Association of the Human Y Chromosome With High Blood Pressure in the General Population

Justine A. Ellis, Margaret Stebbing, Stephen B. Harrap

Abstract—Genetic variation in the Y chromosome has significant effects on male blood pressure in experimental animals, but the effects in humans are unknown. We examined the relationship between blood pressure and a polymorphic HindIII restriction site in the nonrecombining region of the Y chromosome in 409 randomly selected men from the general population. Carefully standardized measures of systolic and diastolic blood pressures were made. The HindIII restriction site was significantly more common (43.2%) in men in the lowest decile of the diastolic blood pressure distribution than men in the highest decile (15.9%, P=0.007). No significant difference in genotype frequency was observed between the lowest and highest deciles for systolic pressure (32.4% versus 27.8%, P=0.66). In the entire group, men with the HindIII restriction site had significantly lower diastolic blood pressures (81.2 mm Hg, SD:8.7, P=0.03). No significant differences in systolic blood pressure (130.6 mm Hg, SD:14.7, versus 128.3 mm Hg, SD: 13.6) were observed in relation to genotypes. Our results indicate that genetic variation in the human Y chromosome is associated with high blood pressure and contributes significantly to the quantitative variation of male diastolic blood pressure in the general population. (Hypertension. 2000;36:731-733.)

Key Words: gender ■ hypertension, genetic ■ risk factors ■ genetics

The well-established difference in cardiovascular risk between the genders is associated with elevated levels of conventional risk factors in men, including higher blood pressure.1,2 In the Victorian Family Heart Study (VFHS), a general population study of 2959 people, we found that men had systolic blood pressures 7 mm Hg greater and diastolic blood pressures 2 mm Hg greater than women (P<0.00001).3 The explanations for these differences are not known exactly, but sex chromosomal contrasts are likely to be relevant.

In an elegant series of breeding experiments, Ely and Turner linked the Y chromosome with high blood pressure in the spontaneously hypertensive rat (SHR) model of hypertension.4 Other investigators5,6 replicated this observation in the stroke-prone spontaneously hypertensive rat. However, to date, no studies have considered the role of the Y chromosome in human blood pressure.

We performed a genetic association study using a biallelic polymorphism contained in the nonrecombining region of the Y chromosome.7 This region comprises the majority of the Y chromosome and is inherited intact by sons from their fathers. The marker in this study is close to the centromere but is in linkage disequilibrium with the entire nonrecombining region. Approximately 400 unrelated males from the parental generation of the VFHS were sampled without regard to blood pressure and divided into Y chromosome allelic groups and their blood pressures were compared.

Methods

Subject Recruitment and Phenotype Measurement

The VFHS is a population-based study of cardiovascular risk.1 A total of 2959 healthy white subjects were recruited between 1991 and 1996. This group comprised 783 families that consisted of 2 parents who were 40 to 70 years of age and at least 1 natural offspring who was 18 to 30 years of age. Recruitment was limited to white families to reduce the possible confounding effect of racially determined genetic differences. A family history of heart disease was not relevant to recruitment because the aim was to enroll a representative sample of families who exhibited a broad cross-section of cardiovascular risk factor levels. For this study, a total of 409 men from the parental generation were selected without regard to blood pressure. These men were from our core set of families for linkage studies; these families were included if they comprised ≥2 natural offspring. Excluded were men who were fathers in families in which there was only 1 child or the only offspring available consisted of monozygotic twins. This selection introduced no significant selection bias in terms of blood pressure distributions.

These studies were approved by the Ethics Review Committee of the Alfred Hospital, Melbourne, and informed consent was obtained from all participants. Blood pressure was measured by carefully trained observers with a standard mercury sphygmomanometer. Systolic blood pressure was taken at the return of arterial sounds (Korotkoff phase I), and diastolic blood pressure was taken at the disappearance of sounds (Korotkoff phase V). Blood pressure measurements were made to the nearest 2 mm Hg. Three measurements of systolic and diastolic blood pressures were taken in both the lying and standing positions. The last 2 readings in each position were combined and averaged to give representative systolic and diastolic pressures. Pulse pressure was calculated as the difference between the genders is associated with elevated levels of conventional risk factors in men, including higher blood pressure.1,2 In the Victorian Family Heart Study (VFHS), a general population study of 2959 people, we found that men had systolic blood pressures 7 mm Hg greater and diastolic blood pressures 2 mm Hg greater than women (P<0.00001).3 The explanations for these differences are not known exactly, but sex chromosomal contrasts are likely to be relevant.

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between systolic and diastolic pressures. Mean arterial pressure was calculated as diastolic plus one third of pulse pressure. Pulse rate was measured for 60 seconds. Detailed information was obtained regarding treatment with oral contraceptive, hormone replacement therapy, antihypertensive medications, and lipid-lowering therapy. Blood was taken for DNA extraction.

Y Chromosome RFLP

Approximately 50 ng of DNA from each participant was used in polymerase chain reactions. The restriction fragment length polymorphism (RFLP) was detected by amplification of an \( \approx \)285-bp fragment of an alphoid satellite sequence in the centromeric region with forward primer \( 5' \)-TCTGAGACACT-TCTTTGTTGGTA-3' and reverse primer \( 5' \)-CGCTCAAATAT-CCACTTTTCAC-3'. DNA was added to a mix that contained 0.5 \( \mu \)mol/L of each primer, 1X polymerase chain reaction buffer (Perkin-Elmer Applied Biosystems), 250 \( \mu \)mol/L dNTP (Perkin-Elmer Applied Biosystems), 1.5 mmol/L MgCl\(_2\) (Perkin-Elmer Applied Biosystems), and 1 U AmpliTaq Gold DNA polymerase (Perkin-Elmer Applied Biosystems) to give a total reaction volume of 20 \( \mu \)L. Thermal conditions required for the reaction were 95°C for 10 minutes (for activation of the AmpliTaq Gold enzyme), followed by 35 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 1 minute, followed by a final extension time of 72°C for 10 minutes. Products were then digested by the addition of 5 U of \( \mathrm{HindIII} \) restriction endonuclease (Roche Diagnostics) at 37°C for 1 hour. Digested products were electrophoresed through a 2% agarose gel that contained ethidium bromide and visualized with a UV transilluminator.

Two copies of the alphoid satellite are amplified with the above primers, but only 1 copy contains the \( \mathrm{HindIII} \) RFLP. In the presence of the \( \mathrm{HindIII} \) recognition site, this copy is cut into 2 fragments of 250 bp and 35 bp, whereas the additional copy remains uncleaved. Therefore, the presence of the restriction site (which we designate \( \mathrm{A} \) genotype) is indicated by a single 285-bp band. \( \mathrm{A} \) genotype was not associated with the \( \mathrm{Y} \) chromosome on blood pressure. Differences between genotype groups were compared by Student’s \( t \) test, and in some analyses, blood pressure was adjusted for age and body mass index by including these phenotypes as covariates in ANOVA. Differences in proportions were tested with the \( \chi^2 \) statistic. Statistical analyses were performed with the SPSS statistical software package (Macintosh version 6.1).

Results

The \( \mathrm{A} \) genotype was found in 31.3% of the men studied (Table 1). There were no significant differences in age or body mass index between genotype groups (Table 1), although age and body mass index correlated significantly (\( P<0.0001 \)) with systolic and diastolic pressures.

In men with diastolic pressure in the lowest decile of the distribution (<71.5 mm Hg), the frequency of the \( \mathrm{A} \) genotype was 43.2%. This was significantly different (\( P=0.007 \)) to the observed \( \mathrm{A} \) genotype frequency of 15.9% in men with diastolic pressures in the highest decile of the distribution (>93.5 mm Hg). The frequency of the \( \mathrm{A} \) genotype was not different (\( P=0.67 \)) between men in the highest (>151 mm Hg, \( \mathrm{A} \) genotype=27.8%) and lowest (<113.5 mm Hg, \( \mathrm{A} \) genotype=32.4%) deciles of the systolic pressure distribution.

### Table 1: Age and Unadjusted Cardiovascular Phenotypes in Men Grouped According to the Presence (\( \mathrm{A} \) Genotype) or Absence (\( \mathrm{B} \) Genotype) of the \( \mathrm{Y} \) Chromosome \( \mathrm{HindIII} \) Restriction Site

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>( \mathrm{A} ) Genotype</th>
<th>( \mathrm{B} ) Genotype</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>128</td>
<td>281</td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>54.8 (5.5)</td>
<td>54.7 (5.4)</td>
<td>0.88</td>
</tr>
<tr>
<td>BMI, kg · m(^{-2})</td>
<td>27.3 (3.2)</td>
<td>26.9 (3.6)</td>
<td>0.33</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>128.3 (13.6)</td>
<td>130.6 (14.7)</td>
<td>0.12</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>81.2 (8.3)</td>
<td>83.2 (8.7)</td>
<td>0.03</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>96.9 (9.0)</td>
<td>99.0 (9.9)</td>
<td>0.04</td>
</tr>
<tr>
<td>PP, mm Hg</td>
<td>47.1 (10.9)</td>
<td>47.4 (10.6)</td>
<td>0.78</td>
</tr>
<tr>
<td>Heart rate, min(^{-1})</td>
<td>69.7 (9.3)</td>
<td>70.4 (10.0)</td>
<td>0.48</td>
</tr>
</tbody>
</table>

Phenotypes are presented as mean values with SD in parentheses. BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; and PP, pulse pressure. \( P \) values are derived from ANOVA.

A total of 73 men (17.8%) were taking antihypertensive medications. These men had significantly higher average blood pressures (137/86 mm Hg) than those not treated for hypertension (128/82 mm Hg, \( P<0.0001 \)). As expected, treated individuals were more commonly found in the highest deciles for diastolic (\( P=0.02 \)) and systolic (\( P<0.001 \)) pressures. However, not all men with high blood pressures were receiving antihypertensive treatment. Only 37% and 42% of men in the highest deciles of diastolic and systolic pressure, respectively, reported treatment. The prevalence of antihypertensive treatment was 14.8% in men with the \( \mathrm{A} \) genotype and 19.2% in those with the \( \mathrm{B} \) genotype (\( P=0.28 \)).

Overall, men with the \( \mathrm{B} \) genotype had higher diastolic pressures than those with the \( \mathrm{A} \) genotype (\( P=0.03 \), Table 1). The difference between the genotype groups was 2.0 mm Hg for unadjusted diastolic pressure (Table 1) and 2.2 mm Hg (\( F=5.8, P=0.02 \)) when adjusted for age (\( F=1.4, P=0.24 \)) and body mass index (\( F=16.8, P<0.0001 \)). The relationships between genotype and diastolic pressures were slightly more prominent for standing than lying measurements (data not shown). A similar difference was observed for systolic pressure (Table 1), but this was not significant. Calculated mean arterial pressure was significantly higher in the \( \mathrm{B} \) genotype group (Table 1), but no significant differences were observed for pulse pressure or pulse rate. The results of the analyses were not altered materially by the inclusion or exclusion of the 73 hypertensive men, with the difference in standing diastolic pressure being 2.1 mm Hg (\( P<0.05 \)) when hypertensive subjects were excluded.

Discussion

In >400 men, \( \mathrm{Y} \) chromosome polymorphism was associated with a significant difference in diastolic blood pressure. This was evident both in comparisons of the frequency of genotypes in subjects with high and low diastolic pressures and when the effects of the genotype on blood pressure were considered in the entire population sample. This dual approach provides a relatively robust analysis of the effects of the \( \mathrm{Y} \) chromosome on blood pressure.

After adjustment for the influences of variation in age and body mass index, the difference in diastolic blood pressure...
was 2.2 mm Hg. This effect is equivalent to \(\approx 26\%\) of the observed standard deviation in diastolic pressure for the population at large and slightly greater than the difference in diastolic blood pressure between men and women in the VFHS. In the context of population variation in pressure, relatively small blood pressure effects have significant effects on community-wide cardiovascular morbidity and mortality. It has been estimated that a reduction of average population diastolic blood pressure by 4 mm Hg would reduce by 50% the prevalence of clinical hypertension. Our observations are consistent with such estimates, because the prevalence of hypertension treatment (although not statistically significant) was 23% less in men with the \textit{HindIII} restriction site and the associated 2.2 mm Hg lower pressure. However, our study did not primarily concern clinical hypertension. In fact in our population survey, classification by treatment overlooks a substantial proportion of subjects with treatable blood pressure levels.

The possibility of false-positive results because of population stratification must always be considered in association studies. All individuals in the VFHS were white, and an analysis of surnames in a subset of male VFHS individuals suggests that there is little variation in ethnic background (data not shown). Therefore, the positive association of the Y chromosome to blood pressure is unlikely to be due to population stratification. Potential founder effects also need consideration. Because most offspring inherit both their father’s surname and Y chromosome, founder effects might be evident if certain surnames were especially frequent. Of the 409 men, 338 had unique surnames. Twenty surnames were repeated twice, 5 surnames were repeated 3 times, 1 surname was repeated 4 times, and 3 surnames were repeated 5 times. This overall diversity does not suggest a significant bias as a result of a founder effect.

Our results parallel findings in the SHR. On a uniform genetic background of low or average blood pressure, the SHR Y chromosome accounted for an increment of \(\approx 15\) mm Hg\(^1\)-\(^6\) but less when interactions with other genes were included.\(^6\) Our study in a human population in which genetic background is highly variable does not allow us to estimate the blood pressure effect of the human Y chromosome alone. Genetic heterogeneity at other blood pressure loci would tend to obscure the isolated Y chromosome effect. On the basis of experimental observations, it would be fair to presume that on a fixed genetic background, the blood pressure effect of the human Y chromosome would be greater than observed in this population analysis.

Localization of causative mutations on the Y chromosome is difficult because the majority of the Y chromosome does not recombine at meiosis. This nonrecombining region is flanked by 2 smaller pseudoautosomal regions (PARs), which recombine with the X chromosome.\(^10\) The mutation that might explain our findings could be located anywhere in the nonrecombining region or within 500 kilobases\(^11\) from the PAR boundaries. The Y chromosome \textit{SRY} gene is a candidate for involvement in blood pressure regulation, given its pivotal role in the determination of male sex and associated hormones.\(^12\)

In summary, this population study has demonstrated for the first time an association between an RFLP on the nonrecombining region of the human Y chromosome and blood pressure. Such an effect is relevant to the sex chromosome–linked effects on blood pressure and cardiovascular risk. The location of Y chromosome genetic variants that affect blood pressure are not yet known but may be found by the use of linkage studies of the PARs and analysis of blood pressure variation in men with Y chromosome deletions.

**Acknowledgments**

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**References**

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