Association of Common Variants in *NPPA* and *NPPB* with Circulating Natriuretic Peptides and Blood Pressure

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Supplementary Material

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Supplementary Note

In the original Framingham Unrelated sample, 21 out of 1561 (1.4%) rs5063 major homozygotes had an ANP level below the lower limit of detection compared with 7 out of 8 (87.5%) rs5063 minor homozygotes. Because rs5063 is a missense SNP encoding an amino acid change within the binding site of the ANP assay (targeted against N-terminal pro-ANP) used in Framingham but not in Malmö or Finrisk97,[1] we hypothesized that the association in Framingham was artifactual. To test this hypothesis, we selected 12 major homozygotes randomly and the 7 minor homozygotes with values below the lower limit of detection in the Framingham Unrelated sample and determined mature ANP levels using an assay that does not bind to the region containing rs5063. Mature ANP levels did not differ significantly between the major and minor homozygotes (geometric means 18.2 vs. 19.3 pg/ml, Kruskal-Wallis $P = 0.97$). Thus, the association of rs5063 with N-ANP concentration in the Framingham samples was likely attributable to an amino acid change within the epitope of the specific assay used, with no alteration in concentration of the mature peptide. Missense SNPs have received particular attention because of their greater likelihood of being functional compared with non-coding SNPs. This example serves as a cautionary tale and illustrates the potential pitfall of relating missense variants (or strongly correlated variants) in a gene with the concentration of its protein product.

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Supplementary Methods

Tag SNP Selection. We characterized linkage disequilibrium (LD) patterns in 93 individuals from the CEPH collection (European ancestry) from 12 multigenerational pedigrees; the 48 founders from the 12 pedigrees included 96 independent chromosomes. We identified SNPs for LD characterization from public databases including dbSNP and Celera, and from exonic resequencing in a multi-ethnic discovery collection of 20 individuals to identify coding variants. We attempted genotyping of 96 SNPs, of which 63 passed quality control, 48 of which had MAF ≥ 0.05 (**Supplementary Table 1**). Using these 48 SNPs spanning 44kb (937bp average interSNP distance), we defined blocks of strong linkage disequilibrium using the 'solid spine of LD' method implemented in HaploView v2.03. We then phased the chromosomes using an expectation-maximization algorithm implemented in TagSNPs and selected a set of SNPs within blocks of strong LD that captured all haplotypes with frequency ≥5% at an r^2 ≥0.8 using TagSNPs.[2] All SNPs in gaps between blocks of strong LD were selected for genotyping. The 13 SNPs selected through this haplotype-tagging approach captured 97% of the 48 SNPs at r^2 >0.5, and 89% at r2>0.8, with a mean maximal r^2 of 0.95, as assessed using Tagger (implemented in HaploView v4.0).[3,4] We genotyped the 13 tag SNPs in the Framingham Unrelated individuals (sample 1).

Determination of circulating natriuretic peptide concentrations. In the Framingham participants, plasma N-terminal pro-ANP and BNP concentrations were measured at the 6th examination cycle (1995-98) using noncompetitive immunoradiometric assays targeting amino acids 26-50 of N-terminal proANP and mature BNP, respectively (Shionogi, Osaka, Japan).[1,5] Fasting samples were centrifuged, and the plasma frozen at -70°C before assay. Inter-assay coefficients of variation were 12.7% for N-ANP and 12.2% for BNP. In a subset analysis, we assayed mature ANP concentrations in 12 major homozygotes and 7 minor homozygotes for the missense SNP rs5063, using a second plasma sample from the Framingham 6th examination cycle. Mature ANP levels were determined using an immunoradiometric sandwich assay targeting amino acids 129-151 (Shionogi, Osaka, Japan).[6,7] In the Malmö and Finrisk97 samples, plasma N-terminal proANP was determined using an immunoluminometric sandwich assay targeted against the mid-region of the peptide, amino acids 53-90 (BRAHMS, AG, Berlin, Germany),[8] with an inter-assay coefficient of variation of 10%. In Malmö, N-terminal pro-BNP was determined using the Dimension RxL N-BNP (Dade-Behring, Germany), with an inter-assay coefficient of variation of 2.7%. In Finrisk97, plasma BNP concentration was determined using the Abbott AxSYM BNP assay, with an inter-assay coefficient of variation of 8.1%. Throughout the text we refer to the above assays as ANP or BNP, except where noted.

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Supplementary Table 1: **SNP genotyping results for 48 polymorphic SNPs in CEPH sample**

SNP #	rs #	Amino acid change	Position (hg17)*	HW p-value	Call rate %	Mendelian inconsistencies	MAF	Alleles	*NPPA* function†	*NPPB* function
1	rs198402		11818932	1	97.8	0	0.17	A:G	3'	3'
2	rs2075538		11830868	1	98.9	0	0.10	A:G	3'	3'
3	rs198403		11830920	1	100	0	0.09	C:T	3'	3'
4	rs198404		11831093	1	97.8	0	0.09	A:T	3'	3'
5	rs198405		11831537	1	95.7	0	0.14	A:G	3'	3'
6	rs198406		11831858	1	93.5	0	0.45	T:C	3'	3'
7	rs2075539		11832024	1	98.9	0	0.05	C:T	3'	3'
8	rs198408		11832396	1	80.6	0	0.44	A:T	3'	3'
9	rs169158		11832494	1	92.5	1	0.14	A:G	3'	3'
10	rs2272803		11833055	1	97.8	0	0.05	C:A	3'	3'
11	rs198409		11833115	1	98.9	0	0.17	A:C	3'	3'
12	rs1023252		11833299	1	95.7	0	0.26	C:A	3'	3'
13	rs198411		11834651	1	98.9	0	0.16	G:A	3'	3'
14	rs198412		11834703	1	100	0	0.09	A:G	3'	3'
15	rs198413		11835068	1	94.6	1	0.14	A:G	3'	3'
16	rs198415		11835858	1	97.8	0	0.17	A:G	3'	3'
17	rs14078		11837276	1	92.5	1	0.10	C:T	3'	3'
18	rs198357		11838151	1	96.8	0	0.16	G:T	3'	3'
19	rs198358		11838342	0.13	100	0	0.27	A:G	3'	3'
20	rs198359		11838450	1	79.6	0	0.15	A:G	3'	3'
21	rs198360		11838835	1	97.8	0	0.17	A:G	3'	3'
22	rs198361		11839899	0.97	100	0	0.19	A:G	3'	3'
23	rs5068		11840240	1	89.2	0	0.06	T:C	3' UTR	3'
24	rs5067		11840247	1	83.9	1	0.15	T:C	3' UTR	3'
25	rs5066		11840261	0.37	78.5	1	0.08	G:T	3' UTR	3'
26	rs5065	*152R	11840334	1	75.3	0	0.15	T:C	Nonsense	3'
27	rs5063	V32M	11841914	1	98.9	0	0.05	G:A	Missense	3'
28	rs198372		11843780	1	97.8	0	0.17	C:T	5'	3'
29	rs198373		11843801	1	96.8	0	0.17	T:C	5'	3'
30	rs632793		11844943	1	97.8	0	0.47	T:C	5'	3'
31	rs577040		11845452	1	100	0	0.18	G:T	5'	3'
32	rs2981953		11846110	0.06	80.6	0	0.41	A:G	5'	3'
33	rs2981954		11846718	0.94	98.9	0	0.19	C:T	5'	3'
34	rs198374		11847835	1	97.8	0	0.18	C:T	5'	3'
35	rs198375		11848023	1	100	0	0.47	A:G	5'	3'
36	rs198378		11848462	1	95.7	0	0.19	T:C	5'	3'
37	rs6668352		11849095	0.75	100	0	0.29	C:T	5'	3'
38	rs198381		11850015	1	98.9	0	0.18	T:C	5'	3'
39	rs616308		11850152	0.94	94.6	1	0.19	C:T	5'	3'
40	rs198387		11851184	1	97.8	0	0.18	T:C	5'	3'
41	rs198388		11851606	0.82	93.5	0	0.50	G:A	5'	3'
42	rs198389		11853537	0.94	97.8	0	0.50	T:C	5'	5'
43	rs3753581		11854455	0.45	77.4	0	0.38	G:T	5'	5'
44	rs3753580		11855314	0.99	97.8	0	0.38	A:G	5'	5'
45	rs12406089		11855447	1	83.9	0	0.37	G:C	5'	5'
46	rs6668659		11856564	0.97	94.6	0	0.36	A:C	5'	5'
47	rs6676300		11859566	0.66	89.2	0	0.37	T:C	5'	5'
48	rs1009592		11862980	1	94.6	0	0.38	G:C	5'	5'

Thirteen SNPs selected for genotyping in the stage 1 Framingham sample are underlined. CEPH = Centre d'Etude du Polymorphisme Humain (96 chromosomes), SNP = single nucleotide polymorphism, HW = Hardy-Weinberg equilibrium, MAF = minor allele frequency. *Position in May 2004 freeze of genome (hg17). †Function relative to cDNA NM_006172 for *NPPA* and NM_002521 for *NPPB*.

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Supplementary Table 2. **Covariate relationships with ANP and BNP in multivariable regression models**

ANP (lnANP)	Framingham Heart Study N=3,398		Malmö Diet and Cancer N=5,203		Finrisk97 N=7,722	
	Beta (SE)	*P*	Beta (SE)	*P*	Beta (SE)	*P*
age (per 10 years)	0.20 (0.01)	<0.0001	0.21 (0.01)	<0.0001	0.19 (0.004)	<0.0001
sex (women compared to men)	0.23 (0.02)	<0.0001	0.13 (0.01)	<0.0001	0.15 (0.01)	<0.0001
body mass index (per 5 kg/m2)	-0.07 (0.009)	<0.0001	-0.04 (0.01)	<0.0001	-0.03 (0.01)	<0.0001
diabetes	-0.15 (0.03)	<0.0001	-0.13 (0.02)	<0.0001	0.02 (0.02)	0.25
systolic blood pressure (per 10 mmHg)	0.03 (0.006)	<0.0001	0.02 (0.01)	<0.0001	0.03 (0.003)	<0.0001
diastolic blood pressure (per 10 mmHg)	-0.10 (0.01)	<0.0001	-0.04 (0.01)	<0.0001	-0.04 (0.01)	<0.0001
antihypertensive therapy	0.14 (0.02)	<0.0001	0.18 (0.02)	<0.0001	0.18 (0.01)	<0.0001
prevalent myocardial infarction	0.34 (0.05)	<0.0001	0.24 (0.04)	<0.0001	0.40 (0.03)	<0.0001
atrial fibrillation	0.52 (0.08)	<0.0001	NA	NA	NA	NA
serum creatinine (per 1 mg/dL)	0.42 (0.05)	<0.0001	NA	NA	NA	NA
BNP (lnBNP)	Framingham Heart Study		Malmö Diet and Cancer		Finrisk97	
	Beta (SE)	*P*	Beta (SE)	*P*	Beta (SE)	*P*
age (per 10 years)	0.34 (0.02)	<0.0001	0.28 (0.02)	<0.0001	0.32 (0.01)	<0.0001
sex (women compared to men)	0.38 (0.04)	<0.0001	0.39 (0.03)	<0.0001	0.46 (0.02)	<0.0001
body mass index (per 5 kg/m2)	-0.07 (0.02)	<0.0001	-0.11 (0.02)	<0.0001	-0.06 (0.01)	<0.0001
diabetes	-0.13 (0.07)	0.07	-0.16 (0.05)	0.001	0.05 (0.05)	0.26
systolic blood pressure (per 10 mmHg)	0.10 (0.01)	<0.0001	0.04 (0.01)	<0.0001	0.07 (0.01)	<0.0001
diastolic blood pressure (per 10 mmHg)	-0.20 (0.03)	<0.0001	-0.05 (0.02)	0.02	-0.14 (0.01)	<0.0001
antihypertensive therapy	0.16 (0.05)	<0.01	0.30 (0.04)	<0.0001	0.30 (0.03)	<0.0001
prevalent myocardial infarction	0.95 (0.11)	<0.0001	0.51 (0.10)	<0.0001	0.85 (0.08)	<0.0001
atrial fibrillation	0.93 (0.17)	<0.0001	NA	NA	NA	NA
serum creatinine (per 1 mg/dL)	0.24 (0.11)	<0.05	NA	NA	NA	NA

Shown are the effect estimates (beta coefficients), standard errors (SE) and P-values for covariates used in natriuretic peptide analyses from multivariable linear regression models of natural log(natriuretic peptide). Atrial fibrillation and creatinine were only available in the Framingham Heart Study. Sample 1 (Framingham Unrelated) and Sample 2 (Framingham Related) are subsets of the 3,398 individuals used to examine clinical covariates shown here. NA = not available.

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Supplementary Table 3. Natriuretic peptide association results (stage 1, Framingham Unrelated Sample)

SNP ID	Position relative to *NPPA*	Position relative to *NPPB*	Minor allele frequency	ANP association p-value	BNP association p-value
rs169158	3'	3'	0.14	$1x10^{-5}$	$6x10^{-4}$
rs1023252	3'	3'	0.25	$5x10^{-7}$	0.07
rs198412	3'	3'	0.08	0.51	0.08
rs198415	3'	3'	0.14	$4x10^{-5}$	0.003
rs198358	3'	3'	0.19	$2x10^{-5}$	0.001
rs5068	3' UTR	3'	0.04	$4x10^{-6}$	0.18
rs5063	Missense	3'	0.04	$7x10^{-55}$	0.003
rs632793	5'	3'	0.39	0.05	$2x10^{-8}$
rs577040	5'	3'	0.13	0.01	$6x10^{-4}$
rs198375	5'	3'	0.40	0.07	2E-06
rs198387	5'	3'	0.14	0.001	$4x10^{-5}$
rs198388	5'	3'	0.42	0.39	$2x10^{-6}$
rs6676300	5'	5'	0.36	0.07	0.007

Results for 13 *NPPA*/*NPPB* tag SNPs with ANP and BNP levels in 1,705 FHS Unrelated participants (sample 1). SNPs were tested individually for association with multivariable-adjusted log ANP and log BNP separately using a two degree-of-freedom general model test, as described in the text.

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Supplementary Table 4: Association results in stage 1 after adjustment for the SNP with lowest p-value for ANP and BNP, separately.

SNP #	rs ID	ANP		BNP	
		p-value	p-value adjusting for rs5063 genotype	p-value	p-value adjusting for rs632793 genotype
9	rs169158	$1.7x10^{-6}$	0.0003	0.001	0.14
12	rs1023252	$3.0x10^{-7}$	0.68	0.12	0.12
14	rs198412	0.32	0.75	0.048	0.47
16	rs198415	$4.1x10^{-6}$	0.0004	0.003	0.33
19	rs198358	$9.8x10^{-7}$	$6.5x10^{-8}$	0.0009	0.40
23	rs5068	$5.2x10^{-7}$	$6.4x10^{-6}$	0.17	0.35
27	rs5063	$4.6x10^{-57}$	-	0.02	0.31
30	rs632793	0.12	0.002	$1.8x10^{-8}$	-
31	rs577040	0.003	0.008	0.0004	0.27
35	rs198375	0.17	0.006	$8.6x10^{-7}$	0.15
40	rs198387	0.0005	0.002	$3.3x10^{-5}$	0.12
41	rs198388	0.54	0.003	$2.8x10^{-7}$	0.71
47	rs6676300	0.18	0.01	0.004	0.07

Association results before and after adjustment for SNP with lowest p-value for ANP and BNP, respectively (rs5063, rs632793). All models adjusted for age, sex, and clinical covariates as detailed in the text. Based on these 2-SNP analyses and on the linkage disequilibrium patterns (data not shown), SNPs rs5068, rs198358, rs5063, and rs632793 were carried forward into stage 2.

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Supplementary Table 5. Association results for natriuretic peptide concentrations under 3 genetic models, by cohort (stage 2).

	Framingham 1 n=1,705 beta (SE) *P*	Framingham 2 n=751 beta (SE) *P*	Malmö n=5,196 beta (SE) *P*	Finrisk97 n=7,091 beta (SE) *P*	Pooled beta (SE)	Pooled P-value
ANP						
rs5068 dominant	0.42 (0.08) $7x10^{-7}$	0.35 (0.20) 0.08	0.41 (0.04) $2x10^{-21}$	0.42 (0.03) $7x10^{-44}$	0.42 (0.02)	$8x10^{-70}$
rs5068 additive	--	--	--	--	--	--
rs5068 recessive	--	--	--	--	--	--
rs198358 dominant	0.21 (0.05) $5x10^{-5}$	-0.11 (0.07) 0.14	0.13 (0.03) $2x10^{-5}$	0.27 (0.02) $1x10^{-28}$	0.20 (0.02)	$8x10^{-30}$
rs198358 additive	0.13 (0.04) 0.002	-0.06 (0.06) 0.34	0.04 (0.03) $1x10^{-4}$	0.25 (0.02) $1x10^{-32}$	0.17 (0.01)	$1x10^{-29}$
rs198358 recessive	-0.10 (0.12) 0.38	0.12 (0.16) 0.48	0.06 (0.07) 0.37	0.44 (0.06) $2x10^{-12}$	0.21 (0.04)	$5x10^{-7}$
rs632793 dominant	-0.01 (0.05) 0.79	-0.09 (0.08) 0.21	0.09 (0.03) 0.002	0.12 (0.02) $1x10^{-6}$	0.08 (0.02)	$9x10^{-7}$
rs632793 additive	-0.05 (0.04) 0.15	-0.07 (0.06) 0.21	0.08 (0.02) $5x10^{-5}$	0.12 (0.02) $2x10^{-11}$	0.08 (0.01)	$2x10^{-10}$
rs632793 recessive	-0.16 (0.07) 0.02	-0.07 (0.11) 0.52	0.14 (0.04) $3x10^{-4}$	0.22 (0.04) $3x10^{-10}$	0.05 (0.03)	0.09

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BNP						
rs5068 dominant	0.10 (0.08) 0.22	-0.02 (0.19) 0.92	0.15 (0.04) 0.001	0.19 (0.03) $8x10^{-10}$	0.17 (0.02)	$3x10^{-12}$
rs5068 additive	--	--	--	--	--	--
rs5068 recessive	--	--	--	--	--	--
rs198358 dominant	0.18 (0.05) $3x10^{-4}$	0.22 (0.07) 0.003	0.07 (0.03) 0.02	0.25 (0.02) $7x10^{-23}$	0.18 (0.02)	$4x10^{-54}$
rs198358 additive	0.16 (0.04) $2x10^{-4}$	0.23 (0.06) $1x10^{-4}$	0.04 (0.03) 0.09	0.22 (0.02) $1x10^{-25}$	0.15 (0.01)	$2x10^{-25}$
rs198358 recessive	0.24 (0.12) 0.05	0.54 (0.16) 0.004	-0.04 (0.07) 0.55	0.38 (0.06) $2x10^{-9}$	0.21 (0.04)	$3x10^{-7}$
rs632793 dominant	0.28 (0.05) $1x10^{-8}$	0.32 (0.07) $1x10^{-5}$	0.16 (0.03) $5x10^{-8}$	0.32 (0.02) $6x10^{-40}$	0.26 (0.02)	$4x10^{-54}$
rs632793 additive	0.19 (0.03) $2x10^{-8}$	0.30 (0.05) $4x10^{-8}$	0.13 (0.02) $1x10^{-9}$	0.27 (0.02) $1x10^{-53}$	0.21 (0.02)	$2x10^{-68}$
rs632793 recessive	0.20 (0.07) 0.003	0.48 (0.11) $1x10^{-4}$	0.17 (0.04) $2x10^{-5}$	0.41 (0.04) $2x10^{-31}$	0.30 (0.03)	$1x10^{-35}$

Shown are the sample-specific effect estimates, standard error (SE) and P value for the tests of association with natriuretic peptide levels under dominant, additive and recessive models for 3 SNPs in samples 1-4 for ANP and BNP (Framingham Unrelated, Framingham Related, Malmö Diet and Cancer, Finrisk97). Meta-analysis was performed using inverse variance weights. Results for rs5063 are not shown (see text

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for discussion). Only dominant models were tested for SNP rs5068 because of its low minor allele frequency and the rarity of minor allele homozygotes. Effect estimates are shown for log-transformed natriuretic peptide concentration after adjustment for covariates (see Methods) on the standard deviation scale. Effects for additive models are shown per minor allele copy. Effects for dominant models are shown for heterozygotes and minor homozygotes compared to major homozygotes. Effects for recessive models are shown for minor homozygotes compared to major homozygotes and heterozygotes. -- = Not available

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Supplementary Table 6. Natriuretic peptide concentration and blood pressure by genotype

		Genotype N (frequency)				ANP Mean (SEM)			BNP Mean (SEM)			SBP Mean (SEM)				DBP Mean (SEM)				Hypertension			
		FHS	MDC	FR97	MPP	FHS	MDC	FR97	FHS	MDC	FR97	FHS	MDC	FR97	MPP	FHS	MDC	FR97	MPP	FHS	MDC	FR97	MPP
rs5068	TT	1,874 92%	5,206 88%	6,227 81%	12,812 88%	379 (5.7)	71.7 (0.5)	50.0 (0.4)	15.5 (0.5)	98.3 (3.0)	24.8 (0.7)	129.0 (0.4)	141.6 (0.3)	136.1 (0.3)	127.2 (0.1)	75.7 (0.2)	87.1 (0.1)	82.5 (0.1)	85.6 (0.1)	0.42	0.64	0.48	0.73
rs5068	TC	156 8%	683 11%	1,347 18%	1,613 11%	452 (21.3)	83.8 (1.7)	58.6 (1.0)	16.7 (1.7)	117.3 (8.0)	27.8 (1.1)	126.4 (1.5)	139.8 (0.7)	135.1 (0.5)	125.1 (0.4)	73.7 (0.8)	86.1 (0.4)	82.3 (0.3)	84.8 (0.2)	0.37	0.59	0.45	0.70
rs5068	CC	5 0.2%	14 0.2%	68 0.9%	44 0.3%	467 (139)	70.7 (6.0)	85.2 (18.6)	8.5 (4.2)	62.4 (22.5)	65.4 (31.8)	134.6 (12.8)	138.4 (4.1)	134.1 (2.5)	127.7 (2.1)	72.8 (3.7)	85.6 (2.1)	80.9 (1.3)	84.1 (1.3)	0.40	0.79	0.49	0.73
rs198358	AA	1386 63%	4,034 69%	4,925 65%	9,317 68%	374 (6.0)	71.8 (0.6)	49.7 (0.5)	0.63 (0.5)	98.0 (2.7)	23.7 (0.7)	128.5 (0.5)	141.8 (0.3)	136.3 (0.3)	127.0 (0.1)	75.5 (0.3)	87.1 (0.1)	82.6 (0.2)	85.5 (0.1)	0.42	0.64	0.49	0.73
rs198358	AG	699 32%	1,563 26%	2,389 32%	3,728 27%	401 (10.7)	75.9 (1.1)	54.8 (1.0)	0.32 (0.9)	110.8 (8.1)	28.6 (1.2)	127.1 (0.7)	140.3 (0.5)	135.4 (0.4)	126.6 (0.2)	75.3 (0.4)	86.8 (0.2)	82.1 (0.2)	85.1 (0.1)	0.39	0.63	0.45	0.71
rs198358	GG	100 5%	276 5%	280 4%	680 5%	397 (29.1)	74.2 (2.1)	65.2 (18.6)	0.046 (2.7)	87.8 (6.1)	39.8 (7.9)	128.9 (2.0)	140.8 (1.1)	134.8 (1.1)	127.3 (0.5)	74.8 (0.9)	85.8 (0.5)	82.5 (0.7)	85.5 (0.4)	0.42	0.62	0.46	0.77
rs632793	TT	833 37%	2,112 35%	3,136 41%	NA	373 (7.7)	71.5 (0.8)	50.2 (0.6)	12.3 (0.5)	93.2 (3.6)	22.4 (1.0)	128.9 (0.7)	141.8 (0.4)	135.9 (0.4)	NA	75.8 (0.3)	87.3 (0.2)	82.4 (0.2)	NA	0.44	0.65	0.48	NA
rs632793	TC	1,089 49%	2,903 49%	3,499 46%	NA	388 (8.0)	73.8 (0.7)	51.9 (0.6)	16.5 (0.7)	103.5 (4.6)	26.5 (0.9)	127.8 (0.6)	141.3 (0.4)	135.8 (0.3)	NA	75.2 (0.3)	87.0 (0.2)	82.4 (0.2)	NA	0.39	0.64	0.47	NA
rs632793	CC	312 14%	933 16%	981 13%	NA	373 (14.1)	76.0 (1.2)	57.9 (1.7)	19.4 (1.5)	112.6 (7.3)	34.4 (2.5)	128.8 (1.1)	140.3 (0.6)	136.7 (0.6)	NA	75.2 (0.5)	86.4 (0.3)	82.8 (0.4)	NA	0.41	0.60	0.48	NA

Shown are the cohort-specific unadjusted means (and standard errors of the mean, SEM) by genotype for rs5068, rs198358, rs632793 of ANP, BNP, systolic blood pressure (SBP), and diastolic blood pressure (DBP). Also shown is the prevalence of hypertension by genotype. Framingham Heart Study (FHS) samples 1 and 2 are combined for display purposes. The small sample sizes of the rs5068 minor homozygote class resulted in high standard errors reflecting imprecise mean estimates. In natriuretic peptide association analyses, dominant models were supported for rs5068 and rs198358 and an additive model for rs632793 (**Supplementary Table 5**).

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Supplementary Table 7. **Association results for blood pressure, by cohort (stages 3 and 4).**

	Framingham Unrelated n=1,705 beta (SE) *P*	Framingham Related n=751 beta (SE) *P*	Malmö n=5,196 beta (SE) *P*	Finrisk97 n=7,549 beta (SE) *P*	Malmö Preventive Project n=14,516 Beta SE *P*	Pooled n=29,717 beta (SE)	Pooled P-value
Systolic blood pressure							
rs5068 dominant	-0.15 (0.09) 0.08	-0.22 (0.18) 0.23	-0.13 (0.04) 0.001	-0.05 (0.03) 0.07	-0.07 (0.03) 0.005	-0.08 (0.02)	$2x10^{-6}$
rs198358 dominant	-0.10 (0.05) 0.05	-0.01 (0.07) 0.85	-0.09 (0.03) 0.002	-0.05 (0.02) 0.03	-0.03 (0.02) 0.10	-0.05 (0.01)	$6x10^{-5}$
rs632793* additive	0.01 (0.04) 0.86	0.06 (0.05) 0.26	-0.06 (0.02) 0.003	0.002 (0.02) 0.88	NA	-0.02 (0.01)	0.16
Diastolic blood pressure							
rs5068 dominant	-0.22 (0.09) 0.01	-0.24 (0.19) 0.21	-0.12 (0.04) 0.002	-0.02 (0.03) 0.46	-0.09 (0.03) $1x10^{-4}$	-0.08 (0.02)	$1x10^{-6}$
rs198358 dominant	-0.11 (0.05) 0.03	-0.07 (0.07) 0.34	-0.07 (0.03) 0.01	-0.04 (0.02) 0.10	-0.04 (0.02) 0.03	-0.05 (0.01)	$5x10^{-5}$
rs632793* additive	-0.02 (0.04) 0.55	-0.02 (0.04) 0.12	-0.06 (0.02) 0.001	0.01 (0.02) 0.70	NA	-0.02 (0.01)	0.12

*Pooled results for rs632793 derive from 15,201 individuals from samples 1-4.
Shown are the effects of minor alleles under either a dominant or additive model (based on best model from natriuretic peptide level analyses) on systolic and diastolic blood pressure residuals after adjustment for age, sex, body mass index, and imputation of blood pressure in individuals on anti-hypertensive treatment. All effect sizes are shown on the standard deviation scale for comparability. Nominal 2-tailed *P*-values are shown. SE = standard error

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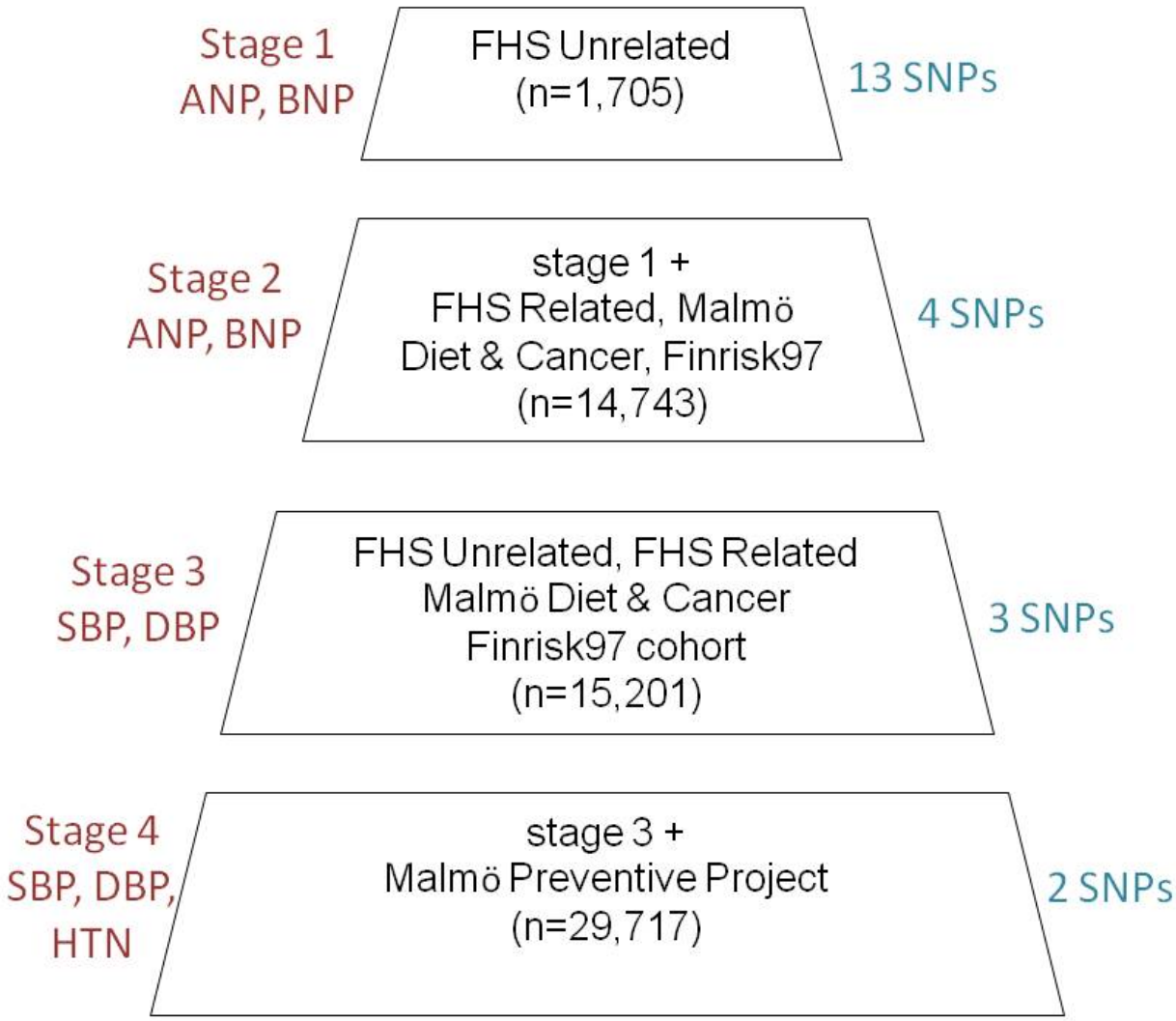
Supplementary Figure 1



Supplementary Figure 1. Study design. In stage 1, 13 SNPs across the *NPPA/NPPB* locus were tested for association with natriuretic peptide concentration in 1,705 Framingham Unrelated individuals (sample 1). In stage 2, three SNPs with nominal evidence of association with ANP and one SNP associated with BNP were tested for natriuretic peptide association in 3 additional samples: Framingham Related (sample 2, n=751), Malmö Diet and Cancer (sample 3, n=5,196), and Finrisk97 (sample 4, n=7,091). In stage 3, the three SNPs that met a stringent significance threshold for association with either ANP or BNP were tested for association with systolic and diastolic blood pressure in all 4 samples, in total comprising 15,201 individuals. In stage 4, two

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SNPs associated with blood pressure were tested in an additional 14,516 individuals from the Malmö Preventive Project (sample 5, n=14,516), with total sample size across 5 samples for blood pressure analyses of 29,717 individuals. ANP = atrial natriuretic peptide, BNP = B-type natriuretic peptide, SBP = systolic blood pressure, DBP = diastolic blood pressure, HTN = hypertension, SNP = single nucleotide polymorphism.

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