Linkage of MN Locus and Erythrocyte Lithium–Sodium Countertransport in Tecumseh, Michigan

Alan B. Weder, Nicholas J. Schork, and Stevo Julius

Human essential hypertension is a family of diseases; one subtype has an increased maximum velocity for red blood cell lithium–sodium countertransport activity. To begin the localization of the gene or genes responsible for this phenotype, we examined the association of blood pressure, lithium–sodium countertransport, and two genetic markers previously associated with hypertension—the MN blood group antigen (chromosome 4) and the plasma protein haptoglobin (chromosome 18) — in a population-based sample of 592 young adults from Tecumseh, Mich., the site of an ongoing cardiovascular epidemiological investigation. Our results suggest that the relation between MN phenotype and systolic blood pressure is significantly different and oppositely directed in men and women. Analysis of data available from previous examinations revealed that similar blood pressure differences related to MN phenotype had been present at least a decade earlier in both men and women. There also was a significant relation between systolic blood pressure and haptoglobin phenotype for the combined group of men and women. In addition to having high systolic blood pressure, men with the MM phenotype had significantly elevated red blood cell lithium–sodium countertransport activity. In studies of brother-brother pairs, we found evidence for significant genetic linkage between the MN locus and red blood cell lithium–sodium countertransport activity. (Hypertension 1991;17:977–981)

There is little doubt that human essential hypertension is heritable, but no specific candidate genes mediating either blood pressure (BP) as a continuous quantitative trait or hypertension as a disease entity have been identified. In part, the slow progress in defining the genetic basis of hypertension results from the complexity of the physiological systems controlling BP. Most evidence suggests that hypertension results not simply from the elaboration of structurally abnormal gene products but rather from dysfunctions at integrative, regulatory levels, and it has proved difficult to find evidence that the complex, continuously distributed, multifactorially determined biochemical and physiological disturbances contributing to hypertension result from allelic differences. Although two recent segregation studies have detected evidence of a major gene effect,1,2 the inheritance of high BP traditionally has been conceived of as representing the additive or multiplicative effects of polygenes, whose individual identification by mathematical or biochemical techniques exceeds current capabilities.

The description by Canessa et al3 of an association of an increased maximum velocity for red blood cell lithium–sodium (RBC Li\(^{+}\)-Na\(^{+}\)) countertransport and essential hypertension has led to studies in which the transport abnormality has been used to probe the genetic basis of the disease. Several features lend appeal to this marker in studies of genetic hypertension: transmembrane Li\(^{+}\)-Na\(^{+}\) countertransport is presumably mediated by a single intrinsic membrane protein, the activity of which is highly heritable4 and stably expressed as a phenotype that can be modeled as a mixture of two normal subpopulations in both sexes at all ages.5 However, because the RBC Li\(^{+}\)-Na\(^{+}\) countertransporter has not been isolated, molecular genetic studies have been limited to examining polymorphisms in the recently cloned Na\(^{+}\)-H\(^{+}\) antiporter gene.6 Two groups now have reported an absence of linkage between allelic variations in the Na\(^{+}\)-H\(^{+}\) antiporter gene or flanking loci on chromosome 1 and either hypertension7,8 or increased RBC Li\(^{+}\)-Na\(^{+}\) countertransport,9 suggesting that the primary factors controlling the expression of increased RBC Li\(^{+}\)-Na\(^{+}\) countertransport and its associated...
hypertension must reside outside the region of this gene.

To further investigate this issue, we examined a population-based sample of the young residents of Tecumseh, Mich., for associations of BP and increased RBC Li\(^+\)-Na\(^+\) countertransport with two well-localized markers previously associated with hypertension, the RBC blood group MN antigen\(^9\) and the plasma protein haptoglobin.\(^10\)

Methods

Participants in the present study were 592 18–42-year-old men and women who reside in the vicinity of Tecumseh, Mich., the site of an ongoing epidemiological study of the antecedents of essential hypertension. None of the individuals in the present report was taking antihypertensive treatment, birth control pills, or estrogen supplements at the time of the study. All were free of overt cardiovascular, renal, endocrine, and hepatic disease on physical and laboratory examinations.

All physiological measurements were performed at a field clinic in the community. Subjects reported in a fasting state, and weight and height were measured with subjects in street clothes without shoes. Subjects then rested in a seated position for at least 2 minutes with the right arm comfortably supported at the level of the heart before BP was determined twice by a physician using a standard sphygmonanometer and a cuff appropriate to arm size. Blood then was drawn by venipuncture for measurement of RBC Li\(^+\)-Na\(^+\) countertransport by the method of Canessa et al.\(^3\)

During earlier examinations, participants in the present study and their parents had undergone anthropometric measurements, BP determination, and phenotyping for the MN blood group antigen and serum haptoglobin as part of the Tecumseh Community Health Study.\(^11\) Countertransport data had not been collected previously for either the study participants or for their parents.

Statistical Methods

Within-sex associations were assessed by univariate and multivariate analysis of variance.\(^12\) Sib-pair linkage analyses for the MN locus with RBC Li\(^+\)-Na\(^+\) countertransport and systolic BP were performed using the method of Haseman and Elston,\(^13\) which makes use of the nonparametric correlation procedures of Kendall and Spearman.\(^14\) Estimates of 95% confidence limits for Spearman's correlation coefficient were obtained from 6,000 bootstrap samples.\(^15\)

Results

Figure 1A shows the significant, but oppositely directed, relations between MN phenotype and systolic BP in men and women. There were no significant differences in age or body mass index related to MN phenotype that could explain the differences in systolic BP. Diastolic BP was not significantly different between MN phenotypes for either men (\(p=0.76\)) or women (\(p=0.20\)). BP also had been determined in the current study participants during previous examinations, and as shown in Table 1, the gender-specific relations between MN phenotypes and systolic BP described in the current study also were evident a decade earlier in men and even in childhood in women.

Systolic BP was significantly different among haptoglobin phenotypes for the entire group (1/1: 117.8±1.6 mmHg; 1/2: 113.5±0.8 mmHg; 2/2: 115.1±1.1 mm Hg, \(p=0.05\) by analysis of variance), but diastolic BP was not (\(p=0.40\)). When stratified by gender, there were no significant differences in the relation of systolic BP to haptoglobin phenotype.

RBC Li\(^+\)-Na\(^+\) countertransport activity was significantly related to MN phenotype only in men (Figure 1B). However, it is evident that in both men and women, the patterns of Li\(^+\)-Na\(^+\) countertransport activity parallel those of systolic BP. Multivariate analysis of variance revealed significant differences among the MN genotypes within and between sex groups for the systolic BP/RBC Li\(^+\)-Na\(^+\) countertransport bivariate profile. There were no significant differences in RBC Li\(^+\)-Na\(^+\) countertransport activity for the different haptoglobin phenotypes (\(p=0.75\).
TABLE 1. Associations Between MN Genotypes of Present Study Subjects and Past Systolic Blood Pressure and Body Mass Index

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th></th>
<th></th>
<th>p</th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MM</td>
<td>MN</td>
<td>NN</td>
<td></td>
<td>MM</td>
<td>MN</td>
<td>NN</td>
</tr>
<tr>
<td>Childhood</td>
<td>n</td>
<td>Age (yr)</td>
<td>SBP (mm Hg)</td>
<td>BMI (kg/m²)</td>
<td>n</td>
<td>Age (yr)</td>
<td>SBP (mm Hg)</td>
</tr>
<tr>
<td>MM</td>
<td>85</td>
<td>8.4±0.2</td>
<td>114.7±1.3</td>
<td>16.8±0.3</td>
<td>75</td>
<td>23.4±0.5</td>
<td>117.7±1.2</td>
</tr>
<tr>
<td>MN</td>
<td>138</td>
<td>8.5±0.3</td>
<td>115.1±1.1</td>
<td>16.7±0.2</td>
<td>130</td>
<td>23.6±0.4</td>
<td>118.9±1.0</td>
</tr>
<tr>
<td>NN</td>
<td>55</td>
<td>7.7±0.5</td>
<td>113.6±1.9</td>
<td>17.2±0.6</td>
<td>49</td>
<td>23.5±0.6</td>
<td>123.0±1.7</td>
</tr>
<tr>
<td>Youth</td>
<td>n</td>
<td>Age (yr)</td>
<td>SBP (mm Hg)</td>
<td>BMI (kg/m²)</td>
<td>n</td>
<td>Age (yr)</td>
<td>SBP (mm Hg)</td>
</tr>
<tr>
<td>MM</td>
<td>69</td>
<td>23.4±0.5</td>
<td>128.4±2.2</td>
<td>24.6±0.4</td>
<td>68</td>
<td>21.6±0.4</td>
<td>117.7±1.2</td>
</tr>
<tr>
<td>MN</td>
<td>103</td>
<td>23.6±0.4</td>
<td>124.8±1.2</td>
<td>24.8±0.4</td>
<td>124</td>
<td>21.4±0.3</td>
<td>118.9±1.0</td>
</tr>
<tr>
<td>NN</td>
<td>41</td>
<td>23.5±0.6</td>
<td>122.4±2.0</td>
<td>25.2±1.2</td>
<td>48</td>
<td>21.2±0.5</td>
<td>123.0±1.7</td>
</tr>
</tbody>
</table>

Values are mean±SEM. SBP, systolic blood pressure; BMI, body mass index (weight [in kg]/height² [in meters]).

*p<0.05, tp<0.01 vs. MM by analysis of variance.

Twenty-five brother pairs with sufficient personal and family data to determine the numbers of shared MN alleles identical by descent (IBD) were included in the current study group. The difference between the brothers for unadjusted RBC Li⁺-Na⁺ countertransport values (Figure 2) and after adjustment for age and body mass index by linear regression showed a significant rank-order correlation with the proportion of IBD alleles (unadjusted: Spearman's ρ=-0.59 [p<0.01], Kendall's T=1.43 [p=0.005]; adjusted: Spearman's ρ=-0.46 [p<0.05], Kendall's T=-0.34 [p<0.05]). Because the analysis is based on a relatively small number of brother pairs, we performed bootstrap resampling to estimate the error of the rank-order correlation coefficients. As shown in Figure 2, the distribution of Spearman's correlation coefficient derived from the bootstrap procedure (unadjusted mean=-0.58 [95% confidence limits, -0.84, -0.32]; adjusted mean=-0.46 [95% confidence limits, -0.78, -0.14]) provide strong support for the significance and robustness of the linkage between the MN locus and RBC Li⁺-Na⁺ countertransport in these sib pairs. Analysis of the same brother pairs did not reveal significant linkage between MN phenotype and systolic BP. Similar analyses did not detect significant linkage of the MN locus and either systolic BP or RBC Li⁺-Na⁺ countertransport activity in sister pairs or in brother-sister pairs.

Discussion

Phenotypic differences at the MN locus previously have been associated with differences in BP¹⁶ (see Reference 9 for review), and our further observation of linkage between RBC Li⁺-Na⁺ countertransport
activity and the MN locus suggests the possibility of identifying the genetic basis of the subtype of essential hypertension characterized by an increased maximum velocity for RBC Li\(^+\)-Na\(^+\) countertransport. Our observations on the association of systolic BP and haptoglobin phenotypes provide some support for earlier observations\(^6\) but do not yield any further insights into the high Li\(^+\)-Na\(^+\) countertransport subtype of hypertension.

Canessa et al\(^{17}\) have argued that RBC Li\(^+\)-Na\(^+\) countertransport is a mode of Na\(^+\)-H\(^+\) exchange and that increased RBC Li\(^+\)-Na\(^+\) countertransport activity in hypertensives represents a functional disorder of allosteric control of the Na\(^+\)-H\(^+\) antiporter by its internally facing H\(^+\)-sensitive modifier site. Although that contention remains unproved, it led to two studies that searched for, but did not find, linkage between alleles at the Na\(^+\)-H\(^+\) antiporter locus\(^s\) and either hypertension\(^7,8\) or elevated RBC Li\(^+\)-Na\(^+\) countertransport.\(^8\) This failure to demonstrate linkage means that it is very unlikely that any of the defined alleles of the only gene presently known to code for the Na\(^+\)-H\(^+\) antiporter\(^s\) cause hypertension. Current evidence does not exclude an epistatic or interactive effect of another locus on this Na\(^+\)-H\(^+\) antiporter gene or its product, and the evidence we present for significant linkage between the MN locus and RBC Li\(^+\)-Na\(^+\) countertransport in brother pairs in the current study participants suggests that a gene of unknown function linked to the MN locus may be a reasonable candidate for such a primary genetic abnormality. It is possible that this as yet unidentified locus may code for the RBC Li\(^+\)-Na\(^+\) countertransport or another isof orm of the Na\(^+\)-H\(^+\) antiporter, or, equally plausibly, it may produce a product that affects expression of the Na\(^+\)-H\(^+\) antiporter gene or causes a posttranslational modification of the Na\(^+\)-H\(^+\) antiporter that alters its function.

There are at least three different ways in which the associations, linkage, and gender differences we observed could be explained, in addition to the statistically unlikely possibility that the findings are spurious. First, loci (or a single locus) contributing to the phenotypic expression of systolic BP and RBC Li\(^+\)-Na\(^+\) countertransport could be in linkage disequilibrium with the MN locus either because of the emergence of a recent mutation that has produced a sex-specific phenotype or because of population stratification resulting from the recent introduction of a novel allele into the population. Such an occurrence would be similar to that described as the “hitchhiking” phenomenon\(^{18}\) and would invalidate the sib-pair linkage results we report, because the method of Haseman and Elson\(^{13}\) assumes linkage equilibrium. Second, the associations in men between the MN locus, systolic BP, and RBC Li\(^+\)-Na\(^+\) countertransport could be due to sex-specific epistatic interactions between the MN phenotypes (or a closely linked locus) and each of the other variables. Finally, RBC Li\(^+\)-Na\(^+\) countertransport, systolic BP, and the MN locus all could be caught in a causal nexus of allelic and epistatic effects, much as has been described in the multilocus, epistatic theories of Hodge\(^{19}\) and Hodge and Spence,\(^{20}\) in which some of the effects manifest themselves in a sex-limited or sex-specific fashion. Such interactions may contribute to gender differences in RBC Li\(^+\)-Na\(^+\) countertransport activity previously reported.\(^4,5\)

This third explanation seems most appealing. It would be hard to reconcile stratification effects with previous studies associating MN genotypes and systolic BP\(^9,16\) given the geographic and racial diversity in those populations. In addition, the recombination fraction between a systolic BP locus and the MN locus would have to be very small for a mutation to have failed to reach linkage equilibrium with the MN locus in the time it took to infiltrate populations in widespread geographic locations. Equally important, it is hard to understand how the expression of the MN antigen could affect systolic BP, although conceivably there could be an effect of the surface antigen on Li\(^+\)-Na\(^+\) countertransport activity. All in all, it seems most reasonable to suppose that the failure to demonstrate significant linkage between the MN locus and systolic BP for both sexes while finding evidence for linkage between the MN locus and RBC Li\(^+\)-Na\(^+\) countertransport activity in brother pairs is the result of factors not yet accounted for that uniquely mediate associations between genes affecting specific MN phenotypes and systolic BP in men and women. Alternatively, it may simply reflect the imprecision of using BP itself as a phenotypic marker for hypertension, particularly when the analysis focuses on younger subjects, in whom the full phenotypic expression of BP potential is not yet realized.

In summary, we have found evidence suggesting a possible association between elevated systolic BP and the MN phenotype in men and the NN phenotype in women. Similar associations also were found when BPs determined in an independent assessment a decade earlier were used. Using a sib-pair technique, we found evidence suggesting that there is significant linkage between RBC Li\(^+\)-Na\(^+\) countertransport, a biochemical marker for hypertension, and the MN locus in men, although the MN locus was not significantly linked to systolic BP itself in sibs. These findings suggest that interest might be directed to the area of the fourth chromosome bearing the MN locus in future studies of the genetic basis of human essential hypertension.

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