Chromosome 2p Shows Significant Linkage to Antihypertensive Response in the British Genetics of Hypertension Study

Sandosh Padmanabhan, Chris Wallace, Patricia B. Munroe, Richard Dobson, Morris Brown, Nilesh Samani, David Clayton, Martin Farrall, John Webster, Mark Lathrop, Mark Caulfield, Anna F. Dominiczak, John M. Connell

Abstract—There is a lack of consistently linked loci influencing blood pressure and hypertension status, and this may be because of genetic or phenotypic heterogeneity. We hypothesize that stratification of subjects by response to antihypertensive drug groups could be used to stringently define subsets that will have reduced genetic and etiologic heterogeneity, by partitioning contrasting mechanisms of hypertension and, thus, enhancing gene finding. We investigated the British Genetics of Hypertension Study population, which is composed of 2142 severely hypertensive white affected sibling pairs. Nonresponse to antihypertensive therapy was defined as an on-treatment blood pressure of >140/90 mm Hg or a difference between prediagnosis and on-treatment blood pressure of <20 mm Hg. Of the nonresponders, there were 89 sibling pairs (AB) who were both on antihypertensive therapy that inhibit the renin–angiotensin system (angiotensin-converting enzyme inhibitors, angiotensin II type-1 receptor blockers, or β-blockers), and 76 sibling pairs (CD) who were both on drugs that do not (calcium channel blockers or diuretics). Nonparametric linkage analysis carried out using markers from a 10-cM genome scan and additional “grid tightening” markers showed significant linkage in the AB group on chromosome 2p (logarithm of odds = 4.84 at 90.68 Kosambi cM) and suggestive linkage for the CD group on chromosome 10q (logarithm of odds = 2.83 at 125.96 Kosambi cM). The AB linkage locus attained genomewide significance after simulation using 10,000 replicates (P = 0.005). This locus may contain a gene for the salt-sensitive form of hypertension and/or a pharmacogenetic locus affecting drug response. We have demonstrated for the first time identification of a significant locus by partitioning different pathways of hypertension using drug response. (Hypertension. 2006;47[part 2]:603-608.)

Key Words: antihypertensive therapy ■ genetics ■ hypertension, genetic ■ hypertension, sodium-dependent ■ renin-angiotensin system ■ race

Optimal blood pressure control is achieved in only one quarter of hypertensives, despite there being >100 antihypertensive drugs available, leaving most with suboptimal blood pressure control and an increased risk of cardiovascular sequelae.1 This can be attributed partly to the heterogeneity of the response to antihypertensive therapy, partly to noncompliance, and partly to side effects that contribute to withdrawal of treatment.2 Antihypertensive drugs lower blood pressure by acting on specific targets in the pathway of blood pressure regulation, although the mode of antihypertensive action of many of the available drugs is complex and multifactorial. Obvious candidate genes that influence drug responses are those that code for components of a system targeted by the drug or components of the counterregulatory systems opposing the drug-induced fall in blood pressure.

Many pharmacogenetic studies have been conducted of association with a priori selected candidate genes in hypertension.3–9 An alternative to gene-specific testing uses panels of SNPs or microsatellites to search the entire genome for “linked” regions likely to harbor such genes. Because no prior knowledge or assumptions are required about gene function, one attractive feature of this approach is the possibility of identifying new genes previously unsuspected to influence the trait. Moreover, the relative strength of linkage evidence accompanied by existing knowledge about functions of genes...
within the linked regions can help prioritize the subsequent search for functional mutations in the positionally implicated genes.10

The theory underpinning the AB/CD algorithm11–13 is that hypertension can be broadly classified as “high renin” or “low renin” based on the vasoconstriction-volume (renin/sodium) model of hypertension.14,15 It is proposed that initial treatment should be with 1 of 2 categories of antihypertensive drug, those that inhibit the renin–angiotensin system [angiotensin converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs; A) or β-adrenoceptor blockers (B)] and those that do not [calcium channel blockers (C) or thiazide diuretics (D)].11–15 Individuals of white European ancestry and <55 years of age tend to have higher renin concentrations than those ≥55 of age or of African descent,11,16–18 A or B drugs are, therefore, generally preferred as initial blood pressure–lowering treatment in younger white patients than C or D drugs. However, C or D drugs are more effective first-line agents for those of African descent of any age or older white patients.19

Our hypothesis was that response to antihypertensive drug groups could be used to stringently define subsets that will have reduced genetic and etiologic heterogeneity by partitioning contrasting mechanisms of hypertension and, thus, enhancing gene finding. Alternatively, this stratification may identify a pharmacogenetic trait locus and, thus, help identify loci of etiologic and therapeutic importance.

### Methods

#### Study Population

The study population was derived from the Medical Research Council British Genetics of Hypertension Study (BRIGHT; see online at http://www.brightstudy.ac.uk).20 This study has completed a 10-cM genomewide scan for hypertension loci among 1599 severely hypertensive sibling pairs of white European ancestry up to the level of grandparents and identified regions of interest on chromosomes 2, 5, 6, and 9.20 Additional “grid tightening” markers have since been genotyped in the regions of interest identified in that initial scan, and the current resource contains 2142 severely hypertensive sibling pairs in 1634 UK families. Each family contained ≥2 affected siblings, in whom onset of hypertension was diagnosed before age 60 years and who had sitting blood pressure values of 150/100 mm Hg based on 1 reading or >145/95 mm Hg as a mean of 3 readings. These criteria correspond to the threshold for the top 5% of the blood pressure distribution in a contemporaneous health screening survey of 5000 UK men and women in 1995.21 Blood pressure was measured as the mean of 3 seated readings, and all of the antihypertensive therapy was recorded.

#### Classification of Subjects by Drug Response

Subjects were categorized as nonresponders if their on-treatment blood pressure was >140/90 mm Hg or if the difference between prediagnosis and on-treatment systolic or diastolic blood pressure was <20 mm Hg. A more stringent threshold of 20 mm Hg was used, because the duration and type of treatment were not identical in all of the individuals. Of the total of 2142 hypertensive sibling pairs, 558 sibling pairs were on exclusively a combination of ACEIs, ARBs, β-blockers, calcium channel blockers, or thiazide diuretics, and 288 sibling pairs (52%) of this group were also concordant for nonresponse (ABCD group).

There were 194 sibling pairs taking exclusively ACEIs, ARBs or β-blockers, and of them, 89 sibling pairs (46%) were concordant for nonresponse (AB group), 29 (15%) were concordant for response, and 76 (39%) were discordant. There were 118 sibling pairs exclusively taking calcium channel blockers or thiazide diuretics, and of them, 74 (64%) were concordant for nonresponse (CD group), 11 (9%) were concordant for response, and 31 (26%) were discordant.

#### Statistical Analysis

Groups were compared using the nonparametric Kruskal–Wallis test. The 3 groups (ABCD, AB, and CD) were analyzed using the affected sibling pair approach. Multipoint nonparametric linkage analysis was performed using MERLIN22 in combination with MLSix to compute multipoint maximum logarithm of odds (lod) score statistics23,24 for each sample of treatment nonresponders. In order to correct for multiple testing, we repeatedly made 10 000 random samples of 288 sibling pairs from the entire resource from which random nonoverlapping subsamples of 89 and 76 sibling pairs were made. Thus, we replicated the structure found in the observed data of 89 AB and 76 CD nonresponse pairs, which were nonoverlapping subsets of the 288 ABCD nonresponse pairs. For each repetition, the maximum lod score over all 3 of the samples was recorded, and these were used to generate the empirical distribution of the maximum lod score from which a multiple-testing corrected, genomewide P value was generated.

### Demographic Characteristics of the Study Population Stratified by Drug Response and Sex

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Males</th>
<th></th>
<th></th>
<th>Females</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AB</td>
<td>CD</td>
<td>P Value</td>
<td>AB</td>
<td>CD</td>
<td>P Value</td>
</tr>
<tr>
<td>No.</td>
<td>94</td>
<td>55</td>
<td>0.001</td>
<td>89</td>
<td>98</td>
<td>0.004</td>
</tr>
<tr>
<td>Anti-HTN drugs</td>
<td>1.12 (0.3)</td>
<td>1.36 (0.6)</td>
<td>0.001</td>
<td>1.1 (0.3)</td>
<td>1.3 (0.6)</td>
<td>0.004</td>
</tr>
<tr>
<td>Age, y</td>
<td>62.6 (10.6)</td>
<td>65.8 (8.9)</td>
<td>0.058</td>
<td>64.7 (8.6)</td>
<td>65.7 (8.8)</td>
<td>0.405</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.7 (0.1)</td>
<td>1.7 (0.1)</td>
<td>0.640</td>
<td>1.6 (0.1)</td>
<td>1.6 (0.1)</td>
<td>0.908</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>84.9 (10.8)</td>
<td>87.1 (14.8)</td>
<td>0.296</td>
<td>70.8 (10.1)</td>
<td>71.1 (11.3)</td>
<td>0.846</td>
</tr>
<tr>
<td>Prediagnosis systolic BP, mm Hg</td>
<td>167.5 (15.9)</td>
<td>170.9 (12.6)</td>
<td>0.233</td>
<td>170.4 (14.9)</td>
<td>168 (14.7)</td>
<td>0.361</td>
</tr>
<tr>
<td>Prediagnosis diastolic BP, mm Hg</td>
<td>102 (6.6)</td>
<td>102.8 (5.4)</td>
<td>0.889</td>
<td>102.9 (5.8)</td>
<td>100.6 (6.2)</td>
<td>0.030</td>
</tr>
<tr>
<td>Heart rate, min</td>
<td>64.7 (10.9)</td>
<td>73.1 (13.8)</td>
<td>&lt;0.001</td>
<td>65.6 (10.9)</td>
<td>73.5 (9.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>On-treatment systolic BP, mm Hg</td>
<td>167.2 (18.9)</td>
<td>162.8 (16.1)</td>
<td>0.143</td>
<td>168 (17.7)</td>
<td>161.9 (16)</td>
<td>0.015</td>
</tr>
<tr>
<td>On-treatment diastolic BP, mm Hg</td>
<td>101.3 (8.4)</td>
<td>98.8 (8.2)</td>
<td>0.078</td>
<td>97.8 (10.3)</td>
<td>95.3 (8.5)</td>
<td>0.075</td>
</tr>
<tr>
<td>BMI</td>
<td>28 (3)</td>
<td>28.6 (4)</td>
<td>0.356</td>
<td>27.4 (3.7)</td>
<td>27.5 (4.1)</td>
<td>0.921</td>
</tr>
<tr>
<td>Waist:hip ratio</td>
<td>0.9 (0.1)</td>
<td>0.9 (0.1)</td>
<td>0.817</td>
<td>0.82 (0.1)</td>
<td>0.83 (0.1)</td>
<td>0.163</td>
</tr>
</tbody>
</table>

HTN indicates hypertension; BP, blood pressure; BMI, body mass index; AB, nonresponders to ACEI and β-blockers; CD, nonresponders to calcium channel blockers and diuretics. Data shown as mean (SD).
Results

Characteristics of the Subjects
The demographic characteristics of the study population are summarized in the Table. The mean age in the nonresponder groups ranged from 62.6 to 65.8 years with no significant difference between AB and CD groups. There was also no significant difference between the groups in terms of body mass index, height, weight, waist:hip ratio, prediagnosis systolic and diastolic pressures, and the on-treatment systolic and diastolic pressures. However, the average number of antihypertensive drugs was significantly lower in the AB group compared with the CD group both among males and females. Subjects on AB therapy had a significantly lower pulse rate than those on CD therapy, an indication of β-adrenoceptor blockade in the AB group.

Genomewide Scan
The multipoint lod scores for the AB, CD, and ABCD groups are shown in Figure 1. Linkage was found in the AB group on chromosome 2 (multipoint lod 4.84 at 90.68 Kosambi cM) near marker D2S2368 with flanking markers D2S337 and D2S286. Suggestive linkage for the CD group was found on chromosome 10 (lod 2.83 at 125.96 cM) near marker D10S597 and the combined ABCD group on chromosome 2 in the same region as the AB group (lod 1.61 at 90.68 cM). Permutation testing using 10,000 replicates showed that the lod score of 4.84 on chromosome 2p is significant at the genome-wide level after allowance for multiple testing (P=0.005).

Discussion
Numerous genome-wide linkage studies have found linked loci influencing blood pressure and hypertension status in a number of populations and ethnic groups across almost all of the chromosomes.20,25–32 Perhaps most striking is the lack of consistently linked loci, and this may be because of heterogeneity present within the data set, because family units may have a different disease etiology both between and within family or different ages of onset.

To our knowledge, this is the first study to identify genomewide significant linkage by partitioning different pathways of hypertension based on drug response. The susceptibility locus

![Figure 1. Multipoint lod scores for the 3 groups of nonresponders to antihypertensive agents. Multipoint lod scores shown on the y axis and chromosomes along the x axis. Significance line at lod 4.84.](image-url)
on chromosome 2p at 90.68 cM in white European nonresponders to the AB group colocalizes to a region found in black hypertensives in the Family Blood Pressure Program who showed evidence of linkage with hypertension status at 93 cM with an lod score of 2.84. The previously reported genome scan from the unstratified population for hypertension status showed an lod score of 1.76 at 165 cM near marker D2S142. Our result suggests that similar pathophysiological or pharmacogenetic mechanisms may underlie the hypertension in these 2 groups. It is known that blacks are more responsive to diuretics and calcium channel blockers and less responsive to β blockers and ACE inhibitors than their white European counterparts. Thus, we postulate that the chromosomal 2p locus independently identified in different populations may contain a gene or genes for the salt-sensitive form of hypertension, which is common among Africans, and the same mechanism may be operative in a subset of white European hypertensives identified by unresponsiveness to the AB group of antihypertensive agents. The potential candidate genes in the susceptibility locus on chromosome 2p are shown in Figure 2. These genes were selected based on presumed function and previous association with blood pressure regulation. Of these genes, the sodium-bicarbonate transporter SLC4A5 appears to be a promising candidate supported by recent reports of consistent association with hypertension in both blacks and whites. ADD2, which encodes the β subunit of adducin, is another potential candidate and was reported previously to be associated with hypertension in women. Additional studies are needed to fine map this region and to identify the causative gene or genes and determine their functional role.

The present study should be seen in the context of its limitations. We have not accounted for concomitant nonantihypertensive therapy, but we assume the level of interaction to be minimal. Our study was not primarily designed to investigate response to therapy; this has been imputed from data held in clinical records, and, therefore, some caution must be observed in the interpretation of the prediagnostic blood pressure data. We have also not replicated the findings in an independent population. On the other hand, the blood pressure changes that we describe relate to sibling pairs who were concordant for the observed response/nonresponse, and this is a powerful observation, which, to our knowledge, has not been described before.

There has been major interest in identifying genes that influence the pharmacodynamic determinants of blood pressure response, because these mechanisms may play the predominant role in determining interindividual variation in blood pressure responses to antihypertensive drugs now in common use. Recent pharmacogenetic studies have looked at specific polymorphisms in candidate genes selected on existing knowledge of the mechanism of action of the drug or disease pathophysiology. For example, sodium- and volume-dependent hypertension have been studied by looking at diuretic-induced genetic differences in response or by stratifying subjects based on treatment subtypes. Whereas some of these associations are negative and others have not been replicated, it would be prudent to point out that currently there is no consensus on pharmacogenetic associations, just as there are problems with linkage consensus. We have demonstrated that drug response is a far more tractable phenotype for genetic studies using the linkage

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**Figure 2.** Potential candidate genes in the susceptibility locus in chromosome 2p. PRKCE indicates protein kinase C, epsilon type; EPAS1, endothelial PAS domain protein 1; ATP6V1E2-ATPase, H+ transporting; RHOO-ρ-related GTP-binding protein RhoQ; CALM2, calmodulin 2; KCNK12, potassium channel subfamily K member 12; PSME4, proteasome; GPR75, G protein–coupled receptor 75; RPS27A, ubiquitin; FANCL, ubiquitin ligase protein PHF8; USP34, ubiquitin-specific protease 34; RAB1A, Ras-related protein Rab-1A; Calcineurin B, calcineurin B subunit isoform 1; PLEK, pleckstrin; ARHGAP25, ρ-GTPase–activating protein 25; ANX4, annexin A4; ADD2, adducin; ATP6V1B1, vacuolar ATP synthase subunit B, kidney isoform; NAT8, N-acetyltransferase 8; MTHFD2, bifunctional methylenetetrahydrofolate dehydrogenase/cyclohydrolase; SLC4A5, sodium bicarbonate transporter; PRSS25, serine protease HTRA2, mitochondrial precursor; DOK1, docking protein 1; USP39, ubiquitin-specific protease 39; FABP1, fatty acid-binding protein, liver.
approach than the complex hypertension that underlies the initial need for treatment and enhances gene finding. One explanation can be that the efficacy of an antihypertensive drug is possibly dependent on fewer physiological variables than is the regulation of blood pressure itself.47

Perspectives

The present study has shown that drug response information is a powerful means of stratifying a complex phenotype to narrow down a region involved in a specific pathway of causation. Although we have not demonstrated causality, additional studies should lead to the identification of genetic variants in this region involved in the pathways of hypertension associated with drug response and salt sensitivity. These types of population stratification studies offer promise to finding genes for intermediate phenotypes of hypertension.

Acknowledgments

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