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# Association of Genetic Variation with Gene Expression and Protein Abundance within the Natriuretic Peptide Pathway

**David E. Lanfear**[1,3,*], **Bipin Sunkara**[1], **Jia Li**[2], **Sharad Rastogi**[1], **Ramesh C. Gupta**[1], **Badri Padhukasahasram**[3], **L. Keoki Williams**[3], and **Hani N. Sabbah**[1]

[1]Department of Internal Medicine, Division of Cardiology, Henry Ford Heart and Vascular Institute, Henry Ford Hospital, Detroit, MI, USA

[2]Public Health Sciences, Henry Ford Hospital, Detroit, MI, USA

[3]Center for Health Services and Policy Research, Henry Ford Hospital, Detroit, MI, USA

## Abstract

The natriuretic peptide (NP) system is a critical physiologic pathway in heart failure with wide individual variability in functioning. We investigated the genetic component by testing the association of single nucleotide polymorphisms (SNP) with RNA and protein expression. Samples of DNA, RNA, and tissue from human kidney (n=103) underwent genotyping, RT-PCR, and protein quantitation (in lysates), for four candidate genes (NP-receptor 1 [*NPR1*], *NPR2, NPR3* and membrane metallo-endopeptidase [*MME*]). The association of genetic variation with expression was tested using linear regression for individual SNPs, and a principal components (PC) method for overall gene variation. Eleven SNPs in *NPR2* were significantly associated with protein expression (false discovery rate ≤0.05), but not RNA quantity. RNA and protein quantity correlated poorly with each other. The PC analysis showed only *NPR2* as significant. Assessment of the clinical impact of *NPR2* genetic variation is needed.

### Keywords

Natriuretic peptide; heart failure; gene expression; pharmacogenomics; nesiritide; genetic polymorphisms

## Background

Heart failure (HF) continues to be an enormous public health problem despite the many advances in its pharmacotherapy over the past 25 years, with a prevalence of 5.7 million individuals affected and an incidence of over 500,000 new cases annually [1], so that better prognostics, improved targeting of current therapies, and novel therapies are still needed. The relevance of the natriuretic peptide (NP) system, particularly B-type NP (BNP), is well known in terms of HF pathophysiology [2,3], diagnosis [4], prognosis [5], and therapy [6,7]. However, the full utility of testing and modulating the natriuretic pathway remains unclear. Part of the difficulty in how to harness this pathway for the benefit of patients is due to substantial inter-individual variability in NP pathway functioning. The optimal diagnostic and prognostic thresholds for testing are uncertain and varying [8], and it has already been shown that a relevant genetic sequence variant can affect BNP test performance [9]. Moreover, the response to extrinsic NP (e.g. nesiritide, carperitide) is also highly variable,

*Corresponding Author: David E. Lanfear, MD, MS, FACC Phone: (313) 916 6375, Fax: (313) 916 8799 dlanfea1@hfhs.org.

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with unclear therapeutic range and the potential for adverse effects [10-12]. This is likely to continue to be a relevant clinical issue as there are numerous investigational NP therapeutics currently under development (e.g. ularitide [13], CDNP [a hybrid designer peptide with aspects of both CNP and DNP] [14]). With all this, and the fact that overall heart failure outcomes continue to be unacceptably poor, greater understanding of NP pathway variability is critical because this knowledge could be leveraged to improve the value of NPs as biomarkers, optimally target use of current NP therapeutic agents, and aid in the development of new interventions for HF.

Genetic variation may hold a key to better understanding of this individual variability [15]. BNP levels are known to be heritable [16], and specific genetic variants in NP pathway genes have been associated with cardiovascular disease [17], BNP level and test performance [18,9], and intracardiac filling pressures [19], and some early data indicates nesiritide clearance is associated with genotype [20]. Yet there is still a lack of systematic knowledge on how genetic variability in the principal genes in this pathway affects gene expression and the production of the relevant protein end-products. The substantial knowledgebase regarding the NP system makes a candidate gene approach initially most reasonable. Briefly, we identified four key genes/proteins that are known to interact with native or infused NPs (Fig. 1) as our principle candidates. Briefly, NPs are thought to act primarily by binding to two membrane-spanning receptors called natriuretic peptide receptor A (NPRA) and B (NPRB), activation of which results in cyclic guanylate mono phosphate (cGMP) production, which is thought to be the key second messenger mediating the NP effects. Active NP is thought to be cleared predominantly by membrane metallo endopeptidase (MME, aka neutral endopeptidase), which can cleave them to inactive forms, and NPRC, a non-catalytic receptor lacking guanylate cyclase domain [15]. These four products are produced by the genes *NPR1, NPR2, MME*, and *NPR3*, respectively. Thus the purpose of this study was to systematically try to determine whether sequence variants in these genes are importantly connected to gene expression and or protein abundance in relevant human tissue, as a way to discover, prioritize, and/or establish biologic plausibility for potential pharmacogenomic associations within the NP system.

## Methods

The study was approved by the Henry Ford Hospital Institutional Review Board. DNA, RNA, and tissue samples from non-cancerous human kidney were obtained via the Alvin J. Siteman Cancer Center at Washington University School of Medicine and Barnes-Jewish Hospital in St. Louis, MO, Tissue Procurement Core lab, under approval from Washington University's Institutional Review Board and with informed consent. Kidney was chosen as target tissue because it is a key site of action for natriuretic peptides, and each of the four candidate genes and proteins are known to be expressed there in significant quantities. We initially sought 100 total subjects each having all three sample types (DNA, RNA, tissue), ideally with equal distribution among African Americans vs. whites and men vs. women. However, due to limitations in availability of suitable samples, we utilized some samples that did not have all 3 types, and a greater proportion of white subjects were included. In total, samples from 103 unique subjects were analyzed; 30% African American, 46% Female. Sixty six individuals had all 3 types of samples, and the number of subjects in each analysis is as follows: DNA:RNA (n= 77), RNA:Protein (n= 70), and DNA:Protein (n= 66).

### Genotyping

DNA samples were genotyped using a custom Illumina Goldengate array which contained candidate-gene coverage relevant to HF including focused attention on the four genes of interest. Single nucleotide polymorphisms (SNP) were chosen for the array by attempting to include all coding variants, and also utilizing HAPMAP to select optimal non-coding (‘tag’)

variants to capture blocks with minor allele frequency >0.1 prevalence in whites or African Americans within the gene regions of interest. After processing requirements for the Goldengate technology and quality control of genotyping, 118 SNPs in the four genes of interest were available for this study. Genotyping calls were made using GenomeStudio software automatic algorithms (Illumina, San Diego, CA), and then individual SNPs were reviewed manually. Sample call rates were >90% and none of the SNPs analyzed deviated significantly from Hardy-Weinberg Equilibrium.

### Gene Expression

Gene expression was assessed by quantifying mRNA for the glyceraldehyde-3-phosphate dehydrogenase gene (comparator), *NPR1, NPR2, NPR3*, and *MME* using real-time reverse transcriptase polymerase chain reaction (PCR), performed in duplicate for each sample. The duplicates were averaged and this result was used in analysis. Commercially available Taqman Gene Expression assays (Life Technologies, Carslbad CA) were utilized for these experiments. These assays are designed so that probes span across an exon junction, ensuring that only cDNA (and not genomic DNA) is detected. The kit numbers used were HS00418568_m1 (*NPR1*), HS00241516_m1 (*NPR2*), Hs01099009_m1 (*NPR3*), and Hs01115451_m1 (*MME*).

### Protein Quantitation

To determine the concentration of the protein targets, tissue samples were made into lysates and then assayed using double antibody sandwich enzyme-linked immunosorbent assays (ELISA). The samples were homogenized by suspending in 1ml phosphate-buffered saline solution and then sonicated. The resulting suspension was centrifuged for 5 minutes at 5000g. The supernatant was then removed and stored at -80°C for testing. The concentration of each marker was determined using commercially available assay kits (USCN Life Science Inc., Missouri City, TX) according to manufacturer protocol and using standard curves and software. Total protein concentrations were determined by using a modified Lowry protein assay. The ratio of target protein to total protein was reported and tested for association with genotype and RNA quantity.

### Statistical Analysis

Following log transformation of the protein and RNA expression data, linear regression was used to test for the association of each SNP with RNA and protein quantity under an additive genetic model. Pearson's correlation coefficient was used to quantify the correlation between RNA and protein expression for each gene. The Locally Weighted Scatterplot Smoothing (LOWSS) was used to help visualize the trend between RNA and protein expressions. A principal components (PC)-based method [21-24] was also used to capture the underlying correlation structure within each gene region and test the association of overall gene variation with RNA and protein quantity. These were performed separately for each candidate gene. We selected top PCs that explain at least 80% of the variation as the gene representation (2 PCs each for *NPR1* and *NPR2*; 7 PCs each for *NPR3* and *MME*). The PCs association with RNA and protein expression was then tested using linear regression. All models were adjusted for gender and race. P values <0.05 were considered of interest in this exploratory study. To account for multiple comparisons we also utilized the method of Hochberg [25] and considered findings with false discovery rate (FDR) ≤0.05 significant (PC analysis was corrected for the number of PCs tested).

## Results

Genotype was obtained for 7 loci in *NPR1*, 18 loci in *NPR2*, 53 loci in *NPR3*, and 40 loci in *MME*. Each SNP was tested individually for association with RNA and protein quantity.

Summary results with linkage maps and unadjusted p-value heat maps are shown in Figure 2. All adjusted and unadjusted p-values are shown in supplementary Table 1. In terms of gene expression, several variants in *MME* and *NPR3* showed crude associations. There were four such SNPs in *MME* (rs1025192, rs1436630, rs10513469, and rs1816558) and one in *NPR3* (rs696831) that showed suggestive associations with RNA levels (all $p<0.05$). However, none of these met significance once adjusted for FDR. There were no significant associations of genotype with gene expression for *NPR1* or *NPR2*.

Considering protein quantitation, we assayed each sample for the ratio of specific protein of interest (NPR1, NPR2, NPR3, and MME) to total protein, and then tested the association of this ratio (i.e. the target protein abundance) with the genotypes within the corresponding gene. There were no significant associations of *NPR1* genotype with protein abundance. There were two sequence variants in *NPR3* (rs696836, rs2062708) and one in *MME* (rs3773895) with significant associations of genotype with protein quantity; however, these did not withstand adjustment for multiple comparisons. Interestingly, 11 SNPs in *NPR2* were significantly associated with protein expression (p values ranging from 0.010 to 0.031), and this association persisted after controlling for multiple comparisons (all FDR = 0.05). Boxplots of protein abundance by genotype for each of the significant loci are shown in Figure 3. There were no SNPs associated with both RNA and protein expression in any of the candidate genes. RNA and protein quantity poorly correlated with each other; NPR1 and MME showed weak but statistically significant positive correlations (Pearson's correlation coefficient=0.23 and 0.26, $p = 0.04$ and 0.03, respectively) while NPR2 and NPR3 did not (Fig. 4).

To try to assess the impact of the composite genetic variation at each candidate gene (as opposed to testing only individual SNPs) we also performed a PC based analysis. After constructing PCs that could account for 80% of genetic variation at a given gene, the PCs were tested in linear regression models for association with RNA and protein expression. The results of PC analyses were broadly consistent with the individual SNP results above. PC1 of *NPR2* (which accounted for 71% of genetic variability) was the only genetic correlate of protein abundance (unadjusted p=0.04), though once adjusted for multiple comparisons this was of borderline significance (FDR = 0.08). The factor loadings for PC1 suggested that it was mainly determined by the same 11 SNPs indicated above, which each had equal weight (data not shown). We also found a crude association between PC5 of *NPR3* and gene expression (p=0.0084, FDR =0.059). The factor loadings indicated that PC5 is contributed by SNPs rs764124, rs1847018, rs10057069, rs6889608, rs696831, and rs2302954.

## Discussion

Our systematic interrogation of genotype, gene expression, and protein quantity correlations revealed that genetic variation may play a key role in determining protein abundance for NPRB. Interestingly, these associations did not seem to occur via changes in gene expression, which did not correlate to either protein quantity or genotype for *NPR2*. This is consistent with existing evidence that gene expression does not always correlate well with protein expression [26], and suggests that post-transcriptional regulation may play an important role. The other genes tested did not show indications of genetic variation importantly affecting gene expression or protein abundance in kidney.

Although numerous studies have examined the relationship of NP pathway genetic polymorphisms to clinical phenotypes, corresponding functional data is less available. While our study is exploratory and descriptive in nature, these data add to the existing knowledge base by describing the potential physiologic impact of candidate variants on gene and

protein expression, and prioritizing these for future investigation. These data could also be used to buttress the biologic plausibility of previously described clinical phenotypes. In terms of prioritizing variants for subsequent clinical interrogation, *NPR2* appears to be the best target. While there were some interesting genotype:gene expression associations for other pathway candidate genes, these did not meet significance and did not correlate to protein abundance. On the other hand, NPR2 genetic variants not only met statistical significance after adjustment, but the variation in abundance across genotypes was roughly two-fold, which conceivably could be in a clinically relevant range. These findings of course require validation and further testing to understand their impact and mechanism. It is interesting to hypothesize regarding possible mechanisms since gene expression did not appear to be the intermediary. This could conceivably be due to translational regulation, post-translational modifications, or via differences in miRNA mediated regulation. In our searches of existing databases of SNP functional impact, we did not identify nearby variants previously known to impact these processes. However, because there was significant linkage disequilibrium in the *NPR2* region, it is possible that an untyped functional variant(s) is in linkage disequilibrium with the typed SNPs.

Several limitations of this study should be considered when evaluating these data. First, the relatively small sample size limited the power. We estimated 90% power to detect at least 2.5-fold variation and minor allele frequency of 0.2. While high-throughput methods are widely available for genotyping, they are less so for real-time PCR, and protein quantification often remains labor intensive and impractical for large sample sizes. Our sample size was designed to accommodate this limitation and yet be able to identify high-impact variants, recognizing that subtle differences in protein or gene expression would likely be undetected. Second, we examined only kidney tissue, and cannot deduce information about gene and protein expression in other tissues that may be of interest such as cardiac tissue. However, kidney was felt to be the best choice when considering the NP pathway physiologically and as it pertains to pharmaceuticals (such as recombinant NPs or endopeptidase inhibitors), because it is a key location for both clearance and effect of NPs and there is expression of all the candidate genes. Another potential concern is that renal tissue is not completely homogenous; whether this impacted our findings is unknown. Finally, we have focused on protein quantitation and have not tested protein function. This remains important to investigate for follow-up studies.

## Conclusion

Our systematic testing of the impact of genetic sequence variants in the NP pathway on gene expression and protein expression indicates that NPRB protein quantity is significantly impacted by genetic variation. In contrast, NPRA, NPRC, and MME expression did not appear to be importantly correlated to genotype. *NPR2* is a strong candidate gene for further investigation in terms of pharmacogenetic interactions of exogenous NPs. Additional studies are also needed to determine whether NPRB quantity correlates to protein function or other clinical phenotypes.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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## References

1. Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Soliman EZ, Sorlie PD, Sotoodehnia N, Turan TN, Virani SS, Wong ND, Woo D, Turner MB. Heart disease and stroke statistics--2012 update: a report from the American Heart Association. Circulation. 2012; 125(1):e2–e220.10.1161/CIR.0b013e31823ac046 [PubMed: 22179539]

2. Maeda K, Tsutamoto T, Wada A, Hisanaga T, Kinoshita M. Plasma brain natriuretic peptide as a biochemical marker of high left ventricular end-diastolic pressure in patients with symptomatic left ventricular dysfunction. Am Heart J. 1998; 135(5 Pt 1):825–832. [PubMed: 9588412]

3. Ellmers LJ, Scott NJ, Piuhola J, Maeda N, Smithies O, Frampton CM, Richards AM, Cameron VA. Npr1-regulated gene pathways contributing to cardiac hypertrophy and fibrosis. J Mol Endocrinol. 2007; 38(1-2):245–257.10.1677/jme.1.02138 [PubMed: 17293444]

4. Maisel AS, Krishnaswamy P, Nowak RM, McCord J, Hollander JE, Duc P, Omland T, Storrow AB, Abraham WT, Wu AH, Clopton P, Steg PG, Westheim A, Knudsen CW, Perez A, Kazanegra R, Herrmann HC, McCullough PA. Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure. N Engl J Med. 2002; 347(3):161–167.10.1056/NEJMoa020233 [PubMed: 12124404]

5. Pascual-Figal DA, Domingo M, Casas T, Gich I, Ordonez-Llanos J, Martinez P, Cinca J, Valdes M, Januzzi JL, Bayes-Genis A. Usefulness of clinical and NT-proBNP monitoring for prognostic guidance in destabilized heart failure outpatients. Eur Heart J. 2008; 29(8):1011–1018.10.1093/eurheartj/ehn023 [PubMed: 18263871]

6. VMAC Investigators (Vasodilation in the Management of Acute CHF). Intravenous nesiritide vs nitroglycerin for treatment of decompensated congestive heart failure: a randomized controlled trial. JAMA. 2002; 287(12):1531–1540. [PubMed: 11911755]

7. Colucci WS, Elkayam U, Horton DP, Abraham WT, Bourge RC, Johnson AD, Wagoner LE, Givertz MM, Liang CS, Neibaur M, Haught WH, LeJemtel TH. Intravenous nesiritide, a natriuretic peptide, in the treatment of decompensated congestive heart failure. Nesiritide Study Group. N Engl J Med. 2000; 343(4):246–253.10.1056/nejm200007273430403 [PubMed: 10911006]

8. Maisel AS, Clopton P, Krishnaswamy P, Nowak RM, McCord J, Hollander JE, Duc P, Omland T, Storrow AB, Abraham WT, Wu AH, Steg G, Westheim A, Knudsen CW, Perez A, Kazanegra R, Bhalla V, Herrmann HC, Aumont MC, McCullough PA. Impact of age, race, and sex on the ability of B-type natriuretic peptide to aid in the emergency diagnosis of heart failure: results from the Breathing Not Properly (BNP) multinational study. Am Heart J. 2004; 147(6):1078–1084.10.1016/j.ahj.2004.01.013 [PubMed: 15199359]

9. Costello-Boerrigter LC, Boerrigter G, Ameenuddin S, Mahoney DW, Slusser JP, Heublein DM, Redfield MM, Rodeheffer RJ, Olson TM, Burnett JC Jr. The Effect of the Brain-Type Natriuretic Peptide Single-Nucleotide Polymorphism rs198389 on Test Characteristics of Common Assays. Mayo Clin Proc. 2011; 86(3):210–218. 86/3/210 [pii] 10.4065/mcp.2010.0708. [PubMed: 21364112]

10. O'Connor CM, Starling RC, Hernandez AF, Armstrong PW, Dickstein K, Hasselblad V, Heizer GM, Komajda M, Massie BM, McMurray JJ, Nieminen MS, Reist CJ, Rouleau JL, Swedberg K, Adams KF Jr, Anker SD, Atar D, Battler A, Botero R, Bohidar NR, Butler J, Clausell N, Corbalan R, Costanzo MR, Dahlstrom U, Deckelbaum LI, Diaz R, Dunlap ME, Ezekowitz JA, Feldman D, Felker GM, Fonarow GC, Gennevois D, Gottlieb SS, Hill JA, Hollander JE, Howlett JG, Hudson MP, Kociol RD, Krum H, Laucevicius A, Levy WC, Mendez GF, Metra M, Mittal S, Oh BH, Pereira NL, Ponikowski P, Tang WH, Tanomsup S, Teerlink JR, Triposkiadis F, Troughton RW, Voors AA, Whellan DJ, Zannad F, Califf RM. Effect of nesiritide in patients with acute decompensated heart failure. N Engl J Med. 2011; 365(1):32–43.10.1056/NEJMoa1100171 [PubMed: 21732835]

11. Sackner-Bernstein JD, Kowalski M, Fox M, Aaronson K. Short-term risk of death after treatment with nesiritide for decompensated heart failure: a pooled analysis of randomized controlled trials. JAMA. 2005; 293(15):1900–1905.10.1001/jama.293.15.1900 [PubMed: 15840865]
12. Sackner-Bernstein JD, Skopicki HA, Aaronson KD. Risk of worsening renal function with nesiritide in patients with acutely decompensated heart failure. Circulation. 2005; 111(12):1487–1491.10.1161/01.cir.0000159340.93220.e4 [PubMed: 15781736]
13. Luss H, Mitrovic V, Seferovic PM, Simeunovic D, Ristic AD, Moiseyev VS, Forssmann WG, Hamdy AM, Meyer M. Renal effects of ularitide in patients with decompensated heart failure. Am Heart J. 2008; 155(6):1012 e1011–1018.10.1016/j.ahj.2008.02.011 [PubMed: 18513512]
14. McKie PM, Sangaralingham SJ, Burnett JC Jr. CD-NP: an innovative designer natriuretic peptide activator of particulate guanylyl cyclase receptors for cardiorenal disease. Curr Heart Fail Rep. 2010; 7(3):93–99.10.1007/s11897-010-0016-6 [PubMed: 20582736]
15. Lanfear DE. Genetic variation in the natriuretic peptide system and heart failure. Heart Fail Rev. 2010; 15(3):219–228.10.1007/s10741-008-9113-y [PubMed: 18850266]
16. Wang TJ, Larson MG, Levy D, Benjamin EJ, Corey D, Leip EP, Vasan RS. Heritability and genetic linkage of plasma natriuretic peptide levels. Circulation. 2003; 108(1):13–16.10.1161/01.cir.0000081657.83724.a7 [PubMed: 12821537]
17. Newton-Cheh C, Larson MG, Vasan RS, Levy D, Bloch KD, Surti A, Guiducci C, Kathiresan S, Benjamin EJ, Struck J, Morgenthaler NG, Bergmann A, Blankenberg S, Kee F, Nilsson P, Yin X, Peltonen L, Vartiainen E, Salomaa V, Hirschhorn JN, Melander O, Wang TJ. Association of common variants in NPPA and NPPB with circulating natriuretic peptides and blood pressure. Nat Genet. 2009; 41(3):348–353.10.1038/ng.328 [PubMed: 19219041]
18. Lanfear DE, Stolker JM, Marsh S, Rich MW, McLeod HL. Genetic variation in the B-type natiuretic peptide pathway affects BNP levels. Cardiovasc Drugs Ther. 2007; 21(1):55–62.10.1007/s10557-007-6007-5 [PubMed: 17340039]
19. Lanfear DE, Stolker JM, Marsh S, Rich MW, McLeod HL. Natriuretic peptide receptor 3 genotype modulates the relationship between B-type natriuretic peptide and left ventricular end-diastolic pressure. Therapy. 2006; 3(6):765–771.10.2217/14750708.3.6.765
20. Lanfear DE, Yang J, Leszczynski K, Sabbah HN. Natriuretic Peptide Receptor 3 Genotype Is Associated with Altered Pharmacokinetics during Nesiritide Infusion. J Card Fail. 2011; 17(8):S62.
21. Gauderman WJ, Murcray C, Gilliland F, Conti DV. Testing association between disease and multiple SNPs in a candidate gene. Genet Epidemiol. 2007; 31(5):383–395.10.1002/gepi.20219 [PubMed: 17410554]
22. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet. 2006; 38(8): 904–909. [PubMed: 16862161]
23. An P, Mukherjee O, Chanda P, Yao L, Engelman CD, Huang CH, Zheng T, Kovac IP, Dube MP, Liang X, Li J, de Andrade M, Culverhouse R, Malzahn D, Manning AK, Clarke GM, Jung J, Province MA. The challenge of detecting epistasis (G × G interactions): Genetic Analysis Workshop 16. Genet Epidemiol. 2009; 33(1):S58–67.10.1002/gepi.20474 [PubMed: 19924703]
24. Oh S, Park T. Association tests based on the principal-component analysis. BMC Proc. 2007; 1(1):S130. [PubMed: 18466473]
25. Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I. Controlling the false discovery rate in behavior genetics research. Behav Brain Res. 2001; 125(1-2):279–284. [PubMed: 11682119]
26. Vogel C, Marcotte EM. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. Nat Rev Genet. 2012; 13(4):227–232.10.1038/nrg3185 [PubMed: 22411467]

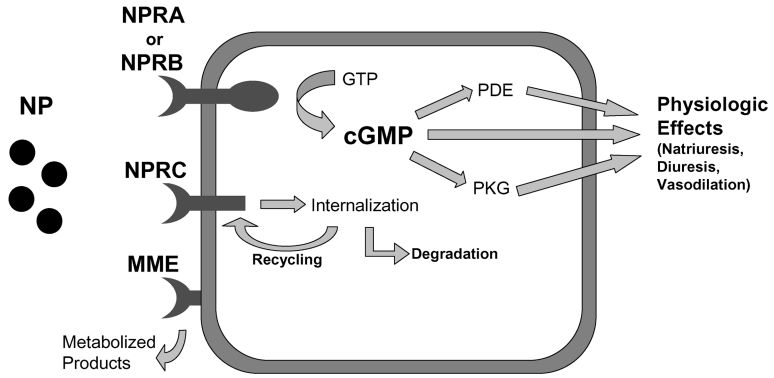


**Fig. 1.**

Schematic of Natriuretic Peptide Signaling and Breakdown. NP= Natriuretic Peptide; NPRA=Natriuretic Peptide Receptor A; MME=Membrane Metallo Endopeptidase; cGMP=cyclic Guanylate Monophospate

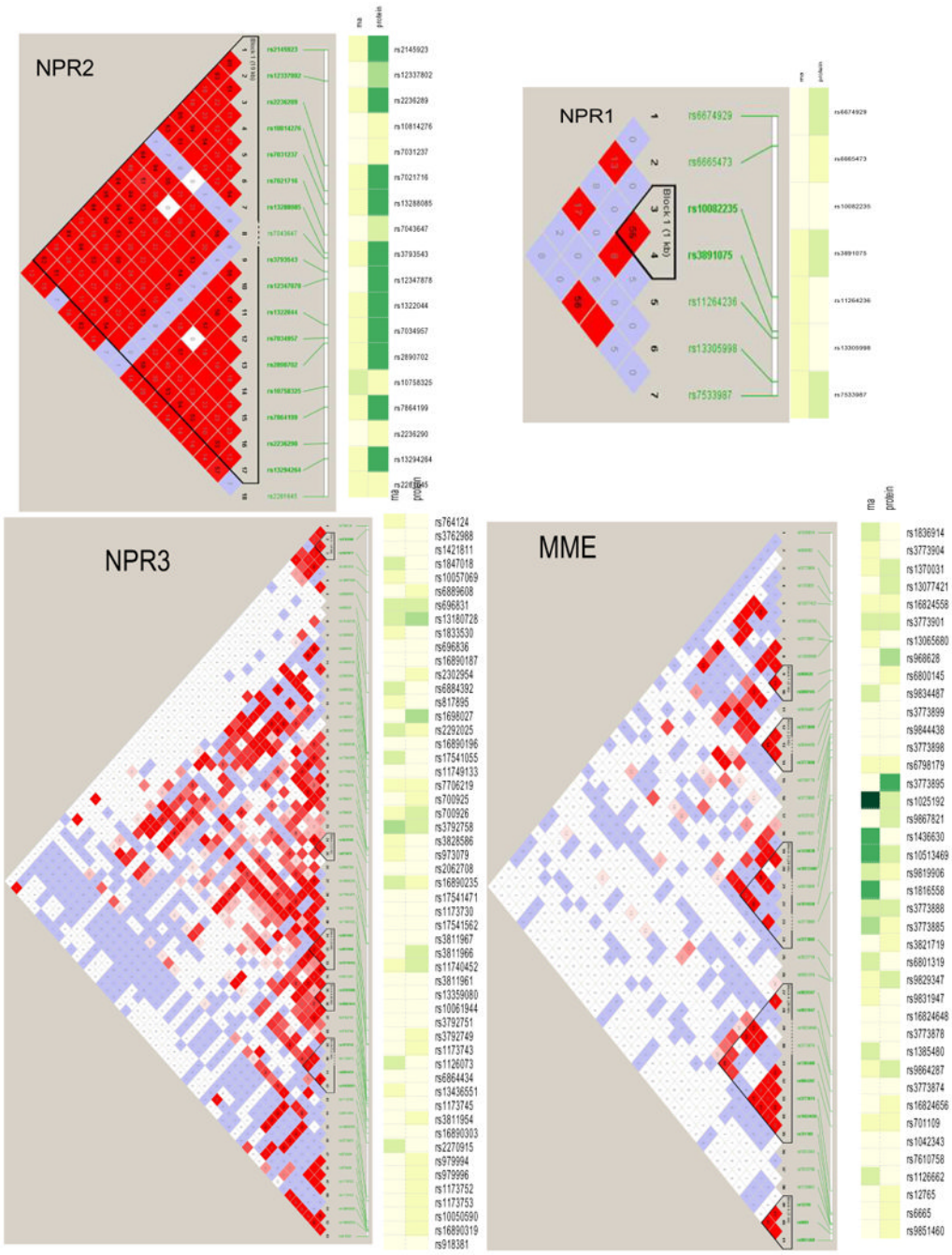


**Fig. 2.**

Linkage disequilibrium (LD) maps and graphical representation of genotype associations with gene expression and protein expression for *NPR1, NPR2, NPR3*, and *MME*. For the triangular LD maps, the numerical values in the boxes are the $r^2$ values between single nucleotide polymorphism (SNP) pairs, with box fill color being shown in darker red for higher $r^2$ values (i.e., higher LD). Each SNP association with protein expression is shown on the rightmost vertical columns, and the SNP association with RNA expression is shown in the adjacent vertical column (left). Each row in the column represents one SNP with more significant associations (i.e., lower unadjusted P-values) shown in darker green. All variants are listed with dbSNP ID#.

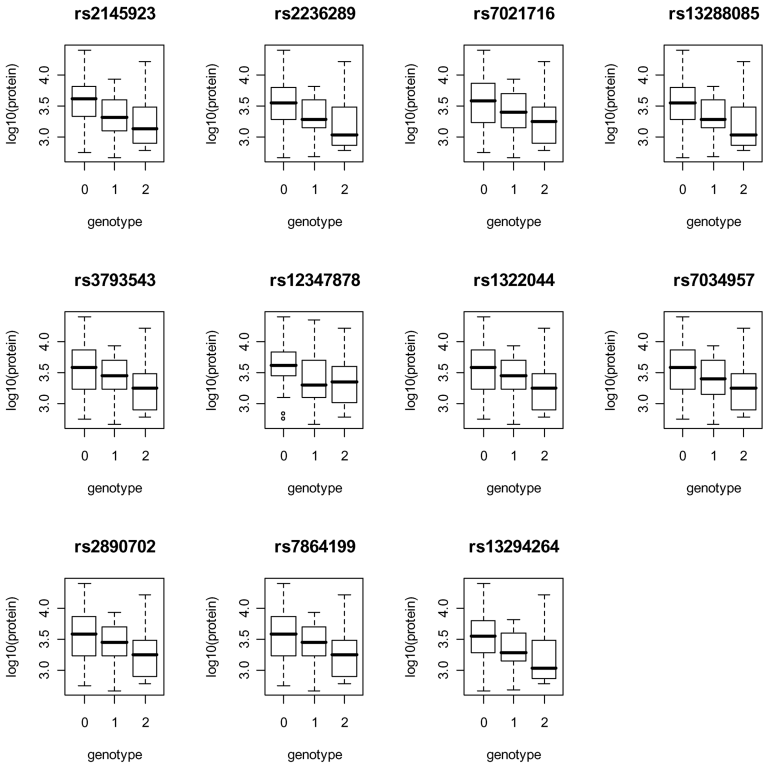


**Fig. 3.**

Target protein abundance by genotype for *NPR2* (loci with FDR≤0.05). (0,1,2) represents the additive coding of the number of copies of the minor allele

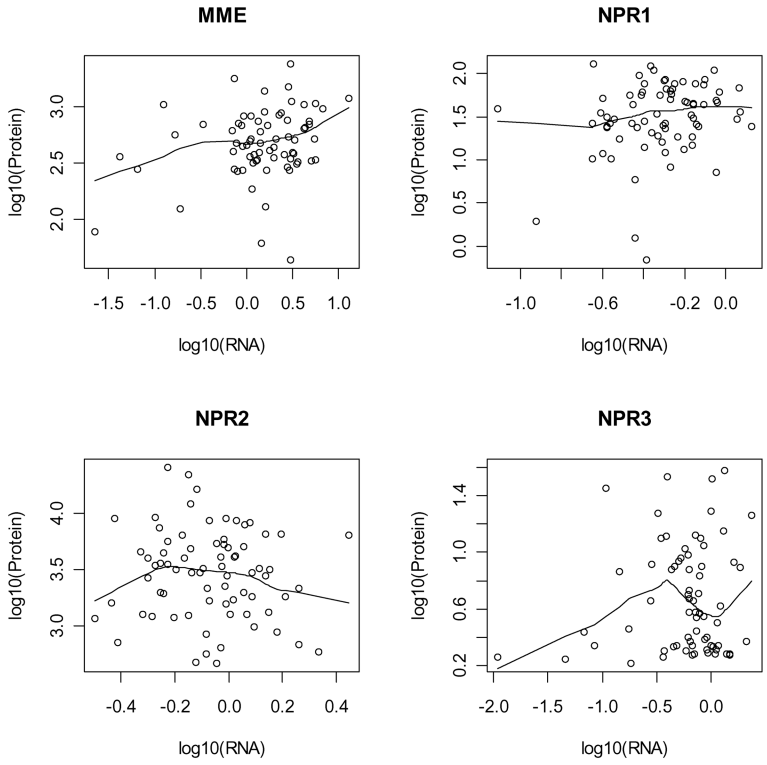


**Fig. 4.**

Scatter plot showing the relation between RNA and protein quantity for each gene. Solid curve represents the smooth fit to better visualize the trends. The fit was generated using locally weighted scatter plot smoother (LOWESS)