

# Genome-Wide Association Study Implicates Atrial Natriuretic Peptide Rather Than B-Type Natriuretic Peptide in the Regulation of Blood Pressure in the General Population

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**Background**—Cardiomyocytes secrete atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) in response to mechanical stretching, making them useful clinical biomarkers of cardiac stress. Both human and animal studies indicate a role for ANP as a regulator of blood pressure with conflicting results for BNP.

**Methods and Results**—We used genome-wide association analysis ( $n=6296$ ) to study the effects of genetic variants on circulating natriuretic peptide concentrations and compared the impact of natriuretic peptide-associated genetic variants on blood pressure ( $n=27059$ ). Eight independent genetic variants in 2 known (*NPPA-NPPB* and *POC1B-GALNT4*) and 1 novel locus (*PPP3CC*) associated with midregional proANP (MR-proANP), BNP, aminoterminal proBNP (NT-proBNP), or BNP:NT-proBNP ratio. The *NPPA-NPPB* locus containing the adjacent genes encoding ANP and BNP harbored 4 independent *cis* variants with effects specific to either midregional proANP or BNP and a rare missense single nucleotide polymorphism in NT-proBNP seriously altering its measurement. Variants near the calcineurin catalytic subunit gamma gene *PPP3CC* and the polypeptide N-acetylgalactosaminyltransferase 4 gene *GALNT4* associated with BNP:NT-proBNP ratio but not with BNP or midregional proANP, suggesting effects on the post-translational regulation of proBNP. Out of the 8 individual variants, only those correlated with midregional proANP had a statistically significant albeit weak impact on blood pressure. The combined effect of these 3 single nucleotide polymorphisms also associated with hypertension risk ( $P=8.2 \times 10^{-4}$ ).

**Conclusions**—Common genetic differences affecting the circulating concentration of ANP associated with blood pressure, whereas those affecting BNP did not, highlighting the blood pressure-lowering effect of ANP in the general population. (*Circ Cardiovasc Genet.* 2017;10:e001713. DOI: 10.1161/CIRCGENETICS.117.001713.)

**Key Words:** blood pressure ■ genes ■ genome-wide association study ■ hypertension ■ natriuretic peptide, brain

The heart secretes atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) into the circulation in response to myocardial stretching. Atrial cardiomyocytes mainly secrete ANP, whereas ventricular cardiomyocytes predominantly produce BNP. Coded by the adjacent genes *NPPA* and *NPPB* in humans, the proANP and proBNP pro-hormones are cleaved to produce an inactive N-terminal fragment and the active hormone. ANP and BNP reduce cardiac load via increased natriuresis, vasorelaxation, and other physiological effects mediated by the natriuretic peptide receptor A.<sup>1</sup> Both the active hormones and the N-terminal fragments may be used as biomarkers of cardiac stress. A particularly valuable clinical application is the use of low

measured BNP or NT-proBNP (N-terminal proBNP) concentration to rule out suspected heart failure.<sup>2</sup> ANP and BNP are, thus, regulators of cardiovascular function and useful clinical biomarkers.

## See Editorial by Armando See Clinical Perspective

Natriuretic peptides are attractive therapeutic targets. Overexpression of either *NPPA* or *NPPB* in mice leads to pronounced hypotension.<sup>3,4</sup> Deleting *NPPA* in mice predisposes them to hypertension, but knocking out *NPPB* triggers cardiac fibrosis instead of inducing hypertension.<sup>5-7</sup> In contrast to mice, the deletion of *NPPB* in a hypertensive rat

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model has been reported to decrease survival and increase both systolic and diastolic blood pressure (BP).<sup>8</sup> In humans, the effects of ANP or BNP infusions depend on baseline status. Infusions of ANP or BNP in patients having heart failure trigger various hemodynamic changes, including a decrease in arterial pressure, but in healthy males only induce natriuresis without affecting arterial pressure.<sup>9–12</sup> Both ANP and BNP have a BP-lowering effect in those having essential hypertension, with BNP surprisingly showing a 2- to 3-fold greater potency than ANP despite similar receptor affinity.<sup>13,14</sup> A lack of association or even a paradoxical negative association of ANP with BP has been reported in obese men.<sup>15</sup> How heart failure, hypertension, or obesity may modify ANP and BNP function is incompletely understood. Recombinant BNP has also failed to show a clear clinical benefit in treating acute decompensated heart failure when used in addition to standard care.<sup>9</sup> A more detailed understanding of ANP, BNP, and their physiological role may aid in successfully exploiting their potential.

Genetic studies of ANP and BNP in humans are of particular interest as a large part of the literature regard knockout animal models and relatively high doses of intravenous infusions. Data on variation in their concentration within the normal physiological range are more scarce but necessary to understand the function of these peptides under nondiseased conditions. The association of human genetic variation with circulating ANP and BNP has been studied for selected single nucleotide polymorphisms (SNPs).<sup>16,17</sup> Four genome-wide association studies (GWAS) have studied circulating BNP or NT-proBNP levels.<sup>18–21</sup> The prior studies have associated variants near *NPPA-NPPB* with proANP, BNP, and NT-proBNP, and the GWAS have associated *trans* loci near *LOXL2*, *SLC39A8*, *KLKB1*, and *GALNT4* with NT-proBNP. No genome-wide studies have been published on ANP. The prior studies, thus, either did not have genome-wide coverage of genetic variation or did not assay ANP, limiting the interpretation of their results. We performed genome-wide association tests of BNP, NT-proBNP, and midregional proANP (MR-proANP) and studied the impact of the natriuretic peptide-associated genetic variants on BP. Because proBNP is processed peripherally into BNP and NT-proBNP that have different circulating half-lives, we also studied the ratio of BNP to NT-proBNP concentrations (BNP:NT-proBNP ratio) as a potential proxy for the processing and degradation of BNP, proBNP, and NT-proBNP.<sup>1,22</sup>

## Materials and Methods

MR-proANP, NT-proBNP, and BNP were measured in the GWAS discovery (n=4932) and replication samples (n=1373), originally recruited for the FINRISK 1997 study. The National FINRISK Study cohorts are collected every 5 years as representative age- and sex-stratified samples of the populations of 5 geographical areas of Finland, described in more detail elsewhere.<sup>23,24</sup> We tested the association of genetic variants with natriuretic peptide traits in the GWAS discovery and replication samples excluding participants who had prevalent diabetes mellitus, heart failure, stroke, or coronary heart disease. We then studied the BP associations of the genetic variants detected in the GWAS in an independent study population, comprising the FINRISK 1992 (n=4920), FINRISK 2002 (n=5,21), FINRISK 2007 (n=4996), the Northern Finland Birth Cohort 1966 (NFBC66, n=5363), the HBCS (Helsinki Birth Cohort Study, n=1619), the YFS

(Young Finns Study, n=2443), and the Health2000 (n=1997) cohorts.<sup>23–28</sup> All study cohorts were population-based samples of Finns, approved by their respective institutional review committees, and participants gave their informed consent.

## Natriuretic Peptide and BP Measurements

Natriuretic peptide concentrations were measured in the MORGAM Biomarker Laboratory, University of Mainz, Germany, using the Abbott Architect i2000 BNP (BNP, UniProt acc. P16860, residues 103–134), Roche Elecsys 2010 proBNP (NT-proBNP, acc. P16860 residues 27–134), and B.R.A.H.M.S. MR-proANP KRYPTOR (MR-proANP, acc. P01160) assays, described in more detail previously.<sup>29</sup> The inter/intra-assay coefficients of variation were 2.11%/4.28% (BNP), 2.58%/1.38% (NT-proBNP), and 3.65%/2.33% (MR-proANP). BP was measured from the participants' right arm, and hypertension was defined as diastolic BP >90 mmHg or systolic BP >140 mmHg or known use of antihypertensive medication.

## Genotyping and Imputation

The GWAS discovery sample and replication samples were genotyped using the Illumina HumanCoreExome beadchip at the Wellcome Trust Sanger Institute (Cambridge, UK) and at the Broad Institute of Harvard and MIT (MA, USA), respectively. The data were prephased and imputed using the 1000 Genomes project phase 1 and 3 haplotypes and a custom haplotype set of 2000 Finnish individuals. After quality control (Hardy–Weinberg equilibrium *P* value <0.01, minor allele frequency <1%, imputation quality <0.9, genotyping success rate <95%) and removal of rare SNPs (minor allele frequency <1%), the discovery phase GWAS data set contained a total of 7358451 SNPs and 4932 samples. Cohorts comprising the BP study population were genotyped on various genome-wide genotyping arrays and imputed using the same methods as used for the GWAS discovery sample (Data Supplement). All genomic coordinates are given using the GRCh37 human reference genome.

## Association Tests

We used multiple imputation to account for any missing values for MR-proANP ( $N_{\text{MISSING}}=0$ ), BNP ( $N_{\text{MISSING}}=131$ ), and NT-proBNP ( $N_{\text{MISSING}}=133$ ) and random-effects meta-analysis to combine results from the different cohorts.<sup>30</sup> We inverse-normal transformed the natriuretic peptide measurements and used linear regression with an additive genetic model adjusted for geographical sampling region, age<sup>2</sup> sex, body mass index (BMI), current smoking (yes/no), systolic BP, glomerular filtration rate estimated using cystatin C and creatinine as proxies, and genotyping batch. We used least absolute shrinkage and selection operator regression implemented in the LLARMA package for fine-mapping the natriuretic peptide-associated loci to identify possible secondary independent variants.<sup>31</sup> The genetic association tests are described in more detail in the Data Supplement.

We used linear regression implemented in the glm function for R to study the association of genetic variants with systolic and diastolic BP. We log-transformed systolic (but not diastolic) BP and set the first 2 genomic principal components, age, sex, BMI, current BP medication use (yes/no, only for systolic and diastolic BP), study year, and genotyping batch as covariates (the latter 2 only for the FINRISK samples). For hypertension, we used logistic regression and the same covariates excluding BP medication.

## Phenotypic Variance Explained by SNPs Genome-Wide

We used autosomal SNPs from the imputed data set to estimate the fraction of phenotypic variance explained by the SNPs genome-wide in the participants of the GWAS discovery sample using PLINK v1.90 and GCTA v1.25.3.<sup>32,33</sup> As a quality control measure, we derived 4 genomic scores corresponding to each of the 4 estimates (for MR-proANP, BNP, NT-proBNP, and BNP:NT-proBNP ratio) and tested the association of the genomic scores with their respective phenotypes in the replication sample (Data Supplement).

### Coassociation With Gene Expression

We investigated the coassociation of SNPs with both natriuretic peptides and gene expression in data from 190 left ventricular tissue samples and 159 atrial appendage samples from the Genotype-Tissue Expression (GTEx) consortium (release V6, October 6, 2016).<sup>34</sup> We used 3 metrics to confirm that the same genetic variants correlated with both gene expression and natriuretic peptide concentrations in a consistent way (Data Supplement): we required that the most statistically significant natriuretic peptide-associated SNPs (lead SNPs) associated with the genes' expression levels and that both the association  $P$  values and the effect estimates ( $\beta_j$ ) were correlated across the SNPs in the natriuretic peptide-associated regions. Because  $P$  values depend on allele frequencies, we used both Spearman rank (for  $P$  values) and Pearson product moment (for  $\beta_j$ ) correlation coefficients as measures of the correlation between the natriuretic peptide and gene expression associations and derived the  $P$  values empirically.

## Results

### Baseline Characteristics

The baseline characteristics of the GWAS discovery sample, the replication sample, and the BP study population are described in Table I in the Data Supplement. The strata were broadly similar, and the main difference was that participants with prevalent cardiovascular disease were not excluded from the BP study population.

### GWAS and Variance Explained by All SNPs

To quantify the total amount of genetic signal present in the data, we first estimated the proportion of variance in the natriuretic peptide traits jointly explained by all SNPs genome-wide. The point estimates were 13.9% for MR-proANP, 13.5% for BNP, 23.0% for NT-proBNP, and 17.9% for BNP:NT-proBNP ratio, but the coarse precision of the estimates prevents ranking the 4 phenotypes in any particular order in terms of variance explained (Table II in the Data Supplement). The magnitude of the 4 estimates nonetheless indicates that the SNPs together explained a moderate proportion of the phenotypic variance.

Having estimated the proportion of variance explained by all SNPs genome-wide, we tested the SNPs individually for association with the phenotypes. Variants in 4 loci near *NPPA-NPPB*, *PPP3CC*, *GALNT4*, and *NCOR12* met the prespecified threshold for genome-wide significance  $P < 5 \times 10^{-8}$  for association (Figure 1; Table 1; Figure I in the Data Supplement; Table III in the Data Supplement). We selected the SNP with the smallest  $P$  value (lead SNP) at each locus for replication. Only the association of rs701041 with MR-proANP near *NCOR12* did not replicate ( $P=0.94$ ). Associations near *NPPA-NPPB* and *GALNT4* have been reported previously, whereas the association of rs7000551 with BNP:NT-proBNP ratio on chromosome 8 near *PPP3CC* is a novel finding.<sup>16–20</sup> Fine-mapping the loci using least absolute shrinkage and selection operator regression identified independent secondary signals near *NPPA-NPPB* and *GALNT4*. Three independent SNPs near *NPPA* associated with MR-proANP levels, whereas 2 independent SNPs near *GALNT4* associated with BNP:NT-proBNP ratio.

Previously detected associations replicated successfully in the present data in terms of the direction of association (Table IV in the Data Supplement). Of these, all but 1 of the *cis* associations near *NPPA-NPPB* also reached statistical

significance. Two of the 3 previously published *trans* associations, rs13107325 in *SLC39A8* and rs3733402 in *KLKB1*, associated with BNP:NT-proBNP ratio in the meta-analysis of the discovery and replication samples (rs13107325  $P=2.19 \times 10^{-9}$ ; rs3733402  $P=0.00277$ ) and the meta-analysis  $P$  value of rs13107325 with NT-proBNP ( $P=0.00496$ ) was also nominally significant. The third, rs6557662 in *LOXL2*, did not reach statistical significance. None of the *trans* loci associated with BNP or MR-proANP.

Most common variants are thought to affect phenotypes by altering gene expression.<sup>35,36</sup> We, thus, studied data from 190 left ventricular tissue samples and 159 atrial appendage samples from the GTEx consortium to identify coassociation of SNPs with both natriuretic peptide traits and gene expression.<sup>34</sup> The results of these tests, together with those of the fine-mapping tests with least absolute shrinkage and selection operator regression, are presented in detail below for the loci meeting genome-wide significance in the present study.

### NPPA-NPPB on Chromosome 1

SNPs associating with the natriuretic peptide on chromosome 1 were located near the *NPPA* and *NPPB* genes (Figure 2; Figure II in the Data Supplement). Previously, associations in this locus have been reported using a GWAS strategy for NT-proBNP and a candidate SNP approach for ANP.<sup>16–20</sup> To extend the previously reported results, we focus here on the extensive panel of SNPs and the more detailed phenotyping, which were not available in the prior studies.

The *NPPA-NPPB* locus contained 3 initial association signals for BNP, NT-proBNP, and BNP:NT-proBNP ratio, depending on which of the phenotypes was tested (Table 1; Table III in the Data Supplement). Rs198379, situated 2055 base pairs downstream from the last exon of *NPPB*, associated with BNP ( $P=4.42 \times 10^{-52}$ ). For NT-proBNP and BNP:NT-proBNP ratio, rs61761991 was the most statistically significant SNP ( $P=8.76 \times 10^{-68}$  and  $P=4.81 \times 10^{-103}$ , respectively), and least absolute shrinkage and selection operator regression detected rs12406089 as a secondary signal for NT-proBNP ( $P=8.31 \times 10^{-48}$ ). However, neither of these 2 SNPs associated with BNP when rs198379 was included in the model. The *NPPA-NPPB* locus, therefore, harbored only 1 variant, rs198379, independently associated with both BNP and NT-proBNP, with every C allele increasing BNP concentration by  $\approx 4.5$  pg/mL and NT-proBNP concentration by 9.6 pg/mL.

Three SNPs associated independently with MR-proANP at the *NPPA-NPPB* locus (Table 1). The most statistically significant was rs3753584 ( $P=3.85 \times 10^{-13}$ ), but the effect sizes of the 3 SNPs were broadly similar. Each allele of the SNPs correlated with a 2.5 to 5.0 pmol/L difference in MR-proANP concentration. The SNPs are found  $\approx 40$  kb downstream from *NPPA* within an area bound by regulatory proteins in human cardiomyocytes (ENCODE: Encyclopedia of DNA Elements, <https://www.encodeproject.org>, experiment ENCSR000ENJ).<sup>37</sup> Because obesity disturbs the association of MR-proANP with BP, we studied the effect of body mass on the SNP associations by introducing body mass\*SNP interaction terms to the regression models.<sup>15</sup> The interaction terms were statistically nonsignificant ( $P>0.05$ ) for both BMI as a continuous variable and obesity (BMI>30) as a categorical



**Table 1. Association of Genetic Variants With Natriuretic Peptides in the Genome-Wide Significant Loci**

Trait	SNP	Chromosome	Position	Alleles* (MAF)	Imputation Quality†	Genes (Distance‡, Location)	Model	$P_{\text{GWAS}}$	$P_{\text{REPLICATION}}$	$\beta$ (SE; 95% CI)	$P_{\text{COMBINED}}$
BNP	rs198379	1	11915467	t/C (0.365)	0.989	<i>NPPB</i> (3.5 kb, 3')	GWAS	$6.85 \times 10^{-41}$	$7.99 \times 10^{-13}$	0.249 (0.0164; 0.217 to 0.282)	$4.42 \times 10^{-52}$
BNP:NT-proBNP	rs61761991	1	11918444	c/T (0.029)	0.996	<i>NPPB</i> (0.5 kb, coding exon)	GWAS	$7.17 \times 10^{-79}$	$5.71 \times 10^{-26}$	1.114 (0.0517; 1.013 to 1.215)	$4.81 \times 10^{-103}$
	rs7000551	8	22276251	a/G (0.369)	0.994	<i>SLC39A14</i> (38.6 kb, intronic) <i>PPP3CC</i> (22.5 kb, 5')	GWAS	$2.16 \times 10^{-8}$	0.0248	0.109 (0.0181; 0.073 to 0.144)	$2.00 \times 10^{-9}$
	rs11105298	12	89876143	t/C (0.211)	0.992	<i>POC1B</i> (59 kb, intronic) <i>GALNT4</i> (43.2 kb, 3')	GWAS	$3.06 \times 10^{-18}$	$4.11 \times 10^{-6}$	0.21 (0.0213; 0.169 to 0.252)	$6.77 \times 10^{-23}$
	rs11105298	12	89876143	t/C (0.211)	0.992	<i>POC1B</i> (59 kb, intronic) <i>GALNT4</i> (43.2 kb, 3')	Conditional-1	$3.67 \times 10^{-20}$	$2.01 \times 10^{-6}$	0.189 (0.02185; 0.189 to 0.275)	$2.96 \times 10^{-26}$
	rs61378614	12	89903654	a/C (0.16)	0.994	<i>POC1B</i> (87 kb, intronic) <i>GALNT4</i> (15.7 kb, 3')	Conditional-1	$1.70 \times 10^{-10}$	0.0453	0.101 (0.03242; 0.101 to 0.228)	$4.13 \times 10^{-7}$
MR-proANP	rs3753584	1	11864586	t/C (0.149)	1	<i>MTHFR</i> (3 kb, intronic) <i>NPPA</i> (43.5 kb, 3')	GWAS	$4.63 \times 10^{-38}$	$3.48 \times 10^{-7}$	0.275 (0.038; 0.201 to 0.35)	$4.19 \times 10^{-13}$
	rs4845875	1	11824133	A/c (0.355)	0.944	<i>C1orf167</i> (11 kb, intronic) <i>NPPA</i> (84 kb, 3')	Conditional-2	$3.53 \times 10^{-7}$	0.0031	-0.156 (0.0198; -0.156 to -0.079)	$3.37 \times 10^{-9}$
	rs6540997	1	11827355	A/g (0.274)	0.995	<i>C1orf167</i> (8 kb, intronic) <i>NPPA</i> (80.8 kb, 3')	Conditional-2	$9.03 \times 10^{-10}$	0.0326	0.074 (0.0195; 0.074 to 0.171)	$7.13 \times 10^{-7}$
	rs3753584	1	11864586	t/C (0.149)	1	<i>MTHFR</i> (3 kb, intronic) <i>NPPA</i> (43.5 kb, 3')	Conditional-2	$1.85 \times 10^{-20}$	$1.80 \times 10^{-4}$	0.162 (0.023; 0.162 to 0.282)	$3.85 \times 10^{-13}$
	rs701041	12	124999344	G/c (0.106)	0.928	<i>NCOR2</i> (126 kb, intronic)	GWAS	$1.23 \times 10^{-8}$	0.9381	-0.088 (0.0782; -0.241 to 0.066)	0.2624
NT-proBNP	rs61761991	1	11918444	c/T (0.029)	0.996	<i>NPPB</i> (0.5 kb, coding exon)	GWAS	$1.72 \times 10^{-51}$	$5.33 \times 10^{-18}$	-0.766 (0.044; -0.853 to -0.68)	$8.76 \times 10^{-68}$
	rs61761991	1	11918444	c/T (0.029)	0.996	<i>NPPB</i> (0.5 kb, coding exon)	Conditional-3	$3.85 \times 10^{-43}$	$1.26 \times 10^{-15}$	-0.782 (0.0425; -0.782 to -0.616)	$1.41 \times 10^{-60}$
	rs12406089	1	11921181	c/G (0.291)	0.995	<i>NPPB</i> (2.2 kb, 5')	Conditional-3	$2.45 \times 10^{-33}$	$3.54 \times 10^{-14}$	0.201 (0.0172; 0.201 to 0.264)	$8.31 \times 10^{-48}$
	rs10858906	12	89934474	c/T (0.21)	0.996	<i>GALNT4</i> (15.2 kb, 5')	GWAS	$1.08 \times 10^{-12}$	0.0388	-0.12 (0.0338; -0.186 to -0.054)	$3.91 \times 10^{-4}$

Association tested with an additive genetic model using single SNP (GWAS) or conditional models which included all SNPs of each model simultaneously. All models adjusted for geographical sampling region, age, age<sup>2</sup>, sex, current smoking status (yes or no), systolic blood pressure, estimated glomerular filtration rate, and genotyping batch. Genomic positions given relative to the GRCh37 reference genome build. BNP indicates B-type natriuretic peptide; MAF, minor allele frequency; MR-proANP, midregional proatrial natriuretic peptide; and NT-proBNP, aminoterminal pro-B-type natriuretic peptide.

\*Alleles given as (reference allele)/(effect allele) with minor alleles in lower case letters.

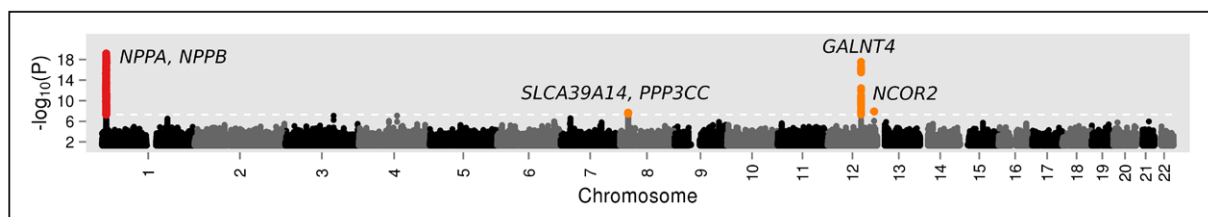
†IMPUTE info metric. Rs4845875 was directly genotyped with missing genotypes imputed.

‡Median distance to the transcription start sites of the candidate gene(s).

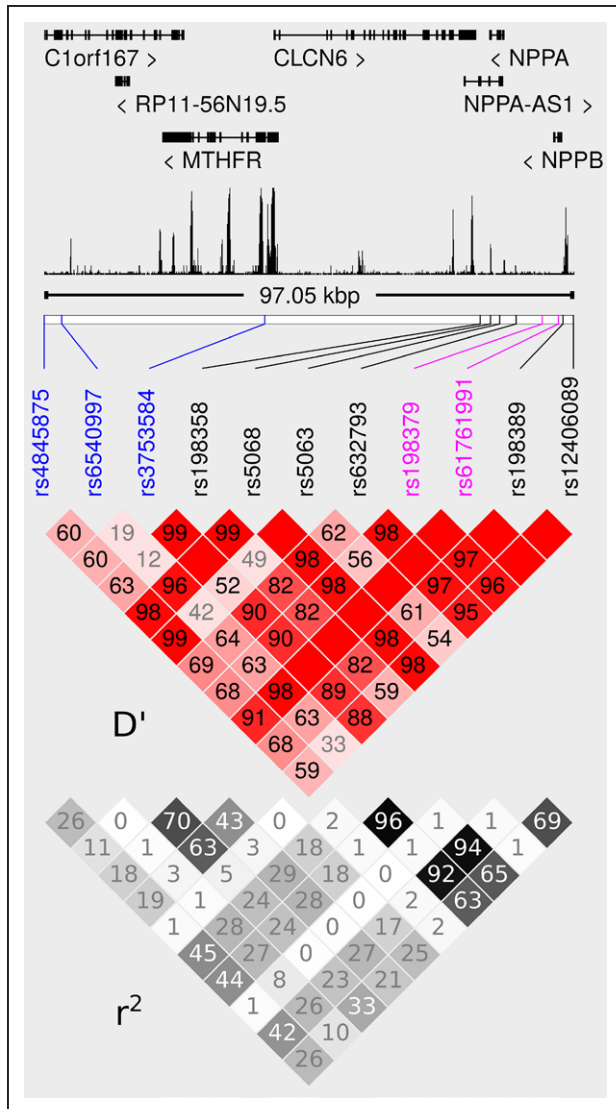
variable. Furthermore, because *NPPA* and *NPPB* are separated by <10 kb, any variant in this region might affect either both genes or only 1 of the 2. We explored this by fitting models containing all of the previously mentioned SNPs of the *NPPA-NPPB* locus and found that SNPs associated with MR-proANP did not associate with BNP or NT-proBNP

and vice versa (Table V in the [Data Supplement](#)), indicating that their effects were specific to either MR-proANP or NT-proBNP (and BNP).

Gene expression profiling in human cardiac tissue samples confirmed that the associations of SNPs with BNP or MR-proANP concentration and with *NPPB* and *NPPA* gene



**Figure 1.** Genome-wide association study  $P$  values.  $P$  values of the genome-wide association tests and their genomic locations. Y axis cut at  $Y=18$ , the peak on chromosome 1 extends to  $Y=80$ .



**Figure 2.** SNPs associated with natriuretic peptides on chromosome 1 near *NPPA* and *NPPB*. Linkage disequilibrium on chromosome 1 near *NPPA* and *NPPB*.  $R^2$  and  $D'$  calculated with Haploview v4.2 from 22374 unrelated Finnish samples. Genes are depicted as annotated in GENCODE v19, potential regulatory regions identified by digital genomic footprinting in human cardiac myocytes (ENCODE: Encyclopedia of DNA Elements, experiment number ENCSR000ENJ) indicated by the black graph. SNPs independently associated with midregional proatrial natriuretic peptide (MR-proANP) colored with blue, SNPs independently associated with B-type natriuretic peptide (BNP) or NT-proBNP (N-terminal pro-B-type natriuretic peptide) colored in pink. SNPs associated with MR-proANP or NT-proBNP in previous studies colored in black.

expression were positively correlated. In left ventricular tissue samples, SNPs associated with circulating MR-proANP concentration also associated with *NPPA* expression level (Figure III in the [Data Supplement](#); Spearman rank correlation of  $P$  values,  $P=0.014$ ), and SNPs associated with BNP concentration also associated with *NPPB* expression ( $P=0.004$ ). Furthermore, the effect estimates for circulating MR-proANP concentration and *NPPA* expression in the left ventricle correlated (Pearson  $r=0.611$ ;  $P=0.024$ ) as did those for BNP and *NPPB* ( $r=0.735$ ;  $P=0.001$ ). A somewhat attenuated trend was

also present in the atrial appendage samples, where the correlations between the effect estimates were statistically significant (MR-proANP versus *NPPA*  $r=0.508$ ;  $P=0.021$  and BNP versus *NPPB*  $r=0.481$ ;  $P=0.033$ ), but the correlations between association  $P$  values were not. In addition to *NPPA* and *NPPB*, the correlations were also significant for *EXOSC10* and ENSG00000272482 (with MR-proANP) and *EXOSC10* and *MTHFR* (with BNP). The regulatory effects underlying the MR-proANP and BNP associations near *NPPA*-*NPPB* may, therefore, be stronger in the left ventricle compared with the atrium and also selectively affect the expression of other nearby genes.

### *PPP3CC* and *GALNT4* on Chromosomes 8 and 12

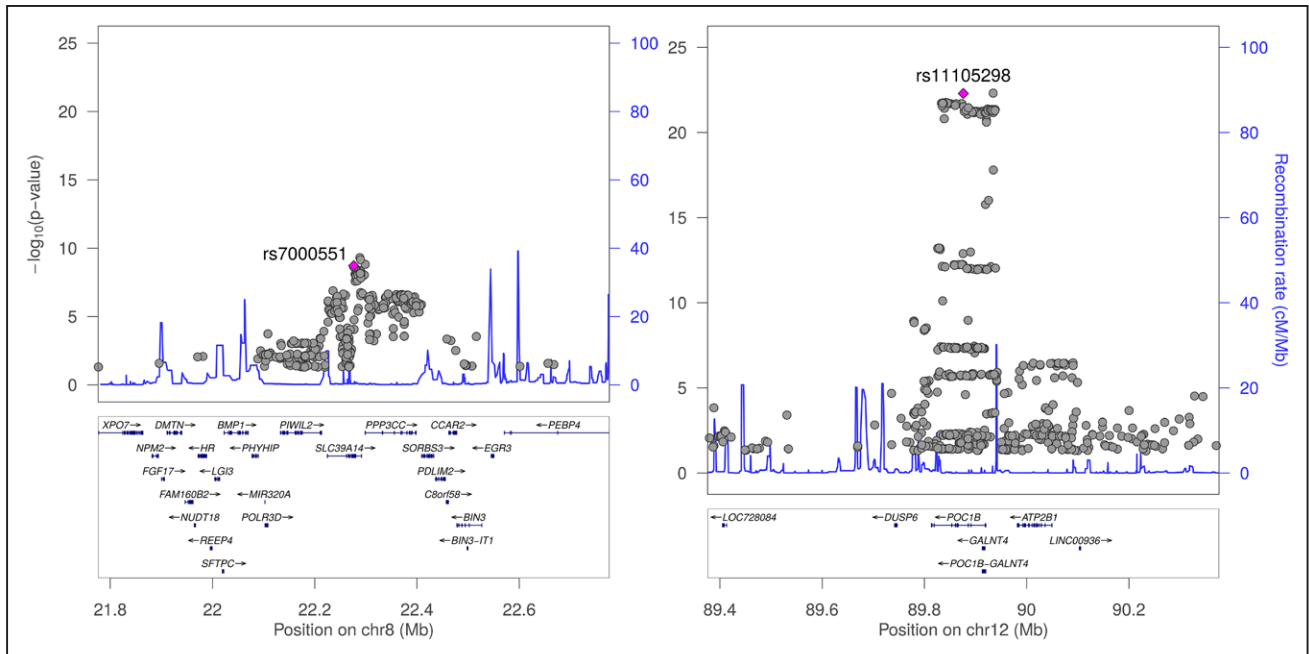
Rs7000551 on chromosome 8 near *PPP3CC* associated with BNP:NT-proBNP ratio ( $P=2.27\times 10^{-9}$ ). This correlation was driven by an effect on the NT-proBNP concentration as rs7000551 associated with NT-proBNP ( $P=3.72\times 10^{-5}$ ) but not with BNP ( $P=0.87$ ) in the discovery GWAS sample. However, only the association of rs7000551 with BNP:NT-proBNP ratio met genome-wide significance and replicated. The genotype-specific mean BNP:NT-proBNP ratios for rs7000551 (AA=0.386; AG=0.412; GG=0.463) suggest an additive or multiplicative genetic effect with each G-allele raising the ratio by  $\approx 0.04$  U or 10%.

The association peak on chromosome 8 extends from the 3' end of *SLC39A14* into the promoter region and 5' end of *PPP3CC*, with rs7000551 itself located in an intron of *SLC39A14* (Figure 3). When we studied the coassociation of SNPs with BNP:NT-proBNP ratio and gene expression, *PPP3CC* and 2 antisense RNA genes ENSG00000245025 and ENSG00000248738 matched the prespecified criteria. SNPs associated with increased BNP:NT-proBNP ratio also associated with reduced expression of *PPP3CC* in both atrial and left ventricular tissue samples (atrial appendage, Pearson  $r=-0.70$ ;  $P=0.006$  and left ventricle,  $r=-0.81$ ;  $P=0.021$ ; Figure III in the [Data Supplement](#)). The coassociation with the 2 RNA genes was significant only in the left ventricular tissue samples. Both the physical location near the promoter of *PPP3CC* and the coassociation with its expression, therefore, suggest that the BNP:NT-proBNP ratio-associated SNPs tag a regulatory variant that alters the expression of *PPP3CC* in the heart.

SNPs near *POC1B* and *GALNT4* on chromosome 12 associated with NT-proBNP and BNP:NT-proBNP ratio. Rs11105298 and rs61378614, located in different introns of the *POC1B* gene (Figure 3), independently associated with the ratio ( $P=1.52\times 10^{-26}$  and  $P=3.98\times 10^{-9}$ , respectively). The genes' expression on chromosome 12 did not show a clear coassociation with BNP:NT-proBNP ratio because none of them was significant for all 3 predefined criteria.

### Association With BP

Having identified the set of SNPs associated with the natriuretic peptide traits in the genome-wide significant loci, we next studied their correlation with systolic BP, diastolic BP, and hypertension in an independent sample. We fitted all SNPs simultaneously in each locus, excluding rs61761991 and rs12406089 on chromosome 1, which did not independently



**Figure 3.** B-type natriuretic peptide (BNP):NT-proBNP (N-terminal pro-B-type natriuretic peptide) associated SNPs on chromosomes 8 and 12. Association of SNPs with BNP:NT-proBNP ratio on chromosomes 8 and 12 after meta-analyzing the results from the genome-wide association study (GWAS) and replication samples with lead SNPs from the GWAS indicated with purple diamonds.

associate with BNP. After genotyping quality control, the study sample contained 27 059 participants with both BP measurements and SNP genotypes available.

The 3 SNPs associated with MR-proANP also associated weakly with BP (Table 2; Figure IV in the [Data Supplement](#)). The point estimates of the MR-proANP increasing alleles' effects were  $\approx 0.25$  mmHg (diastolic BP) and 0.50 mmHg (systolic BP). Only 1 of these SNPs was independently associated with hypertension as a binary end point (rs3753584;  $P=6.8 \times 10^{-4}$ ). To assess the combined effect of the genetic differences in MR-proANP concentration on BP, we formed an allele-counting score of the 3 SNPs. The score explained 2.36% of the variance in MR-proANP concentration and a unit increase in the score associated with a 9% decrease in the odds ratio for hypertension (odds ratio=0.91; SE=0.0283;  $P=8.2 \times 10^{-4}$ ).

In contrast to MR-proANP, none of the SNPs correlated with BNP, NT-proBNP, or BNP:NT-proBNP ratio associated with BP. Rs198379, associated with *NPPB* expression and circulating BNP levels, did not associate with systolic or diastolic BP when adjusted for the nearby MR-proANP-correlated SNPs. SNPs near *PPP3CC* and *GALNT4*, correlated with NT-proBNP and BNP:NT-proBNP ratio, similarly did not associate with BP or hypertension.

## Discussion

We performed a GWAS of circulating MR-proANP, BNP, and NT-proBNP concentration and BNP:NT-proBNP concentration ratio in 4932 samples with replication in 1373 samples. We then studied the effect of the natriuretic peptide-associated loci on systolic BP, diastolic BP, and hypertension in 27 059 additional samples. We detected a novel locus for BNP:NT-proBNP ratio on chromosome 8 near *PPP3CC*

and fine-mapped 2 published loci on chromosomes 1 and 12 for their association with ANP and BNP and BP. The entire genome-wide SNP data explained from 14% to 23% of the variation in the natriuretic peptide traits in our population-based sample. These estimates are similar to those, for example, BMI (14%) or systolic BP (24%) published elsewhere, showing that the natriuretic peptide traits considered here have an additive genetic component comparable to traditional cardiovascular risk factors.<sup>38</sup>

The present study is the first to assess the *NPPA-NPPB* locus with a dense SNP panel simultaneously for MR-proANP, BNP, and NT-proBNP, extending the results of previous investigations.<sup>16-21</sup> We identified 3 statistically independent *cis* variants associated with MR-proANP, and 1 variant associated with BNP and NT-proBNP. Analysis of gene expression data suggests that the protein-level *cis* associations stem from effects on *NPPA* and *NPPB* gene expression, affecting both atrial and ventricular tissue. Furthermore, even if the 2 genes are separated by <10000 bp, their transcriptional regulation is decoupled to the extent that the ANP-associated SNPs had no observable effect on BNP and vice versa. Each of these SNPs, however, correlates with both MR-proANP and BNP concentrations, if the analysis is not adjusted for the other SNPs. This is crucial for the interpretation of results from Mendelian randomization studies using SNPs in this locus as instruments, such as those performed in relation with type 2 diabetes mellitus.<sup>39</sup>

SNPs on chromosome 8 near *SLC39A14* and *PPP3CC* associate with BNP:NT-proBNP ratio. *SLC39A14* belongs to the same large family of solute carrier proteins as *SLC39A8* in the previously detected NT-proBNP associated locus on chromosome 4, but it is difficult to assess whether this is only coincidental.<sup>19,40</sup> SNPs associated with increased BNP:NT-proBNP ratio correlated with decreased expression

**Table 2. Independent Effects of Genetic Variants on Natriuretic Peptides and Blood Pressure**

SNP	Chr	Position	Alleles*	Candidate Genes	GWAS and Replication (n=6296)				Blood Pressure Study Population (n=27 059)		
					BNP, pg/mL	NT-proBNP, pg/mL	BNP:NT-proBNP Ratio	MR-proANP, pmol/L	Diastolic BP, mm Hg	Systolic BP, mm Hg	Hypertension (OR)
rs4845875	1	11824133	A/c	<i>NPPA</i>	0.84, ns.	5.00, ns.	0.01, ns.	-2.40, $P=2.1 \times 10^{-8}$	0.40, $P=0.0033$	0.63, ns.	1.00, ns.
rs6540997	1	11827355	A/g	<i>NPPA</i>	0.11, ns.	-2.20, ns.	-0.01, ns.	3.10, $P=5.8 \times 10^{-7}$	-0.25, ns.	-0.47, $P=0.029$	0.93, ns.
rs3753584	1	11864586	t/C	<i>NPPA</i>	1.50, ns.	8.70, ns.	-0.00, ns.	5.00, $P=1.2 \times 10^{-6}$	-0.38, $P=0.022$	-0.36, ns.	0.88, $P=6.8 \times 10^{-4}$
rs198379	1	11915467	t/C	<i>NPPB</i>	4.50, $P=1.2 \times 10^{-20}$	9.60, $P=7.2 \times 10^{-19}$	-0.00, ns.	0.33, ns.	-0.02, ns.	-0.29, ns.	1.00, ns.
rs7000551	8	22276251	a/G	<i>SLC39A14</i> and <i>PPP3CC</i>	-0.34, ns.	-3.80, ns.	0.03, $P=5.4 \times 10^{-9}$	-0.11, ns.	0.16, ns.	-0.07, ns.	1.00, ns.
rs11105298	12	89876143	t/C	<i>GALNT4</i>	0.66, ns.	-7.40, $P=0.0067$	0.06, $P=3 \times 10^{-26}$	1.30, ns.	-0.01, ns.	-0.04, ns.	1.00, ns.
rs61378614	12	89903654	a/C	<i>GALNT4</i>	0.71, ns.	-4.40, ns.	0.04, $P=4.1 \times 10^{-7}$	1.00, ns.	0.23, ns.	0.38, ns.	0.99, ns.

Independent effects of SNPs from regression models where, per each locus, all SNPs were simultaneously included. Effects estimated using untransformed trait values,  $P$  values derived using untransformed (diastolic BP), inverse-normal transformed (natriuretic peptides), or log-transformed (systolic BP) values. For natriuretic peptide traits, the models were adjusted for geographical sampling region, age, age<sup>2</sup>, sex, current smoking status (yes/no), systolic BP, estimated glomerular filtration rate, and genotyping batch. For BP traits, the models were adjusted for the first 2 genomic principal components, age, sex, BMI, current BP medication use (yes/no, only for systolic and diastolic BP), cohort year, and genotyping batch (the latter 2 only for the FINRISK samples). BP indicates blood pressure; BNP, B-type natriuretic peptide; MR-proANP, midregional proatrial natriuretic peptide; NT-proBNP, aminoterminal pro-B-type natriuretic peptide; and SNP, .

\*Alleles given as (reference allele)/(effect allele) with minor alleles in lower case letters.

of *PPP3CC* in both left ventricular and atrial tissue samples, whereas no such correlation was present for *SLC39A14*. *PPP3CC* codes for 1 of the 3 alternative catalytic subunits of calcineurin, a phosphatase with a wide range of functions including the regulation of cardiac hypertrophic signaling.<sup>41</sup> Originally characterized as a testis-specific calcineurin subunit, *PPP3CC* has been later detected in multiple tissues.<sup>34</sup> Because of its central role in spermatogenesis, drugs inhibiting *PPP3CC* have been suggested as a potential male contraceptive.<sup>42</sup> Our results indicate that systemic inhibition of calcineurin containing the subunit coded by *PPP3CC* may have unintended cardiovascular side effects.

Two independent SNPs near *POC1B* and *GALNT4* associated with NT-proBNP levels and BNP:NT-proBNP ratio in our study. An association of a SNP with NT-proBNP in this locus has been previously reported in whites.<sup>19</sup> Analysis of gene expression in cardiac tissue failed to highlight any of the nearby genes but, as previously noted, *GALNT4* is an attractive candidate.<sup>19</sup> It codes for an aminoacyltransferase that initiates O-linked glycosylation, and proBNP is known to be O-glycosylated.<sup>43,44</sup> According to data presented here, the association near *GALNT4* is specific to NT-proBNP, supporting the hypothesis that proBNP may be a target of *GALNT4*.

Because BNP and NT-proBNP are produced as a single polypeptide, deviations in their circulating concentration ratio should reflect their differential secretion or removal, the processing of proBNP, or factors disturbing the detection of the peptides. The latter is probably the case with rs61761991, located within the region of the NT-proBNP prohormone (NP\_002512.1:p.Arg72His) used as the antigen to prepare the assay's primary antibody.<sup>45,46</sup> The variant, which effectively

blocked the signal of the NT-proBNP assay, is rare or absent in other populations but significantly enriched in Finns, where the frequency of the T allele is  $\approx 3\%$ .<sup>47</sup> One in 20 Finns will, therefore, have a measured concentration of NT-proBNP, which is  $\approx 50\%$  lower than the corresponding C-terminal BNP value, potentially causing false rule-out of suspected heart failure. The associations of SNPs near *GALNT4* with BNP:NT-proBNP ratio may also relate to the detection of NT-proBNP rather than changes in its concentration, if they are indeed linked to the possible glycosylation of proBNP by *GALNT4*. How *PPP3CC* may affect BNP:NT-proBNP ratio is unclear. We adjusted the analysis for the estimated glomerular filtration rate, but confounding by kidney function cannot be ruled out.

Experimental data has pointed to either similar or different cardiovascular effects of ANP and BNP, depending on the experimental setting.<sup>6-8,48</sup> The results of this study are in line with some of the previous studies that identified ANP rather than BNP as an important regulator of BP. Genetically determined increases in ANP concentration decreased systolic and diastolic BP, but a smaller genetic decrease in BNP did not. Obesity did not modify the associations of SNPs with MR-proANP, showing that the transcriptional regulation of *NPPA* is at least partially unaffected by the reported ANP-decreasing effect of high body mass.<sup>15,49</sup> According to data presented here, earlier genetic associations of the *NPPA-NPPB* locus with BP were driven by ANP-associated variants and should not be taken as evidence of any BP lowering effect of BNP.<sup>16</sup> We conclude that there are interesting differences between ANP and BNP in humans that are yet to be fully elucidated and that genetics provides unique insights into the effects of lifelong alterations of these hormones.



## Appendix

From the National Institute for Health and Welfare, Helsinki, Finland (P.P.S., A.S.H., J.K., J.G.E., A.J., V.S., K.K., M.P.); Institute for Molecular Medicine Finland, Helsinki (P.P.S., A.S.H., T.T., K.K., M.P.); Diabetes and Obesity Research Program (K.K., M.P.) and Department of General Practice and Primary Health Care, Helsinki University Hospital (J.G.E.), University of Helsinki, Finland; The Research Centre of Applied and Preventive Cardiovascular Medicine (O.R.) and Department of Clinical Physiology, Turku University Hospital (O.R.), University of Turku, Finland; Department of Clinical Chemistry, Fimlab Laboratories and Finnish Cardiovascular Research Center Tampere, Faculty of Medicine and Life Sciences, University of Tampere, Finland (T.L.); Department of Clinical Physiology, Tampere University Hospital, Finland (M.K.); Department of Clinical Physiology, University of Tampere School of Medicine, Finland (M.K.); Institute for Computational Medicine, Center for Life Course Health Research, Faculty of Medicine (J.K.), Biocenter Oulu (J.K.), and Center for Life Course Health Research, Faculty of Medicine (M.M.), University of Oulu, Finland; Folkhälsan Research Center, Helsinki, Finland (J.G.E.); Department of General and Interventional Cardiology, University Heart Center Hamburg, Germany (S.B., T.Z.); German Center for Cardiovascular research, partner site Hamburg/Lübeck/Kiel, Hamburg, Germany (S.B., T.Z.); and Estonian Genome Center, University of Tartu, Estonia (M.P.).

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## Disclosures

None.

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### CLINICAL PERSPECTIVE

Atrial natriuretic peptide and B-type natriuretic peptide are unique hormones secreted by cardiomyocytes, often used in the diagnostics of heart failure. They bind to the same receptor, but unexpected differences in their effects have been reported in both human and animal models. We used genome-wide association analysis to study genetic variation affecting their circulating concentration, identifying 8 variants near the genes *NPPA*, *NPPB*, *PPP3CC*, and *GALNT4*. Subsequently, we investigated the correlation between the natriuretic peptide-associated genetic variants and blood pressure. Genetic variants lowering the concentration of midregional proatrial natriuretic peptide associated with higher blood pressure, but we did not observe a similar blood pressure correlation with genetic variants affecting B-type natriuretic peptide or NT-proBNP (N-terminal pro-B-type natriuretic peptide). The effect sizes of the midregional proatrial natriuretic peptide correlated genetic variants on blood pressure were small, from 0.25 to 0.50 mmHg per allele. Their combined effect, however, associated with a 9% difference in the odds ratio for hypertension, contributing significantly to the burden of high blood pressure in the general population.