Possible Association of MN Locus Haplotypes with Essential Hypertension

EUGENE R. HEISE, MICHAEL A. MOORE, QUEEN B. REID, AND HAROLD O. GOODMAN

SUMMARY Five multigenerational kindreds with familial hypertension were typed for human leukocyte antigen (HLA) and blood group antigens to investigate genetic factors that influence variability in blood pressure. Pedigree analysis revealed that children of matings in which both parents were hypertensive had a significantly greater risk of hypertension than children of matings in which one parent or neither parent was hypertensive. Blood types N and MN were abnormally distributed among hypertensive as compared with normotensive members of white but not black families. The distribution of ABO and Rh types was not significantly different between hypertensive and normotensive siblings. When all possible pairings of siblings were examined for HLA haplotype sharing, abnormal distributions were observed among hypertensive sib pairs whereas the expected mendelian segregation was observed among hypertensive-normotensive sib pairs and normotensive-normotensive sib pairs. These results suggest the genetic factors controlling variation in blood pressure may include loci in the region of the MN locus on chromosome 4 and, possibly, the major histocompatibility complex on chromosome 6. (Hypertension 9: 634–640, 1987)

KEY WORDS hypertension • family studies • genetic factors • MN blood groups • HLA haplotypes

ALTHOUGH it has been speculated that an interaction of environmental factors acting on particular genotypes is responsible for familial (essential) hypertension, the genetic component is poorly defined and the mode of inheritance has not been determined.1 The occurrence of hypertension and coronary heart disease in multiple family members and in successive generations does not distinguish between inherited and acquired factors. Nevertheless, the role of genetic factors is clear from the human studies2–4 and from animal models of hypertension,5–7 Essential hypertension generally is regarded as a polygenic disorder,8,9 although it has been suggested that a single gene with complex expression over many years might be responsible.10

A sex-related pattern of inheritance is indicated by the increased prevalence of hypertension among female relatives of hypertensive persons as compared with male relatives11 and by the similarity of blood pressures of female relatives.12 However, these results do not establish an X-chromosome linkage with hypertension.

Proposed genetic markers of hypertension include the Na⁺-K⁺ cotransport system,13–15 alleles of the MNS blood groups,16,17 and ABO blood group systems.18,19 Convincing evidence for associations with human leukocyte antigen (HLA)-ABC antigens from population studies has not been obtained.20–26 However, genes in the region of HLA that are not in linkage disequilibrium most likely would not have been detected in these studies. Alleles of properdin factor B and C3 of the complement system have been associated with ischemic heart disease27 and benign essential hypertension (see Kristensen20 for references).

The purpose of the present study was to assess the reported influence of particular blood group alleles on the variability in blood pressure and to perform an HLA haplotype segregation analysis in families with hypertension. Since genetic association or linkage may be dependent on both the group under study and its environment, we included both black and white families in an effort to identify genetic components that are either common or disparate to different ethnic groups living in the same area. These results are consistent
with a genetic relationship between the MN blood group and essential hypertension. Evidence of a possible linkage of HLA haplotypes and essential hypertension in one family will be demonstrated.

Materials and Methods

Pedigree Evaluation

Family histories were obtained from new patients seen in one of four outpatient hypertension clinics at the Bowman Gray School of Medicine, North Carolina Baptist Hospital, and Reynolds Health Center (Winston-Salem, NC, USA). Patients who had living relatives with hypertension in multiple generations were invited to participate. Informed consent was obtained from all participants or their parents (in the case of patients less than 21 years of age). All persons over 12 years of age were included. Blood pressure measurements were obtained by one of us (Q.B.R.) with a mercury manometer while the subject was seated. First and fifth Korotkoff sounds were used for the systolic and diastolic blood pressures, respectively. Blood pressure determinations were obtained on one to two different occasions in each subject. Home visits were required to obtain pedigree data, follow-up blood pressure measurements, and blood specimens from some subjects. Family members were categorized as hypertensive, borderline hypertensive, or normotensive. Persons under 18 years of age were considered hypertensive if their blood pressure was equal to or exceeded the 95% percentile for their age. For those 18 to 40 years of age, hypertension was defined as a blood pressure of 150/90 mm Hg or more. Subjects over 40 years of age, hypertension was defined as a blood pressure of 140/90 mm Hg or more. Subjects over 40 years of age who were receiving treatment. Borderline hypertension was considered to be present in those not taking hypertension medication, with normal blood pressure values on more than one determination, an average systolic pressure between 150 and 160 mm Hg, and no evidence of end-organ disease on the basis of physical or laboratory examination.

Phenotyping

Commercial antisera for A, B, M, N, S, s, C, c, D, E, e, Fy (a), Fy (b), K, k, and Xg were used for blood grouping by a standard tube hemagglutination assay. A local serum panel was used to type for 13 HLA-A, 14 HLA-B, and 6 HLA-C antigens by a two-stage microcytotoxicity assay.

Analytical Methods

HLA haplotypes were deduced from the segregation patterns in two or three generations. The chi-square statistic was used to test the statistical significance of blood group phenotypes in hypertensive and normotensive subjects. Departure from expected mendelian segregation was evaluated by a goodness-of-fit chi-square test. An α value of 0.05 was accepted as the level of significance.

Results

Table 1 shows that of 195 subjects studied, 29% were hypertensive, 3% were borderline hypertensive, and 69% were normotensive. HLA typing was available for 63% of the subjects. To establish the familial nature of hypertension, we compared the prevalence of hypertension among the offspring for three types of matings. Table 2 demonstrates that where 1) both parents were affected, 2) one parent was affected, and 3) neither parent was affected, hypertension was found in 44.8%, 12.8%, and 0% of the offspring, respectively. These differences were statistically significant. When the data were analyzed by sex of the offspring, the correlation with mating type was strongest in female offspring whose parents were both hypertensive. We could not analyze the backcross (i.e., hypertensive × normotensive matings according to the sex of the hypertensive parent) because of the small numbers in the cells.

Blood grouping was available for two white families and two black families. Table 3 shows that there were no significant differences in the distribution of four ABO phenotypes in hypertensive subjects as compared with normotensive subjects in either the black or white families. In contrast, analysis of the MN antigen distribution revealed a statistically significant difference in the distribution of the MN alleles in hypertensive white subjects as compared with normotensive white subjects. This result was caused by an excess of the N phenotype and a deficiency of the M phenotype among hypertensive white subjects. Thus, 14 of 16 (88%) hypertensive white subjects and 11 of 11 (100%) normotensive white subjects were either heterozygous or homozygous for the N allele. In contrast, the M antigen was expressed in only seven of 16 (44%) hypertensive white subjects as compared with 27 of 34 (79%) normotensive white subjects. In blacks, shifts in the same direction were noted but did not reach statistical significance.

Seven different Rh genotypes could be distinguished in the three families based on proven or probable haplotype assignments. Table 4 indicates that there was no significant difference in the distribution of Rh genotypes between hypertensive and normotensive subjects.

Population studies have yielded no consistent HLA antigen associations with hypertension. We reasoned

<table>
<thead>
<tr>
<th>Family</th>
<th>Race</th>
<th>Blood pressure</th>
<th>HLA type</th>
<th>Hypertensive</th>
<th>Borderline</th>
<th>Normotensive</th>
</tr>
</thead>
<tbody>
<tr>
<td>233</td>
<td>White</td>
<td>43</td>
<td>40</td>
<td>14</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>234</td>
<td>Black</td>
<td>79</td>
<td>39</td>
<td>27</td>
<td>1</td>
<td>51</td>
</tr>
<tr>
<td>239</td>
<td>White</td>
<td>15</td>
<td>10</td>
<td>4</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>251</td>
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<td>37</td>
<td>19</td>
<td>5</td>
<td>0</td>
<td>32</td>
</tr>
<tr>
<td>253</td>
<td>White</td>
<td>21</td>
<td>16</td>
<td>7</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>195</td>
<td>124</td>
<td>57</td>
<td>3</td>
<td>135</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Occurrence of Hypertension in Five Kindreds
TABLE 2. Occurrence of Hypertension in the Offspring of Matings in Which Both, One, or Neither Parent Was Hypertensive

<table>
<thead>
<tr>
<th>Gender</th>
<th>Hypertensive x Hypertensive</th>
<th>Hypertensive x Normotensive</th>
<th>Normotensive x Normotensive</th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>9/13 (69.2)</td>
<td>1/14 (7.1)</td>
<td>0/8 (0)</td>
<td>8.83</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>Male</td>
<td>4/16 (25.0)</td>
<td>4/25 (16.0)</td>
<td>0/11 (0)</td>
<td>2.47</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Total</td>
<td>13/29 (44.8)</td>
<td>5/39 (12.8)</td>
<td>0/19 (0)</td>
<td>10.6</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

Percentage of total is shown in parentheses.

TABLE 3. Distribution of ABO and MN Blood Types Among Hypertensive and Normotensive Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hypertensive</th>
<th>Normotensive</th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABO</td>
<td>O A B AB</td>
<td>O A B AB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>3 5 2 3</td>
<td>6 4 1 2</td>
<td>0.69</td>
<td>NS</td>
</tr>
<tr>
<td>Black</td>
<td>2 4 5 0</td>
<td>2 6 4 2</td>
<td>2.18</td>
<td>NS</td>
</tr>
<tr>
<td>Total</td>
<td>8 9 7 3</td>
<td>13 17 8 5</td>
<td>0.59</td>
<td>NS</td>
</tr>
<tr>
<td>MN</td>
<td>M MN N</td>
<td>M MN N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>3 2 5 9</td>
<td>2 11 6 7</td>
<td>6.62</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Black</td>
<td>3 10 9 11</td>
<td>11 12 12</td>
<td>2.34</td>
<td>NS</td>
</tr>
<tr>
<td>Total</td>
<td>5 15 18</td>
<td>22 28 19</td>
<td>6.20</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

TABLE 4. Distribution of Rh Genotypes Among Hypertensive and Normotensive Subjects

<table>
<thead>
<tr>
<th>Probable genotype</th>
<th>Hypertensive</th>
<th>Normotensive</th>
<th>Hypertensive</th>
<th>Normotensive</th>
<th>Hypertensive</th>
<th>Normotensive</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cde/cde</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>CDe/cde</td>
<td>6</td>
<td>8</td>
<td>6</td>
<td>3</td>
<td>12</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>CDe/Cde</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>cD/cde</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>CDe/cDe</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>7</td>
<td>4</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>CDe/CDe</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>CDe/cDE</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>χ²</td>
<td>5.37</td>
<td>3.61</td>
<td>8.25</td>
<td>4.46</td>
<td>6.62</td>
<td>9.02</td>
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<tr>
<td>p</td>
<td>NS</td>
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<td>NS</td>
<td>NS</td>
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</tr>
</tbody>
</table>

Degrees of freedom = 6.

that a haplotype analysis could be informative, as genetic associations often differ between different ethnic or geographic groups and between population and family studies. Haplotype segregation in three kindreds is shown in Figures 1 through 3. We used a sib-sib pair analysis to determine the number of HLA haplotypes that were shared between sibling pairs in which both members of the pair were either hypertensive or normotensive. All possible pairwise combinations of haplotyped siblings were included. Table 5 reveals that hypertensive pairs departed from mendelian segregation, but that the departure was of borderline statistical significance. Thus, an excess of hypertensive sib pairs were found to share one or two HLA haplotypes. In contrast, hypertensive-normotensive pairs and normo-
tensive pairs gave the expected mendelian pattern of 1:2:1 for two, one, and no shared haplotypes. Further inspection of the data revealed that excess haplotype sharing occurred chiefly in one white family (No. 233). In this family, all nine siblings (5 women and 4 men) of the first generation were hypertensive. HLA typing was available from eight of the nine siblings. Five of these eight hypertensive siblings inherited haplotypes ac, two inherited haplotypes bc, and one inherited haplotypes cd (where a = A23,B18; b = A29,B12; c = A2,B12; and d = A26,B27).

To determine whether particular haplotypes were associated with hypertension we examined the distribution of the 61 defined HLA haplotypes among hypertensive and normotensive subjects in the five kindreds using the chi-square statistic for each 2 x 2 comparison. The only statistically significant finding was a decreased frequency of the HLA-A2,B5 haplotype among hypertensive subjects ($\chi^2 = 3.95$), but this haplotype was present in only one of the kindreds. To increase the numbers in each of the cells of the 2 x 2 tables, we then analyzed each of the HLA-A,B antigens separately. Table 6 shows that HLA-B18 was more frequent and HLA-B5 was less frequent as compared with the frequency in normotensive subjects. The pronounced effect of this one family is evident in both the HLA haplotype and the phenotype analyses.

Discussion

Essential hypertension is probably a complex of multifactorial diseases involving combinations of genetic and environmental influences. Wright suggested that the number of leading genes (i.e., those accounting for most of the variability in those who are or are not affected) is probably low in any particular family. His conjecture is supported in hypertension by the relatively large risk for recurrence in siblings, which would not be compatible with segregation at a large number of loci unless the responsible alleles were near fixation in the general population. The results of the present study are consistent with the possibility that at least two marker loci are important factors in blood pressure variability.

The most striking finding concerns the MN locus. Overall, 33 of 38 (87%) hypertensive subjects carried the N allele, whereas 47 of 69 (68%) of normotensive subjects carried this allele. The distribution of the M, MN, and N types among affected and unaffected subjects differed significantly in the white families only (see Table 3). These data may be compared with those of Miller et al., who reported on a population study of U.S. whites and blacks. They found a significantly different distribution of MNSs phenotypes only between white hypertensive and normotensive subjects. When their data were analyzed according to the MN locus alone, as in the present study, no significant differences were found in the distribution of MM, MN,
and NN types when hypertensive subjects were compared with normotensive subjects; however, they found that hypertensive whites had a lower frequency of the S gene and a higher frequency of the s gene than did normotensive whites (p < 0.005).

In 1964, Cruz-Coke et al. observed a tendency for the NN homozygotes to become hypertensive under the influence of environmental factors, whereas the MM homozygotes tended to be normotensive. In the Israeli study reported by Medalie et al., the frequency of NN homozygotes in hypertensive subjects was 35.5% compared with a frequency of approximately 20% in European white populations. A connection with hypertension also is suggested by the association of the MN locus with variances of serum cholesterol, high density lipoprotein cholesterol, non-high density lipoprotein cholesterol, and triglyceride levels.

The M and N genes are known to encode for the amino terminal sequences of glycophorin A, whereas the S and s genes are associated with glycophorin B. Additional studies on other polymorphisms in the MNSs gene region were indicated by Martin et al., who reported that the variance of triglyceride levels was larger in M⁺ (N phenotype) subjects than in M⁻ (M and MN) subjects. We did not type for the closely linked Ss or Gc loci. Recently, we observed a statistically significant increase in the frequency of the Gc 2-2 type of the vitamin D binding protein in a group of white female patients with fibromuscular dysplasia, all of whom were being treated for hypertension (E. R. Heise, unpublished data, 1986).

With respect to the ABO blood group (chromosome 9) our material did not support the weak association (relative risk, 1.5) between the group A phenotype and hypertension reported by others based on population studies. The A allele also has been associated with ischemic heart disease. The dopamine-β-hydroxylase locus has been linked to the ABO locus on chromosome 9 and might provide a plausible hypothesis for the reported association at the population level. To our knowledge, the Rhesus system (chromosome 1) has not been associated with hypertension, and the present study makes it unlikely that marker loci will be found in the region of the Rh locus.

Garay et al. reported a decreased net Na⁺K⁺ flux ratio in patients with essential hypertension and in some normotensive offspring of hypertensive patients. Several investigators have suggested that an abnormality of sodium metabolism plays a role in essential hypertension. The considerable overlap in cotransport values between hypertensive and normotensive subjects has led to conflicting results. Race may ac-
count for part of the variability. Thus far, blood groups apparently have not been considered as a covariable in membrane flux studies.

Analysis of the HLA data revealed that significant deviations in expected mendelian segregation occurred in hypertensive-normotensive pairs and in normotensive-normotensive pairs. In the overall analysis, we observed an excess of hypertensive sib pairs sharing one or two haplotypes. When the data were analyzed by family, most of the deviation was found to originate from one family (see Figure 1). In this white family, all nine siblings (five women, four men) of the first generation were hypertensive, and six of the nine were under treatment for hypertension. HLA typing was available for eight of these siblings. Of these eight hypertensive subjects, five were HLA-haploidentical and the remaining three shared one haplotype with the other five. The predominance of this one family in the overall HLA analysis was also reflected in the frequency distribution of HLA-A,B antigens, where 18 occurred significantly more often in hypertensive subjects as compared with their normotensive cohorts. We do not attribute biological significance to this observation because of the nonrandom nature of the sample. The only other significant result of HLA antigen analysis was a significant decrease of B5 in the hypertensive subjects. HLA-B5 was present in Family 233, but not in the other four families, again showing the predominant effect of this family on the overall HLA analysis.

It was of interest to examine the blood group distribution in the same kindred (No. 233). Ten of 11 hypertensive members (91%) carried the N allele, whereas 25 of 27 normotensive members (93%) carried the M allele. The NN genotype was 5.3 times more frequent in the hypertensive members as compared with normotensive members of this family. In contrast, MM homozygotes were 4.1 times more frequent in the normotensive members than in the hypertensive members. In this kindred, blood group N allele and HLA-B18 were correlated with hypertension, whereas the M allele and HLA-B5 were correlated with normotension.

These data suggest that the MNS regions and possibly, the HLA region may contain genes that contribute to hypertension through either pleiotropic effects of the loci themselves or the action of other neighboring loci on chromosome 4 and chromosome 6, respectively. Further studies will be needed to distinguish between these possibilities.

Acknowledgments

We thank Robin Burgess, R.N., for coordinating the studies in the research unit. Kent Volosin assisted in data collection and preparation of the pedigrees, and Robert C. Elston provided guidance in the study. The staff of the Medical Immunology Laboratory performed the HLA typing. Blood grouping was provided by the Medical Genetics Laboratory.

References

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Hypertension. 1987;9:634-640
doi: 10.1161/01.HYP.9.6.634

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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