Sodium-Lithium Countertransport Genotype and the Probability of Hypertension in Adults

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The objective of the present study was to determine whether information about a biometrically inferred single gene with large effects on erythrocyte sodium-lithium countertransport is useful in predicting the probability of having hypertension. We used multivariate logistic regression to model the relationship between the probability of having hypertension and predictor traits in a sample of 382 unrelated adult women and 347 unrelated adult men from Rochester, Minn. First, we identified a set of demographic, biochemical, and physiological predictors. Second, we analyzed whether the relationship between the probability of having hypertension and the identified predictor traits was heterogeneous between the biometrically inferred single locus genotypes with large effects on sodium-lithium countertransport level. Third, if there was no heterogeneity, we assessed whether sodium-lithium countertransport genotypes made an additional contribution to predicting the probability of having hypertension after other predictors were considered. In women, the predictors of the probability of having hypertension were age, plasma apolipoprotein CIII, body mass index, and an interaction term involving age and body mass index. The relationship between the probability of having hypertension and the identified predictors was not heterogeneous between sodium-lithium countertransport genotypes, and genotype did not contribute to the prediction of the probability of having hypertension after the identified predictors were considered. In men, predictors of the probability of having hypertension were age, plasma levels of high-density lipoprotein cholesterol, apolipoproteins AI and CII, sodium-lithium countertransport level, and sodium-lithium countertransport genotype. The relationship between the probability of having hypertension and sodium-lithium countertransport level and age were heterogeneous between biometrically inferred sodium-lithium countertransport genotypes. For a given increase in sodium-lithium countertransport level, the odds of having hypertension increased 5.2 times more in men with the genotype associated with elevated levels of sodium-lithium countertransport than in men with alternative genotypes. For a given increase in age, the odds of having hypertension increased 3.4 times more in men with the genotype associated with elevated levels of sodium-lithium countertransport than in men with alternative genotypes. These results reject the null hypothesis that information about allelic variation at a single gene that influences sodium-lithium countertransport level does not improve the ability to predict the probability of having hypertension in men after other predictors have been considered. (Hypertension. 1993;22:560-568.)

KEY WORDS • genetics • hypertension, essential • logistic models • epidemiology • sodium • lithium • ion transport

Cross-sectional studies have demonstrated that elevated levels of sodium-lithium (Na-Li) countertransport are associated with essential hypertension (hypertension). Using logistic regression models, Turner et al demonstrated that the level of Na-Li countertransport contributes to the prediction of the probability of having hypertension after other predictors of hypertension are considered. In the general population, the distribution of Na-Li countertransport levels is composed of a mixture of two distributions. In some families, segregation of alleles at a single biometrically inferred gene explains the large differences in mean levels between these distributions.

No previous studies have assessed whether information about allelic variation in a single gene with large effects on Na-Li countertransport level contributes to the prediction of hypertension after other known predictors have been considered. There are two possible ways in which such genetic variation might contribute to prediction. First, different genotypes may be associated with different relationships between the probability of having hypertension and other identified predictors, including the level of Na-Li countertransport. This could be the case if the effects of variation in the single locus genotypes estimated by the analysis of Na-Li countertransport levels are not additive to the effects of variation in the rest of the genome and/or variation in environmental factors that influence levels of the other predictors. Second, if the relationships between the probability of having hypertension and measured pre-
dictors are not associated with variation in the single locus genotypes inferred from Na-Li countertransport levels, such variation may still contribute to prediction if it determines pleiotropic effects on unmeasured biochemical or physiological traits that influence blood pressure and the predisposition to hypertension.

The objective of the present study was to test these hypotheses in a sample of adults representative of the population of Rochester, Minn. First, we asked whether the relationship between the probability of having hypertension and demographic, physiological, and biochemical predictor traits was homogeneous between biometrically inferred single locus genotypes that have large effects on levels of Na-Li countertransport. If this were the case, we might infer that the single locus genotypes have effects that are not additive to the other genetic and/or environmental effects on the trait of interest. Second, if this relationship was not significantly heterogeneous between single locus genotypes, we asked whether an individual's genotype at the locus with large Na-Li countertransport effects made an additional contribution to the prediction of the probability of having hypertension after other predictors, including Na-Li countertransport level, were considered. If this were the case, we might infer that the single locus genotypes have pleiotropic effects on unmeasured traits that influence blood pressure or the predisposition to hypertension.

Methods

Sample

The individuals in this study were adults selected from 276 multigeneration pedigrees having two or more children enrolled in the schools of Rochester, Minn, in 1984. Between 1984 and 1987, 1989 members of these pedigrees participated in the Rochester Family Heart Study.5 Although drawn from the same population, the sample used in the present study is different from those samples considered in previous publications from the Rochester Family Heart Study.2,3 The steps taken to select the sample for the study reported here were as follows: First, all individuals younger than 20 years were excluded (n=711). Next, from the remaining adults older than age 20, individuals were excluded if they were not fasting at the time of their clinic visit (n=25), were normotensive with any evidence of factors that may have lowered blood pressure, or were hypertensive with any evidence of factors that may have raised blood pressure (n=16). Finally, individuals were excluded who were being treated with estrogen or progestosterone (n=46), oral or topical glucocorticoids (n=21), insulin (n=9), oral hypoglycemics (n=20), lipid-lowering drugs (n=6), or cis-retinoic acid (n=1).

After these exclusions were made, a sample of unrelated men and unrelated women was selected in the following manner: First, all biologically unrelated trios, each consisting of a mother-father pair and one offspring, were identified. If a mother-father pair had two or more children, one child was selected at random to join with the parents to form a trio. Second, incomplete trios consisting of parent-offspring pairs, spouse pairs, or individuals not biologically related to anyone else of the same gender in a pedigree were identified. From each complete trio (or when possible for an incomplete trio), one man and one woman were selected at random to form samples of unrelated women and men. After completion of these exclusion and selection procedures, 382 unrelated women and 347 unrelated men were available for the present study. All participants gave informed consent, and the study protocol was approved by institutional review committees at both the Mayo Clinic and the University of Michigan.

Measures

Blood pressure diagnostic category was coded as a discrete trait with outcomes of normotension and hypertension, because most hypertensive individuals in the Rochester population are treated to lower their blood pressures, and a treated individual's blood pressure reading is not an accurate measure of their "native" blood pressure status. When a continuous variable is made discrete, information is generally lost; however, in this case, categorization of blood pressure provides a better measure of an individual's blood pressure status than the continuous blood pressure variable. Determination of blood pressure diagnostic category is coded as a binary trait with outcomes of normotension or hypertension, was based on the average of three blood pressure readings taken at the time of each individual's Mayo Clinic visit and information contained in that person's medical record. Individuals were considered normotensive if their average systolic and diastolic blood pressures were less than 140 mm Hg and less than 90 mm Hg, respectively, at the time of examination. None of these individuals had a previous diagnosis of hypertension, and none were treated with antihypertensive medications at the time of examination. An individual was considered hypertensive if his or her average systolic or diastolic blood pressure was greater than 139 mm Hg or greater than 89 mm Hg, respectively, or if he or she were being treated with antihypertensive medications because of a previous diagnosis of hypertension.

Of those individuals classified as hypertensive in the present study, 120 (58.5%) were treated with blood pressure-lowering medications. Of those hypertensive individuals who were not treated with blood pressure-lowering medications, the mean blood pressure level was 158 mm Hg systolic and 78 mm Hg diastolic. Twenty (23.5%) of the untreated hypertensive individuals had blood pressure readings between 140 and 160 mm Hg systolic or 90 and 95 mm Hg diastolic. The remaining 65 (76.5%) had blood pressure readings greater than 160 mm Hg systolic or 95 mm Hg diastolic.

Other traits measured at the time of physical examination included age (years), weight (kilograms), height (centimeters), and cigarette smoking (ever or never). Body mass index (BMI) was calculated as weight (kg)/height (m^2). Aliquots of a heparinized peripheral blood sample collected at the time of physical examination were used to measure total plasma cholesterol and triglycerides; high-density lipoprotein cholesterol (HDL-C); plasma apolipoproteins A1, AII, CII, CHI, E, and B40-100, and red blood cell Na-Li countertransport.2,3

Na-Li countertransport genotypes were inferred for each individual in the sample from complex segregation analyses carried out on Na-Li countertransport levels determined in a larger sample of adults (over age 20) that participated in the Rochester Family Heart Study between 1984 and 1987.4 Those analyses determined that interindividual variability in Na-Li countertrans-
port may be explained by the effects of polygenes and either a single gene or a nontransmitted environmental factor, each having large effects on Na-Li countertransport level. The single gene was inferred to have two alleles: one associated with lower levels of Na-Li countertransport (L), and the other with associated with higher levels of Na-Li countertransport (H). For the purposes of this study, these individuals in 11 pedigrees that supported the nontransmitted environmental factor hypothesis were assumed to be not segregating for this single gene. The pattern of Mendelian segregation in the remaining 256 pedigrees indicated that the H allele was recessive to the L allele to form two genotypic classes, LL+LH and HH. Genotype assignments from the results of the complex segregation analysis of the 256 pedigrees were made for those individuals in whom the probability of belonging to the LL+LH genotypic class was greater than or equal to .6 or in whom the probability of belonging to the HH genotypic class was greater than or equal to .6. Only nine individuals could not be assigned genotypes because they did not fall into either the LL+LH or HH class with a probability of at least .6. None of these individuals was included in the sample selected for the present analysis. All individuals in the 11 pedigrees that showed evidence for the major effects of a nontransmitted environmental factor were assigned to the LL+LH genotypic class.

Statistical Analyses

The distribution of hypertension diagnostic categories and each of the potential predictor traits was summarized by computing gender-specific means and standard deviations for the quantitative traits and gender-specific proportions for the qualitative traits. Student's t test was used to evaluate differences in means between genders. Satterthwaite's correction of the t-test was used when a variance ratio F test indicated that standard deviations differed between genders. Contingency χ² statistics were used to test differences in proportions of qualitative traits between genders. A null hypothesis that means, standard deviations, or proportions did not differ between genders was rejected when the probability value was less than .05.

A multivariate logistic regression model for a binary outcome was used to define the relationship between the probability of having hypertension and predictor traits. Analyses were carried out separately in women and men. First, a stepwise trait-selection procedure was used to identify a parsimonious set of predictors of the probability of having hypertension. At this initial step, all predictors described above (see "Measures") except Na-Li countertransport level and genotype were considered. Both forward stepwise addition (beginning with an intercept-only model and adding significant predictors) and backward stepwise elimination (beginning with all predictors and retaining only significant predictors) were conducted, with an inclusion and exclusion significance level of .05. Second, Na-Li countertransport was forced into a model containing the significant predictors identified in the stepwise procedure. Forward stepwise trait selection was again carried out to identify pairwise interactions and polynomial terms. Pairwise interactions were considered that only involved traits with a significant main effect. Second-order polynomial effects were considered only for traits with a significant main effect that were not involved in a significant interaction term.

The predictors in the most complete logistic regression model were those identified by the stepwise procedure, Na-Li countertransport, and significant interaction and polynomial terms. Hypotheses were then tested to determine whether the regression of the logarithm of the odds (logit) of having hypertension on each of the predictors in the complete model was homogeneous between genotypes, where the odds is defined as the probability of having hypertension divided by the probability of not having hypertension. The relationship between the probability of having hypertension and each predictor was modeled as

$$
\text{Probability (Hypertension|g,x)} = \frac{\exp(\sum \beta g x)}{1 + \exp(\sum \beta g x)}
$$

(1)

where g indexes the biometrically inferred Na-Li countertransport genotypic class (g=LL+LH or HH), x is the vector of the predictors, and the parameters \(\beta g\) and \(\beta x\) are genotype-specific intercept and regression coefficients, respectively. The test of the null hypothesis that the regression on the ith predictor of the complete logistic regression model was homogeneous between Na-Li countertransport genotypes (Ho: \(\beta_{g=L+L-H} = \beta_{g=L} + \beta_{g=H}\)) was made by comparing Equation 1, the most complete model, with a model in which the estimation of the regression on the ith predictor ignored genotypic class. If the regression of the logit probability of having hypertension on all predictors was not dependent on genotype, a test of the null hypothesis that the intercept was also genotype independent (Ho: \(\alpha_{g=L+L-H} = \alpha_{g=L} + \alpha_{g=H}\)) was carried out. Rejection of either of these hypotheses established that Na-Li countertransport genotype contributed to the prediction of diagnostic category after the other predictors were considered. Finally, if the regression of the logit probability of having hypertension on Na-Li countertransport was not dependent on genotype, a test was made to determine whether Na-Li countertransport level, previously forced into the complete model, was a significant predictor once the other predictors identified by stepwise regression were considered.

Likelihood ratio statistics were used to test all hypotheses about homogeneity between genotypic classes of the regression of the logit probability of having hypertension on predictors. These statistics compared the likelihood of the complete model in which regression coefficients for all predictors were genotype specific with the likelihood of a second model in which the regression coefficient associated with a particular predictor was constrained under the null hypothesis. The likelihood ratio test statistic was computed as minus two times the natural logarithm of the ratio of these two likelihoods. In this application, the likelihood ratio statistic was asymptotically distributed as a \(\chi^2\) with one degree of freedom.

An analysis of model fit was also undertaken. First, the goodness of fit \(\chi^2\) statistic of Hosmer and Lemeshow was computed to evaluate the overall agreement of the data to the fitted logistic curve. Second, an examination of the ability of the model to discriminate hypertensive from normotensive individu-
TABLE 1. Na-Li Countertransport Genotype and Smoking Status by Gender

<table>
<thead>
<tr>
<th>Genotype*Smoking</th>
<th>Women (n=382)</th>
<th>Men (n=347)</th>
<th>Total (n=729)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LL+LH</td>
<td>374 (97.9%)</td>
<td>332 (95.7%)</td>
<td>706 (96.8%)</td>
</tr>
<tr>
<td>HH</td>
<td>8 (2.1%)</td>
<td>15 (4.3%)</td>
<td>23 (3.2%)</td>
</tr>
<tr>
<td>Never smoked</td>
<td>235 (61.5%)</td>
<td>140 (40.3%)</td>
<td>375 (51.4%)</td>
</tr>
<tr>
<td>Ever smoked</td>
<td>147 (38.5%)</td>
<td>207 (59.7%)</td>
<td>354 (48.6%)</td>
</tr>
</tbody>
</table>

*Assigned from complex segregation analysis for genotypic classes with a probability of greater than .6.

**L** indicates lower levels of Na-Li countertransport; and **H**, higher levels, for genotypic classes LL+LH and HH.

Three additional steps were taken to identify and correct conditions contributing to lack of model fit. First, if there was evidence of a poor-fitting model as determined by the $\chi^2_{HL}$ or $D_X$ statistics, marginally significant traits (ie, those with partial regression coefficients significant at .05 < $P$ < .1) were added back to the set of identified predictor traits to ensure that important predictors that might improve the fit of a model were not excluded. Second, the appropriateness of the logistic model for describing the relationship between hypertension and the predictor traits was tested. The statistic of Brown was used to determine whether poor model fit was the result of the use of a logistic model when some other model structure (eg, probit) was more appropriate. Third, regression diagnostic plots were generated to identify observations that may have unduly influenced model fit.

Parameter estimation and likelihood calculations were undertaken using the statistical software of SAS LOGIST and SAS CATMOD. All model checking and summary statistics were computed using the statistical and graphic software of SAS LOGIST and BMDP.

Results

Of the 382 women in the sample, 120 were hypertensive (31.4%); of the 347 men in the sample, 85 were hypertensive (24.5%). The proportion of hypertensive women was significantly greater than the proportion of hypertensive men ($\chi^2=4.31$, $P=.038$). The proportion of women with the HH biometrically inferred Na-Li countertransport genotype was not significantly different from the proportion of men with the HH genotype ($\chi^2=2.955$, $df=1$, $P=.086$, Table 1). The proportion of female smokers was lower than the proportion of male smokers ($\chi^2=32.63$, $df=1$, $P<.0001$, Table 1).

Mean levels of height; HDL-C; apolipoproteins AI, AII, and CII; and Na-Li countertransport differed between women and men (Table 2). Both mean levels and standard deviations of weight, BMI, and triglycerides differed between women and men. The standard deviations but not the mean levels of apolipoproteins E, B, and CIII differed significantly between women and men. Mean levels and standard deviations of age and total cholesterol did not differ significantly between women and men. Because Tables 1 and 2 and previous studies in the Rochester population indicate gender differences in the distribution of many of the traits considered as predictors of the probability of having hypertension, all subsequent analyses were carried out separately for women and men.

In women, stepwise selection identified age, BMI, and apolipoprotein CIII as predictors of the probability of having hypertension. In a model that contained these predictors and Na-Li countertransport level, prediction was improved by the addition of an interaction term between age, BMI, and apolipoprotein CIII.

TABLE 2. Descriptive Summary of Quantitative Traits

<table>
<thead>
<tr>
<th>Trait</th>
<th>Significance*</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Women</td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td>Age, y</td>
<td>54.9</td>
<td>53.3</td>
<td>14.9</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>M</td>
<td>66.3</td>
<td>85.1</td>
</tr>
<tr>
<td>Height, cm</td>
<td>MS</td>
<td>162.4</td>
<td>176.6</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>MS</td>
<td>26.0</td>
<td>27.3</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>M</td>
<td>51.4</td>
<td>40.9</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td></td>
<td>201.2</td>
<td>200.8</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>MS</td>
<td>126.4</td>
<td>183.9</td>
</tr>
<tr>
<td>Apo AI, mg/dL</td>
<td>M</td>
<td>146.3</td>
<td>133.2</td>
</tr>
<tr>
<td>Apo AII, mg/dL</td>
<td>M</td>
<td>34.6</td>
<td>33.8</td>
</tr>
<tr>
<td>Apo E, mg/dL</td>
<td>S</td>
<td>5.5</td>
<td>5.8</td>
</tr>
<tr>
<td>Apo B, mg/dL</td>
<td>S</td>
<td>82.0</td>
<td>83.4</td>
</tr>
<tr>
<td>Apo CII, mg/dL</td>
<td>M</td>
<td>2.5</td>
<td>2.7</td>
</tr>
<tr>
<td>Apo CIII, mg/dL</td>
<td>S</td>
<td>15.5</td>
<td>16.1</td>
</tr>
<tr>
<td>Na-Li CNT, (μmol/L RBC)/h</td>
<td>M</td>
<td>286.9</td>
<td>316.3</td>
</tr>
</tbody>
</table>

HDL-C indicates high-density lipoprotein cholesterol; Apo, apolipoprotein; Na-Li CNT, sodium-lithium countertransport; and RBC, red blood cells.

*Statistically significant differences ($P<.05$ based on $t$ test or $F$ ratio) between women and men for means (M), standard deviations (S), and means and standard deviations (MS).
involved age and BMI. In a model that included these predictors, the interaction term, and Na-Li countertransport, prediction was not further improved by addition of polynomial terms for age, BMI, apolipoprotein CIII, and Na-Li countertransport. The regression of the logit probability of having hypertension on each of these identified predictors was not dependent on genotype. Genotype provided no additional predictive information, as indicated by a test of the equality of the genotype-dependent intercepts. Na-Li countertransport level did not improve the prediction of the probability of having hypertension when added to the model that included age, BMI, apolipoprotein CIII, and the age-by-BMI interaction term. The \( \chi^2_{HL} \) statistic (6.289, \( df=8, P=.615 \)) indicated that this reduced model fit the data well. Somer's \( D_{xy} \) of 0.842 also indicated that there was a high correlation between the observed data and predicted values. Brown's statistic (0.277, \( df=2, P=.893 \)) indicated that the logistic model adequately described the relationship between the probability of having hypertension and the selected predictor traits. No high leverage or outlier observations were detected in regression diagnostic plots (not shown).

In men, stepwise selection identified age, HDL-C, and apolipoproteins AI and CII as predictors of the probability of having hypertension. In a model that included these predictors and Na-Li countertransport, prediction was not improved by the addition of polynomial or interaction terms. The regression of the logit probability of having hypertension on age (\( \chi^2=5.03, df=1, P=.003 \)) and on Na-Li countertransport level (\( \chi^2=5.00, df=1, P=.003 \)) differed significantly between the HH and LL+LH Na-Li countertransport genotypes. Regression of the logit probability of having hypertension on each of the remaining predictors, HDL-C and apolipoproteins AI and CII, was not dependent on genotype. Goodness of fit statistics indicated an adequate fit of this model to the data (\( \chi^2_{HL}=12.177, df=8, P=.143 \)). Somer's \( D_{xy} \) statistic of 0.720 also indicated that there was a high correlation between the observed data and predicted values. Brown's statistic (3.707, \( df=2, P=.157 \)) indicated that the logistic model adequately described the relationship between hypertension and the selected predictor traits. Regression diagnostic plots (not presented) revealed no high leverage or outlier observations.

Estimates of the partial regression coefficient (\( \beta \)) and conditional odds ratio (computed as \( e^\beta \)) associated with each predictor trait are presented in Table 3 for women and Table 4 for men. In women, the odds of having hypertension increased with increases in apolipoprotein CIII and the age-by-BMI interaction term. Because of this interaction, the negative age and BMI coefficients do not have a biologic interpretation. In men, the odds of having hypertension increased with increases in Na-Li countertransport, age, apolipoprotein AI, and apolipoprotein CII. In contrast, the odds of having hypertension decreased with increases in levels of HDL-C.

For a 1-year increase in age, the odds of having hypertension increased 5.82 times in men with the HH genotype but only 1.13 times in men with the LL+LH genotype. For an increase of 10 \( \mu \)mol/L red blood cells per hour in Na-Li countertransport, the odds of having hypertension increased 5.37 times in men with the HH genotype but only 1.11 times in men with the LL+LH genotype. The Figure depicts the probability of having hypertension in men at each level of Na-Li countertransport and age. Equation 1 was used to compute the predicted probabilities under the assumption that the other predictors remain constant at their mean values: 1 year of age, 1 U BMI (kg/m\(^2\)), and 1 mg/dL Apo CIII. No high leverage or outlier observations were detected in regression diagnostic plots (not shown).
The results of our study suggest several inferences about the genetics of hypertension. First, rejection of the null hypothesis that allelic variation at a single locus does not predict interindividual variation in the probability of having hypertension suggests that genetic variation can make a useful contribution to the prediction of hypertension after other known predictors have been considered. Second, finding that the regression relationship between the logit probability of having hypertension and age and Na-Li countertransport level is dependent on the genotype of this single locus in men supports the widely held belief that biologic relationships between the molecular effects of genome variation and development of common chronic diseases such as hypertension are complex. Third, differences between women and men in the contribution of allelic variation in this gene to the prediction of the probability of hypertension suggest interactions of genome effects with biologic and/or environmental effects that are marked by the gender stratification.

A previous study suggests that allelic variation in a single gene that is associated with interindividual variation in Na-Li countertransport level predicts the development of hypertension. Hunt et al assessed the onset of hypertension over a 7-year follow-up period in a cohort of normotensive adults ascertained through individuals with coronary artery disease. The incidence of hypertension in individuals likely to have a genotype associated with increased Na-Li countertransport levels was 18.8%. The incidence of hypertension in individuals likely to have an alternative genotype was significantly lower, at 3.7%. They reported that the incidence of hypertension was not associated with the level of Na-Li countertransport measured at the beginning of the follow-up period. It is not possible to make direct comparisons between the present study and that of Hunt et al because of the different sampling designs and the different analytic methods used in the two studies. In addition, Hunt et al considered only the separate effects of Na-Li countertransport genotype and Na-Li countertransport level in predicting the probability of developing hypertension. They did not consider a model in which an interaction between variation in Na-Li countertransport level and variation in genotype was included. Regardless, both the present study and the one by Hunt et al are consistent in demonstrating an association between variation in a gene responsible for large biometric effects on Na-Li countertransport level and the occurrence of hypertension.

In our cross-sectional study we used an analytic strategy that considered the possibility for an interaction between the effects of genetic variation and variation in continuously distributed intermediate traits that are hypothesized to link genetic and environmental variation to interindividual differences in the risk of hypertension. Our finding that the statistical relationships between the probability of having hypertension and Na-Li countertransport are heterogeneous among the biometrically inferred Na-Li countertransport genotypes calls attention to the complexity of the etiologic connections that exist between variation in causations and interindividual variation in the risk of hypertension. This finding suggests that the primary influence of allelic variation marked by large effects on levels of Na-Li countertransport on the risk of hypertension may be through alterations of the interdependency of the biochemical and physiological processes that are in-

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**Discussion**

The results of our study suggest several inferences about the genetics of hypertension. First, rejection of the null hypothesis that allelic variation at a single locus does not predict interindividual variation in the probability of having hypertension suggests that genetic variation can make a useful contribution to the prediction of hypertension after other known predictors have been considered. Second, finding that the regression relationship between the logit probability of having hypertension and age and Na-Li countertransport level is dependent on the genotype of this single locus in men supports the widely held belief that biologic relationships between the molecular effects of genome variation and development of common chronic diseases such as hypertension are complex. Third, differences between women and men in the contribution of allelic variation in this gene to the prediction of the probability of hypertension suggest interactions of genome effects with biologic and/or environmental effects that are marked by the gender stratification.

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volved rather than on the levels of any particular intermediate trait.

Our ability to study the complexity of such relationships across the hierarchy from the molecular level of the genotype to the clinical level of the hypertension end point is seriously hampered by our ignorance about which genes might be involved. The gene responsible for the large biometric effects on Na-Li countertransport level joins the gene that codes for angiotensinogen as a candidate for determining an individual's predisposition to hypertension. The angiotensinogen gene has been characterized; however, the identification, location, and characterization of the Na-Li countertransport gene remain elusive goals. Furthermore, a top-down phenotype-to-genotype search for the majority of the genes involved in the genetics of hypertension may be even more difficult because most may make a smaller contribution to the determination of the variable in the risk of hypertension than the gene responsible for large effects on Na-Li countertransport.

Interactions of the sort suggested by the present study bring into question the analytic strategy that should be used to detect genetic effects on the probability of hypertension. An analytic strategy that adjusts blood pressure variation, or probability of having hypertension, for variability in traits that are hypothesized to be risk factors before a genetic analysis is carried out may lead to erroneous inferences about the causes of hypertension. When the contribution of a particular trait to prediction is statistically significant in only a small subgroup of the population defined by genotype information, it is probable that one will not observe a significant contribution of variation in this trait to the prediction of hypertension when genetic information is disregarded. A nonsignificant result will certainly be the expectation if the regression relationship between the level of the predictor trait and blood pressure (or probability of having hypertension) is reversed in different genetically defined subgroups. More important for understanding the genetics of hypertension in the population at large is the real possibility that the effect of a particular genetic variation may be different in different subdivisions of the population defined by values of a particular risk factor trait. Perusse et al showed that the effect of a single gene on interindividual variation in blood pressure levels is dependent on the age of the carriers of a particular allelic variation. The effects of the gene were greater in older individuals, suggesting the possibility of interactions with environments correlated with age. Reilly et al have convincingly shown that the effect of allelic variation in the gene coding for the apolipoprotein E molecule on plasma HDL-C levels depends on the waist-to-hip ratio of the individuals being considered. Such effects cannot be considered when the genetic analysis is conducted on residual variation in the dependent variable after an adjustment for variation in other risk factor traits has been carried out.

The range of genetic models available for the analysis of complex traits such as blood pressure is limited. A single-gene, segregation-linkage paradigm that assumes a Cartesian, one-to-one mapping between genotypic variation and discrete phenotypic variation is of limited value in unraveling the genetics of hypertension. Genetic models that do not include the possibility that phenotypic variation may be a consequence of interactions between genotypic and environmental effects rather than an addition of these primary causations have the greatest potential to mislead. The results of the present study suggest that an appropriate model considers hypertension to be a consequence of genetically determined variation in the norm of reaction to variations in the rest of the genome and variations in external environmental factors measured by age in Na-Li countertransport. In such models, different genotypes determine different responses when exposed to a particular array of environmental effects. Studies that do not consider models that include a genotype-by-environment interaction will most likely fail to detect a genetic effect when in fact one does exist.

The biologic complexity implied by the genetic effects found in the present study is not unexpected by those familiar with the study of common, chronic diseases such as hypertension. and are correlated with age. Reilly and Molf have suggested a genetic model that considers Na-Li countertransport and other continuously distributed biochemical and physiological traits, many of which are correlated with age. One such model is the biologic processes that link genetic variation with variation in measures of the blood pressure phenotype. Such a model considers hypertension to be an emergent property of numerous intermediate processes that are each under the influence of many genetic and environmental factors. The contribution of measures of body size, Na-Li countertransport, and plasma lipids and apolipoproteins to the prediction of the probability of having hypertension in our study points to several of the intermediate physiological processes that determine the risk of hypertension. Many polymorphic genes and variation in exposures to many environmental factors are expected to influence the distribution of the measures of each of these intermediate processes. The consequences of such complexity in the hierarchical relationships between genetic variation and variation in the risk of disease are twofold. First, mapping of effects is expected to be one-to-many, from a particular gene to intermediate traits, and many-to-one, from intermediate traits to clinically defined hypertension. Second, genetic variation can be expected to influence relationships between measures of the intermediate processes and the relationships of these measures with the risk of hypertension.

One implication of this etiologic complexity for the prediction of hypertension is illustrated by considering the results presented in Table 4 and the Figure. The probability of having hypertension given the HH genotype spans the same range of values (0 to 1) as the probability of having hypertension given the LL genotype. Two men with different genotypes could have the same probability of having hypertension because they have different ages, Na-Li countertransport levels, or both. Similarly, two men with the same genotype could have different probabilities of having hypertension because they have different ages, Na-Li countertransport levels, or both. Furthermore, because allelic variations in different genes may have similar effects on the traits in the hierarchy from genome to clinical end point and because many polymorphic genes influence the distribution of the intermediate traits that determine the risk of hypertension in the population at large, different subsets of genes are expected to be associated.
with disease in particular families and particular individuals. In the present study, the probability of having hypertension given Na-Li countertransport level differed significantly between Na-Li countertransport genotypes only in men. Estimates of the effects of age and Na-Li countertransport on the probability of having hypertension in men made by Turner et al represent a composite measure of the effects of each genotypic class presented in the present article. Accordingly, the odds ratio estimates of the effects of changes in age and Na-Li countertransport level made by Turner et al fell intermediate to the genotype-specific estimates of the present study. In women, the inferences of Turner et al differed from the present study in that Turner et al found a significant relationship between Na-Li countertransport level and the probability of having hypertension, whereas we found no relationship between Na-Li countertransport genotype or level and the probability of having hypertension in women in the present study. A comparison of the present study with the results of Turner et al suggests that differences in results between genders may be explained by sample size considerations. In the present study, 31% of women ranging from 20 to 89 years of age were hypertensive. In the sample of Turner et al, drawn from the same reference population as the present study, 56% of women ranging from 47 to 89 years of age were hypertensive. Both studies used the same diagnostic definitions for hypertension. Three sample-related differences between the present study and that of Turner et al may explain the observed gender differences. First, the present sample may have had insufficient statistical power to detect an association of Na-Li countertransport with the probability of having hypertension in women, whereas the sample of Turner et al did have sufficient power, with its higher hypertension prevalence. To illustrate this possibility, we used the method of Hsieh to compare the power to detect a conditional odds ratio for Na-Li countertransport significantly different from 1. The conditional odds ratio estimated at one standard deviation above the mean Na-Li countertransport level in women in the present study was 1.26. Given that the prevalence of hypertension in women was 30%, the present sample had less than 70% power to detect a conditional odds ratio of this magnitude. Conversely, the power to detect a conditional odds ratio of 1.26 in the study of Turner et al was approximately 90%. Therefore, the relatively lower prevalence of hypertensive subjects in the present sample may have decreased the power to identify Na-Li countertransport as a predictor of hypertension in women.

Second, misclassification of blood pressure diagnostic status might also explain a decrease in the statistical power to detect a particular odds ratio effect. For example, misclassification of hypertension in women but not in men may explain in part why Na-Li countertransport level was not found to be a significant predictor in women. However, there is no evidence in the present study to suspect a systematic misclassification of hypertension that would bias our results. Hypertension in previously diagnosed or treated individuals was based on medication history and careful review of complete medical records. Hypertension status in previously undiagnosed, nontreated individuals was based solely on blood pressure readings. Therefore, any misclassification of the hypertension end point was nondifferential with respect to the predictor variables and gender.

Third, there may be a specific relationship of Na-Li countertransport and hypertension in women that is manifest only (or predominantly) in older age groups. Finally, because of differences in the predictors and Na-Li countertransport and because many of these predictors are correlated with age, the particular combination of traits identified as significant predictors in each study could account for the differences in prediction by Na-Li countertransport genotype or level in women. Because of these concerns, the results of the present study suggest, but do not conclusively demonstrate, that the role of the Na-Li countertransport gene in predicting the probability of having hypertension is different in women and men.

The results of the present study allow inferences to be made about the proportion of hypertension in the reference population of Rochester, Minn, that may be associated with elevated levels of Na-Li countertransport. A comparison of the present study with the results of Turner et al suggests that differences in results between genders may be explained by sample size considerations. In the present study, 31% of women ranging from 20 to 89 years of age were hypertensive. In the sample of Turner et al, drawn from the same reference population as the present study, 56% of women ranging from 47 to 89 years of age were hypertensive. Both studies used the same diagnostic definitions for hypertension. Three sample-related differences between the present study and that of Turner et al may explain the observed gender differences. First, the present sample may have had insufficient statistical power to detect an association of Na-Li countertransport with the probability of having hypertension in women, whereas the sample of Turner et al did have sufficient power, with its higher hypertension prevalence. To illustrate this possibility, we used the method of Hsieh to compare the power to detect a conditional odds ratio for Na-Li countertransport significantly different from 1. The conditional odds ratio estimated at one standard deviation above the mean Na-Li countertransport level in women in the present study was 1.26. Given that the prevalence of hypertension in women was 30%, the present sample had less than 70% power to detect a conditional odds ratio of this magnitude. Conversely, the power to detect a conditional odds ratio of 1.26 in the study of Turner et al was approximately 90%. Therefore, the relatively lower prevalence of hypertensive subjects in the present sample may have decreased the power to identify Na-Li countertransport as a predictor of hypertension in women.

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tributable to the HH genotype decreases as more hypertension is attributed to the more common LL+LH genotypic class.

The conclusion of the present study is that all genetic variation at a single gene with large effects on levels of Na-Li countertransport contributes information that will be useful in predicting hypertension. However, our study also suggests that this single gene alone is not sufficient to explain all hypertension in the general population. There are several reasons for this latter inference. The first is that the estimates of allele frequencies associated with this single gene will at most explain only a fraction of the prevalence of hypertension in the population. Second, there is evidence for heterogeneity in the relationship between the probability of having hypertension and Na-Li countertransport and age across Na-Li countertransport genotypes. Interactions between the effects of this gene and the effects of environment or other genes that influence the predisposition to hypertension are implicated. This study contributes to a growing awareness that the etiology of common chronic diseases such as hypertension is an emergent property of the interaction of genetic and environmental factors.

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