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Genetics

Common Polymorphisms at the CYP17A1 Locus Associate With Steroid Phenotype
Support for Blood Pressure Genome-Wide Association Study Signals at This Locus

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Abstract—Genome-wide association studies implicate the CYP17A1 gene in human blood pressure regulation although the causative polymorphisms are as yet unknown. We sought to identify common polymorphisms likely to explain this association. We sequenced the CYP17A1 locus in 60 normotensive individuals and observed 24 previously identified single-nucleotide polymorphisms with minor allele frequency >0.05. From these, we selected, for further studies, 7 polymorphisms located ≤2 kb upstream of the CYP17A1 transcription start site. In vitro reporter gene assays identified 3 of these (rs138009835, rs2150927, and rs2486758) as having significant functional effects. We then analyzed the association between the 7 polymorphisms and the urinary steroid metabolites in a hypertensive cohort (n=232). Significant associations included that of rs138009835 with aldosterone metabolite excretion; rs2150927 associated with the ratio of tetrahydrodeoxycorticosterone to tetrahydrodeoxycortisol, which we used as an index of 17α-hydroxylation. Linkage analysis showed rs138009835 to be the only 1 of the 7 polymorphisms in strong linkage disequilibrium with the blood pressure–associated polymorphisms identified in the previous studies. In conclusion, we have identified, characterized, and investigated common polymorphisms at the CYP17A1 locus that have functional effects on gene transcription in vitro and associate with corticosteroid phenotype in vivo. Of these, rs138009835—which we associate with changes in aldosterone level—is in strong linkage disequilibrium with polymorphisms linked by genome-wide association studies to blood pressure regulation. This finding clearly has implications for the development of high blood pressure in a large proportion of the population and justifies further investigation of rs138009835 and its effects. (Hypertension. 2016;67:724-732. DOI: 10.1161/HYPERTENSIONAHA.115.06925.) ★ Online Data Supplement

Key Words: aldosterone ■ blood pressure ■ genome-wide association study ■ hypertension ■ polymorphism, single nucleotide

Hypertension is a major risk factor for cardiovascular and cerebrovascular diseases. The risk of both increases with blood pressure, even within the normal range.1 Blood pressure has a substantial heritable component, and although polymorphic variations in a small number of genes have been shown to associate with blood pressure levels, much of this genetic component remains unidentified. Recently, the International Consortium for Blood Pressure genome-wide association study (GWAS), using 200,000 individuals of European descent, identified 16 novel loci as being significantly associated with systolic blood pressure or diastolic blood pressure2 and also confirmed the association at 12 loci reported by the Cohorts for Heart and Aging Research in Genetic Epidemiology (CHARGE)3 and Global Blood Pressure Genetics (Global BPgen)4 consortia.
Of these 28 loci, 1 on chromosome 10 encompasses a cluster of 5 genes forming a high linkage disequilibrium (LD) block spanning 347 kb. This locus was flagged by all 3 GWASs; the most associated variant was rs1004467, located in intron 3 of CYP17A1 in the CHARGE study, whereas the most associated variant in the other 2 studies was rs11191548, which lies some 250 kb distant from CYP17A1 at the intergenic region between CNNM2 and NT5C2 and is in high LD with rs1004467 (r²=0.91 in the 1000 Genomes CEU population of Utah residents with Northern and Western European ancestry). In each of the studies, this locus was associated with a potential difference in systolic blood pressure of ≈1.1 mm Hg. Subsequent replication of the GWAS findings in East Asian populations has increased the potential global effect of the CYP17A1 locus. A recent study has also associated rs11191548 with left ventricular mass index in hypertensive patients, which is suggestive of a role in cardiac hypertrophy. Rare genetic variants at CYP17A1 are already known to cause hypertension, and so, this locus represents a rare concurrence of blood pressure candidate gene studies with GWAS evidence.

The CYP17A1 gene is located on chromosome 10q24 and encodes a dual-function cytochrome P450 enzyme expressed primarily in the adrenal cortex, ovarian thecal cells, and testes. In the adrenal zona fasciculata, this CYP17A1 enzyme functions as a 17α-hydroxylase, converting pregnenolone and progesterone to 17α-hydroxy pregnenolone and 17α-hydroxyprogesterone, respectively. In the zona reticularis, it acts as a 17,20-lyase on these hydroxylated products, cleaving the C17,20 bond to produce dehydroepiandrosterone and androstenedione. The cofactor cytochrome b5 is an important determinant of the balance between these 2 enzymatic functions. Recently, the substantial heritability of 17α-hydroxylase activity in the general population has been demonstrated as having inverse correlation with adult blood pressure. Deficient 17α-hydroxylation has been known for almost 50 years to result in hypertension, and so, this locus represents a rare concurrence of blood pressure candidate gene studies with GWAS evidence.

Methods

Polymorphism Discovery in Normotensive Subjects

Genetic polymorphisms at the CYP17A1 locus were identified by direct sequencing of genomic DNA from 60 West of Scotland volunteers (27 men and 33 women) recruited for a previous study. Participants were in good health and taking no antihypertensive medication. Median age was 51 years (interquartile range, 32–67 years); median weight was 70 kg (interquartile range, 61–76 kg); median systolic blood pressure was 126 mm Hg (interquartile range, 116–136 mm Hg); and median diastolic blood pressure was 77 mm Hg (interquartile range, 70–84 mm Hg). Ethical approval was granted by the West Glasgow Ethics Committee, and written informed consent was obtained from all participants. Exons, introns, and 3′UTR were each amplified separately by polymerase chain reaction (PCR) using the Thermo-Start Taq DNA Polymerase PCR Enzyme Kit (Thermo Fisher Scientific, Renfrew, United Kingdom), whereas the upstream region was amplified using the Expand High Fidelity PCR System (Roche Diagnostics Ltd, Burgess Hill, United Kingdom), each according to the standard kit protocol. Automated sequencing of PCR products was performed using BigDye Terminator version 3.1 Cycle Sequencing chemistry (Life Technologies, Renfrew, United Kingdom) and the ABI 3730 DNA analyzer (Life Technologies). Further details are available in Tables S2 and S3 in the online-only Data Supplement. Investigations were carried out in accordance with the principles of the Declaration of Helsinki. LD patterns were generated using haploviev software.

Site-Directed Mutagenesis of CYP17A1 Luciferase Reporter Vector

To create the plGL3-17 control vector, 2898 bp of human genomic DNA immediately upstream of the CYP17A1 coding region was inserted into the empty plGL3-basic vector (Promega UK Ltd, Southampton, United Kingdom), thereby fusing this sequence to a firefly luciferase reporter gene. To investigate the polymorphic effects on CYP17A1 promoter activity, the plGL3-17 control vector was then mutated separately at each of the 7 single-nucleotide polymorphism (SNP) positions using the QuikChange Site-Directed Mutagenesis standard kit protocol (Agilent Technologies UK Ltd, United Kingdom) and specific primers (Eurolife MWG Operon, Ebersberg, Germany; Table S4). The resulting 7 vectors were each used to transform JM109 competent cells (100 μL; Promega, United States) by heat shock and purified using the QIAprep Spin Miniprep kit (QIAGEN, Crawley, United Kingdom). Direct sequencing of the entire insert and flanking regions confirmed the correct incorporation of the desired polymorphism in each of the 7 plasmids and the absence of additional unintended mutations.

Luciferase Reporter Gene Assays

H295R cells (a kind gift from Professor William E. Rainey, University of Michigan) were grown in DMEM/F12 medium supplemented with 2.5% Ultroser G serum (Pall Bioscience, Saint-Germain-en-Laye, France), 1% insulin–transferrin–selenium (BD Biosciences, Oxford, United Kingdom), and 1% penicillin–streptomycin (1 IU penicillin and 100 μg/mL streptomycin; Life Technologies) at 37°C, 5% CO2, and transfected with the plGL3-17 control vector or 1 of its 7 mutated derivative vectors using siPORT NeoFX transfection agent (Life Technologies) according to the manufacturer’s protocol at a final cell density of 8×104 cells per well. A pGL4.73 (renilla luciferase;
Genotype/Phenotype Associations in the Hypertensive British Genetics of Hypertension Cohort

The Medical Research Council British Genetics of Hypertension (BRIGHT) cohort is a large multicentre study, with white and British ancestry confirmed to the grand-parental level for all participants. Data presented here are related to 232 unrelated and successfully genotyped hypertensive individuals drawn randomly from the BRIGHT sibling-pair group (Table 1) for whom 24-hour urinary corticosteroid metabolite measurements, previously generated by gas chromatography/mass spectrometry,19 were available. Recruitment to the study required blood pressure values of 150/100 mm Hg or higher, based on 1 reading, or 145/90 mm Hg, as a mean of 3 readings, with onset of hypertension diagnosed before the age 60 years in at least 1 sibling; subjects with body mass index >30 were excluded.19 Ethical approval for this study was granted by the local ethics committees of the participating centers, and fully informed written consent of the subjects was obtained. The 2.4-kb upstream region was amplified using the Expand High Fidelity PCR System (Roche Diagnostics Ltd), according to the standard kit protocol. Automated sequencing of PCR products was performed using BigDye Terminator version 3.1 Cycle Sequencing chemistry (Life Technologies) and the ABI 3730 DNA analyzer (Life Technologies). Further details are available in Table S5. Measurement of urinary corticosteroid metabolites by gas chromatography/mass spectrometry was conducted as previously described.18,20

Statistics

In vitro experiments were performed in quadruplicate on 4 independent occasions (analyzed as n=4), with data presented as the mean and SEM. The activity of pGL3-basic and pGL3-17 control constructs (Figure 2A) was compared using 1-way ANOVA and Bonferroni post hoc tests on log-transformed values; mutated luciferase vector activity (Figure 2B and 2C) was analyzed by 1-sample Student t test on log-transformed values. For analysis of the BRIGHT subjects, demographic and steroid excretion data were not normally distributed and were, therefore, analyzed by the nonparametric Mann–Whitney U test. Comparisons of genotype with steroid excretion were conducted using the dominant model where heterozygote and minor allele homozygote data are grouped and compared against major allele homozygote data. In all instances, 95% confidence intervals were generated and a P value of <0.05 was set as the threshold for significance.

Results

Polymorphism Distribution Across the CYP17A1 Locus

The entire CYP17A1 locus, including exons, introns, and a 2-kb region upstream of the transcription start site (TSS), was sequenced using genomic DNA sourced from 60 healthy white volunteers, identifying 36 polymorphisms with minor allele frequencies ranging from 0.008 to 0.322 (Table S1). All polymorphisms within the coding region were synonymous, and none was observed at intronic splice sites. Pairwise LD was analyzed between the 24 most common polymorphisms (all minor allele frequencies, >0.05) using Haploview software (Figure 1A). We focused on 7 common polymorphisms located upstream of the CYP17A1 coding region with the potential to influence gene transcription (Figure 1B). Analysis of pairwise LD between these SNPs has identified 2 distinct polymorphic blocks in this area, which we termed LD blocks 1 and 2. LD block 1 is comprised of 6 of the 7 SNPs, which display a high degree of pairwise LD with one another (Figure 1C). LD block 2 includes the remaining seventh SNP, rs138009835, which is less strongly linked to the other 6 but is in high LD with 3 further variants that span the CYP17A1 locus and include rs1004467, the intron 3 SNP associated with blood pressure in the CHARGE GWAS (Figure 1D).3

Effect of Common Polymorphisms on CYP17A1 Transcription

The effect of the 7 upstream polymorphisms on CYP17A1 gene transcription was assessed in vitro through transfection
of the H295R adrenocortical cell line with reporter constructs containing 2.9 kb of the CYP17A1 5′ upstream region fused to a firefly luciferase reporter gene. Reporter gene activity was measured under basal conditions and after 24-hour stimulation with dibutyryl cAMP to mimic the intracellular activation of cAMP by adrenocorticotropic hormone. Dibutyryl cAMP caused a 2- to 3-fold increase in the activity of the pGL3-17 control vector relative to basal (Figure 2A). Site-directed mutagenesis of this vector generated 7 further plasmids, each varying from the control sequence only at a single polymorphic base. Under basal conditions, 3 polymorphisms resulted in differential transcriptional activity: the minor C allele at position −362 (rs2486758) significantly increased transcriptional activity relative to the control T allele, whereas the minor A allele at position −1877 (rs138009835) and the major G allele at position −2205 (rs2150927) each reduced the activity relative to their control forms (Figure 2B). The polymorphisms at the other 4 sites had no significant effect on transcription.

Incubation of H295R cells with dibutyryl cAMP stimulated the transcription of all constructs relative to basal conditions. The same 3 polymorphisms resulted in significantly different transcriptional activity under these stimulated conditions as they had under basal conditions (Figure 2C). Although the magnitude of change relative to the control vector was similar under basal and stimulated conditions for the −362

Figure 1. Pairwise linkage disequilibrium (LD) plots, generated using default settings in Haploview software, showing common polymorphisms (minor allele frequency, >0.05) at the CYP17A1 locus in a cohort of 60 normotensive volunteers. The various LD plots show variants found across the entire human CYP17A1 locus (A), the 7 variants subjected to subsequent detailed analysis in vitro and in the British Genetics of Hypertension (BRIGHT) study (B), the 6 highly linked variants comprising LD block 1 (C), and the 4 highly linked variants comprising LD block 2 (D). D′ values (%) are displayed for each pair of single-nucleotide polymorphisms in the intersecting square; squares not displaying a figure are D′=100%. Red squares indicate high D′ and a logarithm of the odds (LOD) score of ≥2; blue squares indicate high D′ and an LOD score of <2; white squares indicate low D′ (≤80%) and an LOD score of ≥2.
Steroid Phenotype in a Hypertensive Population

Association of CYP17A1 Polymorphisms With Steroid Phenotype in a Hypertensive Population

A subset of 232 hypertensive BRIGHT study subjects (106 men and 126 women) for whom urinary corticosteroid excretion data were available was genotyped for the 7 polymorphisms. Age, systolic blood pressure, diastolic blood pressure, and body mass index did not vary significantly with genotype. (Table S5) or with sex (Table 1) although levels of tetrahydroaldosterone, total corticosterone metabolites (Total B), total cortisol metabolites (Total F), and total androgen metabolites were all significantly lower in women (all \( P < 0.01 \); Table 1).

To provide sufficient power to identify significant associations between corticosteroid biosynthesis and CYP17A1 genotype, data were analyzed using a dominant model, grouping heterozygotes with minor allele homozygotes for comparison against major allele homozygotes (Table 2). None of the 7 SNPs departed significantly from Hardy–Weinberg equilibrium, and stratification of the study subjects by genotype revealed no association with key demographic characteristics, including blood pressure and body mass index (data not shown). All significant associations identified between the SNP genotype and the steroid phenotype are detailed below.

Although none of the 7 SNPs associated with significant differences in Total B, total androgen metabolites, or Total F, when the study population were analyzed as a whole, women homozygous for the major alleles at rs10786713 and at rs2150927 (A and G, respectively) had significantly lower levels of Total F metabolites (each \( P < 0.05 \)) when analyzed separately as did men homozygous for the major T allele at rs2486758 (\( P < 0.05 \) relative to heterozygotes only; the cohort contained no men homozygous for the minor allele at this locus; Figure 3).

The ratios of tetrahydrodeoxy cortisol to tetrahydrodeoxycorticisol (THDOC:THS) and Total B:Total F serve as indices of 17α-hydroxylase activity. Subjects homozygous for the major alleles of rs743572, rs10786713, and rs2150927 all associated with a significantly higher THDOC:THS ratio (each \( P < 0.05 \)), suggesting less efficient 17α-hydroxylation (Figure 4). For each of these SNPs, this effect was more pronounced in women than in the total population (each \( P < 0.01 \)) and was not significant in men alone, when analyzed separately (all \( P > 0.05 \)). Female subjects homozygous for the major forms of 2 of these SNPs—rs10786713 A and rs2150927 G—had a higher Total B:Total F ratio (\( P < 0.05 \)), again indicating less efficient hydroxylation; no such difference was detectable in men alone or in the total cohort (Figure 5).

Ratios of THS:dehydroepiandrosterone and Total F:total androgen metabolites, selected as indices of 17α,20β-lyase activity, showed no significant association with any of the 7 SNPs (data not shown).

Major allele homozygotes for rs138009835 (GG genotype) had significantly higher levels of the aldosterone metabolite tetrahydroaldosterone (\( P < 0.05 \)) in comparison with combined heterozygote and minor allele subjects (Figure 6). In separate analyses of men and women, there was no significant difference in tetrahydroaldosterone between these allele groups (\( P = 0.14 \) and \( P = 0.34 \), respectively).

Discussion

Our systematic sequencing of a normotensive population identified 24 common (minor allele frequency, >0.05) polymorphisms spanning the CYP17A1 locus. None is located within the coding region of CYP17A1 or at positions liable to disrupt mRNA splicing. Seven common SNPs located ≤2.2 kb from the TSS form 2 distinct and independent linkage blocks. Three of these SNPs disrupt in vitro CYP17A1 transcription:
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2 from LD block 1 (rs2486758 and rs2150927) and 1 from LD block 2 (rs138009835). Previous reporter construct studies found that the 227 bp lying immediately upstream of the human CYP17A1 TSS account for ≈60% to 80% of basal transcriptional activity at that locus; none of our 7 common SNPs lies in that area (rs743572 lies between the TSS and the start codon of CYP17A1). Therefore, a great deal of the basal—not to mention cAMP activated—transcriptional activity can be attributed to the region further upstream where 6 of the common SNPs are found. Our own previous studies show that transcriptional activity of steroidogenic genes can be significantly altered by functional polymorphisms lying some 1500 to 2000 bases upstream of the TSS, which alter transcription factor–binding affinity. We propose that the blood pressure association identified by GWAS at this locus is, therefore, most likely to result from ≥1 of the common SNPs that significantly alter in vitro transcription: rs2486758, rs138009835, and rs2150927.

Our analysis associates the major forms of the 2 functional polymorphisms found in LD block 1, rs2486758 and rs2150927, with sex-dependent changes in the corticosteroid excretion rate profiles in hypertensive individuals. (The LD block 1 SNP, rs10786713, also shows the same association although this had no functional effect in vitro.) These changes in the phenotype are consistent with altered 17α-hydroxylase efficiency; the similarity of these effects together with the close linkage of these SNPs suggests a common underlying factor. There is no associated effect on the aldosterone excretion rate. Conversely, the sole transcriptionally functional LD block 2 SNP, rs138009835, shows no association with apparent 17α-hydroxylase efficiency but is significantly associated with changes in the aldosterone excretion rate. The influence of the LD block 1 polymorphisms on steroid profile is consistent with their effects on CYP17A1 transcription in vitro: the major alleles rs2486758 T and rs2150927 G each reduce transcription relative to their alternative forms and associate with less efficient hydroxylation in vivo, as reflected in lower Total F for both SNPs and higher Total B:Total F and THDOC:THS ratios for rs2150927. The rs10786713 A allele has similar associations in vivo but no significant effect on transcription, implying that its association with the steroid profile is the result of linkage to one or the other of the functional block 1 polymorphisms. On the basis of the observed steroid ratios, 17-lyase efficiency is unaffected by any of the 7 analyzed SNPs.

The altered 17α-hydroxycorticosteroid:17-deoxycorticosterone ratios in subjects carrying the major alleles at selected SNPs imply that the will have higher adrenocorticotropic

Table 2. Genotype Data for Selected SNPs Upstream of CYP17A1

| SNP   | ID  | CYP17A1 Location | Chromosomal Location | Alleles | Minor Allele Frequency | HWE (P Value) | % Genotyped |
|-------|-----|------------------|----------------------|---------|------------------------|---------------|-------------|
| rs743572 | 1   | -34              | 10:104597152         | A/G     | 0.420                  | 0.9276        | 96.9        |
| rs2486758 | 2   | -362             | 10:104597480         | T/C     | 0.183                  | 0.9940        | 96.9        |
| rs10883784 | 3   | -804             | 10:104597922         | C/T     | 0.304                  | 0.7485        | 100.0       |
| rs10786713 | 4   | -1204            | 10:104598322         | A/G     | 0.417                  | 0.9121        | 99.6        |
| rs10786714 | 5   | -1488            | 10:104598606         | G/C     | 0.304                  | 0.6991        | 99.6        |
| rs138009835 | 6   | -1877            | 10:104598995         | G/A     | 0.109                  | 1.0000        | 100.0       |
| rs2150927 | 7   | -2205            | 10:104599323         | G/A     | 0.413                  | 0.9276        | 99.1        |

Genotypes were generated from a subset (n=232) of the hypertensive British Genetics of Hypertension (BRIGHT) population. Major alleles are listed first, and the given bases are from the forward strand sequence. The CYP17A1 location is relative to the first codon. HWE indicates Hardy–Weinberg equilibrium; and SNP, single-nucleotide polymorphism.

Figure 3. Box–whisker plots of 24-hour total cortisol metabolites (Total F) in the British Genetics of Hypertension (BRIGHT) study subgroup (n=232), stratified by rs10786713 genotype (B), rs2150927 genotype (B), and rs2486758 genotype (C). Total F is the sum of tetrahydrocortisol, allotetrahydrocortisol, and tetrahydrocortisone. Plots show the median within the interquartile range box, with whiskers extending to the fifth and 95th percentiles; data points beyond the whiskers are displayed as dots. Groups were compared by Mann–Whitney nonparametric test; *P<0.05.
hormone drives to maintain normal cortisol levels. There is a clear sex difference in the steroid effects of block 1 polymorphisms, with women tending to have the more altered intermediate phenotype. This may be related to the fact that the women in this cohort have a tendency to higher blood pressure relative to men although this does not achieve significance ($P=0.07$; Table 1). Nevertheless, if impaired 17α-hydroxylase function results in increased blood pressure, it seems legitimate to conclude that such an effect is due—at least in part—to sustained changes in the adrenal steroid profile. In classical 17α-hydroxylase deficiency, massive DOC excess causes an easily recognizable mineralocorticoid hypertension. Whether the small differences in the proportion of DOC in our population—even persisting over the course of a lifetime—could account for the small but significant blood pressure effects identified by GWAS is debatable although the potency of DOC relative to aldosterone remains the subject of discussion.22 Alternatively, corticosterone could be responsible. In our study group, women carrying the major alleles at rs10786713 and rs2150927 had higher corticosterone:cortisol ratios. Previously, Soro et al23 found levels of corticosterone to be higher in subjects with hypertension. This may be related to the easier access corticosterone has to the brain when compared with cortisol24; its concentration relative to cortisol in cerebrospinal fluid is much higher than in plasma,25 and it may be preferentially retained in specific regions of the brain,26 where it occupies both mineralocorticoid and glucocorticoid receptors. Recently, Morris27 has argued that corticosterone is not merely a minor glucocorticoid subsidiary to cortisol but has distinct properties (eg, higher mineralocorticoid activity and lower susceptibility to 11β-hydroxysteroid dehydrogenase types 1 and 2) that might cause it and its 5α-metabolites to affect blood pressure significantly.

Of the 7 analyzed SNPs, only rs138009835 is found in LD block 2 and is, therefore, strongly linked to the blood pressure GWAS variants at this locus. It shows no association with our chosen indices of steroid 17α-hydroxylation efficiency in vivo, but its major G allele—which causes increased $CYP17A1$ transcription in vitro—does associate with higher levels of aldosterone in a nonsex-dependent manner, unrelated to the effects of the LD block 1 polymorphisms. Given that the zona glomerulosa does not express $CYP17A1$ and has no obvious direct interaction with the zona fasciculata, the influence of this SNP on aldosterone levels is not open to a simple explanation. Regardless of the precise mechanism, we demonstrate here that the rs138009835 G allele associates with increased $CYP17A1$ transcription in vitro and with raised aldosterone levels in vivo. Given its strong linkage (via LD block 2) to the A allele of rs1004467—itself significantly associated with increased blood pressure—and the critical role of aldosterone

Figure 4. Box–whisker plots of 24-hour tetrahydrodeoxycorticosterone to tetrahydrodeoxycortisol (THDOC:THS) ratio in the British Genetics of Hypertension (BRIGHT) study subgroup (n=232), stratified by rs743572 genotype (A), rs10786713 genotype (B), and rs2150927 genotype (C). Plots show the median within the interquartile range box, with whiskers extending to the fifth and 95th percentiles; data points beyond the whiskers are displayed as dots. Groups were compared by Mann–Whitney nonparametric test; *$P<0.05$ and **$P<0.01$.

Figure 5. Box–whisker plots of 24-hour total corticosterone metabolite (Total B) to total cortisol metabolite (Total F) ratio in the British Genetics of Hypertension (BRIGHT) study subgroup (n=232), stratified by rs10786713 genotype (A) and rs2150927 genotype (B). Total B is the sum of tetrahydrocorticosterone, allotetrahydrocorticosterone, and tetrahydro-11-dehydrocorticosterone. Total F is the sum of tetrahydrocortisol, allotetrahydrcortisol, and tetrahydrcortisone. Plots show the median within the interquartile range box, with whiskers extending to the 5th and 95th percentiles; data points beyond the whiskers are displayed as dots. Groups were compared by Mann–Whitney nonparametric test; *$P<0.05$. 
in blood pressure homeostasis, this finding clearly warrants further investigation.

This study had limitations and was not designed with the intention of detecting direct associations of CYP17A1 genotype with blood pressure. Given that the BRIGHT study subjects were all hypertensive and on various forms of antihypertensive therapy, the lack of association between blood pressure and any of the 7 SNPs—including rs138009835—is, therefore, unsurprising. It is possible that antihypertensive treatments influenced steroid excretion although previous analysis of the 512 BRIGHT subjects from which this subset was drawn found no evidence that these drugs systematically affected excretion rates of cortisol, aldosterone, or androgens. Finally, this study did not adjust for multiple testing. As such there is a danger that some of the results deemed statistically significant may be false positives. However, for an exploratory study, such as this, it is recognized that adjusting for multiple testing increases the chance that real differences will be missed and may not be advisable. Further investigation in a different study population is now recommended to confirm these findings.

Perspectives

Thorough analysis of the CYP17A1 locus in a control human population reveals a high degree of genetic variation, including 2 distinct LD blocks, each containing common upstream SNPs. Several of these SNPs significantly affect in vitro gene expression and associate with in vivo steroid intermediate phenotype in a hypertensive population. Although this study was not designed with the intention of directly analyzing blood pressure effects, our identification of the functional rs138009835 SNP might account for the significant blood pressure influence at this locus reported by multiple GWAS. The processes by which such alterations in CYP17A1 transcriptional regulation influence steroid profile and, ultimately, blood pressure require further investigation. If subsequent studies confirm CYP17A1 as a significant factor in population blood pressure variation, it has the potential to serve as a prominent target in the treatment and control of human hypertension.

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Disclosures

M.J. Caulfield is the chief scientist for Genomics England, a UK Government company. The other authors report no conflicts.

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Novelty and Significance

What Is New?

- Previous genome-wide association studies have linked the CYP17A1 locus with blood pressure variation.
- This study presents the first evidence of common functional CYP17A1 gene polymorphisms. Of these, the transcriptional, phenotypic, and linkage characteristics of the rs138009835 polymorphism suggest that it could underlie the blood pressure associations identified at this locus.

What Is Relevant?

- Genome-wide association studies have identified several loci as being significantly associated with blood pressure variation, however, few of the molecular mechanisms underlying these associations have been identified.
- This study provides functional evidence that could explain the known blood pressure associations identified at this region of human chromosome 10. The identification of blood pressure–related pathways affected by this locus is potentially of high clinical benefit.

Summary

We report that common genetic polymorphisms at the CYP17A1 locus alter its expression in vitro and associate with changes in steroid levels in vivo. One such variant, rs138009835, is in strong linkage disequilibrium with polymorphisms previously identified by genome-wide association studies as having significant influence on blood pressure. These associations of rs138009835 with transcriptional activity and intermediate steroid phenotype provide a plausible mechanism to explain the known blood pressure associations at this locus.
Common Polymorphisms at the CYP17A1 Locus Associate With Steroid Phenotype: Support for Blood Pressure Genome-Wide Association Study Signals at This Locus

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COMMON POLYMORPHISMS AT THE CYP17A1 LOCUS ASSOCIATE WITH STEROID PHENOTYPE: SUPPORT FOR BLOOD PRESSURE GWAS SIGNALS AT THIS LOCUS.

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Table S1: Genotype data for the 36 polymorphisms identified across the CYP17A1 locus in 60 normotensive volunteers.

SNP: single nucleotide polymorphism; ID: Haploview identification number; Location: location of the polymorphism relative to the first CYP17A1 codon; Position: chromosomal position from ENSEMBL Homo sapiens version 72.37 (GRCh37); Alleles: the major polymorphic form is listed first and the given bases are from the forward strand sequence; MAF: minor allele frequency; HWE: Hardy-Weinberg equilibrium p-value. * denotes that SNP -638 currently has no allocated rs number.

| SNP          | Location | Position | Alleles | MAF   | HWE (p) | Genotyped |
|--------------|----------|----------|---------|-------|---------|-----------|
| rs2150927    | -2205    | 10:104599323 | G/A     | 0.306 | 0.0973  | 100       |
| rs117574307  | -1933    | 10:104599051  | G/A     | 0.032 | 1.0000  | 100       |
| rs138009835  | -1877    | 10:104598995  | G/A     | 0.089 | 1.0000  | 100       |
| rs183906459  | -1722    | 10:104598840  | A/T     | 0.008 | 1.0000  | 100       |
| rs10786714   | -1488    | 10:104598606  | G/C     | 0.210 | 0.1584  | 100       |
| rs544168523  | -1366    | 10:104598484  | G/A     | 0.008 | 1.0000  | 100       |
| rs10786713   | -1204    | 10:104598322  | A/G     | 0.306 | 0.2835  | 100       |
| rs545495641  | -1077    | 10:104598195  | G/A     | 0.008 | 1.0000  | 100       |
| rs10883784   | -804     | 10:104597922  | C/T     | 0.210 | 0.1584  | 100       |
| rs190440742  | -734     | 10:104597852  | C/T     | 0.016 | 1.0000  | 100       |
| -638*        | -638     | 10:104597756  | G/A     | 0.016 | 0.0163  | 100       |
| rs61752856   | -626     | 10:104597744  | G/A     | 0.016 | 1.0000  | 100       |
| rs2486758    | -362     | 10:104597480  | T/C     | 0.242 | 0.9939  | 100       |
| rs743572     | -34      | 10:104597152  | A/G     | 0.282 | 0.0973  | 100       |
| rs61754263   |         | 10:104597057  | C/T     | 0.008 | 1.0000  | 100       |
| rs6162       |         | 10:104596981  | G/A     | 0.315 | 0.1449  | 100       |
| rs6163       |         | 10:104596924  | C/A     | 0.306 | 0.0249  | 100       |
| rs10786712   |         | 10:104596396  | C/T     | 0.293 | 0.0232  | 93.5      |
| rs45463800   |         | 10:104596356  | C/T     | 0.017 | 1.0000  | 95.2      |
| rs754283774  |         | 10:104596011  | C/T     | 0.008 | 1.0000  | 95.2      |
| rs3824755    |         | 10:104595849  | G/C     | 0.052 | 1.0000  | 93.5      |
| rs284847     |         | 10:104595828  | G/A     | 0.068 | 0.4480  | 95.2      |
| rs3781286    |         | 10:104595719  | C/T     | 0.302 | 0.0396  | 93.5      |
| rs3781287    |         | 10:104595420  | T/G     | 0.322 | 0.1374  | 95.2      |
| rs4919687    |         | 10:104595240  | G/A     | 0.241 | 0.0247  | 93.5      |
| rs743575     |         | 10:104594906  | T/G     | 0.208 | 0.4270  | 96.8      |
| rs1004467    |         | 10:104594507  | A/G     | 0.083 | 1.0000  | 96.8      |
| rs544961235  |         | 10:104593194  | C/T     | 0.008 | 1.0000  | 95.2      |
| rs3740397    |         | 10:104592675  | G/C     | 0.292 | 0.1196  | 96.8      |
| rs4919686    |         | 10:104592249  | A/C     | 0.220 | 0.0448  | 95.2      |
| rs284848     |         | 10:104592125  | G/A     | 0.212 | 1.0000  | 95.2      |
| rs45609333   |         | 10:104591639  | G/A     | 0.025 | 1.0000  | 95.2      |
| rs17115100   |         | 10:104591393  | G/T     | 0.085 | 1.0000  | 95.2      |
| rs284849     |         | 10:104591182  | G/T     | 0.234 | 0.3853  | 100       |
| rs10883783   |         | 10:104591152  | T/A     | 0.218 | 0.2194  | 100       |
| rs139275291  |         | 10:104590871  | +/35bp  | 0.218 | 0.2194  | 100       |
Table S2: Primers used to amplify and sequence CYP17A1 exons in normotensive subjects.

| Target Exon | Primer Type | Primer Name   | Sequence 5’-3’                |
|-------------|-------------|---------------|-------------------------------|
| Exon 1      | PCR Primers | cyp17ex1_f1   | CCACTGCTGTCTATCTTTGCC        |
|             |             | cyp17ex1_r1   | TGAAGACCTGAAACAATCCCA        |
|             | Sequencing Primers | cyp17ex1_f1 | CCACTGCTGTCTATCTTTGCC        |
|             |             | cyp17ex1_r1   | TGAAGACCTGAAACAATCCCA        |
|             |             | 17Exon1F      | CAGAGGGTGATCAACTGAC          |
|             |             | 17Exon1R      | CTAGGGCATGGTCTGAAGAGC        |
| Exons 2 & 3 | PCR Primers | cyp17ex2+3_f1 | GGTGTTGAGATTCTTCACAGCC       |
| (+ intron 2) |             | cyp17ex2+3_r1 | TCTACTAGAACCTGAGAGCC         |
|             | Sequencing Primers | cyp17ex2+3_f1 | GGTGTTGAGATTCTTCACAGCC       |
|             |             | cyp17ex2+3_r1 | TCTACTAGAACCTGAGAGCC         |
|             |             | cyp17ex2_r1   | TCCTAACCCCTTACCCTTG          |
|             |             | cyp17ex3_f1   | TGGTACAGAAGGGGAAGAAGG        |
| Exon 4      | PCR Primers | cyp17ex4_f1   | GGTGGGATAGGAACCTCCAG         |
|             |             | cyp17ex4_r2   | TCCACCCCTGCTCTTGTTGAT        |
|             | Sequencing Primers | cyp17ex4_f1 | GGTGGGATAGGAACCTCCAG         |
|             |             | cyp17ex4_r1   | TGTCGACAGCTTCTGCTTG          |
|             |             | cyp17ex4_r2   | AGCTAAGATCCGCTCCAGC          |
|             |             | cyp17ex4_r2   | TCCACCCCTGCTCTTGTTGAT        |
| Exons 5 & 6 | PCR Primers | cyp17ex5+6_f1 | TGCCAGAGGTGTCACAGATG         |
| (+ intron 5) |             | cyp17ex5+6_r1 | TGAATGCATCATGGGCTAG          |
|             | Sequencing Primers | cyp17ex5+6_f1 | TGCCAGAGGTGTCACAGATG         |
|             |             | cyp17ex5_f1   | TGCCAGAGGTGTCACAGATG         |
|             |             | cyp17ex5_r1   | TGCCAGAGGTGTCACAGATG         |
|             |             | cyp17ex6_f1   | ACACACTAGTCACACTCCAC         |
|             |             | cyp17ex6_r1   | TGAATGCATCATGGGCTAG          |
| Exons 7 & 8 | PCR Primers | cyp17ex7+8_f1 | TTCCCTTTCCACTCTGGAG          |
| (+3’UTR)    |             | cyp17ex8utr_r1 | GAATGAGTGAGCAAATGAAATAC     |
|             | Sequencing Primers | cyp17ex7+8_f1 | TTCCCTTTCCACTCTGGAG          |
|             |             | cyp17ex8utr_r1 | GAATGAGTGAGCAAATGAAATAC     |
|             |             | cyp17ex7_r1   | TTGGCAAGAGGTGAAAGGGTA       |
|             |             | cyp17ex8_r1   | GCCACATAGGTGGCAACAGG         |
|             |             | cyp17ex8_f1   | TCAACACAGGGCAGAAACCATG       |
|             |             | cyp17ex8_r1   | TGTGTGGTGAGGGCCACATAG       |
Table S3: Primers used to amplify and sequence *CYP17A1* introns in normotensive subjects.

| Target Intron | Primer Type       | Primer Name | Sequence 5’-3’                  |
|---------------|-------------------|-------------|---------------------------------|
| Intron 1      | PCR Primers       | intron1F    | CACCAAGACTACAGTGATTG            |
|               |                   | intron1R1   | GTGCCGAGGTCCAGCGGAAG            |
|               | Sequencing Primers| intron1F    | CACCAAGACTACAGTGATTG            |
|               |                   | intron1R1   | GTGCCGAGGTCCAGCGGAAG            |
|               |                   | intron1s1f  | CTTAGCGTAGCAACCAGCAC            |
|               |                   | intron1s1r  | AGCTGGAAAATAGCACCAGGA           |
|               |                   | intron1s2f  | CCAATCTCAGCTACTGCAA             |
|               |                   | intron1s2r  | CTAAGGTGGAGGTAGATCGA            |
|               |                   | intron1s4f  | ACCCTGTACAGAAAAAGAC             |
|               |                   | intron1s4r  | TTGCCCTTACACTCTGTC              |
|               |                   | intron1s3f  | CTTCAGGGTCAGAAATGGA             |
|               |                   | intron1s3r  | AGGCTGAGACTGACAGAT              |
| Intron 3      | PCR Primers       | cyp17ex3_f1 | TGGTACAGAGAGGGGTGAAG            |
|               |                   | cyp17ex4_r1 | TGGTCCAGTTCTCTGCTTG             |
|               | Sequencing Primers| Intron3s1f  | TGGTGAAGGTAGATGCTGA             |
|               |                   | Intron3s2f  | AAGCATCTATACTGAGATATTCA         |
|               |                   | Intron3s1r  | AAGCTCTTACACTCCGCTAA            |
|               |                   | cyp17ex3_f1 | TGGTACAGAGAGGGGTGAAG            |
|               |                   | Intron3s2r  | GACCTTCAGCCAGAATGGA             |
| Intron 4      | PCR Primers       | Intron4F1   | AAGCAGAGAACCTGGCAGAT            |
|               |                   | Intron4R1   | TCACTCCGAATTTCTCCTG             |
|               | Sequencing Primers| Intron4F1   | AAGCAGAGAACCTGGCAGAT            |
|               |                   | Intron4R1   | TCACTCCGAATTTCTCCTG             |
|               |                   | Intron4s1r  | CTTGCTGGCTAGTTTTTGG             |
|               |                   | Intron4s1f  | CAGCTATATTGGAGGTGAGT            |
| Intron 6      | PCR Primers       | cyp17ex6_f1 | ACACACTAGTCACCTCCAAC            |
|               |                   | cyp17ex7+8_r1| GCCACATAGGGTGAGACAG             |
|               | Sequencing Primers| Intron6s1f  | GCTGGCAGCCTAAAGTCCGC           |
|               |                   | Intron6s1r  | ATCTTGGCTACACTGACTC             |
|               |                   | Intron6s2r  | GGAAGGCTTCCTGCTGGAAGTC          |
|               |                   | Intron6s3r  | GGGACTTCAGTACTGCTTC             |
| Intron 7      | PCR Primers       | cyp17ex7+8_f1| TTTCTCTTCTCAGCTCTGAGC          |
|               |                   | cyp17ex8utr_r1| GAATGAGTGAGGAAATGACAT         |
|               | Sequencing Primers| cyp17ex8_r1| TGGTGTTGAGGGCCACATAG           |
|               |                   | cyp17ex7+8_r1| GCCACATAGGGTGAGACAG             |
|               |                   | intron7s1r  | GCCCTTACAGCAACAGAGGA           |
|               |                   | Introns7s2r | CAGGCCATGATGAGGAGAAG            |
| SNP            | Primer | Sequence 5'-3' | Base Change |
|----------------|--------|----------------|-------------|
| rs2150927      | Sense  | ATGGGAAATGGTCAAAAGGACACCTTCTGGGTAGGG | T → C       |
|                | Antisense | CCCTACCCAGAGGTGCCTTTTCGACCATTCCCCAT |             |
| rs138009835    | Sense  | AAGGGAGAGATGTTGTGGGAAGTCAGGGACC | C → T       |
|                | Antisense | GTCCCTGACTTCCACACAATCTCTCCCTT |             |
| rs10786714     | Sense  | GAGAGAGGCTATAATGGGAAGTAGGAAGATAT | C → G       |
|                | Antisense | ATATCTTCTCTACTGATCTCCATTTATAGCTCTC |             |
| rs10786713     | Sense  | TGTAAAAAGTTTATCAAGACAGTACGTGCACCGCTGAAC | T → C       |
|                | Antisense | GTTCACGGGTGCACGTACTGTCTTTGATAACACCTTAAACA |             |
| rs10883784     | Sense  | GTCTCTCTTTTATTTCTGACCCAGCTACATGAGAAGAAC | G → A       |
|                | Antisense | GTTCTTCTATAAGTGTCAGCTGCTGAGAAATAAAGAGAC |             |
| rs2486758      | Sense  | CCAGTGTATTTTGTATTTGAGCCTAGTTCCCGCTTTT | A → G       |
|                | Antisense | AAGGCTTGGAACTTTCATGCTGCAAAAATCAAAATCAGTGG |             |
| rs743572       | Sense  | CAGCTCTCTACTCCACTGCTGCTATCTCTGAGCCTG | C → T       |
|                | Antisense | CAGGCAAGATAGACAGCAGGAGTGAAGAGAGCTG |             |
Table S5: Primers used to amplify and sequence 2.4kb CYP17A1 upstream region in hypertensive BRIGHT subjects.

| Primer Type       | Primer Name     | Sequence 5’-3’                                      |
|-------------------|-----------------|-----------------------------------------------------|
| PCR Primers       | cyp17promF2     | ATGCAGTTCGATTTGCAACAC                                |
|                   | cyp17promR2     | TGGGCAAAAACAAATAAGC                                 |
| Sequencing Primers| cyp17promR2     | TTGGGCAAAAACAAATAAGC                                 |
|                   | cyp17-1935F     | AGCTGAGGCGTTAGATCAGG                                 |
|                   | cyp17-1525F     | GTCACTTTAGGACCACCAGTGTA                             |
|                   | cyp17-1213F     | CGGCTCTGGGAATGTCTATC                                 |
|                   | cyp17-884F      | TTGTCTTTCTCCTCAGAAGC                                |
|                   | cyp17-603F      | GAAATATGGGGTGTTGGTC                                 |
|                   | cyp17-159F      | CCCAGATACCATTCGCACTC                                 |
|                   | cyp17-1697R     | GACTTCCCCGCAACCATCTCTC                              |
|                   | cyp17-1388R     | TGTTTGTCCCCCTTGTCC                                  |
|                   | cyp17-1021R     | CAGCGTGACATACTGTCT                                  |
|                   | cyp17-720R      | TACCACCTCGAGCCTAAAC                                 |
|                   | cyp17-374R      | TAAAGGCGCTTGTTATAGG                                 |
|                   | cyp17-117R      | CTCCCCATGCTTTGAAATGACT                              |
|                   | cyp17+67R       | GGCCTACCTTGACTTTACCTTT                              |
|                   | cyp17-112R      | GTCACTTTGATACCCCTCTGA                               |
Table S6: BRIGHT study subgroup demographics data stratified by SNP genotype.

Demographic data (median and interquartile ranges) of the BRIGHT study subgroup stratified by the 7 SNP genotypes. Demographic characteristics were compared by the non-parametric Mann-Whitney test using the dominant model. SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index; WHR: waist-hip ratio.

| SNP      | genotype | N  | Age (Years) | SBP (mm/Hg) | DBP (mm/Hg) | BMI (kg/m2) | WHR          | p-value (genotype vs. genotype + genotype) |
|----------|----------|----|-------------|-------------|-------------|-------------|--------------|------------------------------------------|
| rs743572 | AA       | 72 | 63 (58-67)  | 157 (153-187) | 102 (98-107) | 27 (24-30)  | 0.86 (0.83-0.92) | 0.99                  |
|          | AG       | 109| 62 (55-70)  | 183 (153-190) | 103 (99-110) | 27.5 (25-30) | 0.88 (0.81-0.93) |                       |
|          | GG       | 37 | 64 (58-68)  | 155 (152-191) | 103 (98-110) | 27 (25-31)  | 0.91 (0.83-0.94) |                       |
| rs2486758| TT       | 145| 63 (56-67.25)| 157 (153-190)| 103 (99-108) | 27 (24-30)  | 0.89 (0.81-0.93) | 0.52                  |
|          | TC       | 66 | 63 (56-70)  | 181.5 (152-190)| 103 (98-110.75)| 27 (25-29)  | 0.89 (0.81-0.93) |                       |
|          | CC       | 7  | 63 (60.5-65)| 186 (169-189.5)| 99 (98-103)  | 30 (27-30)  | 0.79 (0.76-0.85) |                       |
| rs10883784| CC       | 107| 63.5 (58.25-68)| 157 (153-190)| 103 (98-108) | 27 (24-30)  | 0.86 (0.80-0.92) | 0.43                  |
|          | CT       | 99 | 62 (54.5-70)| 157 (153-190) | 103 (98-110) | 27 (25-30)  | 0.88 (0.81-0.93) |                       |
|          | TT       | 19 | 63 (60.5-67)| 153 (152-186.5)| 102 (98-110) | 28 (26-31)  | 0.92 (0.89-0.94) |                       |
|          |          |    |             |              |             |             |              |                          |

Table S6 continued over
Table S6 (continued)

| rs10786713 | AA N=75 | AG N=111 | GG N=38 | p-value AA vs. AG+GG |
|------------|---------|---------|---------|----------------------|
| Age (Years) | 63 (58-67) | 63 (55-70) | 64.5 (58.5-68) | 0.94 |
| SBP (mm/Hg) | 157 (153-187) | 183 (153-190) | 156 (152-191) | 0.76 |
| DBP (mm/Hg) | 102 (98-107) | 103 (99-110) | 103 (98.25-109) | 0.58 |
| BMI (kg/m2) | 27 (24-29.75) | 28 (25-30) | 27 (24.25-30.75) | 0.36 |
| WHR | 0.86 (0.81-0.91) | 0.88 (0.81-0.93) | 0.91 (0.82-0.94) | 0.34 |

| rs10786714 | GG N=107 | GC N=98 | CC N=19 | p-value GG vs. GC+CC |
|------------|---------|---------|---------|----------------------|
| Age (Years) | 63.5 (58.25-68) | 62.5 (55-70) | 63 (60.5-67) | 0.49 |
| SBP (mm/Hg) | 157 (153-190) | 169 (153-190) | 153 (152-186.5) | 0.81 |
| DBP (mm/Hg) | 103 (98-108) | 103 (98-110) | 102 (98-110) | 0.98 |
| BMI (kg/m2) | 27 (24-30) | 27 (25-30) | 28 (26-31) | 0.39 |
| WHR | 0.86 (0.80-0.92) | 0.88 (0.81-0.93) | 0.92 (0.89-0.94) | 0.26 |

| rs138009835 | GG N=178 | GA N=45 | AA N=2 | p-value GG vs. GA+AA |
|-------------|---------|---------|--------|----------------------|
| Age (Years) | 63 (56-68) | 65 (58-70) | 67 (66-68) | 0.29 |
| SBP (mm/Hg) | 157 (153-190) | 183 (153-197) | 156 (155.5-156.5) | 0.61 |
| DBP (mm/Hg) | 102.5 (98-110) | 103 (100-110) | 106 (103-109) | 0.72 |
| BMI (kg/m2) | 27 (25-30) | 27 (24-30) | 25.5 (25-26) | 0.51 |
| WHR | 0.88 (0.81-0.93) | 0.87 (0.79-0.92) | 0.99 (0.96-1.01) | 0.66 |

| rs2150927 | GG N=76 | GA N=110 | AA N=37 | p-value GG vs. GA+AA |
|-----------|-------|--------|------|----------------------|
| Age (Years) | 63 (58-67) | 63 (55-70) | 64 (58-68) | 0.94 |
| SBP (mm/Hg) | 157 (153-187) | 182 (153-190) | 155 (152-191) | 0.90 |
| DBP (mm/Hg) | 102.5 (98-107) | 103 (99-110) | 103 (98-110) | 0.64 |
| BMI (kg/m2) | 27 (24-30) | 28 (25-30) | 27 (25-31) | 0.42 |
| WHR | 0.86 (0.81-0.91) | 0.88 (0.80-0.93) | 0.91 (0.83-0.94) | 0.30 |