Original Article

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

Perttu P. Salo, MSc; Aki S. Havulinna, PhD; Taru Tukiainen, PhD; Olli Raitakari, MD, PhD;
Terho Lehtimäki, MD, PhD; Mika Kähönen, MD, PhD; Johannes Kettunen, PhD;
Minna Männikkö, PhD; Johan G. Eriksson, MD, PhD; Antti Jula, MD, PhD;
Stefan Blankenberg, MD, PhD; Tanja Zeller, PhD; Veikko Salomaa, MD, PhD;
Kati Kristiansson, PhD*; Markus Perola, MD, PhD*

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 ($P=8.2\times10^{-4}$).

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 prohormones 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.[1] 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.[2] 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.[3,4] Deleting *NPPA* in mice predisposes them to hypertension, but knocking out *NPPB* triggers cardiac fibrosis instead of inducing hypertension.[5–7] In contrast to mice, the deletion of *NPPB* in a hypertensive rat

model has been reported to decrease survival and increase both systolic and diastolic blood pressure (BP).[8] 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.[9–12] 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.[13,14] A lack of association or even a paradoxical negative association of ANP with BP has been reported in obese men.[15] 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.[9] 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).[16,17] Four genome-wide association studies (GWAS) have studied circulating BNP or NT-proBNP levels.[18–21] 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.[1,22]

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.[23,24] 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.[23–28] 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.[29] 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 mm Hg or systolic BP >140 mm Hg 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 7 358 451 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 ($N_{MISSING}$=0), BNP ($N_{MISSING}$=131), and NT-proBNP ($N_{MISSING}$=133) and random-effects meta-analysis to combine results from the different cohorts.[30] We inverse-normal transformed the natriuretic peptide measurements and used linear regression with an additive genetic model adjusted for geographical sampling region, age[2] 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.[31] 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.[32,33] 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).[34] 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 (β_s) 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 β_s) 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 $P<5\times10^{-8}$ 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.[16–20] 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 $P=2.19\times10^{-9}$; 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.[35,36] 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.[34] 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.[16–20] 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 ($P=4.42\times10^{-52}$). For NT-proBNP and BNP:NT-proBNP ratio, rs61761991 was the most statistically significant SNP ($P=8.76\times10^{-68}$ and $P=4.81\times10^{-103}$, respectively), and least absolute shrinkage and selection operator regression detected rs12406089 as a secondary signal for NT-proBNP ($P=8.31\times10^{-48}$). 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 ≈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 ($P=3.85\times10^{-13}$), 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 ≈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).[37] 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.[15] 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_{GWAS}	$P_{REPLICATION}$	β (SE; 95% CI)	$P_{COMBINED}$
BNP	rs198379	1	11915467	t/C (0.365)	0.989	NPPB (3.5 kb, 3′)	GWAS	$6.85×10^{-41}$	$7.99×10^{-13}$	0.249 (0.0164; 0.217 to 0.282)	$4.42×10^{-52}$
BNP:NT-proBNP	rs61761991	1	11918444	c/T (0.029)	0.996	NPPB (0.5 kb, coding exon)	GWAS	$7.17×10^{-79}$	$5.71×10^{-26}$	1.114 (0.0517; 1.013 to 1.215)	$4.81×10^{-103}$
	rs7000551	8	22276251	a/G (0.369)	0.994	SLC39A14 (38.6 kb, intronic) PPP3CC (22.5 kb, 5′)	GWAS	$2.16×10^{-8}$	0.0248	0.109 (0.0181; 0.073 to 0.144)	$2.00×10^{-9}$
	rs11105298	12	89876143	t/C (0.211)	0.992	POC1B (59 kb, intronic) GALNT4 (43.2 kb, 3′)	GWAS	$3.06×10^{-18}$	$4.11×10^{-6}$	0.21 (0.0213; 0.169 to 0.252)	$6.77×10^{-23}$
	rs11105298	12	89876143	t/C (0.211)	0.992	POC1B (59 kb, intronic) GALNT4 (43.2 kb, 3′)	Conditional-1	$3.67×10^{-20}$	$2.01×10^{-6}$	0.189 (0.02185; 0.189 to 0.275)	$2.96×10^{-26}$
	rs61378614	12	89903654	a/C (0.16)	0.994	POC1B (87 kb, intronic) GALNT4 (15.7 kb, 3′)	Conditional-1	$1.70×10^{-10}$	0.0453	0.101 (0.03242; 0.101 to 0.228)	$4.13×10^{-7}$
MR-proANP	rs3753584	1	11864586	t/C (0.149)	1	MTHFR (3 kb, intronic) NPPA (43.5 kb, 3′)	GWAS	$4.63×10^{-38}$	$3.48×10^{-7}$	0.275 (0.038; 0.201 to 0.35)	$4.19×10^{-13}$
	rs4845875	1	11824133	A/c (0.355)	0.944	C1orf167 (11 kb, intronic) NPPA (84 kb, 3′)	Conditional-2	$3.53×10^{-7}$	0.0031	−0.156 (0.0198; −0.156 to −0.079)	$3.37×10^{-9}$
	rs6540997	1	11827355	A/g (0.274)	0.995	C1orf167 (8 kb, intronic) NPPA (80.8 kb, 3′)	Conditional-2	$9.03×10^{-10}$	0.0326	0.074 (0.0195; 0.074 to 0.171)	$7.13×10^{-7}$
	rs3753584	1	11864586	t/C (0.149)	1	MTHFR (3 kb, intronic) NPPA (43.5 kb, 3′)	Conditional-2	$1.85×10^{-20}$	$1.80×10^{-4}$	0.162 (0.023; 0.162 to 0.282)	$3.85×10^{-13}$
	rs701041	12	124999344	G/c (0.106)	0.928	NCOR2 (126 kb, intronic)	GWAS	$1.23×10^{-8}$	0.9381	−0.088 (0.0782; −0.241 to 0.066)	0.2624
NT-proBNP	rs61761991	1	11918444	c/T (0.029)	0.996	NPPB (0.5 kb, coding exon)	GWAS	$1.72×10^{-51}$	$5.33×10^{-18}$	−0.766 (0.044; −0.853 to −0.68)	$8.76×10^{-68}$
	rs61761991	1	11918444	c/T (0.029)	0.996	NPPB (0.5 kb, coding exon)	Conditional-3	$3.85×10^{-43}$	$1.26×10^{-15}$	−0.782 (0.0425; −0.782 to −0.616)	$1.41×10^{-60}$
	rs12406089	1	11921181	c/G (0.291)	0.995	NPPB (2.2 kb, 5′)	Conditional-3	$2.45×10^{-33}$	$3.54×10^{-14}$	0.201 (0.0172; 0.201 to 0.264)	$8.31×10^{-48}$
	rs10858906	12	89934474	c/T (0.21)	0.996	GALNT4 (15.2 kb, 5′)	GWAS	$1.08×10^{-12}$	0.0388	−0.12 (0.0338; −0.186 to −0.054)	$3.91×10^{-4}$

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, age², 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
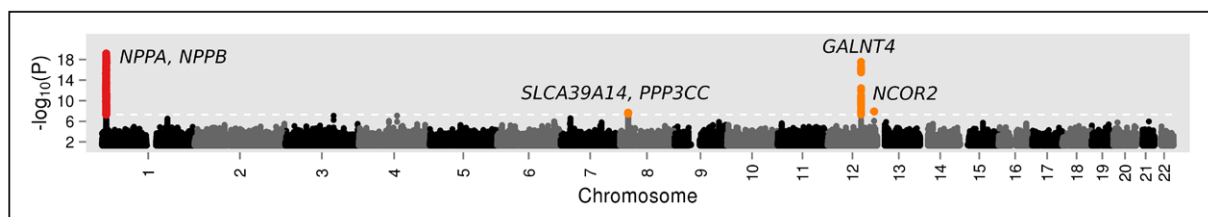
Figure 1. Genome-wide association study *P* values. *P* values of the genome-wide association tests and their genomic locations. Y axis cut at Y=18, the peak on chromosome 1 extends to Y=80.
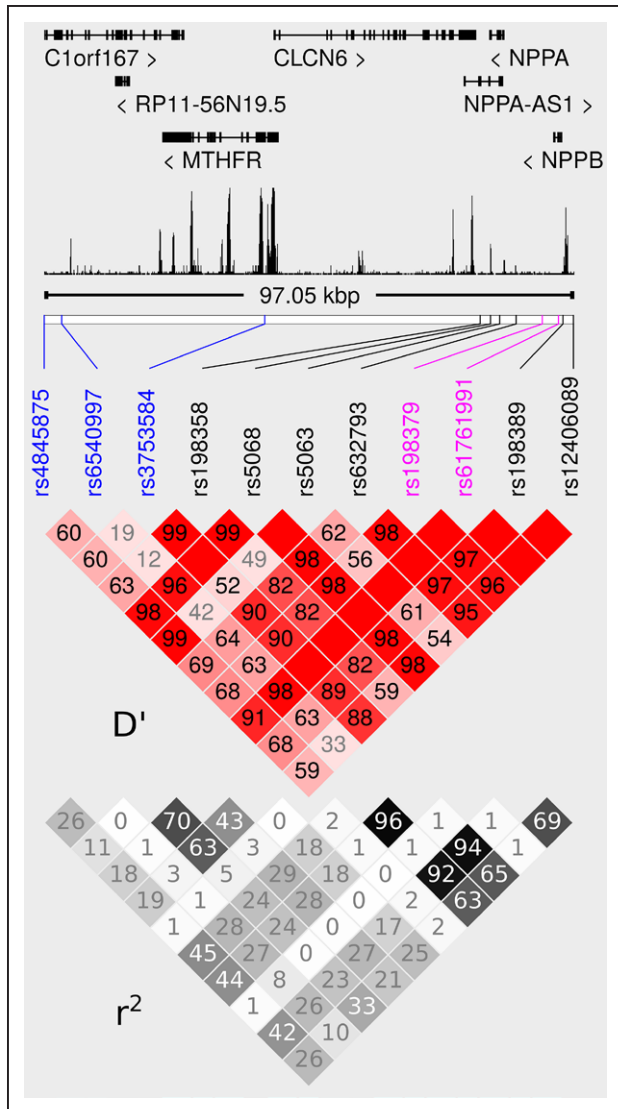
Figure 2. SNPs associated with natriuretic peptides on chromosome 1 near *NPPA* and *NPPB*. Linkage disequilibrium on chromosome 1 near *NPPA* and *NPPB*. R^2 and D′ calculated with Haploview v4.2 from 22 374 unrelated Finnish samples. Genes are depicted as annotated in GENCODE v19, potential regulatory 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 ($P=2.27\times10^{-9}$). This correlation was driven by an effect on the NT-proBNP concentration as rs7000551 associated with NT-proBNP ($P=3.72\times10^{-5}$) 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 atio by ≈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 ($P=1.52\times10^{-26}$ and $P=3.98\times10^{-9}$, 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
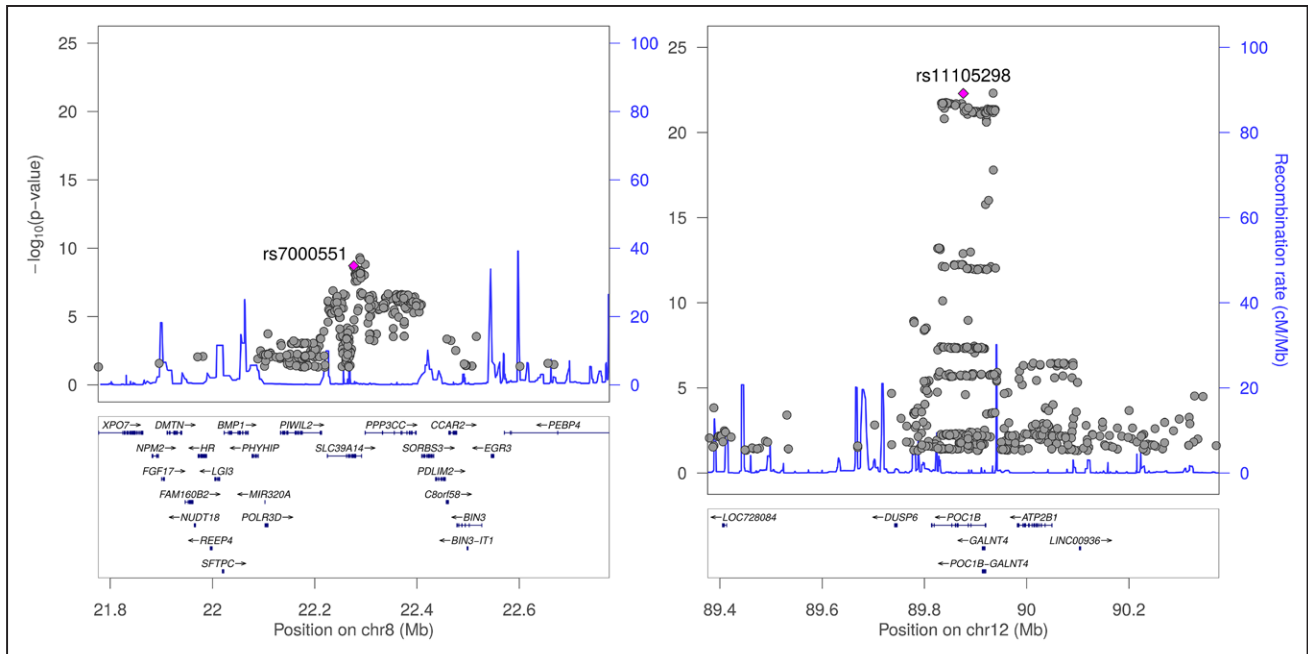
Figure 3. B-type natriuretic peptide (BNP):NT-proBNP (N-terminal pro-B-type natriuretic peptide) associated SNPs on chromosomes 8 and 12. Association of SNPs with BNP:NT-proBNP ratio on chromosomes 8 and 12 after meta-analyzing the results from the genome-wide association study (GWAS) and replication 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 ≈0.25 mm Hg (diastolic BP) and 0.50 mm Hg (systolic BP). Only 1 of these SNPs was independently associated with hypertension as a binary end point (rs3753584; P=6.8×10^{-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=8.2×10^{-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.[38]

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.[16–21] 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 <10 000 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.[39]

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.[19,40] 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	*NPPA*	0.84, ns.	5.00, ns.	0.01, ns.	−2.40, $P=2.1\times10^{-8}$	0.40, $P=0.0033$	0.63, ns.	1.00, ns.
rs6540997	1	11827355	A/g	*NPPA*	0.11, ns.	−2.20, ns.	−0.01, ns.	3.10, $P=5.8\times10^{-7}$	−0.25, ns.	−0.47, $P=0.029$	0.93, ns.
rs3753584	1	11864586	t/C	*NPPA*	1.50, ns.	8.70, ns.	−0.00, ns.	5.00, $P=1.2\times10^{-6}$	−0.38, $P=0.022$	−0.36, ns.	0.88, $P=6.8\times10^{-4}$
rs198379	1	11915467	t/C	*NPPB*	4.50, $P=1.2\times10^{-20}$	9.60, $P=7.2\times10^{-19}$	−0.00, ns.	0.33, ns.	−0.02, ns.	−0.29, ns.	1.00, ns.
rs7000551	8	22276251	a/G	*SLC39A14* and *PPP3CC*	−0.34, ns.	−3.80, ns.	0.03, $P=5.4\times10^{-9}$	−0.11, ns.	0.16, ns.	−0.07, ns.	1.00, ns.
rs11105298	12	89876143	t/C	*GALNT4*	0.66, ns.	−7.40, $P=0.0067$	0.06, $P=3\times10^{-26}$	1.30, ns.	−0.01, ns.	−0.04, ns.	1.00, ns.
rs61378614	12	89903654	a/C	*GALNT4*	0.71, ns.	−4.40, ns.	0.04, $P=4.1\times10^{-7}$	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, age², 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.[41] Originally characterized as a testis-specific calcineurin subunit, *PPP3CC* has been later detected in multiple tissues.[34] Because of its central role in spermatogenesis, drugs inhibiting *PPP3CC* have been suggested as a potential male contraceptive.[42] 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.[19] Analysis of gene expression in cardiac tissue failed to highlight any of the nearby genes but, as previously noted, *GALNT4* is an attractive candidate.[19] It codes for an aminoacyltranferase that initiates O-linked glycosylation, and proBNP is known to be O-glycosylated.[43,44] 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.[45,46] 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 ≈3%.[47] One in 20 Finns will, therefore, have a measured concentration of NT-proBNP, which is ≈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.[6–8,48] 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.[15,49] 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.[16] 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.).

Sources of Funding

Disclosures

None.

References

1. Kerkelä R, Ulvila J, Magga J. Natriuretic peptides in the regulation of cardiovascular physiology and metabolic events. *J Am Heart Assoc*. 2015;4:e002423. doi: 10.1161/JAHA.115.002423.
2. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, et al; Authors/Task Force Members. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J*. 2016;37:2129–2200. doi: 10.1093/eurheartj/ehw128.
3. Steinhelper ME, Cochrane KL, Field LJ. Hypotension in transgenic mice expressing atrial natriuretic factor fusion genes. *Hypertension*. 1990;16:301–307.
4. Ogawa Y, Itoh H, Tamura N, Suga S, Yoshimasa T, Uehira M, et al. Molecular cloning of the complementary DNA and gene that encode mouse brain natriuretic peptide and generation of transgenic mice that overexpress the brain natriuretic peptide gene. *J Clin Invest*. 1994;93:1911–1921. doi: 10.1172/JCI117182.
5. John SW, Krege JH, Oliver PM, Hagaman JR, Hodgin JB, Pang SC, et al. Genetic decreases in atrial natriuretic peptide and salt-sensitive hypertension. *Science*. 1995;267:679–681.
6. Tamura N, Ogawa Y, Chusho H, Nakamura K, Nakao K, Suda M, et al. Cardiac fibrosis in mice lacking brain natriuretic peptide. *Proc Natl Acad Sci USA*. 2000;97:4239–4244. doi: 10.1073/pnas.070371497.
7. John SW, Veress AT, Honrath U, Chong CK, Peng L, Smithies O, et al. Blood pressure and fluid-electrolyte balance in mice with reduced or absent ANP. *Am J Physiol*. 1996;271(1 pt 2):R109–R114.
8. Holditch SJ, Schreiber CA, Nini R, Tonne JM, Peng KW, Geurts A, et al. B-type natriuretic peptide deletion leads to progressive hypertension, associated organ damage, and reduced survival: novel model for human hypertension. *Hypertension*. 2015;66:199–210. doi: 10.1161/HYPERTENSIONAHA.115.05610.
9. O'Connor CM, Starling RC, Hernandez AF, Armstrong PW, Dickstein K, Hasselblad V, et al. Effect of nesiritide in patients with acute decompensated heart failure. *N Engl J Med*. 2011;365:32–43. doi: 10.1056/NEJMoa1100171.
10. Crozier IG, Nicholls MG, Ikram H, Espiner EA, Gomez HJ, Warner NJ. Haemodynamic effects of atrial peptide infusion in heart failure. *Lancet*. 1986;2:1242–1245.
11. Jensen KT, Carstens J, Pedersen EB. Effect of BNP on renal hemodynamics, tubular function and vasoactive hormones in humans. *Am J Physiol*. 1998;274(1 pt 2):F63–F72.
12. Hynynen M, Kupari M, Salmenperä M, Tikkanen I, Heinonen J, Fyhrquist F, et al. Hemodynamic effects of alpha-human atrial natriuretic peptide in healthy volunteers. *J Cardiovasc Pharmacol*. 1988;11:711–715.
13. Pidgeon GB, Richards AM, Nicholls MG, Espiner EA, Yandle TG, Frampton C. Differing metabolism and bioactivity of atrial and brain natriuretic peptides in essential hypertension. *Hypertension*. 1996;27:906–913.
14. Suga S, Nakao K, Hosoda K, Mukoyama M, Ogawa Y, Shirakami G, et al. Receptor selectivity of natriuretic peptide family, atrial natriuretic peptide, brain natriuretic peptide, and C-type natriuretic peptide. *Endocrinology*. 1992;130:229–239. doi: 10.1210/endo.130.1.1309330.
15. Asferg CL, Andersen UB, Linneberg A, Hedley PL, Christiansen M, Goetze JP, et al. Serum proatrial natriuretic peptide does not increase with higher systolic blood pressure in obese men. *Heart*. 2017;103:154–158. doi: 10.1136/heartjnl-2016-309462.
16. Newton-Cheh C, Larson MG, Vasan RS, Levy D, Bloch KD, Surti A, et al. Association of common variants in NPPA and NPPB with circulating natriuretic peptides and blood pressure. *Nat Genet*. 2009;41:348–353. doi: 10.1038/ng.328.
17. Pereira NL, Tosakulwong N, Scott CG, Jenkins GD, Prodduturi N, Chai Y, et al. Circulating atrial natriuretic peptide genetic association study identifies a novel gene cluster associated with reduced NT-proANP, increased stroke and higher diastolic blood pressure. *BMC Pharmacol Toxicol*. 2015;16:A37.
18. Musani SK, Fox ER, Kraja A, Bidulescu A, Lieb W, Lin H, et al. Genome-wide association analysis of plasma B-type natriuretic peptide in blacks: the Jackson Heart Study. *Circ Cardiovasc Genet*. 2015;8:122–130. doi: 10.1161/CIRCGENETICS.114.000900.
19. Johansson Å, Eriksson N, Lindholm D, Varenhorst C, James S, Syvänen AC, et al; PLATO Investigators. Genome-wide association and Mendelian randomization study of NT-proBNP in patients with acute coronary syndrome. *Hum Mol Genet*. 2016;25:1447–1456. doi: 10.1093/hmg/ddw012.
20. Del Greco M F, Pattaro C, Luchner A, Pichler I, Winkler T, Hicks AA, et al. Genome-wide association analysis and fine mapping of NT-proBNP level provide novel insight into the role of the MTHFR-CLCN6-NPPA-NPPB gene cluster. *Hum Mol Genet*. 2011;20:1660–1671. doi: 10.1093/hmg/ddr035.
21. Folkersen L, Fauman E, Sabater-Lleal M, Strawbridge RJ, Frånberg M, Sennblad B, et al; IMPROVE study group. Mapping of 79 loci for 83 plasma protein biomarkers in cardiovascular disease. *PLoS Genet*. 2017;13:e1006706. doi: 10.1371/journal.pgen.1006706.
22. Semenov AG, Tamm NN, Seferian KR, Postnikov AB, Karpova NS, Serebryanaya DV, et al. Processing of pro-B-type natriuretic peptide: furin and corin as candidate convertases. *Clin Chem*. 2010;56:1166–1176. doi: 10.1373/clinchem.2010.143883.
23. Vartiainen E, Jousilahti P, Alfthan G, Sundvall J, Pietinen P, Puska P. Cardiovascular risk factor changes in Finland, 1972-1997. *Int J Epidemiol*. 2000;29:49–56.
24. Vartiainen E, Laatikainen T, Peltonen M, Juolevi A, Männistö S, Sundvall J, et al. Thirty-five-year trends in cardiovascular risk factors in Finland. *Int J Epidemiol*. 2010;39:504–518. doi: 10.1093/ije/dyp330.

25. Eriksson JG, Forsén T, Tuomilehto J, Winter PD, Osmond C, Barker DJ. Catch-up growth in childhood and death from coronary heart disease: longitudinal study. *BMJ*. 1999;318:427–431.

26. Järvelin MR, Sovio U, King V, Lauren L, Xu B, McCarthy MI, et al. Early life factors and blood pressure at age 31 years in the 1966 northern Finland birth cohort. *Hypertension*. 2004;44:838–846. doi: 10.1161/01. HYP.0000148304.33869.ee.

27. Johansson JK, Puukka PJ, Niiranen TJ, Varis J, Peltonen M, Salomaa V, et al. Health 2000 score–development and validation of a novel cardiovascular risk score. *Ann Med*. 2016;48:403–409.

28. Viikari J, Rönnemaa T, Seppänen A, Marniemi J, Porkka K, Räsänen L, et al. Serum lipids and lipoproteins in children, adolescents and young adults in 1980-1986. *Ann Med*. 1991;23:53–59.

29. Blankenberg S, Zeller T, Saarela O, Havulinna AS, Kee F, Tunstall-Pedoe H, et al; MORGAM Project. Contribution of 30 biomarkers to 10-year cardiovascular risk estimation in 2 population cohorts: the MONICA, risk, genetics, archiving, and monograph (MORGAM) biomarker project. *Circulation*. 2010;121:2388–2397. doi: 10.1161/CIRCULATIONAHA.109.901413.

30. Viechtbauer W. Conducting meta-analyses in R with the metafor package. *J Stat Softw*. 2010;36:1–48.

31. Sabourin J, Nobel AB, Valdar W. Fine-mapping additive and dominant SNP effects using group-LASSO and fractional resample model averaging. *Genet Epidemiol*. 2015;39:77–88. doi: 10.1002/gepi.21869.

32. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81:559–575. doi: 10.1086/519795.

33. Yang J, Lee SH, Goddard ME, Visscher PM. GCTA: a tool for genome-wide complex trait analysis. *Am J Hum Genet*. 2011;88:76–82. doi: 10.1016/j.ajhg.2010.11.011.

34. GTEx Consortium. Human genomics. the genotype-tissue expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science*. 2015;348:648–660.

35. Nicolae DL, Gamazon E, Zhang W, Duan S, Dolan ME, Cox NJ. Trait-associated SNPs are more likely to be eQTLs: annotation to enhance discovery from GWAS. *PLoS Genet*. 2010;6:e1000888. doi: 10.1371/journal.pgen.1000888.

36. Maurano MT, Humbert R, Rynes E, Thurman RE, Haugen E, Wang H, et al. Systematic localization of common disease-associated variation in regulatory DNA. *Science*. 2012;337:1190–1195. doi: 10.1126/science.1222794.

37. ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. *Nature*. 2012;489:57–74.

38. Vattikuti S, Guo J, Chow CC. Heritability and genetic correlations explained by common SNPs for metabolic syndrome traits. *PLoS Genet*. 2012;8:e1002637. doi: 10.1371/journal.pgen.1002637.

39. Pfister R, Sharp S, Luben R, Welsh P, Barroso I, Salomaa V, et al. Mendelian randomization study of B-type natriuretic peptide and type 2 diabetes: evidence of causal association from population studies. *PLoS Med*. 2011;8:e1001112. doi: 10.1371/journal.pmed.1001112.

40. He L, Vasiliou K, Nebert DW. Analysis and update of the human solute carrier (SLC) gene superfamily. *Hum Genom*. 2009;3:1.

41. Molkentin JD, Lu JR, Antos CL, Markham B, Richardson J, Robbins J, et al. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. *Cell*. 1998;93:215–228.

42. Miyata H, Satouh Y, Mashiko D, Muto M, Nozawa K, Shiba K, et al. Sperm calcineurin inhibition prevents mouse fertility with implications for male contraceptive. *Science*. 2015;350:442–445. doi: 10.1126/science. aad0836.

43. Bennett EP, Hassan H, Mandel U, Mirgorodskaya E, Roepstorff P, Burchell J, et al. Cloning of a human UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase that complements other GalNAc-transferases in complete O-glycosylation of the MUC1 tandem repeat. *J Biol Chem*. 1998;273:30472–30481.

44. Schellenberger U, O'Rear J, Guzzetta A, Jue RA, Protter AA, Pollitt NS. The precursor to B-type natriuretic peptide is an O-linked glycoprotein. *Arch Biochem Biophys*. 2006;451:160–166. doi: 10.1016/j. abb.2006.03.028.

45. Analytical characteristics of commercial MR-proANP, BNP and NT-proBNP assays as per the manufacturer. http://www.ifcc.org/media/276711/ IFCC%20NP%20Assay%20Table%20November%202014.pdf.

46. Aken BL, Ayling S, Barrell D, Clarke L, Curwen V, Fairley S, et al. The ensembl gene annotation system. *Database (Oxford)*. 2016;2016. doi: 10.1093/database/baw093.

47. Lek M, Karczewski K, Minikel E, Samocha K, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. *BioRxiv*. 2016;030338.

48. Hunt PJ, Espiner EA, Nicholls MG, Richards AM, Yandle TG. Differing biological effects of equimolar atrial and brain natriuretic peptide infusions in normal man. *J Clin Endocrinol Metab*. 1996;81:3871–3876. doi: 10.1210/jcem.81.11.8923831.

49. Wang TJ, Larson MG, Levy D, Benjamin EJ, Leip EP, Wilson PW, et al. Impact of obesity on plasma natriuretic peptide levels. *Circulation*. 2004;109:594–600. doi: 10.1161/01.CIR.0000112582.16683.EA.

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 mm Hg 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.

Circulation
Cardiovascular Genetics

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

Perttu P. Salo, Aki S. Havulinna, Taru Tukiainen, Olli Raitakari, Terho Lehtimäki, Mika Kähönen, Johannes Kettunen, Minna Männikkö, Johan G. Eriksson, Antti Jula, Stefan Blankenberg, Tanja Zeller, Veikko Salomaa, Kati Kristiansson and Markus Perola

The online version of this article, along with updated information and services, is located on the World Wide Web at:

http://circgenetics.ahajournals.org/content/10/6/e001713
Free via Open Access

Data Supplement (unedited) at:

http://circgenetics.ahajournals.org/content/suppl/2017/12/12/CIRCGENETICS.117.001713.DC1

SUPPLEMENTAL MATERIAL

Supplemental Methods

Genotyping and imputation

The GWAS discovery sample was genotyped using the Illumina HumanCoreExome beadchip at the Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK. SNPs with clustering probability < 95%, genotyping success rate < 95%, minor allele frequency (MAF) < 1%, or $P < 10^{-6}$ for an exact test of Hardy-Weinberg equilibrium (HWE) were removed. Samples with more than 5% of the genotypes missing or a mismatch in reported and genotype-determined sex were excluded. The data was then pre-phased with SHAPEIT v1 and imputed with IMPUTE v2.2.2 using the 1000 Genomes phase 1 (September 2013) reference haplotypes.[1-3] After imputation, we removed SNPs with more than 5% of genotypes missing, minor allele frequency (MAF) <1%, imputation quality <0.9 (IMPUTE INFO metric), and SNPs with P<0.01 for a test on HWE.

The GWAS replication sample was genotyped at the Broad Institute of Harvard and MIT, Cambridge, MA, US, with the Illumina HumanCoreExome beadchip. Before imputation, we removed samples which were outliers in terms of genomic principal components or heterozygosity, or had a sex mismatch or more than 2% of the genotypes missing. In order to produce a uniformly imputed dataset, we pooled the genotypes of the replication sample with all other genotyped FINRISK samples and used the same exclusion criteria as before with one exception. Because the number of samples was greater, we included rarer reliably genotyped SNPs (MAF<5% and call rate>99%) with MAF>0 (Illumina HumanCoreExome Beadchip array) or MAF>(10/number of samples genotyped on the array) (all other genotyping arrays). We next pre-phased the genotypes with SHAPEIT v2.r727 and imputed the data with IMPUTE v2.3.2 using the 1000 Genomes Phase 3 haplotypes augmented with a custom haplotype set of 2,000 Finnish individuals. We then used genotypes from this uniformly imputed FINRISK dataset for all analyses subsequent to the discovery GWAS.

The additional cohorts in the blood pressure study population were genotyped on various genotyping arrays: FINRISK 1992, FINRISK 2002, and FINRISK 2007 were genotyped using the Affymetrix Genome-Wide Human SNP Array, and the Illumina Human610-Quad, HumanCoreExome-24, and HumanOmniExpress genotyping arrays. NFBC66 was genotyped on the Illumina HumanHap CNV 370k array, HBCS and Health2000 on the Illumina HumanHap610 quad array, and YFS was genotyped using the Illumina HumanHap 670k array. The non-FINRISK cohorts were imputed using the same methods as used for the GWAS discovery sample.

Association tests

We used multiple imputation methods in the "mice" package of R to account for any missing values for MR-proANP (N=0), BNP (N=131), and NT-proBNP (N=133).[4] We imputed the rank-normalized data 500 times to be able to ensure convergence and thinned the back-transformed imputations to 10 datasets for the analyses. We analyzed the 10 resulting datasets separately and pooled the results using base R implementing the previously described equations (for the GWAS's) or the R package "mice" (all other tests) in all statistical tests of the natriuretic peptide traits.[4,5]

We tested the association of genetic variants with MR-proANP, BNP, NT-proBNP, and BNP:NT-proBNP ratio using linear regression. To identify and exclude outliers, we log-transformed the measurements and excluded values differing more than 4 standard deviation units from the trait mean. We then inverse-normal transformed the natriuretic peptide measurements and modeled them with a linear regression model adjusted for geographical sampling region, age, age^2, sex, BMI, current smoking status (yes/no), systolic blood pressure, glomerular filtration rate estimated using cystatin C and creatinine as proxies (eGFR), and genotyping batch.[6] We extracted the residuals from those linear regression models and tested the association of the genetic variants with the residuals using SNPTEST.[7]

To combine the results from the discovery and replication samples, we used random-effects meta-analysis as implemented in the "rma.uni" function of the "metafor" package for R with the restricted maximum-likelihood estimator of heterogeneity.[8]

We used ordinary linear regression and LASSO regression to search for multiple independent association signals at the genome-wide significant loci. Per locus, we first fitted all SNPs simultaneously in the discovery sample using LASSO regression with fractional model averaging implemented in the LLARMA package for R, with an additive genetic model and default parameter values.[9] To form a candidate multi-SNP model for each locus, we selected the top two SNPs in terms of their fractional resample model inclusion probabilities and all additional SNPs with an inclusion probability of 0.10 or greater, averaging the inclusion probabilities from the multiply imputed datasets. Next, we tested the candidate models in the replication sample using ordinary linear regression and declared the SNPs significant if the point-wise P-values were smaller than 0.05 in both the discovery and replication samples. Finally, we fitted models with the significant SNPs only and combined the results from the two samples using the previously described meta-analysis model.

We used linear regression implemented in the "glm" function for R to study the association of genetic variants with systolic and diastolic blood pressure. We log-transformed systolic (but not diastolic) blood pressure and set the first two genomic principal components, age, sex, and BMI as covariates for all tests. For FINRISK samples we additionally included study year and genotyping batch as covariates. To test the genetic variants for association with hypertension, we used logistic regression and the same covariates excluding blood pressure medication. We then combined the results from the different cohorts using the same meta-analysis model as previously.

Co-association with gene expression

We used the meta-analysis P-values and effect estimates from the present study (for natriuretic peptide traits) and those from the GTEx eQTL analysis (release V6, Oct 6th 2016; for gene expression) as measures of association. Because linkage disequilibrium patterns (LD) and allele frequencies are mostly similar across independent samples, both the association P-values and effect estimates for any two traits may be expected to correlate even under the null hypothesis of no co-association. We thus derived the P-values corresponding to the Pearson and Spearman correlation coefficients empirically by permuting the genotype-phenotype relationship 1,000 times for the natriuretic peptide traits and analyzing the permuted replicates exactly as done for the GWAS.

To define the regions of association for each genome-wide significant natriuretic peptide-associated locus, we first located the lead SNP. We then defined the region by sliding a 500 SNP-wide window away from the lead SNP one variant at a time and stopped extending the region once the average P-value of association within the window reached 0.5, the expected mean P-value under the null hypothesis of no association. The regions defined in this way ranged in size from 585,134 to 1,335,097 bp.

Because the GWAS results were exclusively from Finnish samples while the GTEx studied a cosmopolitan sample of mostly white Caucasians, we excluded variants with the most dissimilar LD patterns between Finns and other Europeans. For each SNP in a given region, we calculated its correlation coefficients r_i with the i adjacent SNPs at most 100,000 bp away in both Finns and other Europeans using publicly available 1000 genomes project data.[3] We then derived the root-mean square deviation (RMSD) in r_i between the two populations. Having calculated the RMSD of all SNPs in the region, we excluded the top 100 SNPs with the greatest RMSD and repeated the procedure until the average RMSD of the remaining SNPs was smaller than 0.075. This cutoff was selected based on plotting

the mean RMSD of the retained SNPs against the range of possible cutoff values (from 0 to 2 by increments of 0.001). For values greater than 0.075, the mean RMSD increased sharply in a non-linear manner indicating the inclusion of SNPs with particularly high population differences in LD. For values smaller than 0.075, the relationship was approximately linear.

Phenotypic variance explained genome-wide

We used the uniformly imputed dataset to estimate the fraction of phenotypic variance explained by the SNPs but modified the imputation quality and HWE test P-value thresholds. Because imputation quality is positively correlated with MAF, we included SNPs with IMPUTE INFO metric > 0.3 in order to avoid unnecessarily penalizing rarer SNPs. Due to the increase in the size of the dataset as compared to the discovery phase GWAS, we used a numerically smaller HWE P-value limit by excluding SNPs with P < 0.005 for a test on HWE. We estimated the genetic relationship matrix (GRM) of the samples using PLINK v1.90.[10] Next, we used the GRM to estimate the phenotypic variance explained by the genotypes with the GCTA v1.25.3 program in the participants of the GWAS discovery sample, setting aside 863 samples for which duplicate genotypes from other genotyping arrays were available to be used in quality control.[11]

In order to assess the reliability of the estimates of phenotypic variance explained, we derived four genomic scores corresponding to each of the four estimates and studied the association of the genomic scores with their respective phenotypes in the replication sample as follows: For each phenotype, we derived the contribution of the individual SNPs to the total genetic effect using GCTA and used these as weights to estimate the total genetic effect or genomic score for each of the study participants for the four traits. The genomic scores were technically robust as the correlation between the 863 genotyping replicates was high (Pearson's product-moment correlation r > 0.99) for all

phenotypes. We then tested the association of the genomic scores of MR-proANP, NT-proBNP, BNP, and

BNP:NT-proBNP ratio with said traits in the replication sample using linear regression. We confirmed a

statistically significant association (P < 0.05) with NT-proBNP, BNP, and BNP:NT-proBNP ratio but not

with MR-proANP (Supplemental Table 2).

Supplemental Tables

Supplemental Table 1. Sample characteristics

	GWAS Sample	Replication Sample	Blood Pressure Study Sample
N	4,932	1,373	27,059
Age (years)	46.18 (21.39)	48.67 (20.5)	42.41 (25.42)
Females (n/%)	2,592 (52.55%)	711 (51.78%)	14,377 (53.13%)
Body Mass Index (kg/m2)	25.73 (5.473)	25.97 (5.116)	25.69 (5.714)
Diastolic Blood Pressure (mm Hg)	82 (15)	82 (14)	80 (16)
Systolic Blood Pressure (mm Hg)	132 (26)	134 (26)	130 (25)
Hypertension (n/%)	2,090 (42.38%)	605 (44.06%)	10,404 (39.3%)
Smoking (n/%)	1,230 (24.94%)	300 (21.85%)	3,893 (24.9%)
Prevalent Heart Failure (n/%)	0 (0%)	0 (0%)	na.
Incident Heart Failure (n/%)	289 (5.86%)	84 (6.118%)	na.
NT-proBNP (pg/ml)	39.26 (56.49)	46.75 (57.2)	na.
MR-proANP (pmol/L)	41.3 (25.2)	43.4 (25.4)	na.
BNP (pg/ml)	12.9 (17.6)	14.9 (19.6)	na.

Quantitative variables: median (interquartile range)

Qualitative variables: number (proportion)

BNP: B-type natriuretic peptide, MR-proANP: mid-regional proatrial natriuretic peptide, NT-proBNP:

aminoterminal pro-B-type natriuretic peptide

Supplemental Table 2. Trait variance explained by the genome-wide genotype data

Trait	Variance Explained (SE)	Genomic Score Association*
MR-proANP	0.139 (0.071)	Beta=1.60, P=0.31
BNP	0.135 (0.070)	Beta=2.70, P=0.0049
NT-proBNP	0.230 (0.072)	Beta=9.40, P=0.015
BNP:NT-proBNP	0.179 (0.071)	Beta=0.03, P=0.0055

Proportion of phenotypic variance explained by autosomal SNPs in the GWAS discovery sample and the association of the corresponding genomic scores with the traits in the replication sample.

BNP: B-type natriuretic peptide, MR-proANP: mid-regional proatrial natriuretic peptide, NT-proBNP: aminoterminal pro-B-type natriuretic peptide, SE: standard error

* Beta coefficients given as dimensionless units (BNP:NT-proBNP ratio), pg/ml (BNP and NT-proBNP), or pmol/L (MR-proANP) per one standard deviation difference in the genomic score

Supplemental Table 3. Association of genome-wide significant genetic variants with natriuretic peptide traits

Trait	SNP*	Chr	Position	Alleles**	P_{GWAS}	$P_{REPLICATION}$	Beta (95% CI)	$P_{COMBINED}$
BNP	rs3753584	1	11864586	T/C	8.49×10^{-17}	1.33×10^{-4}	0.2047 (0.1609 to 0.2486)	5.71×10^{-20}
	rs198379	1	11915467	T/C	6.85×10^{-41}	7.99×10^{-13}	0.2494 (0.2172 to 0.2816)	4.42×10^{-52}
	rs61761991	1	11918444	C/T	0.0079	0.0904	-0.1465 (-0.2378 to -0.0552)	0.0017
	rs7000551	8	22276251	A/G	0.3909	0.4384	-0.0046 (-0.0414 to 0.0322)	0.8069
	rs11105298	12	89876143	T/C	0.3759	0.2768	0.0032 (-0.056 to 0.0623)	0.9168
	rs10858906	12	89934474	C/T	0.1767	0.3064	-0.0022 (-0.0699 to 0.0654)	0.9482
	rs701041	12	124999344	G/C	0.0047	0.084	-0.0011 (-0.1767 to 0.1745)	0.9905
BNP:NT-proBNP	rs3753584	1	11864586	T/C	0.061	0.3392	-0.0523 (-0.1011 to -0.0036)	0.0354
	rs198379	1	11915467	T/C	0.0709	0.7244	-0.0321 (-0.0679 to 0.0037)	0.0786
	rs61761991	1	11918444	C/T	7.17×10^{-79}	5.71×10^{-26}	1.1138 (1.0125 to 1.215)	4.81×10^{-103}
	rs7000551	8	22276251	A/G	2.16×10^{-8}	0.0248	0.1085 (0.0731 to 0.144)	2.00×10^{-9}
	rs11105298	12	89876143	T/C	3.06×10^{-18}	4.11×10^{-6}	0.2103 (0.1685 to 0.2521)	6.77×10^{-23}
	rs10858906	12	89934474	C/T	2.06×10^{-17}	5.87×10^{-7}	0.2105 (0.1686 to 0.2524)	7.27×10^{-23}
	rs701041	12	124999344	G/C	0.3886	0.5158	-0.0313 (-0.0886 to 0.0261)	0.2858
MR-proANP	**rs3753584**	1	11864586	T/C	4.63×10^{-38}	3.48×10^{-7}	0.2752 (0.2008 to 0.3495)	4.19×10^{-13}
	rs198379	1	11915467	T/C	3.97×10^{-7}	0.0016	0.0914 (0.0614 to 0.1214)	2.46×10^{-9}
	rs61761991	1	11918444	C/T	1.97×10^{-4}	0.3494	-0.1562 (-0.253 to -0.0595)	0.0016
	rs7000551	8	22276251	A/G	0.6066	0.6892	-0.004 (-0.0337 to 0.0258)	0.7944
	rs11105298	12	89876143	T/C	0.0369	0.0109	0.0217 (-0.1111 to 0.1545)	0.7489
	rs10858906	12	89934474	C/T	0.0457	0.0225	0.0179 (-0.1046 to 0.1404)	0.7746
	rs701041	12	124999344	G/C	1.23×10^{-8}	0.9381	-0.0876 (-0.2408 to 0.0656)	0.2624
NT-proBNP	rs3753584	1	11864586	T/C	1.29×10^{-21}	5.65×10^{-6}	0.2235 (0.182 to 0.2649)	4.23×10^{-26}
	rs198379	1	11915467	T/C	4.36×10^{-47}	5.76×10^{-16}	0.2565 (0.2261 to 0.287)	2.30×10^{-61}
	rs61761991	1	11918444	C/T	1.72×10^{-51}	5.33×10^{-18}	-0.7663 (-0.8526 to -0.68)	8.76×10^{-68}
	rs7000551	8	22276251	A/G	3.72×10^{-5}	0.8706	-0.0442 (-0.1086 to 0.0201)	0.1781
	rs11105298	12	89876143	T/C	2.54×10^{-11}	0.0929	-0.107 (-0.1796 to -0.0344)	0.0039
	rs10858906	12	89934474	C/T	1.08×10^{-12}	0.0388	-0.1199 (-0.1862 to -0.0536)	3.91×10^{-4}
	rs701041	12	124999344	G/C	0.0139	0.0366	0.0135 (-0.1602 to 0.1871)	0.8792

Association of SNPs with natriuretic peptides in the GWAS. Association tested with an additive genetic

model adjusted for geographical sampling region, age, age², 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: B-type natriuretic peptide, MR-proANP: mid-regional proatrial natriuretic peptide, NT-proBNP: aminoterminal pro-B-type natriuretic peptide

* Genome-wide significant lead SNPs for each trait and locus are marked with bold underlined text

** Alleles given as [reference allele]/[effect allele]

Supplemental Table 4. Replication of previously published associations.

Snp	Chr	Pos	Alleles*	NT-proBNP			MR-proANP			BNP			BNP:NT-proBNP		
				Beta	P	AP**	Beta	P	AP**	Beta	P	AP**	Beta	P	AP**
rs1023252	1	11899033	G/T	0.2277	4.71×10^{-43}	T[12]	0.0834	3.33×10^{-7}		0.1755	1.31×10^{-23}		-0.1112	1.13×10^{-8}	
rs198358	1	11904076	T/C	0.1724	6.71×10^{-20}		0.2008	4.60×10^{-27}	C[13]	0.1945	1.94×10^{-22}	C[13]	0.0156	0.4823	
rs5063	1	11907648	C/T	0.2532	1.30×10^{-9}		-0.055	0.1813	C[14]	0.1904	0.0041		-0.1637	0.1528	
rs198389	1	11919271	A/G	0.2535	1.03×10^{-60}	G[15]	0.0905	2.79×10^{-9}		0.2462	2.48×10^{-51}		-0.0328	0.0705	
rs35207557	1	11917620	T/TA	0.2502	2.98×10^{-58}		0.0919	2.20×10^{-9}		0.2448	5.32×10^{-50}	TA[16]	-0.0284	0.1585	
rs5068	1	11905974	A/G	0.2213	4.26×10^{-6}		0.3298	7.68×10^{-30}	G[13]	0.1782	4.27×10^{-11}	G[13]	-0.1265	0.0097	
rs549596	1	11916095	T/C	0.2474	4.69×10^{-58}		0.0932	8.76×10^{-10}		0.2417	8.42×10^{-50}	C[16]	-0.027	0.1366	
rs632793	1	11910677	A/G	0.254	3.80×10^{-59}		0.0938	1.30×10^{-9}	G[13]	0.2448	1.81×10^{-49}	G[13]	-0.034	0.0647	
rs13107325	4	103188709	C/T	0.1824	0.005	T[17]	-0.0498	0.4356		-0.0658	0.3373		-0.4549	2.19×10^{-9}	
rs3733402	4	187158034	G/A	0.0053	0.9054		0.0136	0.7592		-0.033	0.3429	G[18]	-0.0529	0.0028	
rs6557662	8	23230898	A/G	-0.0061	0.7792	A[16]	-0.0189	0.6837		-0.0251	0.3838		-0.0217	0.4283	
rs11105306	12	89897388	C/T	-0.1159	2.77×10^{-5}	C[17]	0.0213	0.7416		-0.0002	0.9935		0.2084	9.55×10^{-22}	

Association of previously published SNPs with natriuretic peptide traits in the meta-analysis of the GWAS and replication samples. All models adjusted for geographical sampling region, age, age^2, sex, current smoking status (yes or no), systolic blood pressure, estimated glomerular filtration rate, and genotyping batch.

BNP: B-type natriuretic peptide, MAF: minor allele frequency, MR-proANP: mid-regional proatrial natriuretic peptide, NT-proBNP: aminoterminal pro-B-type natriuretic peptide

* Alleles given as [reference allele]/[effect allele]
** AP: Previously published trait increasing-allele

Trait	SNP	Beta	SE	P
BNP	rs198379_C	0.2385	0.03445	4.42×10^{-12}
BNP	rs3753584_C	0.03386	0.02994	0.2581
BNP	rs6540997_G	0.01056	0.02655	0.6909
BNP	rs4845875_C	0.01701	0.02356	0.4703
BNP	rs61761991_T	-0.03811	0.0527	0.4696
BNP	rs12406089_G	0.03313	0.05278	0.5302
MR-proANP	rs198379_C	-0.03905	0.03207	0.2234
MR-proANP	rs3753584_C	0.1937	0.05636	5.88×10^{-4}
MR-proANP	rs6540997_G	0.1249	0.02474	4.47×10^{-7}
MR-proANP	rs4845875_C	-0.1264	0.02197	8.60×10^{-9}
MR-proANP	rs61761991_T	0.01139	0.04926	0.8171
MR-proANP	rs12406089_G	0.06408	0.05276	0.2246
NT-proBNP	rs198379_C	0.1347	0.05406	0.0127
NT-proBNP	rs3753584_C	0.00616	0.02794	0.8255
NT-proBNP	rs6540997_G	0.01158	0.04709	0.8057
NT-proBNP	rs4845875_C	-0.01402	0.02191	0.5223
NT-proBNP	rs61761991_T	-0.6503	0.04891	2.42×10^{-40}
NT-proBNP	rs12406089_G	0.1222	0.05417	0.0241
BNP:NT-proBNP	rs198379_C	0.1083	0.04947	0.0286
BNP:NT-proBNP	rs3753584_C	0.02899	0.04006	0.4694
BNP:NT-proBNP	rs6540997_G	-0.02637	0.0508	0.6037
BNP:NT-proBNP	rs4845875_C	0.04523	0.02601	0.0821
BNP:NT-proBNP	rs61761991_T	1.085	0.05833	3.33×10^{-77}
BNP:NT-proBNP	rs12406089_G	-0.1126	0.03479	0.0012

Multivariate models of SNP-natriuretic peptide association with all identified SNPs on chromosome included simultaneously. Trait association tested using geographical sampling region, age, age^2, sex, current smoking status (yes or no), systolic blood pressure, estimated glomerural filtration rate, and
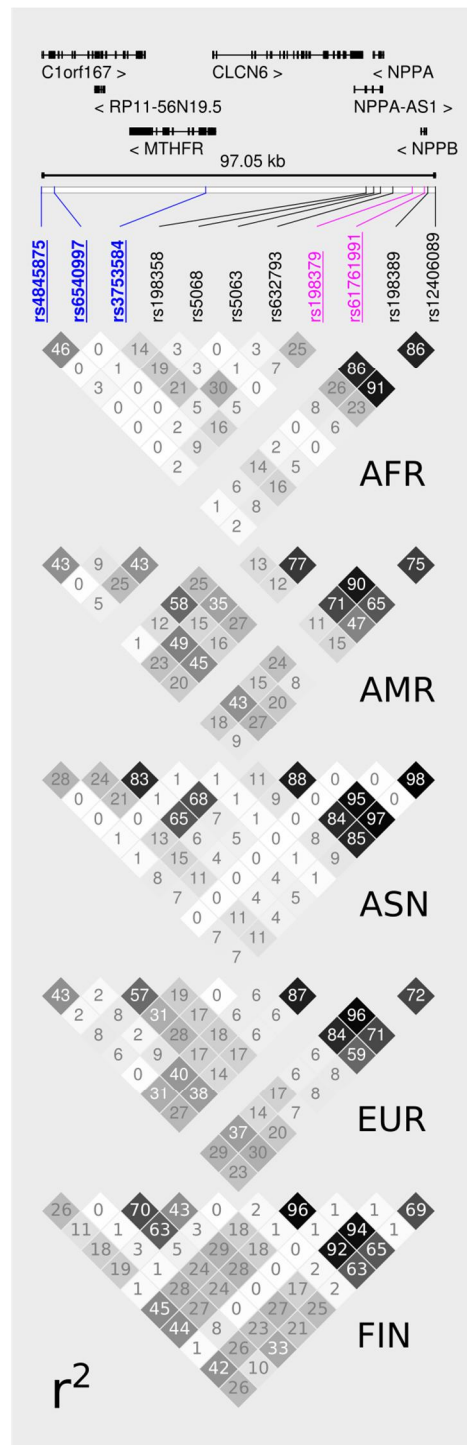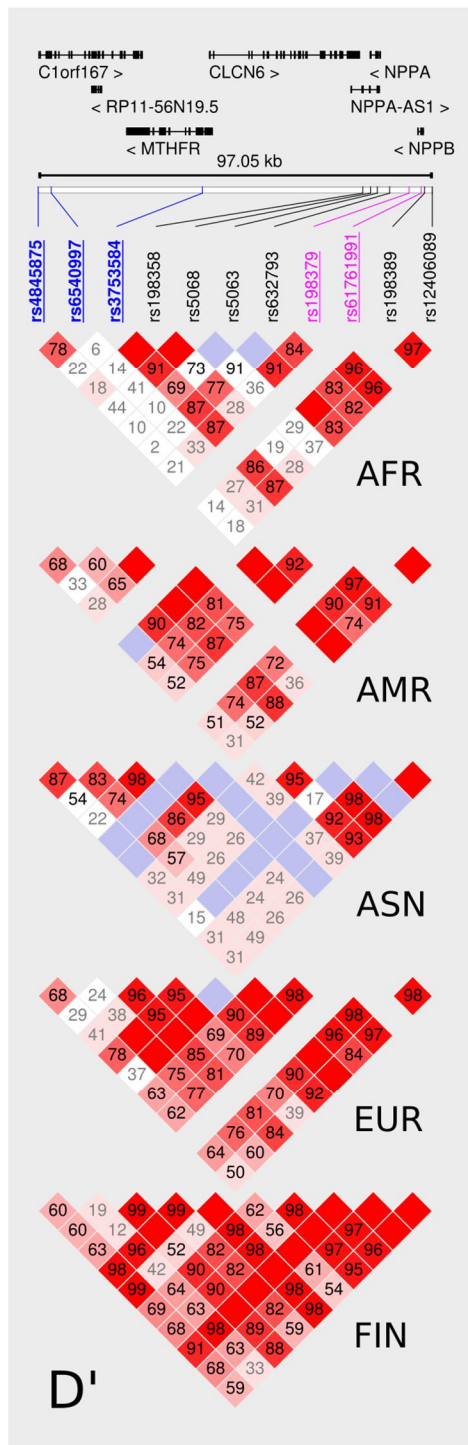
genotyping batch, and the SNPs (rs198379, rs3753584, rs6540997, rs4845875, rs61761991 and

rs12406089) as the independent variables.
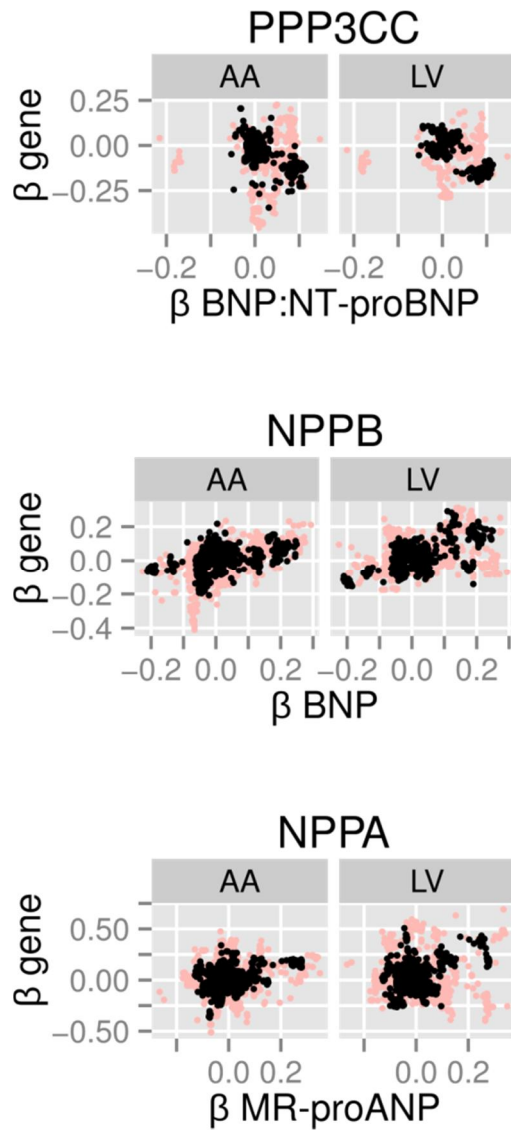
Supplemental Figures
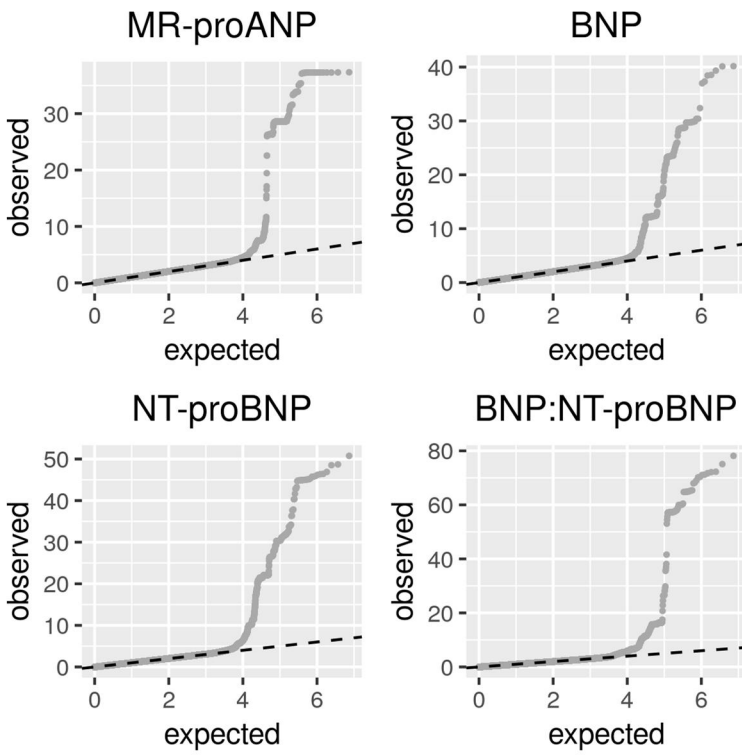
Supplemental Figure 1. Expected and observed -$\log_{10}$(P-value) distributions of the GWAS.

Supplemental Figure 2. Linkage disequilibrium on chromosome 1 near *NPPA* and *NPPB*

Linkage disequilibrium between SNPs on chromosome 1 near *NPPA* and *NPPB*. Genes are depicted as annotated in GENCODE v19 in Ensembl. R-squared and D' calculated with Haploview v4.2 from 22,374 unrelated Finnish samples for Finns and from genotype data published by the 1000 Genomes Project for people of African (AFR), American (AMR), Asian (ASN), and European (EUR) ancestry. SNPs independently associated with MR-proANP colored with blue, SNPs independently associated with BNP or NT-proBNP colored in pink. SNPs associated with MR-proANP or NT-proBNP in previous studies colored in black.

Supplemental Figure 3. Correlation of SNP effects on natriuretic peptides and gene expression

The effect estimates or betas of SNPs on natriuretic peptides (X-axis) and gene expression (Y-axis). Data

is shown for samples from atrial appendages (AA) and left ventricles (LV). SNPs excluded from the

analysis due to population differences in linkage disequilibrium patterns are shown in light red, SNPs

included in the analysis are shown in black.
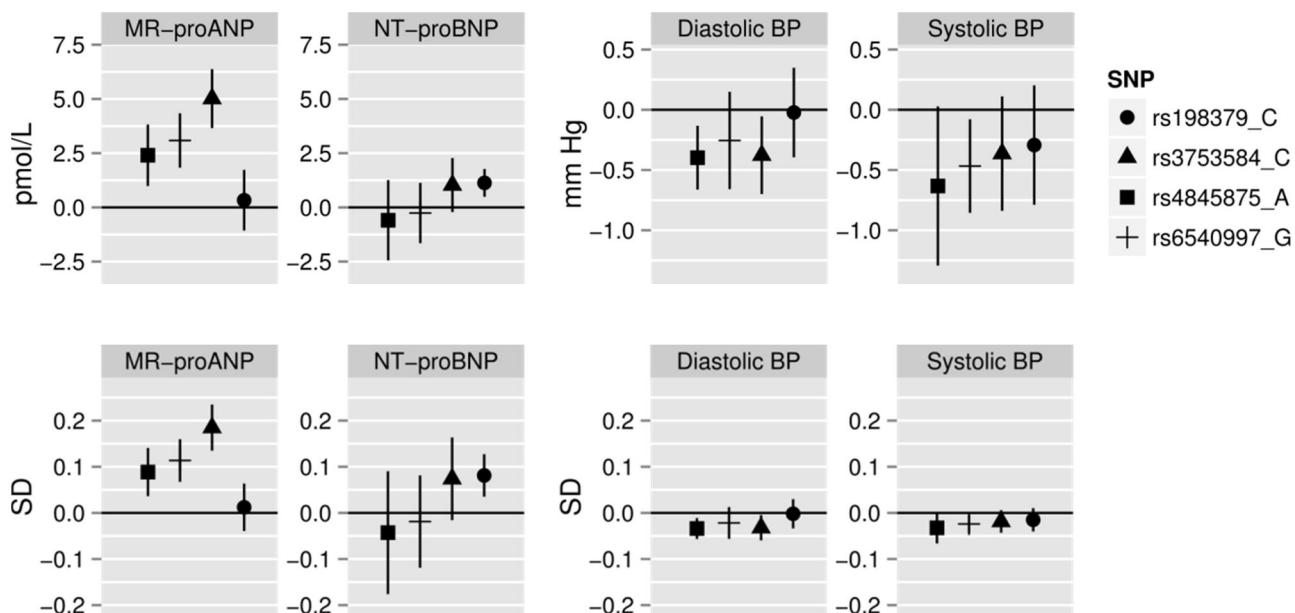
Supplemental Figure 4. Independent relative effects of cis SNPs on natriuretic peptides and blood pressure

The effects of *cis* genetic variants near *NPPA* and *NPPB* on natriuretic peptides and blood pressure (BP)

in regression models including all four SNPs simultaneously. The point estimates of the effects,

represented by the markers, were estimated using untransformed trait values and the 95% confidence

intervals, depicted by the vertical lines, were derived assuming normal distribution of the estimates. NT-

proBNP values were scaled from pg/ml to pmol/L using 0.118 as the scaling factor. For the figures on the

bottom row, the estimates were divided by the standard deviations of each trait. For MR-proANP and

NT-proBNP, the models were adjusted for geographical sampling region, age, age^2, sex, current smoking

status (yes/no), estimated glomerural filtration rate, and genotyping batch. For systolic and diastolic BP,

the models were adjusted for the first two genomic principal components, age, sex, BMI, current BP

medication use (yes/no), cohort year and genotyping batch (the latter two only for the FINRISK

samples).

SD: standard deviation, BP: blood pressure, MR-proANP: mid-regional proatrial natriuretic peptide, NT-

proBNP: aminoterminal pro-B-type natriuretic

Supplemental References

1. Marchini J, Howie B. Genotype imputation for genome-wide association studies. *Nat. Rev. Genet.* 2010;11:499-511.

2. Delaneau O, Marchini J, Zagury J. A linear complexity phasing method for thousands of genomes. *Nature Methods.* 2012;9:179-81.

3. 1000 Genomes Project Consortium, Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, et al. An integrated map of genetic variation from 1,092 human genomes. *Nature.* 2012;491:56-65.

4. Buuren S, Groothuis-Oudshoorn K. Mice: Multivariate imputation by chained equations in R. *Journal of statistical software.* 2011;45:.

5. Schafer JL. Multiple imputation: A primer. *Stat. Methods Med. Res.* 1999;8:3-15.

6. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N. Engl. J. Med.* 2012;367:20-9.

7. Marchini J, Howie B, Myers S, McVean G, Donnelly P. A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat. Genet.* 2007;39:906-13.

8. Viechtbauer W. Conducting meta-analyses in R with the metafor package. *J Stat Softw.* 2010;36:1-48.

9. Sabourin J, Nobel AB, Valdar W. Fine-Mapping additive and dominant SNP effects using Group-LASSO and fractional resample model averaging. *Genet. Epidemiol.* 2015;39:77-88.

10. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, et al. PLINK: A tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* 2007;81:559-75.

11. Yang J, Lee SH, Goddard ME, Visscher PM. GCTA: A tool for genome-wide complex trait analysis. *The American Journal of Human Genetics.* 2011;88:76-82.

12. Del Greco MF, Pattaro C, Luchner A, Pichler I, Winkler T, Hicks AA, et al. Genome-wide association analysis and fine mapping of NT-proBNP level provide novel insight into the role of the MTHFR-CLCN6-NPPA-NPPB gene cluster. *Hum. Mol. Genet.* 2011;20:1660-71.

13. Newton-Cheh C, Larson MG, Vasan RS, Levy D, Bloch KD, Surti A, et al. Association of common variants in NPPA and NPPB with circulating natriuretic peptides and blood pressure. *Nat. Genet.* 2009;41:348-53.

14. Pereira NL, Tosakulwong N, Scott CG, Jenkins GD, Prodduturi N, Chai Y, et al. Circulating atrial natriuretic peptide genetic association study identifies a novel gene cluster associated with reduced NT-proANP, increased stroke and higher diastolic blood pressure. *BMC Pharmacology and Toxicology.* 2015;16:A37.

15. Pfister R, Sharp S, Luben R, Welsh P, Barroso I, Salomaa V, et al. Mendelian randomization study of B-type natriuretic peptide and type 2 diabetes: Evidence of causal association from population studies. *PLoS Med.* 2011;8:e1001112.

16. Folkersen L, Fauman E, Sabater-Lleal M, Strawbridge RJ, Frånberg M, Sennblad B, et al. Mapping of 79 loci for 83 plasma protein biomarkers in cardiovascular disease. *PLoS Genetics.* 2017;13:e1006706.

17. Johansson A, Eriksson N, Lindholm D, Varenhorst C, James S, Syvanen AC, et al. Genome-wide association and mendelian randomization study of NT-proBNP in patients with acute coronary syndrome. *Hum. Mol. Genet.* 2016;25:1447-56.

18. Musani SK, Fox ER, Kraja A, Bidulescu A, Lieb W, Lin H, et al. Genome-wide association analysis of plasma B-type natriuretic peptide in blacks: The jackson heart study. *Circ. Cardiovasc. Genet.* 2015;8:122-30.