Heritability and Genetic Linkage of Plasma Natriuretic Peptide Levels

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Background—Natriuretic peptides play a critical role in the maintenance of salt and water homeostasis and regulation of vascular tone. Thus, interindividual variation in plasma natriuretic peptide levels may contribute to variation in susceptibility to volume overload and hypertension. It is unknown to what extent genetic factors contribute to variation in plasma natriuretic peptide levels.

Methods and Results—We studied 1914 Framingham Study participants (mean age 57 years, 53% women) who underwent routine echocardiography and testing for plasma N-terminal proatrial natriuretic peptide (N-ANP) and brain natriuretic peptide (BNP). We estimated sex-specific multivariable models and used variance-components methods, implemented in SOLAR (Sequential Oligogenic Linkage Analysis Routines), to estimate heritability. Multipoint linkage analyses were performed using data from a 10-cM-density genome scan. Age, clinical, and echocardiographic variables accounted for 42% and 40% of the variation in log N-ANP and log BNP levels, respectively, in men. Corresponding values in women were 27% and 21%. Multivariable-adjusted heritabilities were 0.44 for log N-ANP and 0.35 for log BNP (P<0.0001). Genome-wide linkage analyses, based on 1142 participants from the 314 largest families, revealed 2 regions of suggestive linkage for log N-ANP and log BNP on chromosomes 2p25 (log-of-odds score 2.40) and 12p13 (log-of-odds score 2.13), respectively.

Conclusions—In this community-based sample, a substantial proportion of the unexplained variation in plasma natriuretic peptide levels was attributable to additive genetic effects. Additional studies using candidate gene approaches may provide insight into the genetic loci that regulate plasma natriuretic peptide levels in humans. (Circulation. 2003;108:13-16.)

Key Words: natriuretic peptides ■ genetics ■ epidemiology ■ atrial natriuretic factor

Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) play a critical role in sodium homeostasis and regulation of peripheral vascular tone.1 Experimental data based on transgenic mouse models have demonstrated that underexpression of natriuretic peptide (NP) genes is associated with high blood pressure and left ventricular (LV) hypertrophy, whereas overexpression is associated with low blood pressure.2,3 These investigations raise the possibility that interindividual variability in circulating NP levels may be related to genetic influences, which may in turn contribute to the differential susceptibility to hypertension and other diseases. Thus, it is important to understand the genetic influences on plasma NP levels, but data from humans are limited.

The Framingham Study is a large, community-based cohort of individuals from multiple extended families. The availability of routine plasma NP measurements and echocardiograms in this study provides a unique opportunity to assess the contribution of genetic factors to NP levels (ie, heritability) in a relatively unbiased manner. We also report results of genome-wide linkage analyses using NP levels as quantitative traits.

Methods

The Framingham offspring cohort was initiated in 1971 with the recruitment of 5124 children (and their spouses) of the original Framingham Study participants.4 The 6th examination (1995–1998) was attended by 3532 (78%) of 4518 living members. Subjects underwent a routine physical examination, laboratory assessment of cardiovascular risk factors, and echocardiography. Plasma levels of BNP and the N-terminal component of pro-ANP (N-ANP) were measured using high-sensitivity immunoradiometric assays (Shionogi).5

Subjects were excluded from the investigation because of unavailable plasma NP levels (n=80), serum creatinine >2.0 mg/dL (n=21), and history of heart failure (n=33). After exclusions, 3398 subjects (96% of attendees) remained eligible. The heritability analyses were based on 1914 subjects belonging to 588 extended families with ≥2 members. The sample included 1669 sibling pairs.
Heritability denotes the proportion of phenotypic variance after—assumed to be normally distributed and to be mutually independent—residual error. The additive genetic effects and residual error are incorporated into a mixed-effects model with fixed covariate effects, additive genetic effects, and maximum-likelihood estimation is applied to a variance-components model for estimating heritability of plasma NP levels. With this approach, Routines (SOLAR) package to fit a variance-components model for analyses. We used the Sequential Oligogenic Linkage Analysis (marker set 8, heterozygosity 0.77; available at http://research.marshfieldclinic.org/genetics). Details of DNA extraction and genotype data cleaning have been reported previously.

Statistical Analyses
We logarithmically transformed NP levels because of their skewed distributions. Initially, we performed sex-specific multivariable regression analyses to assess the contribution of clinical covariates to log N-ANP and log BNP; these analyses were done on all 3398 subjects to obtain covariate estimates with the greatest precision and least bias. Covariates were selected on the basis of prior reports and included age, myocardial infarction, clinical valve disease, atrial fibrillation, diabetes, systolic blood pressure, diastolic blood pressure, antihypertensive therapy, body mass index, heart murmur, and creatinine. Because of truncation by the lower detection limit of the assays, regression models for left-censored data (tobit models) were used (LIFEREG procedure in SAS).

We estimated partial correlation coefficients for plasma NP levels in sibling and spousal pairs, using sex-specific residuals and adjusting for age, clinical, and echocardiographic characteristics noted to be significantly associated with NP levels in the initial multivariable analyses. We used the Sequential Oligogenic Linkage Analysis Routines (SOLAR) package to fit a variance-components model for estimating heritability of plasma NP levels. With this approach, maximum-likelihood estimation is applied to a mixed-effects model that incorporates fixed covariate effects, additive genetic effects, and residual error. The additive genetic effects and residual error are assumed to be normally distributed and to be mutually independent. Heritability denotes the proportion of phenotypic variance—after accounting for covariates—explained by additive genetic effects. The analyses were performed on sex-specific residuals from models adjusting for (1) age; (2) age and clinical characteristics; and (3) age, clinical, and echocardiographic characteristics. We adjusted for covariates hierarchically because some of these covariates have a genetic basis.

Multipoint quantitative trait linkage analyses were conducted with the residuals from fully adjusted models using the variance-components model implemented in SOLAR. This model makes no assumptions about mode of transmission and uses all available data from the extended families. Linkage is assessed by comparing a polygenic model that does not incorporate genetic marker information with a model that does incorporate genotype data (ie, identity by descent status). Results are expressed as the base 10 log of the ratio of likelihoods (log-of-the-odds score [LOD]) from each model.

Results
The 1914 participants (1005 women) in the heritability sample had a mean age of 57 years. Mean N-ANP and BNP levels were 328 pmol/L and 13.6 pg/mL in men and 388 pmol/L and 16.1 pg/mL in women, respectively. In multivariable regression analyses, clinical and echocardiographic variables accounted for 42% and 40% of the variation in log N-ANP and log BNP, respectively, in men. Corresponding values in women were 27% and 21%.

Familial Correlations of Plasma NP Levels
Sibling correlations were substantially higher than spousal correlations for both log N-ANP and log BNP (Table). After multivariable adjustment, sister-sister correlations were 0.26 and 0.26, brother-brother correlations were 0.23 and 0.21, and sister-brother correlations were 0.22 and 0.14 for log N-ANP and log BNP, respectively.

Variance-Components Heritability Estimates
Estimated heritabilities, after accounting for age and sex, were 0.45 for log N-ANP and 0.34 for log BNP (Table). After further adjustment for clinical and echocardiographic variables, estimated heritabilities were 0.44 for log N-ANP and 0.35 for log BNP. All heritability estimates were significant ($P<0.0001$).

Linkage Analyses
We performed linkage analyses using fully adjusted data from the 1142 participants who underwent a genome scan. Maximal multipoint LOD scores are shown in the Figure (A and B). The highest multipoint LOD scores were 2.40 for log
Discussion

To our knowledge, the present study is the first report of the heritability and genetic linkage of NP levels in humans. An understanding of the genetic contribution to variation in NP levels is important because these molecules play a vital role in the response to cardiac overload. The results of experimental studies using transgenic mice and genetic crosses of inbred rats further underscore the potential usefulness of studying NP levels, which are important “proximal” phenotypes in the pathway between genes and overt cardiac disease.

Heritability of Plasma NP Levels

Clinical and echocardiographic features account for only 20% (women) to 40% (men) of the total variation in NP levels. A substantial proportion of the remaining variation, roughly 40% for N-ANP and BNP, appears to be attributable to additive genetic effects. Our estimates suggest that NP levels are as heritable as other cardiovascular risk factors such as blood pressure, cholesterol, and body mass index. Prior studies suggest that plasma levels of other neurohormones, such as renin and norepinephrine, may also be heritable, although these studies have been based on smaller samples and have accounted for fewer potential confounders.

The results of our multivariable analyses suggest that the genes influencing NP levels may operate independently of vascular risk factors, existing cardiovascular disease, and alterations in LV structure and function. A genetic predilection for low plasma NP levels may increase susceptibility to diseases characterized by impaired salt handling. The demonstration that NP levels are heritable in an unselected sample motivates further analyses to identify the specific loci responsible for interindividual variation in NP levels.

Linkage Analyses

Interestingly, we did not find evidence for significant genetic linkage to chromosomal regions containing genes for ANP (1p36), BNP (1p36), or their receptors (1q21–22 and 5p14–13). Some previous studies have found little association between ANP polymorphisms and ANP levels or hypertension. Thus, although genetic factors appear to contribute substantially to NP levels, this contribution may be attributable to the influence of genes other than those encoding the
NPs themselves. The activation of NP transcription by mechanical strain appears to involve cell-matrix interactions and several signaling pathways. Genes influencing any of these pathways or involving non-receptor-mediated clearance may contribute to variation in NP levels.

We report 2 regions of suggestive linkage for plasma N-ANP and BNP levels on chromosomes 2p25 and 12p13, respectively. Known genes in these regions include lysine-deficient protein kinase-1 (PRKWNK1) and guanine nucleotide binding β3 (GNB3), which have been associated with rare and common forms of hypertension. Additional studies will be required to investigate whether variation in plasma NP levels can be mapped to known genes.

It is important to acknowledge that linkage analyses inherently have limited statistical power, particularly in the setting of multiple genes with modest effects. Thus, our findings do not exclude a role for the pro-ANP, pro-BNP, or NP receptor genes in the regulation of plasma NP levels. Complementary candidate gene studies are warranted to test this hypothesis.

Strengths and Limitations

The large community-based sample that was not enrolled on the basis of a disease condition (such as hypertension), the large number of sibling and spousal pairs, the routine ascertainment of all covariates, and the use of multivariable analyses strengthen our investigation.

Nonetheless, it can be challenging to distinguish the influences of shared early environment from that of shared genes. We addressed this by adjusting for multiple characteristics that were either known to influence NP levels or appeared to be plausible determinants of NP levels. We also examined spousal correlations to provide some insight into the influence of shared environment. Caution should be used when extrapolating our results to other ethnicities, because the Framingham cohort is white. Furthermore, it is possible that nonattendance at the index examination may have biased our results, although the attendance rate was relatively high.

Although we used high-sensitivity immunoradiometric assays, circulating NP levels in ambulatory subjects are frequently below the lower detection limit of the assays. We found that 3% of N-ANP levels and 31% of BNP levels were censored by the lower detection limits. There is limited information regarding the influence of censoring on heritability estimates, although results of computer simulations indicate that the true heritability may be underestimated by 7% to 10%. The greater degree of censoring of BNP may contribute to the lower heritability estimates for BNP compared with N-ANP.

Conclusion

In our community-based sample, we found evidence of substantial heritability of plasma NP levels. Genes other than those encoding the NPs or their receptors may contribute to interindividual variability in NP levels. Given the fundamental role of NPs in salt homeostasis and regulation of vasomotor tone, it is conceivable that such genes may play an important role in determining the susceptibility to hypertension and heart failure.

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