Antihypertensive Treatments Obscure Familial Contributions to Blood Pressure Variation

Jisheng S. Cui, John L. Hopper, Stephen B. Harrap

Abstract—The linkage and association between inherent blood pressure and underlying genotype is potentially confounded by antihypertensive treatment. We estimated blood pressure variance components (genetic, shared environmental, individual-specific) in 767 adult volunteer families by using a variety of approaches to adjusting blood pressure of the 244 subjects (8.2%) receiving antihypertensive medications. The additive genetic component of variance for systolic pressure was 73.9 mm Hg$^2$ (SE, 8.8) when measured pressures (adjusted for age by gender within each generation) were used but fell to 61.4 mm Hg$^2$ (SE, 8.0) when treated subjects were excluded. When the relevant 95th percentile values were substituted for treated systolic pressures, the additive genetic component was 81.9 mm Hg$^2$ (SE, 9.5), but individual adjustments in systolic pressure ranged from $-53.5$ mm Hg to $+64.5$ mm Hg (mean, +17.2 mm Hg). Instead, when 10 mm Hg was added to treated systolic pressure, the additive genetic component rose to 86.6 mm Hg$^2$ (SE, 10.1). Similar changes were seen in the shared environment component of variance for systolic pressure and for the combined genetic and shared environmental (ie, familial) components of diastolic pressure. There was little change in the individual-specific variance component across any of the methods. Therefore, treated subjects contribute important information to the familial components of blood pressure variance. This information is lost if treated subjects are excluded and obscured by treatment effects if unadjusted measured pressures are used. Adding back an appropriate increment of pressure restores familial components, more closely reflects the pretreatment values, and should increase the power of genomic linkage and linkage disequilibrium analyses. (Hypertension. 2003;41:207-210.)

Key Words: antihypertensive therapy ■ blood pressure ■ genetics ■ human ■ epidemiology

Family studies can be used to estimate the relative contribution of genes and environment to blood pressure (BP) variation$^{1,2}$ and are the substrate for genomic discovery based on linkage and association analyses.$^{3-9}$ The key to successful genetic discovery is to use phenotypes that reflect the underlying genotype as closely as possible. BP is usually adjusted by regression methods for covariates such as age and gender. The usual regression techniques used to adjust for covariates are inappropriate for adjusting BP for the effects of antihypertensive treatment because they result in treated levels having an average of zero residuals rather than the extreme residuals they deserve, given their pretreatment pressures. Yet, no standardized approach for dealing with pressure measurements for treated individuals has emerged.

Among genome scans, some use measured pressure,$^1$ but more commonly, treated individuals are excluded.$^4-7$ Underlying hypertension is sometimes presumed and hypertensive values substituted for treated pressures.$^7,8$ Observed blood pressure ranking is lost in these approaches, and adjustments do not necessarily reflect possible treatment effects. One study used a nonparametric algorithm to adjust BP while taking ranking into account.$^9$ More recently, we have used a method by which we arbitrarily added 10 mm Hg and 5 mm Hg, respectively, to the measured systolic BP (SBP) and diastolic BP (DBP) pressures for medicated individuals in an attempt to reflect the anticipated effect of treatment.$^{10}$ In this study, we use the data from the Victorian Family Heart Study (VFHS) to examine the effects on estimates of variance components when different approaches are used to adjust BP in treated subjects.

Methods

The details of the community-based recruitment of volunteer adult families enriched with twins and standardized phenotype measurements have been published previously.$^2$ The details of BP-lowering drugs were recorded specifically. The study was approved by the Ethics Review Committee of the Alfred Hospital, Melbourne, and informed consent was obtained from all participants.

The 767 nuclear families included in this analysis comprised 2912 individuals (1431 male, 1481 female) with 805 sibling pairs (237 sister-sister, 200 brother-brother, 368 brother-sister). The mean age was 55.2 years (SD, 6.4) for fathers, 52.5 years (SD, 5.8) for mothers, and 24.0 years (SD, 3.7) for offspring. The sample included 150 offspring twin pairs, of whom 66 were monozygotic (MZ) and 84 dizygotic (DZ). A total of 244 subjects (8.4% of total) reported receiving BP-lowering medications. These comprised 16.7%...
of fathers, 14.6% (n=112) of mothers, and 0.3% (n=4) of offspring.

Phenotype Adjustment
Mean SBP and DBP were initially adjusted for age by gender within each generation by using linear regression methods. The residuals from these analyses comprise what we term the original data. For untreated subjects, their original data were used in all variance component analysis. For treated subjects, we used either their original data or other approaches to adjustment, namely: (a) Exclusion: Treated subjects were excluded from analyses by coding their phenotypes as missing values. (b) Fixed substitution: Fixed values equivalent to the 95th percentile of the distribution for the relevant age, gender, and generation were substituted for treated subjects. (c) Random substitution: Randomly generated SBP values from a normal distribution of mean 150 mm Hg (SD, 5), constrained to boundaries of 90 mm Hg and 100 mm Hg, were substituted for treated subjects. (d) Fixed addition: Based on the known average treatment effects, fixed increments of 10 mm Hg SBP and 5 mm Hg DBP were added to treated pressures. After addition, SBP and DBP were adjusted for age by gender within each generation. (e) Stepped addition: To account for the number of drugs, stepped increments of 8/4 mm Hg, 14/10 mm Hg, and 20/16 mm Hg were added to measured SBP/DBP of treated subjects taking 1, 2, and 3 drug classes, respectively. These stepped increments were chosen to achieve an average increase of 10/5 mm Hg in treated subjects. After addition, SBP and DBP were adjusted for age by gender within each generation.

Statistical Methods
Variance component analyses were conducted under the assumption of a multivariate normal distribution within a family with the use of FISHER software. Standard errors and 95% CIs were calculated by using large sample normal approximations. The total variance was decomposed into additive genetic ($\sigma_a^2$), shared environmental ($\sigma_e^2$), and individual-specific ($\sigma_n^2$) variance components. The details of these methods have been published elsewhere. Descriptive statistics of phenotypes were calculated with the use of S.A.G.E. software (S.A.G.E. 4.2, Department of Epidemiology and Biostatistics, Case Western Reserve University).

Results
The measured SBP and DBP were on average higher in treated subjects than untreated subjects ($P<0.0001$), but there was considerable overlap in the individual values between these two groups (Figure and Table 1). Most treated subjects (165/244=68%) were receiving only one class of BP-lowering drug.

Variance Component Analyses
For both SBP and DBP, the individual-specific component of variance ($\sigma_n^2$) is relatively unchanged across the different methods of adjustment for treatment (Table 2). Compared with the original data, excluding treated subjects, resulted in a decrease in total variance, mostly caused by a decrease in the additive genetic component ($\sigma_a^2$), both for SBP (by 12.5 mm Hg$^2$) and to a lesser extent for DBP (by 2.7 mm Hg$^2$). It also resulted in a decrease in the shared environmental component ($\sigma_e^2$) for SBP (by 8.6 mm Hg$^2$). The total familial component ($\sigma_a^2 + \sigma_e^2$) decreased by 21.1 mm Hg$^2$ for SBP.

In contrast, compared with the original data, fixed substitution resulted in an increase in total variance caused by increases in both $\sigma_a^2$, by 11.5 mm Hg$^2$ and 2.8 mm Hg$^2$ and in $\sigma_e^2$, by 10.8 mm Hg$^2$ and 2.4 mm Hg$^2$ for SBP and DBP, respectively. Similar comments apply to random substitution, fixed addition, and stepped addition.

As a result of fixed substitution, the average increase of SBP was 17.2 mm Hg (SD, 17.6), but changes in individual

**TABLE 1. Blood Pressures of Treated and Untreated Subjects**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>SBP (SD)</th>
<th>DBP (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>2668</td>
<td>121.5 (13.4)</td>
<td>74.6 (10.1)</td>
</tr>
<tr>
<td>Treated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 drug</td>
<td>165</td>
<td>134.9 (16.7)</td>
<td>84.1 (9.0)</td>
</tr>
<tr>
<td>2 drugs</td>
<td>64</td>
<td>134.7 (17.7)</td>
<td>83.3 (10.1)</td>
</tr>
<tr>
<td>≥3 drugs</td>
<td>15</td>
<td>146.3 (16.2)</td>
<td>87.4 (14.4)</td>
</tr>
<tr>
<td>Total</td>
<td>244</td>
<td>135.5 (17.1)</td>
<td>84.1 (9.7)</td>
</tr>
</tbody>
</table>

Data are presented as mean (SD). SBP indicates systolic blood pressure; DBP, diastolic blood pressure.

All pressures in treated individuals are significantly higher ($P<0.0001$) than in untreated subjects.

Histograms of SBP (left panels) and DBP (right panels) in subjects who reported no use (untreated, above) or use (treated, below) of BP-lowering drugs.
TABLE 2. Estimated Variance Components for Different Adjustment Methods Applied to Subjects Who Reported Receiving BP-Lowering Drugs

<table>
<thead>
<tr>
<th>Adjustment</th>
<th>$\sigma^2$</th>
<th>$\sigma_w^2$</th>
<th>$\sigma_e^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None (original data)</td>
<td>73.9 (8.8)</td>
<td>47.9 (10.1)</td>
<td>46.4 (8.2)</td>
</tr>
<tr>
<td>Exclusion</td>
<td>61.4 (8.0)</td>
<td>39.3 (9.8)</td>
<td>48.4 (8.3)</td>
</tr>
<tr>
<td>Fixed substitution</td>
<td>85.4 (10.1)</td>
<td>58.7 (10.5)</td>
<td>44.0 (7.7)</td>
</tr>
<tr>
<td>Random substitution</td>
<td>81.9 (9.5)</td>
<td>53.2 (10.4)</td>
<td>45.1 (8.0)</td>
</tr>
<tr>
<td>Fixed addition</td>
<td>86.6 (10.1)</td>
<td>59.7 (10.6)</td>
<td>43.9 (7.7)</td>
</tr>
<tr>
<td>Stepped addition</td>
<td>88.3 (10.3)</td>
<td>61.4 (10.7)</td>
<td>43.5 (7.6)</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None (original data)</td>
<td>22.4 (16.5)</td>
<td>17.0 (13.0)</td>
<td>36.3 (5.9)</td>
</tr>
<tr>
<td>Exclusion</td>
<td>19.7 (15.5)</td>
<td>17.3 (12.1)</td>
<td>35.5 (5.7)</td>
</tr>
<tr>
<td>Fixed substitution</td>
<td>25.2 (17.1)</td>
<td>19.4 (13.7)</td>
<td>36.1 (5.9)</td>
</tr>
<tr>
<td>Random substitution</td>
<td>26.7 (17.9)</td>
<td>21.3 (14.5)</td>
<td>36.6 (6.1)</td>
</tr>
<tr>
<td>Fixed addition</td>
<td>25.3 (17.7)</td>
<td>18.8 (14.2)</td>
<td>36.9 (6.2)</td>
</tr>
<tr>
<td>Stepped addition</td>
<td>26.6 (18.7)</td>
<td>19.8 (15.0)</td>
<td>37.6 (6.5)</td>
</tr>
</tbody>
</table>

The model estimates the additive genetic ($\sigma^2$), shared environment ($\sigma_w^2$), and individual-specific ($\sigma_e^2$) components of total phenotype variance. Units are mm Hg$^2$. Standard errors of estimates are given in parentheses. Methods of adjustment applied to subjects receiving BP-lowering medication are explained in the text.

SBP ranged from a reduction of 53.5 mm Hg to an increase of 64.5 mm Hg. For DBP, the range was $-25.0$ mm Hg to $+35.5$ mm Hg (average, $+9.6$ mm Hg; SD, 9.7). With random substitution, changes in SBP were from $-56.7$ mm Hg to $+59.2$ mm Hg (average, $+14.4$ mm Hg; SD, 17.3) and for DBP were from $-22.5$ mm Hg to $+39.0$ mm Hg (average, $+10.9$ mm Hg; SD, 10.1).

Discussion

Our analyses show that subjects treated with antihypertensive medications contribute important information regarding the familial components of BP variance. This is illustrated when treated subjects are excluded by the reduction in both genetic and shared environmental components of variance compared with all other approaches. Not only is important information lost, but statistical power is weakened because of reduced numbers of subjects. Unfortunately, many linkage studies have excluded treated subjects.\textsuperscript{4–7} The impact of excluding treated subjects is illustrated in a recent genome scan\textsuperscript{7} by the disappearance of a quantitative trait locus (QTL) for SBP on chromosome 6 when the 27% of the study population on antihypertensive medications were removed.

If exclusion of treated subjects should be avoided, what should be done? The use of measured pressures from treated subjects is not ideal; they represent a biased distortion in a quantitative analysis because treatment lowers BP and is usually applied to those with the highest values. Indeed, treatable subjects might be expected to provide genetic and environmental clues to high BP, discoveries that depend on unadulterated relations between BP phenotypes and familial factors, including genotypes. A potential solution is to adjust measured pressures in treated subjects so that they better reflect the inherent untreated levels.

One approach is to assume that treated patients have underlying hypertension and substitute values typical of hypertensives for measured pressures. When we applied methods of either fixed or random substitution that others have used, there was an increase in mean BP and increases in both genetic and shared environmental components of variance. This indicates that treatment effects were obscuring the familial contributions to BP. The corollary is that treatment counteracts mechanisms controlled by these same genetic and shared environmental factors.

Although the substitution method increased the variance attributable to familial factors, the individual adjustments to BP were of concern. Because the mean measured pressures in treated subjects fell below 150/95 mm Hg, the average increases in pressures were relatively large (SBP, 17.3 mm Hg; DBP, 10.9 mm Hg) and exceeded the anticipated BP-lowering effects of an average of 1.5 BP-lowering drugs per treated individual.\textsuperscript{11} This questions the assumption that all treated subjects had underlying hypertension. These classes of drugs are also commonly used for other conditions such as myocardial ischemia, heart failure, fluid retention, and tremor. Furthermore, a proportion of treated patients might have had white-coat hypertension with otherwise normal pressures.\textsuperscript{15} Second and more importantly, BP ranking was scrambled with substitution, and some changes were extreme (up to 3 SD) and many of the treated pressures were replaced with lower values. This resulted from a compression of wide range of measured pressures in treated subjects (from 91.5 to 209.5 mm Hg for SBP and from 59.5 to 117.0 mm Hg for DBP) to a 95th percentile or a constrained distribution of $\approx 150/95$ mm Hg. Indeed, for individual BP, the substitution approach can obscure the relations between phenotype and genotype and greatly weaken genetic linkage and linkage disequilibrium analyses that depend on precise individual pressure measures.\textsuperscript{16}

Our addition method makes no assumptions regarding the underlying reasons for treatment and is based on reasonable expectations of treatment effects.\textsuperscript{11} Furthermore, the method avoids major or misdirected adjustments to BP and retains the ranking of treated subjects. Genetic and shared environmental variance components were maximized and individual-specific components minimized when we applied the addition methods, especially the stepped addition method that took into account number of drug classes an individual received.

These findings also have implications for the power of linkage and linkage disequilibrium studies, including genome scans. The likely polygenic nature of BP determination implies that the contribution of individual QTLs might be small. Therefore, strategies to maximize the genetic signal and the power of linkage and linkage disequilibrium mapping are important. The greater the heritability (the proportion of total variance attributable to the additive genetic component), the smaller the sample size required. A change in heritability from 0.4 to 0.5 might halve the number of sibling pairs required.\textsuperscript{17}

Perspectives

The elimination of subjects treated with BP-lowering drugs from quantitative family analyses excludes an informative
subgroup and diminishes sample size. Estimates of genetic and shared environmental effects become unreliable, and statistical power suffers. A more logical approach is one in which appropriate pressure increments are added to treated pressure levels, especially if commensurate with drug treatment. This approach not only retains subjects in the analyses but augments the familial genetic and shared environmental signals without increasing the noise from individual-specific sources of variation.

Acknowledgments
This study was supported by the Victoria Health Promotion Foundation, the National Health and Medical Research Council of Australia and the Australian Twin Registry, and a University of Melbourne Early Career Researcher Grant. The authors thank Margaret Stebbing and Professor Graham G. Giles for their contributions to the Victorian Family Heart Study.

References
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*Hypertension*. 2003;41:207-210; originally published online December 23, 2002;
doi: 10.1161/01.HYP.0000044938.94050.E3

*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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World Wide Web at:
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In the editorial commentary “Loosening the Cuff: Important New Advances in Modeling Antihypertensive Treatment Effects in Genetic Studies of Hypertension” by Palmer (Hypertension 2003;41:197–198), an error was made in referring to an article by Cui et al in the final section of the editorial. The final section, headed “New Approaches to Modeling Antihypertensive Treatment Effects,” was referring to an article by Cui et al that appears in the same issue of the Journal: “Antihypertensive Treatments Obscure Familial Contributions to Blood Pressure Variation” (Hypertension 2003;41:207–210), not to a previous article by Cui et al that was cited earlier in the editorial. The Journal regrets any confusion that may have resulted from this error.