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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 pressure[2] 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.

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Of these 28 loci, 1 on chromosome 10 encompasses a cluster of 5 genes forming a high linkage disequilibrium (LD) block spanning 347 kb.[5] 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 (r^2=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.[6–9] A recent study has also associated rs11191548 with left ventricular mass index in hypertensive patients, which is suggestive of a role in cardiac hypertrophy.[10] 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-hydroxypregnenolone and 17α-hydroxyprogesterone, respectively.[11] 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.[12] Recently, the substantial heritability of 17α-hydroxylase activity in the general population has been demonstrated as has its inverse correlation with adult blood pressure.[13] Deficient 17α-hydroxylation has been known for almost 50 years to result in hypertension, with mutation of the *CYP17A1* gene now estimated to account for ≈1% of congenital adrenal hyperplasia cases.[14,15] Causative mutations usually occur in exons or at intronic splice sites, resulting in defective androgen production—which disrupts sexual development and maturation—and a markedly reduced ability to synthesize cortisol. The consequent sustained surge in adrenocorticotropic hormone production stimulates high secretion of the weak mineralocorticoid, 11-deoxycorticosterone (DOC), causing hypertension, hypokalemia, and suppression of aldosterone synthesis. Simultaneously, high levels of corticosterone compensate for cortisol deficiency. A valuable biochemical indicator is, therefore, an abnormal excess of 17-deoxycorticosteroids (ie, DOC and corticosterone) and lowered 17α-hydroxycorticosteroids (eg, 17-OH-pregenenolone and 17-OH-progesterone).

We hypothesized that common *CYP17A1* polymorphisms exist, which influence the blood pressure and account for the significant association identified at this locus. In this study, therefore, we took the crucial next step of identifying common polymorphic variations within *CYP17A1* and investigating their potential effects on biochemical function. We first characterized the polymorphic frequency and distribution in normotensive individuals and investigated the effects of selected polymorphisms on gene transcription in vitro. We then tested for the association between these candidate polymorphisms and intermediate corticosteroid phenotype in a large hypertensive cohort. As a result, we demonstrate that common *CYP17A1* polymorphisms significantly alter gene function and correlate with changes in intermediate corticosteroid phenotype—including a significant effect on aldosterone levels—that could account for the strong association of this locus with population blood pressure variation.

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.[16] 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); 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 Haploview software.[17]

Site-Directed Mutagenesis of CYP17A1 Luciferase Reporter Vector

To create the pGL3-17 control vector, 2898bp of human genomic DNA immediately upstream of the *CYP17A1* coding region was inserted into the empty pGL3-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 pGL3-17 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 (Eurofins 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% CO_2, and transfected with the pGL3-17 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 8×10^4 cells per well. A pGL4.73 (renilla luciferase;

Promega) construct was cotransfected at a ratio of 50:1 to control for transfection efficiency. After 24 hours, transfectant was removed and replaced with complete medium or complete medium containing 1 mmol/L dibutyryl cAMP for 24 hours. The Dual Luciferase Reporter Assay system (Promega) was used to measure firefly and renilla luciferase activity in cell lysates containing 1× passive lysis buffer, according to the manufacturer's instructions, on a Lumat LB 9507 tube luminometer (Berthold Technologies, United Kingdom).

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,[18] 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

Table 1. Demographic and Urinary Corticosteroid Data (Median and Interquartile Ranges) for the British Genetics of Hypertension Study Subgroup

Urinary Steroid Metabolite (µg per 24 h)	All Subjects (n=232)	Men (n=106)	Women (n=126)	*P* Value (Men vs Women)
Age, y	63 (56–69)	63 (56–68)	64 (56–69)	0.47
SBP, mm Hg	157 (153–190)	157 (151.25–187)	181.5 (153–191)	0.07
DBP, mm Hg	103 (98–110)	103 (98–110)	102 (98–109.5)	0.43
BMI, kg/m^2	27 (25–30)	28 (25–30.75)	27 (25–30)	0.26
WHR	0.88 (0.81–0.93)	0.93 (0.90–0.97)	0.82 (0.78–0.86)	<0.001
Corticosterone (Total B: THB+aTHB+THA)	103 (61–188)	140 (79–228)	86.5 (54–156)	<0.001
Cortisol (Total F: THF+aTHF+THE)	1467 (759–2559)	2081 (1130–3526)	1118 (661–1989)	<0.001
Androgens (DHEA+Aetio+Andro)	613 (322–1227)	1008 (503–1852)	447 (225–815)	<0.001
Aldosterone (THAldo)	3 (1–5)	3 (2–6)	2 (1–4)	0.002

Men and women were compared by the nonparametric Mann–Whitney *U* test. Aetio indicates aetiocholanolone; Andro, androsterone; aTHB, allotetrahydrocorticosterone; aTHF, allotetrahydrocortisol; BMI, body mass index; DBP, diastolic blood pressure; DHEA: dehydroepiandrosterone; SBP, systolic blood pressure; THA, tetrahydro-11-dehydrocorticosterone; THAldo, tetrahydroaldosterone; THB, tetrahydrocorticosterone; THE, tetrahydrocortisone; THF, tetrahydrocortisol; and WHR: waist:hip ratio.

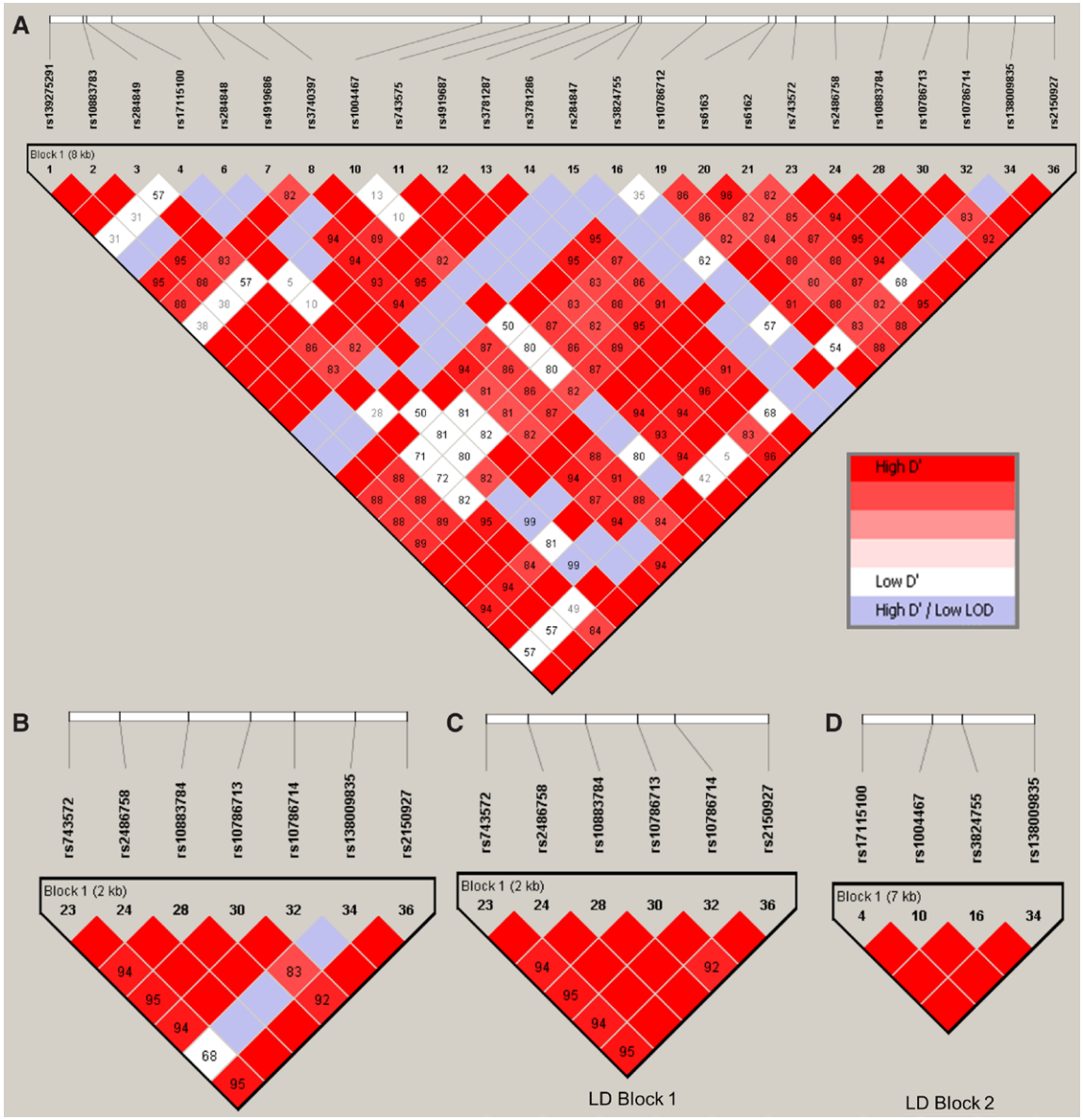

Figure 1. Pairwise linkage disequilibrium (LD) plots, generated using default settings in Haploview software, showing common polymorphisms (minor allele frequency, >0.05) at the *CYP17A1* locus in a cohort of 60 normotensive volunteers. The various LD plots show variants found across the entire human *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.

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

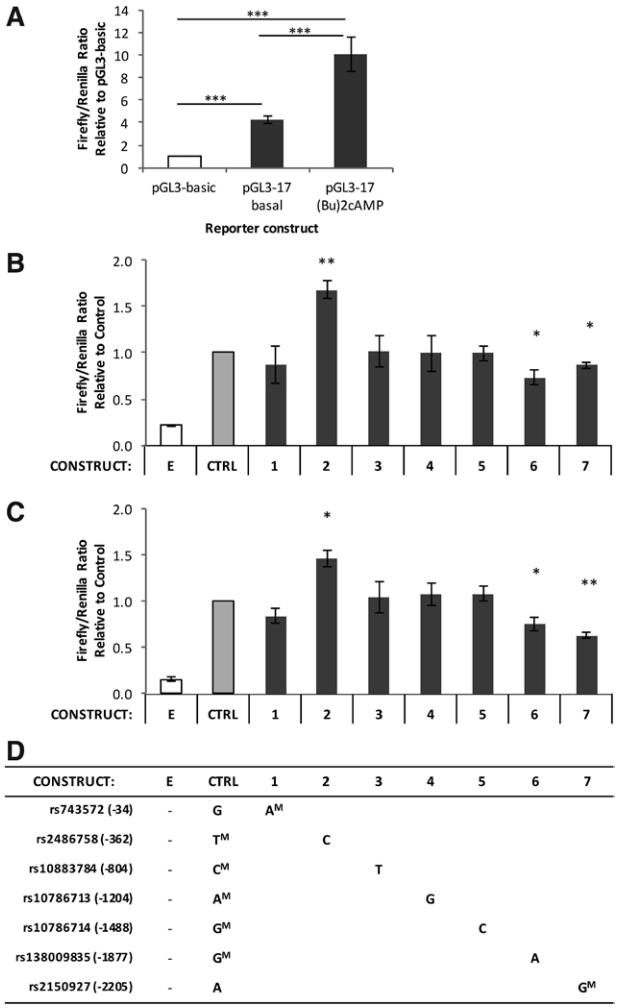

Figure 2. A, H295R cells were transfected with pGL3-basic or pGL3-17 control reporter constructs and grown for 24 hours post transfection under basal or 1 mmol/L dibutyryl cAMP–stimulated conditions. The transcriptional activity of each reporter construct is displayed as a proportion of pGL3-basic (empty vector) activity (normalized to 1). **B** and **C**, H295R cells were transfected with reporter constructs varying in sequence from the pGL3-17 control vector (CTRL) at a single base and then grown for 24 hours post transfection under either basal (**B**) or 1 mmol/L dibutyryl cAMP–stimulated (**C**) conditions. The transcriptional activity of each reporter construct and of a pGL3-basic empty construct (E) is displayed as a proportion of pGL3-control activity (normalized to 1). **D**, Table of reporter construct genotypes; major alleles are indicated by a superscript M. Vector alleles match the control unless otherwise indicated. All data are expressed as the mean (±SEM) of 4 independent experiments (n=4), each performed in quadruplicate.

(rs2486758) and −1877 (rs138009835) vectors, the differences between the alternative forms of the −2205 (rs2150927) vector were more pronounced under stimulation.

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[20] 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 tetrahydrodeoxycorticosterone to tetrahydrodeoxycortisol (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 17,20 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 urinary 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:

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.

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[21]; 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.[16,18] 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-deoxycorticosteroid ratios in subjects carrying the major alleles at selected SNPs imply that they will have higher adrenocorticotropic

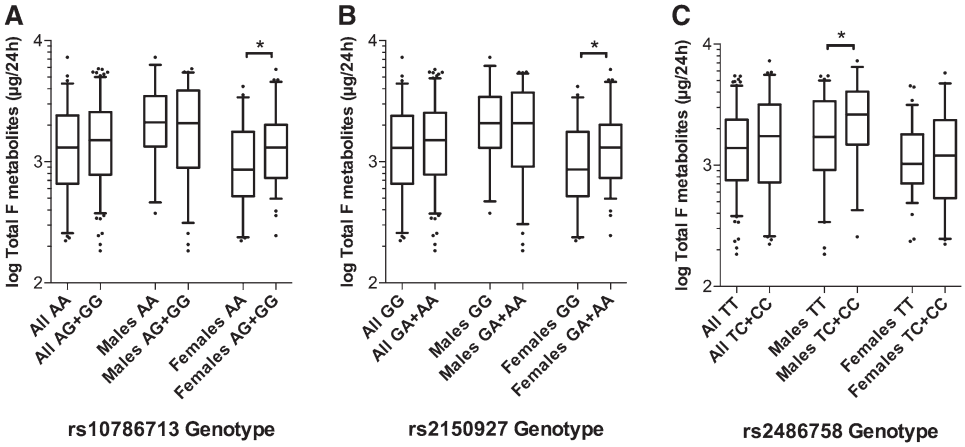

Figure 3. Box–whisker plots of 24-hour total cortisol metabolites (Total F) in the British Genetics of Hypertension (BRIGHT) study subgroup (n=232), stratified by rs10786713 genotype (B), rs2150927 genotype (**B**), and rs2486758 genotype (**C**). Total F is the sum of tetrahydrocortisol, allotetrahydrocortisol, 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.

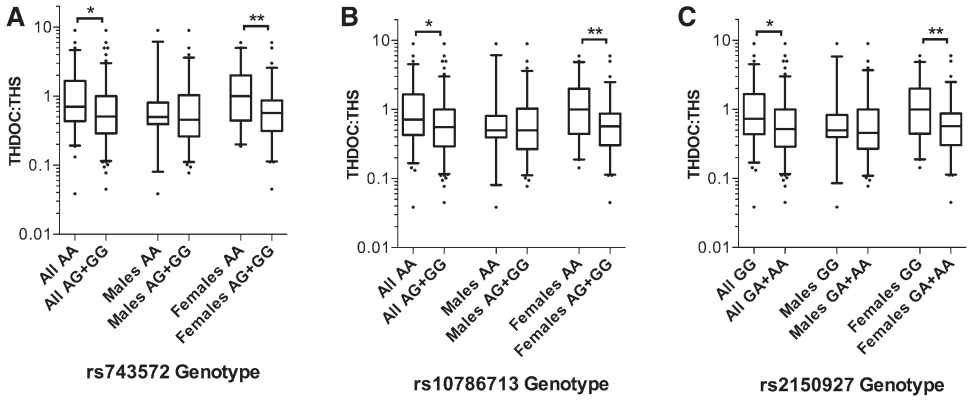

Figure 4. Box–whisker plots of 24-hour tetrahydrodeoxycorticosterone to tetrahydrodeoxycortisol (THDOC:THS) ratio in the British Genetics of Hypertension (BRIGHT) study subgroup (n=232), stratified by rs743572 genotype (**A**), rs10786713 genotype (**B**), and rs2150927 genotype (**C**). Plots show the 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.

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 al[23] 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 cortisol[24]; 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, Morris[27] 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

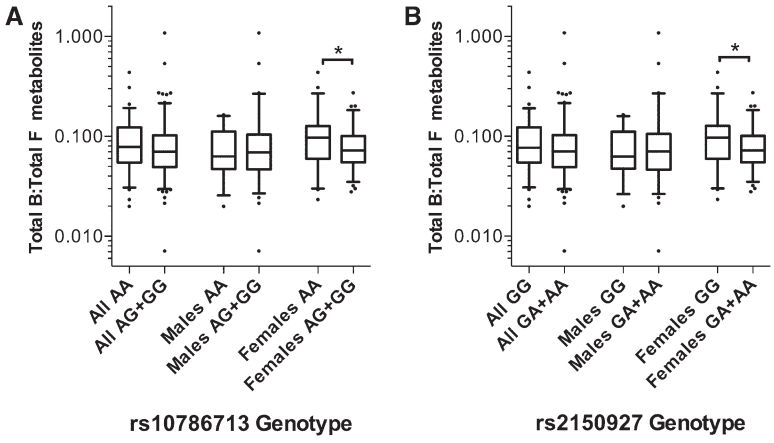

Figure 5. Box–whisker plots of 24-hour total corticosterone metabolite (Total B) to total cortisol metabolite (Total F) ratio in the British Genetics of Hypertension (BRIGHT) study subgroup (n=232), stratified by rs10786713 genotype (**A**) and rs2150927 genotype (**B**). Total B is the sum of tetrahydrocorticosterone, allotetrahydrocorticosterone, and tetrahydro-11-dehydrocorticosterone. Total F is the sum of tetrahydrocortisol, allotetrahydrocortisol, and tetrahydrocortisone. 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.

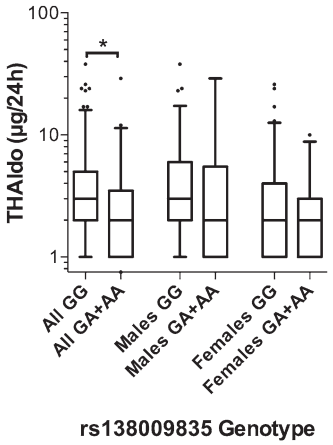

Figure 6. Box–whisker plot of 24-hour tetrahydroaldosterone (Thaldo) excretion rate in the British Genetics of Hypertension (BRIGHT) study subgroup (n=232), stratified by rs138009835 genotype. Plot shows the median within the interquartile range box, with whiskers extending to the 5th and 95th percentiles; 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.[20] 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.[28] 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. 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.
- This study also adds to the substantial evidence base highlighting the importance of adrenal steroid biosynthesis in the development of hypertension.

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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ONLINE SUPPLEMENT

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	Exon 1	10:104597057	C/T	0.008	1.0000	100
rs6162	Exon 1	10:104596981	G/A	0.315	0.1449	100
rs6163	Exon 1	10:104596924	C/A	0.306	0.0249	100
rs10786712	Intron 1	10:104596396	C/T	0.293	0.0232	93.5
rs45463800	Intron 1	10:104596356	C/T	0.017	1.0000	95.2
rs754283774	Intron 1	10:104596011	C/T	0.008	1.0000	95.2
rs3824755	Intron 1	10:104595849	G/C	0.052	1.0000	93.5
rs284847	Intron 1	10:104595828	G/A	0.068	0.4480	95.2
rs3781286	Intron 1	10:104595719	C/T	0.302	0.0396	93.5
rs3781287	Intron 1	10:104595420	T/G	0.322	0.1374	95.2
rs4919687	Intron 1	10:104595240	G/A	0.241	0.0247	93.5
rs743575	Intron 2	10:104594906	T/G	0.208	0.4270	96.8
rs1004467	Intron 3	10:104594507	A/G	0.083	1.0000	96.8
rs544961235	Intron 4	10:104593194	C/T	0.008	1.0000	95.2
rs3740397	Intron 5	10:104592675	G/C	0.292	0.1196	96.8
rs4919686	Intron 6	10:104592249	A/C	0.220	0.0448	95.2
rs284848	Intron 6	10:104592125	G/A	0.212	1.0000	95.2
rs45609333	Intron 6	10:104591639	G/A	0.025	1.0000	95.2
rs17115100	Intron 6	10:104591393	G/T	0.085	1.0000	95.2
rs284849	Intron 7	10:104591182	G/T	0.234	0.3853	100
rs10883783	Intron 7	10:104591152	T/A	0.218	0.2194	100
rs139275291	Intron 7	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	CCACTGCTGTCTATCTTGCC
		cyp17ex1_r1	TGAAGACCTGAACAATCCCA
	Sequencing Primers	cyp17ex1_f1	CCACTGCTGTCTATCTTGCC
		cyp17ex1_r1	TGAAGACCTGAACAATCCCA
		17Exon1F	CAGAGGGTGATCAACTGAC
		17Exon1R	CTAGGCATGGTCTGAAGAC
Exons 2 & 3 (+ intron 2)	**PCR Primers**	cyp17ex2+3_f1	GGTGTGAGATTCCTACAGCC
		cyp17ex2+3_r1	TCTACTAGAACCTGAAGGCAG
	Sequencing Primers	cyp17ex2+3_f1	GGTGTGAGATTCCTACAGCC
		cyp17ex2+3_r1	TCTACTAGAACCTGAAGGCAG
		cyp17ex2_r1	TCCTAACCCTTACCCCTG
		cyp17ex3_f1	TGGTACAGAGAGGGGGTAAG
Exon 4	**PCR Primers**	cyp17ex4_f1	GGTGGAGTAGGAACTTCCAG
		cyp17ex4_r2	TCCACCCTGCTCTTGTGATT
	Sequencing Primers	cyp17ex4_f1	GGTGGAGTAGGAACTTCCAG
		cyp17ex4_r1	TGTGCCAGGTTCTCTGCTTG
		cyp17ex4_f2	AGCTAAGATCCGCCTCCAG
		cyp17ex4_r2	TCCACCCTGCTCTTGTGATT
Exons 5 & 6 (+ intron 5)	**PCR Primers**	cyp17ex5+6_f1	TGGCAGGAGTGTCACAGATG
		cyp17ex6_r1	TGAATGCATCATGGGGCTAG
	Sequencing Primers	cyp17ex5+6_f1	TGGCAGGAGTGTCACAGATG
		cyp17ex5_f1	GGCAGGAGTGTCACAGATG
		cyp17ex5_r1	TGGGGTCTAGGATCAATGAG
		cyp17ex6_f1	ACACACTAGTCACCTCCAAC
		cyp17ex6_r1	TGAATGCATCATGGGGCTAG
Exons 7 & 8 (+3'UTR)	**PCR Primers**	cyp17ex7+8_f1	TTCCTCTTCCACTCTGGAGC
		cyp17ex8utr_r1	GAATGAGTGAGCAAATGAATAC
	Sequencing Primers	cyp17ex7+8_f1	TTCCTCTTCCACTCTGGAGC
		cyp17ex8utr_r1	GAATGAGTGAGCAAATGAATAC
		cyp17ex7_r1	TTGGCAGAGGTGAAGGGGTA
		cyp17ex7+8_r1	GCCACATAGGGTGGACAGG
		cyp17ex8_f1	TCAACCAGGGCAGAACCATG
		cyp17ex8_r1	TGTGTTGTGGGGCCACATAG

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	GTGCGCCAGAGTCAGCGAAG
	Sequencing Primers	intron1F	CACCAAGACTACAGTGATTG
		intron1R1	GTGCGCCAGAGTCAGCGAAG
		intron1s1f	CTTAGCCTAGCACCCAGCAC
		intron1s1r	AGCTGGAAATAGCACCAGGA
		intron1s2f	CCAATCTCAGCTCACTGCAA
		intron1s2r	CTAAGGTGGGAGGATCACGA
		intron1s4f	ACCCTGTCAGCGAAAAGAAC
		intron1s4r	TTGCCCTTACACCTCTGGTC
		intron1s3f	CTTCAGGGTCAGGAAATGGA
		intron1s3r	AGGCTGAGGACTGCACAGAT
Intron 3	**PCR Primers**	cyp17ex3_f1	TGGTACAGAGAGGGGGTAAG
		cyp17ex4_r1	TGTGCCAGGTTCTCTGCTTG
	Sequencing Primers	Intron3s1f	TGGTTGAAGGTGAGATGCTG
		Intron3s2f	AAGCATTCTATACGCATTCATCG
		Intron3s1r	AAGCTCCTTAACCCCGCTAA
		cyp17ex3_f1	TGGTACAGAGAGGGGGTAAG
		Intron3s2r	GACCTTCAGCCAGAATGGAA
Intron 4	**PCR Primers**	Intron4F1	AAGCAGAGAACCTGGCACAT
		Intron4R1	TCACTCCGGAATTTCTCCTG
	Sequencing Primers	Intron4F1	AAGCAGAGAACCTGGCACAT
		Intron4R1	TCACTCCGGAATTTCTCCTG
		Intron4s1r	CTTGCCTGGCCTAGTTTTTG
		Intron4s1f	CAGCTATTTGGGAGGCTGAG
Intron 6	**PCR Primers**	cyp17ex6_f1	ACACACTAGTCACCTCCAAC
		cyp17ex7+8_r1	GCCACATAGGGTGGACAGG
	Sequencing Primers	Intron6s1f	GCTGGCCAACCTAAAGTCAG
		Intron6s1r	ATCTTGGCTCACTGCAACCT
		Intron6s2r	GGAAGCTCCTCTGGGAAGTC
		intron6s3r	GGGACTTCGTACTCCCTTCC
Intron 7	**PCR Primers**	cyp17ex7+8_f1	TTCCTCTTCCACTCTGGAGC
		cyp17ex8utr_r1	GAATGAGTGAGCAAATGAATAC
	Sequencing Primers	cyp17ex8_r1	TGTGTTGTGGGGCCACATAG
		cyp17ex7+8_r1	GCCACATAGGGTGGACAGG
		intron7s1r	GCCCTTAACGACACAGAGGA
		Intron7s2r	CAGGCCATGATGAGGAAGAG

Table S4: Primers used for site-directed mutagenesis of pGL3-17 Control reporter construct.

SNP	Primer	Sequence 5'-3'	Base Change
rs2150927	Sense	ATGGGAAATGGTCAAAGGACACCTTCTGGGTAGGG	T → C
	Antisense	CCCTACCCAGAAGGTGTCCTTTGACCATTTCCCAT	
rs138009835	Sense	AAGGGAGAGATGTTGTGGGAAGTCAGGGACC	C → T
	Antisense	GGTCCCTGACTTCCCACAACATCTCTCCCTT	
rs10786714	Sense	GAGAGAGGCTATAAATGGAGATGCAAGTAGGGAAGATAT	C → G
	Antisense	ATATCTTCCCTACTTGCATCTCCATTTATAGCCTCTCTC	
rs10786713	Sense	TGTTTAAGGTGTTTATCAAGACAGTACGTGCACCGCTGAAC	T → C
	Antisense	GTTCAGCGGTGCACGTACTGTCTTGATAAACACCTTAAACA	
rs10883784	Sense	GTCTCTCTTTATTTCTCAGCCAGCTGACACTTATAGAAAGAAC	G → A
	Antisense	GTTCTTTCTATAAGTGTCAGCTGGCTGAGAAATAAAGAGAGAC	
rs2486758	Sense	CCAGTGATTTTGATTTTGCAGCATGGAAAGTTCCAAGCCTT	A → G
	Antisense	AAGGCTTGGAACTTTCCATGCTGCAAAATCAAAATCACTGG	
rs743572	Sense	CAGCTCTTCTACTCCACTGCTGTCTATCTTGCCTG	C → T
	Antisense	CAGGCAAGATAGACAGCAGTGGAGTAGAAGAGCTG	

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	ATGCAGTTCGATTGCAACAC
	cyp17promR2	TTGGGCCAAAACAAATAAGC
Sequencing Primers	cyp17promF2	ATGCAGTTCGATTGCAACAC
	cyp17promR2	TTGGGCCAAAACAAATAAGC
	cyp17-1935F	AGCTGAGGCGTTAGATCAGG
	cyp17-1525F	GTCACTTCAGGACCACTGTGA
	cyp17-1213F	CCGCTCTGGGAATGTCTATC
	cyp17-884F	TTGTCCTTTCCCTCAGAAGC
	cyp17-603F	GAAATATTGGGGGTGGGTTC
	cyp17-159F	CCCAGATACCATTCGCACTC
	cyp17-1697R	GACTTCCCGCAACATCTCTC
	cyp17-1388R	TGGTTGTTTTCCCTTGTTCC
	cyp17-1021R	CAGCGGTGCACATACTGTCT
	cyp17-720R	TACCCACCTGAGCCTCAAAC
	cyp17-374R	TAAGGGCTGTGGGTTAATGG
	cyp17-117R	CTCCCCATGCTTGAATGACT
	cyp17+67R	GGCTACCCTGATCTTCACCTT
	cyp17-112R	GTCAGTTGATCACCCTCTGA

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.

rs743572	**AA N=72**	**AG N=109**	**GG N=37**	**p-value (AA vs. AG+GG)**
Age (Years)	63 (58-67)	62 (55-70)	64 (58-68)	0.99
SBP (mm/Hg)	157 (153-187)	183 (153-190)	155 (152-191)	0.53
DBP (mm/Hg)	102 (98-107)	103 (99-110)	103 (98-110)	0.51
BMI (kg/m2)	27 (24-30)	27.5 (25-30)	27 (25-31)	0.47
WHR	0.86 (0.83-0.92)	0.88 (0.81-0.93)	0.91 (0.83-0.94)	0.49

rs2486758	**TT N=145**	**TC N=66**	**CC N=7**	**p-value TT vs. TC+CC**
Age (Years)	63 (56-67.25)	63 (56-70)	63 (60.5-65)	0.52
SBP (mm/Hg)	157 (153-190)	181.5 (152-190)	186 (169-189.5)	0.42
DBP (mm/Hg)	103 (99-108)	103 (98-110.75)	99 (98-103)	0.94
BMI (kg/m2)	27 (24-30)	27 (25-29)	30 (27-30)	0.81
WHR	0.89 (0.81-0.93)	0.89 (0.81-0.93)	0.79 (0.76-0.85)	0.44

rs10883784	**CC N=107**	**CT N=99**	**TT N=19**	**p-value CC vs. CT+TT**
Age (Years)	63.5 (58.25-68)	62 (54.5-70)	63 (60.5-67)	0.43
SBP (mm/Hg)	157 (153-190)	157 (153-190)	153 (152-186.5)	0.76
DBP (mm/Hg)	103 (98-108)	103 (98-110)	102 (98-110)	0.97
BMI (kg/m2)	27 (24-30)	27 (25-30)	28 (26-31)	0.38
WHR	0.86 (0.80-0.92)	0.88 (0.81-0.93)	0.92 (0.89-0.94)	0.30

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