Genetic Studies of Cation Tests and Hypertension

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AND K. OWEN ASH

SUMMARY Several tests of cation concentration and transport are being studied among members of large Utah pedigrees as part of a study of the genetic and environmental determinants of essential hypertension. Corrected urinary sodium excretion and plasma sodium concentration correlated well in spouses and siblings ($r = 0.21-0.54$, $p < 0.001$), suggesting the effects of shared family environment (e.g., sodium intake). Intraerythrocytic sodium concentration and sodium-lithium countertransport showed no significant correlation in spouses and very significant correlations between siblings and between parents and offspring ($r = 0.34-0.58$, $p < 0.001$), suggesting mostly genetic determination. Using maximum likelihood tests of different genetic models, both sodium-lithium countertransport and intraerythrocytic sodium showed predominantly polygenic determination ($H^2 = 70\%$) and some possible major gene determinants ($H^2 = 18-25\%$) for a total heritability of 89 to 95% for these characteristics. These data suggest both genes and shared family environment contribute to the familiality of cation tests. They also illustrate the need and utility of quantitative methods for objective analysis of pedigree data. (Hypertension 10 [Suppl I]: I-37-I-41, 1987)

KEY WORDS: biochemistry • essential hypertension • genetics • epidemiology • pathophysiology

FOR several decades studies have indicated that essential hypertension is a familial condition. Recent studies of special cation tests suggest they may help identify some genetically determined homogeneous subgroups of essential hypertension. Family studies can help to answer the following questions:

1. Is there evidence of familial aggregation or correlation of a specific trait (in this case, special cation tests)?
2. If observed, is familiality due to genes, shared environment, or both?
3. Is significant heritability present due to major genes, polygenes, or mixtures?
4. How is the expression of genetic effects modified by environmental factors or other genes?

Five cation tests summarized in Table 1 have been reported to show distinctive values in hypertensive subjects compared to controls and also in normotensive individuals with a positive family history of hypertension. Sodium-lithium countertransport has been studied and reported most widely. Higher mean values for this test have been generally observed in persons with essential hypertension and in their normotensive first-degree relatives. In one study, children who had one hypertensive parent did not show elevated values.

Different populations have shown different values for sodium-potassium cotransport; values in hypertensive subjects and their relatives were low in some studies and high in others.

Elevated values have been reported in hypertensive subjects and in their normotensive first-degree relatives for $Na^+\cdotK^+$-adenosine triphosphatase (ATPase) pump, intralymphocytic sodium, and intraerythrocytic sodium.

These reported studies suggest that cation measurements are associated with hypertension and may provide biochemical manifestations of a developing familial syndrome before blood pressure abnormalities are manifest. Further family and pedigree studies are being carried out in several locations to see if shared family environment, major genes, or polygenes have an effect on cation tests. To illustrate the methodology and provide some developing results, data from Utah pedigrees are presented.
Subjects and Methods

Protocol

From 1980 to 1985, 2548 individuals in 98 Utah kindred participated in a 4-hour research evaluation in the Cardiovascular Genetic Research Clinic at the University of Utah. Pedigrees were ascertained from population-based computer files of Utah deaths or participants in the Hypertension Detection and Follow-up Program (HDFP) and fell into one of four categories:

1. Descendants of sibships with two or more stroke deaths before age 74 years (eight kindred with 553 persons).
2. Descendants of sibships with two or more coronary deaths before age 55 years (19 kindred with 1233 persons).
3. Relatives of hypertensive probands from the HDFP study (53 kindred with 646 persons).
4. Families of normotensive probands from the HDFP study (19 kindred with 116 persons).

During a 4-hour screening clinic, data collection included careful genealogical data, family medical history, personal habits and medical history, several measurements of blood pressure and anthropometrics, a physician’s history and physical examination, fasting blood samples, and three 12-hour overnight urine samples.

Biochemical tests performed in the clinical and research laboratories of the Pathology Department at the University of Utah Medical Center included plasma sodium, urine sodium and creatinine, intraerythrocytic sodium, Na\(^+\),K\(^+\) cotransport, Na\(^+\),K\(^+\)-ATPase pump, and laboratory determinations were reported previously. 2, 8, 17-22

Statistical Analyses

Because our prior studies\(^{23}\) had shown that sodium-lithium countertransport as well as electrolyte measurements are dramatically affected by pregnancy, pregnant women and those 4 months past delivery were excluded from analyses, as were women taking oral contraceptive or menopausal hormones. Any subjects taking diuretic or antihypertensive medications were also excluded because of the potential confound-

ing effects of these pharmacological agents on the study tests.

The possible confounding effects of age, sex, body size, and variability and completeness of urine collection were evaluated by multiple linear regression\(^{24}\) of electrolyte measurements on age, sex, height, weight, skinfold thicknesses, ponderal index, and creatinine excretion. When significant associations were found, the residuals from these regression analyses were used as the corrected variables for tests of familiality and genetic transmission.

Correlation analyses were used to test for similarity of quantitative variables between specific pairs of family members. When several relatives such as siblings were available, all possible pairs were tested and significance values were reduced to adjust for the inflation of sample size.

The frequency distribution of values was tested for possible significant bimodality or trimodality.\(^{25}\) In other words, observed data were tested to see if they fit two or three normal distributions better than a single normal distribution.

Maximum likelihood pedigree analysis\(^{26-32}\) was used to test the following 12 models: random (sporadic), three simple major gene models with one locus and two alleles (recessive, dominant, codominant), pure polygenic, three models of mixed major gene and polygenic effects (recessive-mixed, dominant-mixed, codominant-mixed), two models of mixed major environmental factors and polygenic (2 modes, 3 modes), and two unrestricted models (2 modes, 3 modes). Parameters estimated in these models using maximum likelihood analyses included the frequency and means of each distribution representing either a major-gene genotype or the mode of a major environmental factor, the phenotypic standard deviation, the heritability due either to polygenes or major genes, and the transmission parameters that represent the probability that a particular factor (either genetic or environmental) will be transmitted from parent to offspring.

To avoid bias in the genetic analyses, ascertainment corrections were carried out\(^{25}\) to account for the probability of observing given individuals based on the method for selecting the pedigrees.

Results

Different patterns of correlations between family members were observed for sodium concentration in plasma and erythrocytes and for excretion of sodium in the urine. All values had been adjusted for potential confounders such as body size, anthropometrics, and urinary creatinine excretion before correlation analyses were performed.\(^{24}\) As shown in Table 2, similar low-level correlations were observed for urinary sodium in spouses and siblings living together, and less for siblings living apart. This suggests that environmental factors rather than genes play the prominent role in the familial correlations for urinary sodium.

For plasma sodium concentration, highly significant correlations were noted between spouse pairs and siblings living together, but not for siblings living apart.

<table>
<thead>
<tr>
<th>Cation Test</th>
<th>High BP*</th>
<th>FH†</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na(^+)-Li(^+) countertransport</td>
<td>↑</td>
<td>↑</td>
<td>1-10</td>
</tr>
<tr>
<td>Na(^+)-K(^+) cotransport</td>
<td>↓</td>
<td>↓</td>
<td>12</td>
</tr>
<tr>
<td>Na(^+),K(^+)-ATPase pump</td>
<td>↑</td>
<td>↑</td>
<td>14</td>
</tr>
<tr>
<td>Intralymphocytic Na(^+)</td>
<td>↑</td>
<td>↑</td>
<td>15</td>
</tr>
<tr>
<td>Intraerythrocytic Na(^+)</td>
<td>↑</td>
<td>↑</td>
<td>16</td>
</tr>
</tbody>
</table>

Arrows indicate higher or lower levels than controls. BP = blood pressure; + FH = positive family history.

*Results in hypertensive adults.
†Results in normotensive adults or children with hypertensive first-degree relatives.
This strongly suggests that shared current environmental factors account for plasma sodium correlations rather than genes.

Intracellular sodium showed very different results. Highly significant correlations were found between siblings living together, with lower correlations in siblings living apart and in spouses. This suggests these familial correlations are due to genes and environmental factors.

Familial correlations for sodium-lithium countertransport also strongly suggested genetic effects (Table 3). Significant correlations were observed between siblings and between parents and their offspring, but not between spouses. An even higher correlation between the midparent average and offspring values suggests possible polygenic effects.

Table 4 summarizes the major results from detailed maximum-likelihood pedigree analysis for sodium-lithium countertransport. The results differed depending upon whether or not the original data were transformed to produce a normal distribution before analysis. The untransformed data were skewed, and significantly fit two distributions better than a single distribution. In this case, the best-fitting model was the mixed polygenic-recessive model with a total heritability of 89%, of which 18% was associated with the major gene component and 71% with the polygenic component. When data were transformed to produce a normalized distribution before pedigree analysis, the pure polygenic model fit best with a 71% overall heritability. In either case, evidence for genetic determination and a large polygenic component was strong.

The means of the phenotypes are biologically reasonable. A mean of 0.23 for one phenotype corresponds to an ordinary value in the normal population, and the value of 0.44 mmol/L of red blood cells per hour corresponds to a value commonly seen among hypertensive individuals. The gene frequencies would also indicate that about 10% of individuals in the general population could carry a genotype predisposing to hypertension (some of which may not be expressed due to protective environmental factors).

Similar analyses have been carried out for intracellular sodium. The results appear to be very analogous, with a total heritability of 95%, of which about 25% appears to be due to major gene effects and 70% due to polygenic effects.

**Discussion**

This report has two major objectives. First we present evidence for strong familial and genetic effects for cation tests of interest to the pathophysiology of essential hypertension. Second, and perhaps even more important, we illustrate a quantitative, methodologic approach to collecting and analyzing family data.

Analyses of four different sodium tests (urine, plasma, intracellular, and countertransport) illustrate a broad spectrum of findings. All show strong familial correlations. Urine and plasma sodium appear to be strongly affected by shared family environment, while intracellular sodium and countertransport appear to be strongly affected by genetic factors. For these two variables, polygenic factors appear to be most prominent, but some significant major gene effects would appear also to be present. To determine the degree to which genetic expression is modified by environmental factors or other genes will require further detailed analyses. This can be accomplished most efficiently if a genetic linkage marker is found for the major gene effects.

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**Table 2. Sodium Correlations Between Family Members in Utah**

<table>
<thead>
<tr>
<th>Family Member Pairs</th>
<th>Pearson Correlation Coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sodium Na⁺</td>
</tr>
<tr>
<td>Spouses</td>
<td></td>
</tr>
<tr>
<td>(shared current environment)</td>
<td>0.29* (191)</td>
</tr>
<tr>
<td>Siblings, &lt;20 yr (shared genes and current environment)</td>
<td>0.38* (641)</td>
</tr>
<tr>
<td>Siblings, &gt;20 yr (shared genes and past environment)</td>
<td>0.10 (241)</td>
</tr>
<tr>
<td>Suggested reasons</td>
<td>Current</td>
</tr>
<tr>
<td>Numbers in parentheses are numbers of pairs. Sodium excretion in 12-hour urine sample was adjusted for creatinine excretion. *p &lt; 0.001.</td>
<td></td>
</tr>
</tbody>
</table>

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**Table 3. Sodium-Lithium Countertransport Correlations in Utah Families**

<table>
<thead>
<tr>
<th>Family Member Pairs</th>
<th>r</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spouses</td>
<td>-0.02</td>
<td>81</td>
</tr>
<tr>
<td>Siblings</td>
<td>0.34*</td>
<td>267</td>
</tr>
<tr>
<td>Parent with offspring</td>
<td>0.33*</td>
<td>386</td>
</tr>
<tr>
<td>Mid-parent average with offspring</td>
<td>0.44*</td>
<td>131</td>
</tr>
</tbody>
</table>

*p < 0.001.

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**Table 4. Maximum Likelihood Test of Genetic Models for Na⁺-Li⁺ Countertransport in Utah Pedigrees**

<table>
<thead>
<tr>
<th>Best Fitting Models</th>
<th>Mixed-recessive</th>
<th>Pure Polygenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total heritability</td>
<td>89%</td>
<td>71%</td>
</tr>
<tr>
<td>Major gene</td>
<td>18%</td>
<td>0%</td>
</tr>
<tr>
<td>Polygenic</td>
<td>71%</td>
<td>71%</td>
</tr>
<tr>
<td>Means of phenotypes</td>
<td>0.23</td>
<td>0.25</td>
</tr>
<tr>
<td>Gene frequencies</td>
<td>0.67</td>
<td>0.33</td>
</tr>
</tbody>
</table>

N = 434 persons in 10 pedigrees. A mixed-dominant model also fits well. All listed models were 10⁴ more likely than a random model. RBC Na⁺ showed similar results with 95% total heritability (about 25% major gene and 70% polygenic).
Performing and interpreting genetic analyses should be carried out with great caution. In this study, several important steps have been taken to avoid confusing or misleading results. Population-based families were chosen using objective selection criteria and ascertainment corrections. If only anecdotal families are studied, they may be quite nonrepresentative of the general population and mislead the investigators to feel that they have a characteristic observation. If a particular trait is largely nonpenetrant in the general population, anecdotal families coming to the attention of investigators will likely be those in whom the trait has been expressed, as opposed to others who carry genetic tendencies that are not expressed.

If objective selection criteria are used for choosing families, ascertainment corrections can be carried out and can provide important insights. For example, choosing families based on the observation of an affected proband and an affected parent produces bias in favor of selecting families that have a dominant trait with typical vertical transmission. Mathematical corrections for this ascertainment scheme may help prevent such misleading interpretations.

All four variables analyzed in this report showed significant correlations with some other potential confounders including age, sex, or anthropometric measurements. Siblings and spouses, as groups, are more similar in age than other randomly selected individuals in the general population. Factitious familial correlations could result purely due to similarity in age for any variable that is strongly affected by age (such as systolic blood pressure). Regressing the variable of interest versus the potential confounders produces residual values that can then be used as the unconfounded variable for familial analyses.

Quantitative methods such as likelihood pedigree analysis for comparing genetic models are challenging and generally used only by those with training in population genetics. Many medical scientists are not familiar with these methods and do not use them. They are familiar with the standard mendelian inheritance of traits such as ABO blood type, and often expect to find similar simple models for other variables they study. While simple, straightforward, mendelian inheritance has been found for a variety of rare medical conditions ranging from birth defects to familial hypercholesterolemia, this is unlikely to be the case for any medical condition as common and heterogeneous as essential hypertension. Our understanding of the pathophysiology of hypertension involves many hormones, enzymes, receptors, physiological mechanisms, and biochemical factors. Each of these processes could potentially be affected by one or more genetically determined characteristics. It is logical to assume a polygenic influence plays a dominant role.

For a specific biochemical variable such as a membrane transport test, it is not unreasonable to hope that it might show major gene effects. Our results provide some encouragement by showing a suggestion of major gene effects for sodium-lithium countertransport, but this is also accompanied by an even more pervasive polygenic influence. These results for sodium-lithium countertransport are consistent with several other careful quantitative genetic analyses that report discrete modes, strong familiality, and major gene and/or polygenic effects.

It is also likely that some major gene traits will be associated with more than two different alleles or with more than one locus. Such models can be constructed and tested using the same quantitative methods illustrated here. Careful and time-consuming computer analyses must be carried out to investigate such models. It is hoped that in an iterative and careful fashion, models can be constructed and tested that will eventually lead to a clear picture of the actual underlying genetics and biology.

The best way to identify major gene effects is to use a gene marker in linkage analysis. The recent explosive development of DNA probes for major genes should provide important help. Even here, the heterogeneity of hypertension leads to challenges not usually encountered in other linkage analyses. In most studies, a single large pedigree with affected individuals contains only those individuals whose condition is related to a single major gene of interest. Because hypertension is so common and so heterogeneous, however, even a single large pedigree is likely to have several different types of essential hypertension represented. Thus, even a gene linked to a specific form of hypertension will not be carried by some hypertensive members within a study pedigree (due to other causes of hypertension).

It would appear that combining special biochemical tests, such as sodium-lithium countertransport and DNA probes, may provide powerful tools. Linkage of the DNA probe to biochemical variables will help eliminate some of the heterogeneity of essential hypertension and then identify individuals with specific subtypes of essential hypertension.

In summary, some cation tests are very familial, apparently due to both genes and shared environment. In large pedigrees carefully studied with quantitative genetic analyses, evidence is good for both polygenic and major gene effects. In view of the potential difficulties involved, investigators studying such tests in families should be careful to avoid any claims of major gene effects based purely on visual inspection of a few anecdotaly ascertainment nuclear families. Careful quantitative methods developed by population geneticists were applied in this analysis. They provide an example of study methods that can be used in similar family studies of hypertension and cation transport.

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Genetic studies of cation tests and hypertension.
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Hypertension. 1987;10:137
doi: 10.1161/01.HYP.10.5_Pt_2.I37

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