Genetic Studies of Cation Tests and Hypertension

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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 • biochemistry

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 normoten-

sive 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 \( \text{Na}^+ \), \( \text{K}^+ \)-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 (18 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, and sodium-lithium countertransport. The methods for the selection, clinical screening, and laboratory determinations were reported previously.2,8,17-22

Statistical Analyses

Because our prior studies23 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-
This strongly suggests that shared current environmental factors account for plasma sodium correlations rather than genes.

Intraerythrocytic 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 intraerythrocytic 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.
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 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 anomalously ascertained 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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