurred since the discovery of the existence of an atrial natriuretic factor [A-type natriuretic peptide (ANP)[2]] in the early 1980s by de Bold and his group *(1)*. Since then, our understanding of the importance of the NPs has moved well beyond awareness of their role as regulators of renal function *(2)*. Growing evidence now indicates the strong involvement of NPs in the control of myocardial development and function as well as cardiovascular regulation *(2)*. Accordingly, application of NPs for diagnostic and prognostic purposes has spread in clinical cardiology. Specifically, commercial assays for the measurement of the B-type NP (BNP) and the N-terminal portion of the B-type hormone (NT-proBNP), obtained after cleavage of the prohormone produced by mRNA translation, are largely available for clinical evaluation in the cardiovascular setting (Fig. 1) *(2)*.

Many aspects in this field remain to be elucidated, including what determinants may affect expression, synthesis, release, and metabolism of these peptides, as well as what factors are involved in receptor-mediated biological activity. Of particular interest is increasing knowledge about the function in the natriuretic system–related genes of single-nucleotide polymorphisms (SNPs), which might play substantial roles in the etiology and pharmacotherapy of cardiovascular disease. The aim of this review is to discuss recent discoveries and insights in this field and their potential clinical implications.

The Natriuretic System in Cardiovascular Disease: Basic Biochemical and Physiologic Principles

NPs are a family of hormones that includes ANP, BNP, and C-type NP (CNP), all presenting a similar chemical structure and sharing biological functions *(3)*.

Specifically, all NP family components are characterized by the presence of a 17–amino acid ring, stabilized by a cysteine bridge, with 11 of the 16 residues being conserved in their molecular structure across the different peptides. Two terminal amino acid tails with widely different lengths and compositions are also present (Fig. 2). ANP and BNP are mainly produced by and released from cardiomyocytes (the ANP preferentially from the atria and BNP from the ventricles) *(3)*. CNP, the third natriuretic peptide identified, is primarily expressed in the central nervous system and vascular endothelium, where it exerts autocrine and paracrine effects on vascular tone and muscle cell growth *(2)*.

NP biological effects are mediated by 3 membrane receptors: NP receptor A, preferentially activated by ANP and BNP, and NP receptor B, with higher affinity for CNP than ANP and BNP, and NP receptor C, which

[1] Fondazione G. Monasterio and Institute of Clinical Physiology, Italian National Research Council, Pisa, Italy.
* Address correspondence to this author at: G Monasterio Foundation and Institute of Clinical Physiology, Via Moruzzi 1, I-56124, Pisa, Italy. Fax +39-050-3152166; e-mail cristina.vassalle@ifc.cnr.it.
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[2] Nonstandard abbreviations: ANP, A-type natriuretic peptide; BNP, B-type NP; NT-proBNP, N-terminal portion of the B-type hormone; SNPs, single-nucleotide polymorphisms; CNP, C-type NP; HF, heart failure; UTR, untranslated region; preproBNP, the 26–amino acid signal peptide and the first 18 amino acids of proBNP; VNTR, variable number of tandem repeat.

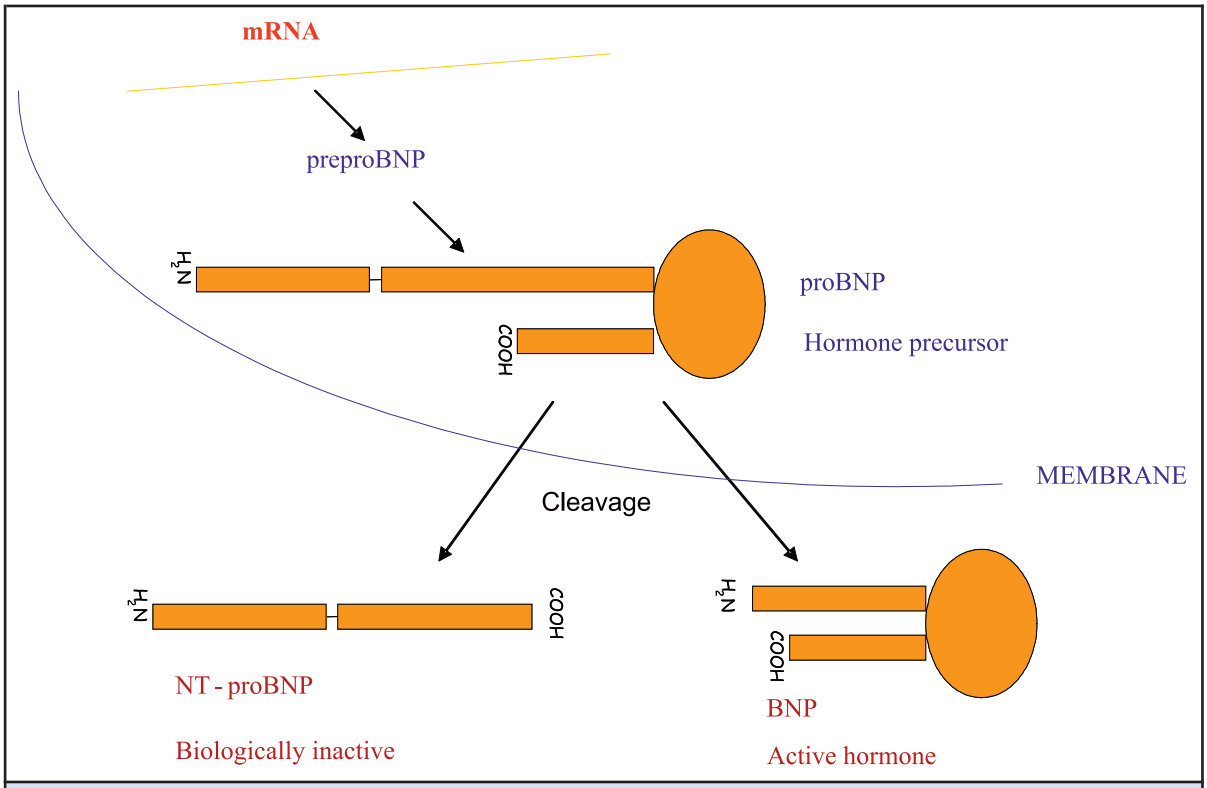

Fig. 1. Schematic representation of BNP, NT-proBNP, and the processing and secretion of precursor peptides.

is involved in the clearance of all NP from the blood (Fig. 2) *(3)*. Degradation by neutral endopeptidase contributes to NP clearance *(3)*.

All the biologically active peptides of the natriuretic hormone family share the same physiological actions, including potent diuretic, natriuretic, and vascular smooth muscle–relaxing effects, as well as complex interactions with the hormonal, nervous, and immunological systems *(2)*. Other important actions, all directed at protecting the cardiovascular system from the consequences of volume and pressure overload, have been identified and are summarized in Table 1.

Clinical Utility of NP Testing

Since the development of the first methods for measuring ANP *(4, 5)* and the documentation of the importance of the NP system in the regulation of blood pressure, NP has been widely investigated in the physiopathology and treatment of hypertension. Plasma concentrations of NP appear lower in normotensive individuals with positive family history, whereas higher NP concentrations are associated with better diastolic function *(6, 7)*. The possible protective role of NP in healthy individuals is otherwise confirmed by direct NP infusion, which improves left ventricular diastolic performance *(8, 9)*. Conversely, abnormal concentrations of NP have been found in hypertensive patients *(4, 5)*. High ANP concentrations have been observed in hypervolemic hypertension, in which ANP secretion is primarily stimulated by atrial myocardial stretch *(10)*. Moreover, BNP concentration appears to increase in relation to hypertension severity, particularly in the presence of left ventricular hypertrophy, presumably due to the increase in ventricular mass and the associated higher BNP synthesis and secretion from ventricular tissue *(2)*.

Increased concentrations of NP have been seen in acute coronary syndromes, in which the amount of increase in concentration is related to the size of the infarct and the extent of ventricular dysfunction *(11)*. Increased NP concentration is a risk factor independent of all other traditional variables in patients with unstable and those with stable coronary artery disease *(11, 12)*. Moreover, elevation of NP concentrations is a strong and independent adverse prognostic indicator of heart failure (HF) or death in acute coronary syndrome as well as in stable coronary artery disease *(11, 12)*. Interestingly, increased expression of NP has been found in human atherosclerotic vessels, suggest-

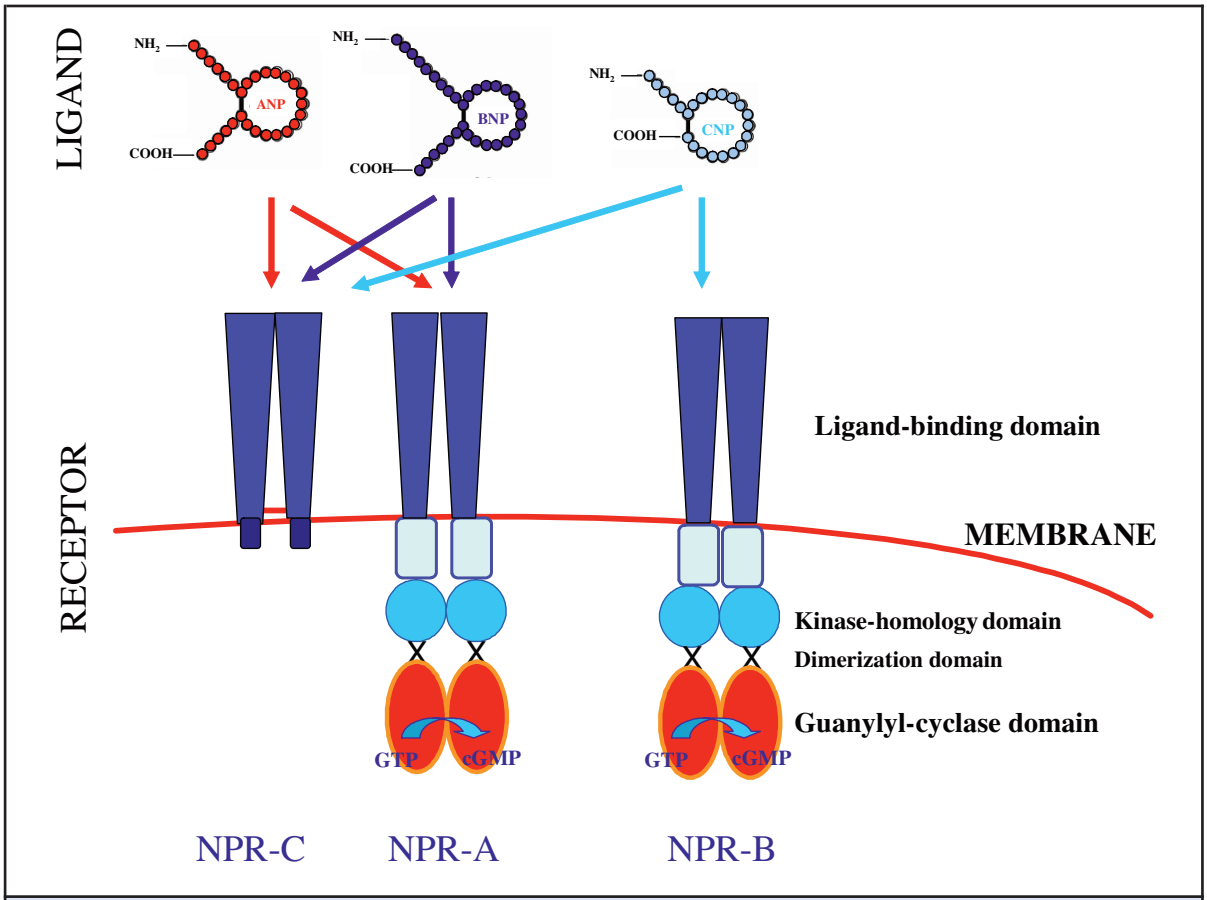

Fig. 2. Schematic representation of natriuretic peptide primary structure, natriuretic peptide receptors, and ligand selectivity.

NPR-A, NPR-B, and NPR-C, represent natriuretic peptide receptors A, B, and C, respectively GTP, guanosine triphosphate; cGMP, cyclic guanosine monophosphate.

Table 1. Physiological effects of natriuretic peptides.

Natriuresis	>
Diuresis	>
Venous tone	<
Arterial tone	<
Cardiac and vascular hypertrophy and remodeling	<
Sympathetic nerve activity	<
Parasympathetic nerve activity	>
Aldosterone concentration	<
Fibrosis	<
Renin secretion	<
Glomerular filtration rate and filtration fraction	>
Lipolysis	>
Inflammation	<
Platelet activation	<

ing that the plaque may contribute to elevation of circulating NP, plaque development, and vascular remodeling *(13)*.

The measurement of BNP and NT-proBNP has emerged as an important clinical tool in the evaluation and management of HF *(14, 15)*. The importance of NP measurements in HF has been underlined in a recent metanalysis, which demonstrated the high diagnostic accuracy and clinical relevance of BNP and NT-proBNP assays for both acute and chronic HF *(16)*. A systematic review found NP to be a strong independent prognostic indicator in acute and chronic HF *(17)*.

Other clinical applications of NPs that are under investigation include screening for asymptomatic ventricular dysfunction and guiding therapy. In particular, on the basis of observations reported to date it is reasonable to expect that treatment of HF guided by BNP and NT-proBNP concentrations could improve clini-

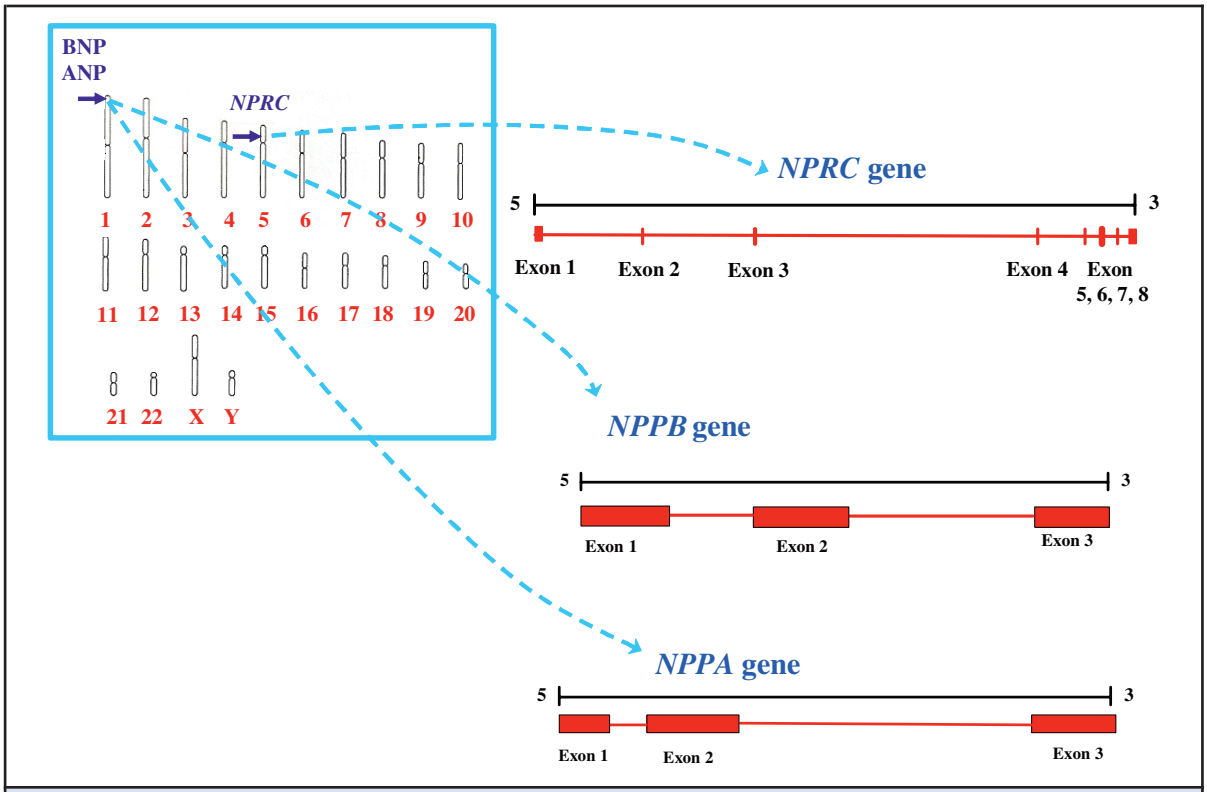

Fig. 3. Chromosome localization and organization of human ANP (*NPPA*), BNP (*NPPB*) and CNP (*NPRC*) genes.

cal outcomes, and large-scale randomized controlled trials are currently in progress to verify the efficacy of such treatment strategies *(18–20)*.

The Natriuretic Precursor Peptide A Gene and Its Polymorphisms

GENETIC LOCATION AND GENE CHARACTERISTICS

The human ANP gene, natriuretic peptide precursor A (*NPPA*),[3] is found on chromosome 1p36.2, in close proximity to the BNP gene , natriuretic peptide precursor B (*NPPB*) (*NPPA* is separated by 8 kb from the 5′ extremity of *NPPB*) (Fig. 3). The ANP gene, like the BNP gene, contains 3 exons and 2 introns *(21)*. Exon 1 codes for the first 16 residues, exon 2 for the rest of the amino acid sequence, except for the C-terminal tyrosine *(21)*. Thus the third exon encodes for this single residue followed by the 3′ untranslated region (UTR) of the mRNA *(21)*.

[3] Human genes: *NPPA*, natriuretic peptide precursor A; *NPPB*, natriuretic peptide precursor B; *NPR1*, natriuretic peptide receptor A/guanylate cyclase A (atrionatriuretic peptide receptor A); *NPR2*, natriuretic peptide receptor B/guanylate cyclase B (atrionatriuretic peptide receptor B); *NPPC*, natriuretic peptide precursor C; *NPR3* natriuretic peptide receptor C/guanylate cyclase C (atrionatriuretic peptide receptor C).

BIOLOGICAL SIGNIFICANCE AND CLINICAL RELEVANCE

Since the beginning of the 1990s, various polymorphisms have been identified at this locus and associated with various conditions *(22–28)*. Results reported more recently have been related to progress in the field of cardiovascular diseases *(29–42)*. A list of the main SNPs of the ANP gene that have been associated with cardiovascular disease is shown in Table 2, with most of these studies generally investigating the presence and features of hypertension, diabetic nephropathy, and ischemic stroke.

The most studied SNP is the transition T2238C in the ANP gene, which introduces a stop codon and leads to extension of the protein from 28 amino acids to 30 by the addition of 2 arginines *(23)*. This variant has been found to be associated with nonfatal myocardial infarction, stroke, coronary artery disease, hypertension, and left ventricular hypertrophy *(29, 34, 37)*. The functional significance of T2238C polymorphisms has not been fully described, however, and there is no clear evidence that the minor C-allele is associated with decreased plasma ANP concentrations *(28, 30)*.

Recently, we investigated the possible impact of the T2238C polymorphism in HF, finding that patients

Table 2. More common SNPs in the *NPPA* gene.

Variant	Restriction site	Position	Mutation	References
T2238C	ScaI	Exon 3	Stop codon loss: protein extended from 28 to 30 amino acids	Rubattu et al. *(27)*, Nannipieri et al. *(28)*, Rubattu et al. *(29)*, Nannipieri et al. *(31)*, Rahmutula et al. *(32)*, Gruchala et al. *(34)*, Nannipieri et al. *(35)*, Roussell et al. *(36)*, Rubattu et al. *(37)*, Conen et al. *(39)*, Vassalle et al. *(41)*
G664A	RsaI	5′ UTR	Vat7Met substitution	Rubattu et al. *(29)*, Kato et al. *(30)*, Rahmutula et al. *(32)*, Kato et al. *(33)*, Rubattu et al. *(37)*, Zhang et al. *(38)*, Conen et al. *(39)*, Iemitsu et al. *(42)*
C708T	BstXI	Intron 1		Nannipieri et al. *(28)*, Nannipieri et al. *(31)*, Nannipieri et al. *(35)*, Roussell et al. *(36)*
G1837A	SmaI	Intron 2		Rubattu et al. *(27)*, Rubattu et al. *(29)*, Rahmutula et al. *(32)*, Rubattu et al. *(37)*

with the T allele have higher concentrations of not only ANP but also of BNP and NT-proBNP *(41)*. It is therefore plausible that the presence of the T2238C polymorphism might be an additive factor influencing disease progression and prognosis in severe HF, although these findings need further confirmation.

A recent multicenter randomized clinical trial conducted on 38 462 participants with hypertension revealed evidence of a pharmacogenetic association of the ANP precursor gene T2238C variant with clinical outcome after randomization to treatment *(43)*. Specifically, patients who were carriers of the minor C-allele presented better prognosis when assigned to receive a diuretic, whereas patients carrying TT had fewer adverse events when randomized to receive a calcium-channel blocker *(43)*. These findings suggest that individuals with a higher-risk genotype would have more favorable outcomes when taking a diuretic compared with their counterparts taking other classes of antihypertensive medications *(43)*. Further studies are needed to better define the physiologic effects of T2238C before introducing into clinical practice individualized treatment based on genetic testing prior to medication administration.

The *NPPB* Gene and Its Polymorphisms

GENETIC LOCATION AND GENE CHARACTERISTICS

The human BNP gene, *NPPB*, is located on chromosome 1p36.2 and contains 3 exons and 2 introns (Fig. 3). The complete nucleotide sequence of BNP was first described at the end of the 1980s *(44)*. Exon 1 of the human BNP gene encoded the 5′ UTR and a part of preproBNP (the 26–amino acid signal peptide and the first 18 amino acids of proBNP); exon 2, the amino acids from 45 to129; and exon 3, the 5 terminal amino acids (from 130 to134) and the 3′ UTR *(44)*. Several years later, the 5′ flanking region located upstream from a UTR was described *(45)*.

BIOLOGICAL SIGNIFICANCE AND CLINICAL RELEVANCE

In the past, very few studies have explored the role of SNPs of the BNP gene in the pathogenesis and development of diseases. In 2000, the CARDIGENE study evaluated a possible association between the nucleotide substitution C-1563T in the BNP gene and the predisposition to idiopathic dilated cardiomyopathy, but study results did not indicate any relationship between this SNP and susceptibility to this disease *(46)*.

Subsequently, a microsatellite marker localized within the 3′ end of the BNP gene was analyzed in a population of essential hypertensive patients to evaluate any possible association with hypertension-related modification of the left ventricle as assessed by echocardiography *(29)*. The findings showed no significant effect of BNP gene variants on ventricular remodeling in human essential hypertension.

A very recent study evaluated the association between the BNP promoter T–381C polymorphism and risk of type 2 diabetes and metabolic and BNP expression traits in samples from several populations. The study included participants of the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) study and 4 other case–control studies, for a total of 3593 cases and 6646 controls *(47)*. The results indicated that individuals carrying the –381CC

genotype presented lower concentrations of plasma glucose and lower risk of type 2 diabetes. Moreover, the –381C allele was associated with higher BNP concentration and higher BNP promoter activity in reporter gene assays. Interestingly, BNP concentrations were found to be particularly increased in –381C allele carriers evaluated in a subpopulation of stable HF patients *(47)*.

Eight polymorphisms in *NPPB*, including the T–381C variant, and their association with BNP concentrations were also evaluated in a large general population of adult Japanese persons, revealing significant associations of the variants with BNP concentration *(48)*. In this study, both single-SNP analysis and haplotype-based analysis revealed an association between the presence of *NPPB* SNPs with circulating BNP concentrations. In particular, the findings for the promoter SNP T–381C showed a codominant effect of the minor C-allele on elevating plasma BNP concentrations.

These findings suggest a functional role for the T–381C variant that may lead to changes in the predictive use of BNP for diagnosis and prognosis. In another study in a selected population of patients undergoing elective cardiac catheterization, the T–381C variants were evaluated together with the *NPPB* 777 G>A (3′ flanking region) and other variants of 5 genes involved in the BNP pathway *(49)*. The results of this study indicated that individuals who carried the TT variant had lower BNP concentrations.

The association between 2 polymorphisms (–381T/C and 1551G/A) of the BNP gene was also assessed according to NT-proBNP concentrations and mortality in 380 type 1 diabetic patients with and without diabetic nephropathy *(50)*. Carriers of the –381T/C and 1551G/A polymorphisms had higher concentrations of NT-proBNP without presenting prevalent overt diabetic nephropathy. These variants did not predict all-cause or cardiovascular mortality in type 1 diabetic patients with or without diabetic nephropathy.

Finally, a novel variable number of tandem repeat (VNTR) polymorphism in the 5′ flanking region of the *NPPB* gene was discovered. This polymorphism was associated with essential hypertension in female patients *(51)*.

Natriuretic Peptide Receptor C/Guanylate Cyclase C (Atrionatriuretic Peptide Receptor C) Gene and Its Polymorphisms

GENETIC LOCATION AND GENE CHARACTERISTICS

The human natriuretic peptide receptor C/guanylate cyclase C (atrionatriuretic peptide receptor C) (*NPR3*) gene is located on chromosome 5p14-p13 *(55)*. It spans approximately 70 kb, and contains 8 exons and 7 introns (Fig. 3) *(52)*.

Several polymorphisms in the *NPR3* gene have been described, including the C(–55)A polymorphism in the promoter region of the *NPR3* gene and a VNTR polymorphism consisting of a 6-nucleotide repeat 4 bp upstream from the major transcriptional initiation site in the 5′ flanking region of the *NPR3* gene *(49, 41, 42, 52–57)*.

BIOLOGICAL SIGNIFICANCE AND CLINICAL RELEVANCE

The C(–55)A and the VNTR polymorphisms have been found to be associated with hypertension and obese hypertension; C(–55)A is also related to lower ANP concentrations and higher systolic and mean blood pressure *(53, 56)*.

Moreover, C-allele carriers in a general population were found to have significantly lower prevalence of overweight, obesity, and abdominal adiposity compared with the A(–55)A carriers *(57)*. The C(–55) allele of the *NPR3* gene was found also to be associated with family history of hypertension *(54)*. However, our recent data did not show the C(–55) *NPR3* polymorphism to be an independent determinant of NP concentration in HF *(41)*.

Genes and Polymorphisms Relative to Other Members of the Natriuretic System

GENETIC LOCATIONS AND GENE CHARACTERISTICS

The natriuretic peptide receptor A/guanylate cyclase A (atrionatriuretic peptide receptor A) (*NPR1*) gene is located on chromosome 1q21–22 and has a similar structure to the natriuretic peptide receptor B/guanylate cyclase B (atrionatriuretic peptide receptor B) (*NPR2*) gene, the latter located at 9p21-p12 *(58, 59)*. These genes are about 16 kb long, and they have 64% cDNA homology *(58)*. They are composed of 22 exons each *(58)*.

The gene encoding CNP, natriuretic peptide precursor C (*NPPC*), is localized to human chromosome 2 and consists of 2 exons separated by 1 intron *(60)*.

BIOLOGICAL SIGNIFICANCE AND CLINICAL RELEVANCE

Some polymorphic sites were identified in the *NPR1* gene, especially in the 5′-flanking region and other noncoding regions, that may influence the transcriptional activity of the gene and may be thus potentially involved in the pathogenesis of essential hypertension and other cardiovascular disease *(37, 54, 61–68)*.

In the Japanese population, an 8-bp deletion in the 5′ flanking region of *NPR1* was found to be associated with essential hypertension and left ventricular hypertrophy *(62)*. In another study by the same authors, a missense mutation, M341I, consisting of a methionine (ATG) to isoleucine (ATC) substitution at nucleotide 1023 in exon 3, was identified and shown to be associated with essential hypertension *(63)*. Amino acid residue 341 is in the loop between 2 α-helices, and therefore the M341I substitution may change the con-

formation of the hydrophobic core, influencing receptor activity *(63)*. Interestingly, the M341I SNP was also reported to be associated with higher risk for myocardial infarction in the Japanese population *(64)*.

The 8-bp deletion in the 5′ flanking region was also evaluated in an Italian population, in which this microsatellite marker of the *NPR1* gene was found to be significantly associated with left ventricular mass index and left ventricular septal thickness, suggesting that the functional relevance of the mutant alleles may be related to a reduced NP receptor A activity *(29)*.

Recently, Knowles et al. sequenced the entire human *NPR1* gene and identified the (CT)n dinucleotide repeat site (n = 6, 10, or 11) at nucleotide −293 in the promoter region as well as 9 other polymorphic sites in the noncoding region of the gene *(66)*. In particular, 3 common haplotypes in the 5′ and 3′ regions of the receptor gene have been identified, and 3 of these have been shown to have quantitative effects on the expression of the receptor in vitro *(66)*.

These haplotypes in the *NPR1* gene have been recently evaluated in 402 unrelated white individuals, including healthy controls and patients with acute coronary syndrome and HF *(67)*. Results indicated that the "4-minus" haplotype of the *NPR1* gene is related to high NT-proBNP concentration and represents a genetic determinant of between-person variability in the BNP system in healthy individuals but not in patients with cardiovascular diseases.

The (CT)n polymorphism in the 5′-flanking region of the *NPR1* gene also was found to be significantly associated with essential hypertension, probably through the downregulation of *NPR1* gene transcription *(68)*. However, the authors did not observe any significant association between the 8-bp deletion in the 5′ UTR and essential hypertension *(68)*.

The structural organization of the human *NPR2* gene was studied, and several polymorphic sites were found in *NPR2*, including a GT-repeat polymorphism in intron 2, a C2077T transition in exon 11, and a 9-bp deletion in intron 18 *(59, 69–71)*. These studies showed that the GT-repeat polymorphism was associated with essential hypertension, whereas the I/D deletion did not show any significant relationship either with this condition or with stroke *(59, 69, 70)*. In addition, no significant association was found between the C2077T polymorphism and myocardial infarction *(71)*.

To our knowledge, there is only a single report that focuses on the associations between CNP, its genetic variants, and blood pressure *(72)*. Specifically, 4 genetic variants have been identified in the CNP gene (*NPPC*). The G2628A variant in the 3′ UTR of the gene was found to be associated with essential hypertension in a Japanese population *(72)*. Accordingly, investigation of the association between this genetic variant and the presence of hypertension in other populations is necessary.

Critical Remarks and Future Perspectives

In summary, growing evidence indicates that specific genetic factors may result in altered properties in the NP system that influence the risk for cardiovascular disease and/or response to drug treatments.

Specifically, some noteworthy relationships have emerged, and evidence of potentially important roles exists for the ANP precursor gene T2238C variant, the –T381C polymorphism in the BNP promoter, and the (CT)n polymorphism in the *NPR1* gene, as summarized in Table 3.

The effect of a single sequence variant might be small, however, and many association studies suffer from a lack of adequate power because of small sample size. The number of individuals needed to achieve adequate power is dependent on the frequency of the polymorphism, and a small effect will require much larger sample sizes to detect a difference, especially if the SNP is rare *(73)*. For instance, a sample size of about 700 cases and 2100 controls is needed to detect a minimum odds ratio of 1.3 in a study with an allele frequency of 30%, with a power of 80% at a significance of 95% *(74)*.

When these factors are taken into account, it is clear that few of the studies listed in Table 3 were sufficiently powered. Therefore, additional data are needed to test the possible contribution of each SNP and potential gene–gene interaction to the susceptibility for heart disease. Future studies should test the simultaneous genotyping of the ANP precursor gene T2238C variant, the –T381C polymorphism in the BNP promoter, and the (CT)n polymorphism in the *NPR1* gene. Moreover, further efforts should also involve collaborations to increase population sizes and maximize power. Several issues must be carefully managed during the design of such studies, including definition of the phenotype, selection of controls, measurement of plasma concentrations of NPs, and adjustment for multiple comparisons and population stratification.

With respect to pharmacogenetics, it is widely expected that in the future drug treatment will be stratified on the basis of genetic information. Gene–drug interactions are complex, but between-person variation in drug responses to treatments appears in many cases to have a simpler genetic basis than that found for common diseases *(75)*. Thus it is crucial to integrate evidence across studies because it is likely that multiple genes operating in pathways will determine response to pharmacological treatment.

Table 3. Possible functional SNPs of the NP system in cardiovascular disease.

Gene variant	Ethnicity	Study population	SNP–disease association	NP levels–SNP association	Ref.
NPPA T2238C	White	Stroke/controls (n = 347/346)	No	Not reported	Rubattu et al. *(27)*
	White	Stroke/controls (n = 206/236)	Yes	Not reported	Rubattu et al. *(37)*
	White	T1 DM[a]/controls (n = 454/58)	Yes	ANP levels A2A2>A2A1	Nannipieri et al. *(28)*
	White	DM/EH/controls (n = 807/121/105)	Yes	Not reported	Nannipieri et al. *(31)*
	Mexican	T2 DM/EH/controls (n = 112/191/985)	Yes	Not reported	Nannipieri et al. *(35)*
	Japanese	EH/controls (n = 233/213)	No	Not reported	Rahmutula et al. *(32)*
	White	MI/noMI (n = 504/340)	Yes	Not reported	Gruchala et al. *(34)*
	White	CAD severity (n = 847)	Yes	Not reported	Gruchala et al. *(34)*
	White	T1 DM with/without DN (n = 489/301)	Yes	Not reported	Roussell et al. *(36)*
	White	HF severity (n = 124)	—	ANP, BNP and NT-proBNP A2A2<A1allele	Vassalle et al. *(41)*
NPPB T-381C	White	T2 DM/controls (n = 3331/4161)	Yes	BNP levels TT<TC+CC	Meirhaeghe et al. *(47)*
	Japanese	General population (n = 2970)	—	BNP levels TT<TC<CC	Takeishi et al. *(48)*
	African/white	Elective cardiac catheterized pts (n = 147)	—	BNP levels TT<TC<CC	Lanfear et al. *(49)*
	African/white	T1 DM with/without DN (n = 197/183)	No	NTpro-BNP levels TT<TC+CC	Layer et al. *(50)*
NPRA (CT)6	Japanese	EH/controls (n = 177/170)	Yes	No	Usami et al. *(68)*

[a] T1 DM, type 1 diabetes mellitus; EH, essential hypertension; T2 DM, type 2 DM; MI, myocardial infarction; CAD, coronary artery disease; DN, diabetic nephropathy.

Actually, a paucity of information is available regarding interactions of NP genes with the effects of standard therapies. Well-conducted clinical studies will be required to evaluate the impact of selected gene variants on the efficacy of a drug treatment as well as on the risk for adverse outcomes.

A potential experimental design strategy for these studies is the limitation of assessed cases to only those cases nested within randomized controlled trials; such a "case-only" design would provide a possible means to decrease the cost of genotyping when dichotomous outcomes are being investigated *(76, 77)*. In epidemiology and genetics, there is a growing literature about case-only study designs as a means of investigating gene–environment interaction in disease etiology *(78–80)*. In the pharmacogenetic context, environmental exposure would correspond to the treatment and genotype to the covariate. The outcome of interest would have to be dichotomous, and study participants who develop this outcome during follow-up would be defined as cases *(78, 79)*.

By design, genotype and treatment are independent, because the latter is randomly allocated within controlled clinical trials. As a result, the case-only odds ratio is also a measure of gene–treatment interaction *(78, 79)*. Such an approach may be especially suitable for studying gene–drug interactions, providing greater statistical power than a case–control study of the same sample size.

In conclusion, the complexity of the NP system requires more appropriate investigations with very large sample sizes and robust genetic approaches, such as the use of high-throughput genotyping platforms, to improve the understanding of gene–gene interactions of NP proteins and related neurohormonal pathways. Nonetheless, the identification and characterization of functional SNPs that influence NP activity is surely an important research goal toward integrating DNA-based testing into clinical strategies for diagnosis and personalized treatment of at-risk individuals.

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