Natriuretic peptide receptor 3 genotype modulates the relationship between B-type natriuretic peptide and left ventricular end-diastolic pressure

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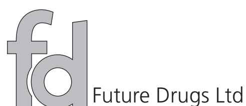
Background: B-type natriuretic peptide levels are associated with left ventricular end-diastolic pressure (LVEDP) and patient outcomes. There is documented variation in the genetic sequence of natriuretic peptide receptor (NPR)3, which is a primary clearance mechanism for B-type natriuretic peptide (BNP). **Methods:** DNA was extracted from 147 patients, aged 60 years or over undergoing elective left-heart catheterization for any indication, excluding acute myocardial infarction or severe valvular disease. Genotype was determined at four loci in the *NPR3* gene. The logBNP:LVEDP ratio was compared between genotype groups. Linear and logistic regression models of LVEDP were generated. **Results:** The LogBNP:LVEDP ratio was significantly different among NPR3 IVS2–84 A allele carriers compared with G allele homozygotes ($p = 0.008$), with the A allele carriers showing a higher BNP level for a given level of LVEDP. The other variants did not alter this relationship (all $p > 0.4$). When added to the optimal linear regression model for LVEDP, NPR3 IVS2–84 genotype incrementally added to the model ($p = 0.024$, model $r = 0.54$). This was also true of the optimal logistic regression model of LVEDP $\geq$ 20 mmHg ($p = 0.026$). **Conclusions:** NPR3 IVS2–84 G>A genotype is associated with altered logBNP:LVEDP ratio and provides incremental value to predictive models of LVEDP. Further studies should address whether this or other variants in the BNP pathway modify the clinical importance of endogenous or exogenous BNP.

Keywords: B-type natriuretic peptide, genetic polymorphisms, left ventricular end-diastolic pressure, natriuretic peptide receptor 3



Heart failure is a modern epidemic and is one of few cardiovascular diseases that currently has increasing prevalence [1,2]. The importance of B-type natriuretic peptide (BNP) as a diagnostic and therapeutic modality in heart failure is well known [3,4]. Our group has demonstrated previously that BNP levels are predictive of left ventricular end-diastolic pressure (LVEDP) [5] and risk of hospitalization among elderly cardiac patients [6]. However, the optimal interpretation of native BNP is controversial [7,8]. BNP levels correlate imperfectly with cardiac filling pressures and the factors that govern BNP levels remain incompletely understood [8,9]. Common genetic sequence variants are increasingly being recognized as determinants of disease risk or drug response [10,11] and may help explain a portion of the interindividual variation in the physiology and pharmacology of the human BNP system.

It has been shown that approximately 40% of variation in native BNP levels is heritable [12]. Furthermore, our group and others have described common polymorphisms and/or haplotype structure of key genes in the BNP pathway [13–16], and some of these variants have already shown *in vivo* and *in vitro* functional activity [17–20]. Natriuretic peptide receptor 3 (NPR3) is a noncatalytic receptor that specifically binds atrial natriuretic peptide (ANP) and BNP. It is one of two primary clearance mechanisms for natriuretic peptides. Binding leads to peptide uptake and subsequent degradation with receptor recycling. The *NPR3* gene is approximately 75 kbp long and is located on chromosome 5 (**Figure 1**). Several common variants have been documented previously [13,15], although associated phenotypes have not yet been described.

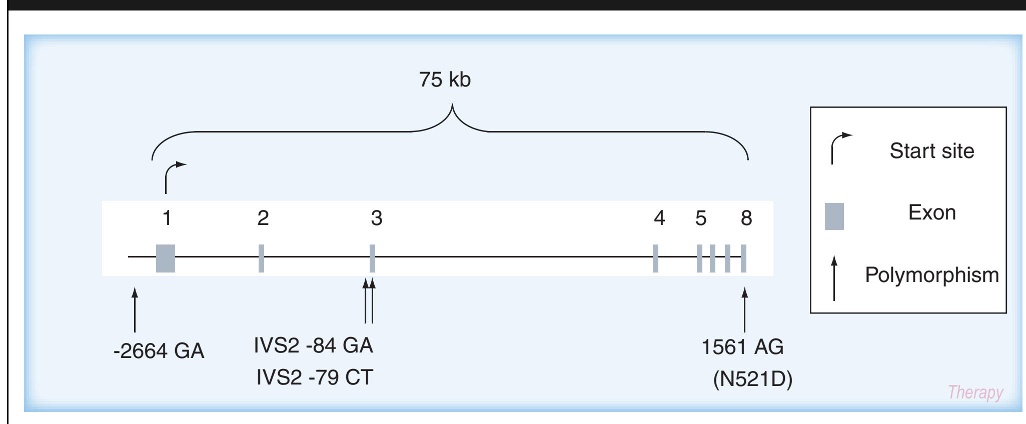
In order to begin to dissect the impact of genetic variation on the BNP system, we set out to assess the impact of sequence variants within *NPR3* on the relationship between BNP and LVEDP. We hypothesized that these variants may be associated with an altered BNP:LVEDP ratio and may affect the predictive ability of measured BNP levels.

Methods

Subjects

The study was approved by the Washington University Human Studies Committee. Written informed consent was obtained for all

Figure 1. Natriuretic peptide receptor 3 gene structure depicting exons, start site and variant loci.



patients. Population data was obtained from 190 anonymous blood donors who were self-identified as being of African or European descent (95 of each). For the clinical study, 147 consecutive patients aged 60 years or older referred for diagnostic left heart catheterization at our institution were enrolled in the study. Patients could be referred for any reason, the most common being chest pain and/or abnormal stress tests or shortness of breath. Exclusion criteria included abnormal troponin values, acute myocardial infarction or percutaneous coronary intervention within the prior 6 months, any previous cardiac surgery, presence of an implantable cardioverter defibrillator, history of known severe valvular heart disease or hypertrophic cardiomyopathy, end-stage renal or hepatic disease or unwillingness to participate. Detailed clinical and demographic data were collected in all patients, including catheterization data, standard echocardiograms and BNP levels. Echocardiographic data assessed included early (E) and late (A) mitral inflow velocity, corresponding tissue Doppler measurements (E_M and A_M, respectively), as well as other standard parameters. All clinical data were assessed by an investigator blinded to the genetic data. Patients were self-identified in terms of race. A total of 122 were self-identified as European descent, 24 were self-identified as African descent and one subject was from Sri Lanka.

Polymorphism selection & genotyping

Polymorphisms were selected using the program PolyMAPr (Polymorphism Mining and Annotation Programs) [21] due to their presence in publicly available databases and the plausibility of functional consequence based on their position (i.e., regulatory sequence, coding

Table 1. Polymerase chain reaction and Pyrosequencing® primers and conditions.

Polymorphism	Primer	Annealing temperature (°C)
Promoter -2664 GA	Forward 5′-Bioteg-gtccaaaagctgcggagagg-3′ Reverse 5′-cgcgtgtggtgtgggttta-3′ Internal 5′-acgcctgtcccatc-3′	62
Intron 2 -84 GA (IVS2–84 G>A)	Forward 5′-cgttgtcccatcatacctaagtgc-3′ Reverse 5′-Bioteg-tttataattccaagccagccacc-3′ Internal 5′-tgccaaaagtaacatgc-3′	69
Intron 2 -79 CT (IVS2–79 G>A)	Forward 5′-cgttgtcccatcatacctaagtgc-3′ Reverse 5′-Bioteg-tttataattccaagccagccacc-3′ Internal 5′-tgccaaaagtaacatgc-3′	69
Non-Syn Coding 1561 AG	Forward 5′-Bioteg-ggcactctttgtaagagaaaccatg-3′ Reverse 5′-ggggcttcctttaagctactga-3′ Internal 5′-cgatgttttccaaggt-3′	60

Bioteg: 5′-biotin tag required for Pyrosequencing®.

sequence or within intron/exon splice sites). Specifically the -2664 G>A variant was chosen because it was in the 5′ flanking region possibly representing a regulatory sequence; two variants within intron 2 were chosen owing to their proximity to the 30′ splice site (84 and 79 bp upstream, respectively); the 1561 A>G variant was chosen because it was the only common nonsynonymous coding variant identified. Genomic DNA was isolated using the Pure-Gene® extraction kit (Gentra). Genotyping was accomplished using Pyrosequencing® (Biotage). DNA containing the region of interest was amplified with the polymerase chain reaction (PCR). PCR primers were designed using Primer Express® version 1.5 (ABI). Pyrosequencing primers were designed using the Pyrosequencing SNP Primer Design Version 1.01 software [101]. Pyrosequencing was carried out as previously described using the PSQ hs96A instrument and software [22]. Primers and reaction conditions are shown in Table 1. Genotype calls were made by the Pyrosequencing software with review by an investigator unaware of the clinical data. Pairwise linkage (D′) and haplotype analysis was carried out using the Polymorphism and Haplotype Analysis Suite [23,102] among African–Americans and European–Americans separately.

Statistics

Hardy–Weinberg equilibrium and population genotype frequencies were assessed using the chi square statistic. The logBNP:LVEDP ratio was compared between genotype groups using the Mann–Whitney or Kruskall–Wallace tests, as appropriate. This ratio was used (as opposed to BNP level alone) due to the strong influence LVEDP is known to have on BNP levels and since it would serve to adjust for differences in loading conditions between patients. Log transformation of BNP was used because it provided a more normal distribution and a stronger bivariate association with LVEDP in the entire cohort than raw BNP level. Baseline characteristics were compared between IVS2–84 genotype groups using the Mann–Whitney test or Fisher exact test, as appropriate.

Regression models for LVEDP (continuous) and LVEDP 20 mmHg or more (dichotomous) were generated using linear and logistic regression, respectively. Variables with bivariate associations with LVEDP with a p value of less than 0.1 were considered for regression modeling. Possible input variables were grouped according to subtype (echo, clinical, demographic and laboratory) and the multivariate predictors from each subgroup were then identified for inclusion into regression models. The final regression models were constructed from multivariate predictors with p values of 0.05 or less. All analyses were carried out using SAS version 9.1.3.

Table 2. *NPR3* genotype frequencies among 190 population samples.

Polymorphism (dbSNP number if listed)	Genotype	Freq (%) AA	Freq (%) EA
Promotor	G/G	74	53
-2664 G>A*	G/A	26	39
	A/A	0	8
Intron 2	G/G	89	91
IVS2–84 G>A	G/A	10	9
(rs2292025)	A/A	1	0
Intron 2	C/C	83	70
IVS2–79 G>A	C/T	11	29
(rs2292026)	T/T	6	1
Non-syn coding	A/A	80	57
1561 A>G*	A/G	19	39
(rs2270915)	G/G	1	5

95 AA and 95 EA subjects were used in the study.
**p-value < 0.05 for comparison between races.*
AA: African–American; EA: European–American.

Results

The polymorphisms under consideration were first validated in population samples of 95 African Americans and 95 European Americans. Resulting genotype frequencies are shown in Table 2. All four polymorphisms had minor allele frequencies of 4% or higher in both populations. There were significant differences in genotype frequency between races for the -2664 G>A and 1561 A>G variants ($p = 0.037$ and $p = 0.045$, respectively). The 1561 A>G and IVS2–79 C>T polymorphisms were in strong linkage disequilibrium among European–Americans ($D' = 0.84$, $p < 0.001$), but only weakly among African–Americans ($D' = 0.19$, $p = 0.044$). The IVS2–79 C>T polymorphism was outside Hardy–Weinberg equilibrium in African–Americans only ($p < 0.001$).

Baseline and demographic description of the overall clinical cohort (and divided by *NPR3* IVS2–84 G>A genotype) is summarized in Table 3. Genotype was obtained for 98–100% of subjects for each polymorphism studied. LVEDP was obtained in 136 subjects. There was no difference in BNP, LVEDP or

Table 3. Baseline characteristics: overall and divided by IVS2–84 G>A genotype.

Characteristic	Overall population (n = 147)		NPR3 IVS2–84 GG (n = 124)		NPR3 IVS2–84 GA (n = 23)		p-value
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	
Age	70.3	6.84	70.17	6.84	71.15	6.93	0.56
Male (%)	51		49		65		0.18
European–American (%)	83		83		85		0.99
New York Heart Association class	2.03	1.36	1.96	1.39	2.50	1.00	0.15
Systolic blood pressure	145.96	22.26	145.67	21.84	147.75	25.29	0.61
Body mass index	29.79	6.74	29.83	6.89	29.57	5.82	0.90
Coronary artery disease (%)	47		42		70		0.022
Heart failure (%)	23		20		35		0.13
Hypertension (%)	86		84		95		0.2
History of atrial fibrillation (%)	20		19		30		0.27
Hemoglobin	13.48	1.54	13.53	1.48	13.16	1.90	0.64
Blood urea nitrogen	19.81	6.96	19.62	6.56	21.00	9.18	0.55
Estimated creatinine clearance*	84.04	30.90	84.11	31.16	83.64	29.97	0.81
Echocardiographic E:A ratio	0.95	0.37	0.93	0.33	1.07	0.52	0.56
Deceleration time	241.53	58.55	241.53	59.26	241.50	55.37	0.98
Left atrial volume index	40.32	13.33	40.74	13.79	37.62	9.69	0.43
Echocardiographic septal $E:E_M$ (tissue Doppler E wave) ratio	12.75	5.16	12.54	5.15	14.04	5.15	0.19
Left ventricular systolic function‡	1.48	0.91	1.42	0.85	1.90	1.17	0.03
Mitral regurgitation grade (1–4)	1.12	0.35	1.12	0.35	1.15	0.37	0.64
Left ventricular end-diastolic dimension (cm)	4.9	0.72	4.9	0.67	5.3	0.92	0.071
Left ventricular end-diastolic pressure (n = 136)	20.17	7.10	20.42	6.90	18.68	8.27	0.13
B-type natriuretic peptide (pg/ml)	133.52	189.60	131.43	197.42	146.60	133.71	0.06
logBNP	1.86	0.47	1.84	0.48	2.01	0.39	0.06

**Estimated by Cockcroft–Gault equation.*

‡Left-venticular systolic function was assessed in ranges as follows: 1: Normal (≥50%); 2: Mildly reduced (ejection fraction [EF] 40–49%); 3: Moderately reduced (EF 30–39%); 4: Severely reduced (EF < 30%).

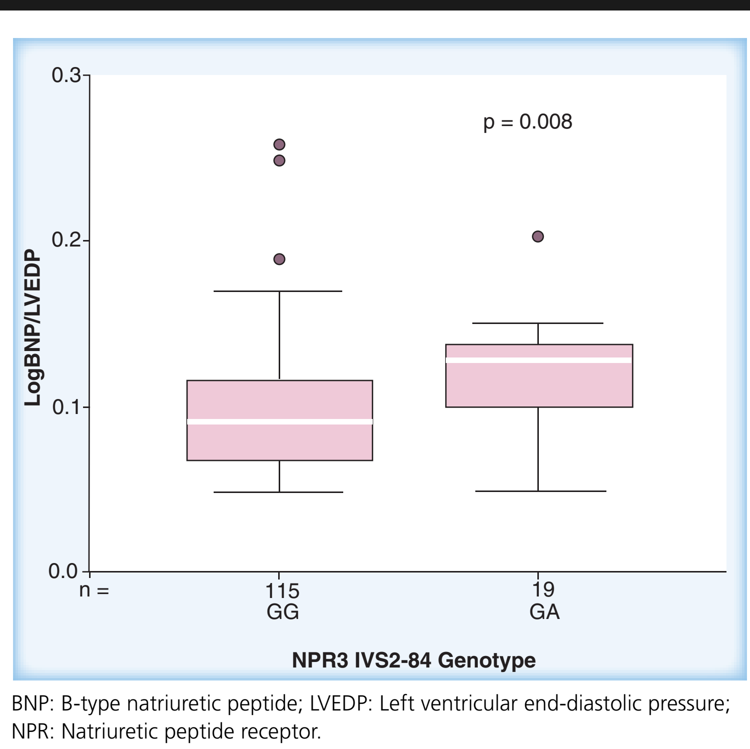
E:A ratio: Early to late ventricular filling ratio; NPR: Natriuretic peptide receptor; SD: Standard deviation.

logBNP:LVEDP ratio by race (European American vs African–American, p = 0.98, 0.39 and 0.35, respectively). The *NPR3* IVS2–84 genotype groups differed only in their assessment of left ventricular (LV) systolic function, with A allele carriers tending to have slightly worse LV function on average (LV function grade 1.4 vs 1.9; p = 0.03).

Of the four polymorphisms examined, only the IVS2–84 G>A variant was significantly associated with logBNP:LVEDP ratio (p = 0.008; all others p > 0.4) (**Figure 2**). No A allele homozygotes were identified in the study population. As indicated above, this variant was not significantly linked to any of the other variants in either African–Americans or European–Americans.

In order to take into account other factors that may affect the BNP–LVEDP relationship and to assess the clinical utility of this genotype (for noninvasive determination of volume status), we assessed the incremental value of IVS2–84 G>A genotype in regression models for prediction of LVEDP. Optimal noninvasive models were generated using all collected data, excluding the genetic data [5]. LogBNP was one of the strongest predictors of LVEDP. The optimal linear regression model for LVEDP included echocardiographic mitral E:A ratio, body mass index and logBNP. The optimal logistic regression model for predicting LVEDP 20 mmHg or over included body mass index, heart rate and logBNP as input variables. When added to these linear and logistic models,

Figure 2. Boxplot depicting median and interquartile range of logBNP/LVEDP ratio in GG versus GA subjects.



BNP: B-type natriuretic peptide; LVEDP: Left ventricular end-diastolic pressure; NPR: Natriuretic peptide receptor.

IVS2–84 G>A genotype incrementally added to both models (p-values 0.025 and 0.026, respectively) (Table 4). Since the severity of left ventricular dysfunction was associated with genotype ($p = 0.03$) (Table 2), we also tested models that included this variable (in addition to the other independent variables above). This did not significantly alter the results ($p = 0.036$ and 0.044 for linear and logistic models, respectively).

Discussion

Our data indicate a gene–environment interaction, where *NPR3* IVS2–84 G>A genotype is associated with changes in the BNP-LVEDP relationship and incrementally adds to noninvasive prediction of LVEDP. This is the first report of a genetic variant affecting this relationship or helping to predict LVEDP noninvasively. Noninvasive prediction of LVEDP is important because LVEDP is an important indicator of cardiac physiological state and pathology, and currently it can only be measured invasively. The most common surrogate measure, the pulmonary capillary wedge pressure, also requires an invasive procedure. Noninvasive data, such as echocardiographic parameters and BNP levels, have been previously correlated with cardiac filling pressures, but our data are the first to demonstrate incremental value from genetic analyses for predicting elevations in left ventricular filling pressures.

The fact that the A allele carriers demonstrated higher BNP levels for each level of LVEDP could suggest impaired clearance receptor function in these subjects relative to G allele homozygotes. The possible functional consequences of this variant have not, to our knowledge, been explored and, hence, the mechanism of this effect remains unexplained. Since this variant is intronic, one could hypothesize that it is in linkage disequilibrium with the true functional variant, in which case the true functional variant remains to be identified. Alternatively, the IVS2–84 GA genotype may alter mRNA splicing or stability. However, despite lying relatively close to a splice junction, it does not alter a consensus splice donor/acceptor sequence and is not predicted to be a functionally significant variant by the commonly used computer algorithms JASPAR or ESEfinder [24,25,103,104].

Our study has significant limitations. Not all factors affecting BNP levels or ventricular pressure could be accounted for, but we have attempted to characterize our population in terms of the commonly accepted clinical and physiological factors that may affect BNP, LVEDP and their relationship. Given the size of our cohort, the fact that it is an experience from a single center, and that only patients aged over 60 years were studied, one must use caution when interpreting the findings and applying them to other patients.

Our findings have potential implications for BNP-based diagnostic testing and therapeutic applications. The fact that a variant in a BNP pathway gene is associated with alterations in BNP's predictive ability for LVEDP suggests that this variant, along with others in relevant genes, may improve the predictive abilities of BNP for diagnosis and prognosis in heart failure. For example, currently, there is a range of BNP values that don't clearly indicate the presence or absence of heart failure. If the genetic variation in the BNP pathway is better understood it may allow clinicians to more clearly interpret the significance of BNP levels since the individual's genetic make-up will inform what values are expected for that person. In addition, since the IVS2–84 variant we identified lies within the clearance receptor, it could impact dosing and adverse events when using BNP infusions as heart failure therapy. This is especially important in light of the recent reports highlighting the potential for adverse impact of exogenous BNP therapy, such as hypotension and renal dysfunction [26]. Further study

Table 4. Linear and logistic regression models of LVEDP.

LVEDP (linear)		LVEDP ≥ 20 mmHg (logistic)	
Factor	***p-value***	***Factor***	***p-value***
BMI	0.0011	BMI	0.0009
Echocardiographic E:A ratio	0.0051	Heart Rate	0.0251
logBNP	0.0001	logBNP	0.0038
NPR3 IVS2–84 genotype	0.0246	NPR3 IVS2–84 genotype	0.0262

Model r = 0.54 (Linear)
BMI: Body mass index; BNP: B-type natriuretic peptide; E:A ratio: Early to late ventricular filling ratio; LVEDP: Left ventricular end-diastolic pressure; NPR: Natriuretic peptide receptor.

of the functional impact of this variant, as well as broader genetic characterization of the entire BNP pathway, is required in order to optimize the clinical use of BNP levels and improve targeting of exogenous BNP therapy.

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Conflict of interest

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Highlights

- B-type natriuretic peptide (BNP) is a hormone that is a key diagnostic and prognostic factor in patients with heart failure, and is also available in synthetic form as a therapy for acutely decompensated heart failure.
- Natriuretic peptide receptor (NPR)3 is a primary clearance mechanism for BNP.
- Genetic variation in *NPR3* appears to modify the usual physiological relationship of BNP to cardiac stretch (i.e., cardiac filling pressures).
- Genetic variation in *NPR3* may have an important impact on the proper interpretation of BNP levels, or the optimal use of BNP as therapy for heart failure.

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