A multilocus approach to the antihypertensive pharmacogenetics of hydrochlorothiazide

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Objectives **To assess the influence of variations in multiple candidate genes on inter-individual variation in diastolic blood pressure (DBP) response to hydrochlorothiazide.**

Methods **A community-based sample of 585 adults with essential hypertension underwent monotherapy with hydrochlorothiazide for 4 weeks. In a nested case–control design, 195 individuals in the highest tertile of DBP response (responders) and 195 individuals in the lowest tertile of DBP response (non-responders) were genotyped for 45 polymorphisms in 19 candidate genes. For those polymorphisms where the set association approach found to be significantly associated with DBP response, logistic regression was performed to estimate the odds ratio (OR) for response associated variation in the identified genotype/haplotype.**

Results **Two polymorphisms in the sodium channel γ-subunit promotor gene, and a polymorphism in the endothelial nitric oxide synthase gene, were significantly associated with blood pressure response to hydrochlorothiazide. In the final experiment for the set association model $P=0.038$. In the logistic regression analyses, compared to subjects with the CT/CT haplotype of the SCNN1G gene, those with the GA/GA haplotype had OR 5.21 of being a DBP responder [95% CI 1.65–16.47]. Compared to subjects with the GT genotype of the ENOSA_rs1799983 polymorphism, those with the GG genotype had an OR 2.19 of being a DBP responder [95% CI 1.27–3.77].**

Conclusions **Two polymorphisms in the sodium channel γ-subunit promotor gene, and a polymorphism in the endothelial nitric oxide synthase gene, were associated with significant differences in odds of DBP response to hydrochlorothiazide. Follow-up studies are needed to define the functional genetic variations and their mechanisms of pharmacogenetic effects. *Pharmacogenetics and Genomics* 15:287–293 © 2005 Lippincott Williams & Wilkins.**

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Introduction

Hypertension is a common disorder afflicting approximately 20% of the adult populations of most developed countries [1]. It is a major risk factor for cardiovascular cerebrovascular, and renal disease [2]. The recommended first-line drug treatment for hypertension is a thiazide diuretic. However, treatment with a thiazide diuretic is associated with large inter-individual differences in blood pressure reduction [3]. Interpreting variation in outcome of drug therapy should take into account a patient's general health, prognosis, disease severity, drug prescribing and dispensing, compliance with prescribed pharmacotherapy, and metabolic and genetic profiles [4,5].

Pharmacogenetics aims to identify and characterize genetic factors contributing to inter-individual differences in drug response [6]. Multiple counteractive blood pressure regulating systems with different genetic regulation contribute to blood pressure regulation and responses to antihypertensive drugs. Although many proteins and many genes may influence the blood pressure lowering effect of thiazide diuretics, most current approaches evaluate only one single nucleotide polymorphism (SNP) at a time, focusing on its marginal effect on blood pressure response. It would be more appropriate to search for sets of marker loci in different genes and analyze these markers jointly rather than testing each marker in isolation.

Several individual polymorphisms have already been studied for association with blood pressure response in patients treated with diuretics. We previously reported

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that the C825 T polymorphism in the gene encoding the β_3-subunit of the G-protein, the Glu298Asp polymorphism of nitric oxide synthase, and polymorphisms of the renin angiotensin-aldosterone system predicted interindividual blood pressure responses to hydrochlorothiazide [7–9]. Other studies have reported that the 460W allele of the G460W α-adducin polymorphism was associated with a greater blood pressure reduction in response to treatment with diuretics [10–12]. In each of these studies the contributions to predicting blood pressure responses were small, usually $< 4\%$.

In the present study, 45 polymorphisms were measured in 19 hypertension candidate genes. The selected genes play roles in the renin–angiotensin–aldosterone system (aldosterone synthase, angiotensin-II receptor type 1, angiotensinogen, angiotensin-I-converting enzyme, renin), in sodium transport systems (α-adducin, chloride channel Kb, potassium inwardly-rectifying channel, protein kinase lysine-deficient l, sodium channel γ-subunit promotor, sodium channel, non-voltage-gated 1, beta solute carrier family 12), and in other systems that modulate vasoconstriction and volume (sympathetic nervous system [adrenergic β-1- and β-2-receptors], G-protein β3 subunit, lipoprotein lipase, cytochrome p450 family 17, endothelial nitric oxide synthase, nuclear receptor subfamily 3 group C, member 2 ligand-activated). These genes were ones repeatedly associated with measures of blood pressure levels and those genes causing rare Mendelian forms of hypertension.

Therefore, the aim of the present study was to use modern statistical genetic approaches to consider the effect of a comprehensive list of candidate gene variations taken together on the response to a thiazide diuretic. The approach that was used controlled for multiple comparisons.

Methods

Subjects

African-Americans were recruited at Emory University in Atlanta, GA, USA, and non-Hispanic whites were recruited at the Mayo Clinic in Rochester, MN, USA. Essential hypertension was defined clinically by blood pressure levels greater than 140/90 mmHg in the absence of known causes of hypertension, or by a previous diagnosis of essential hypertension and current use of prescription antihypertensive drugs. Potential subjects were considered ineligible for study for the following reasons: diseases causing secondary hypertension; more than three antihypertensive medications for blood pressure control; allergy to hydrochlorothiazide; inability to discontinue antihypertensive medications; use of non-steroidal anti-inflammatory medications including daily aspirin > 325 mg per day; congestive heart failure, liver or renal disease (serum creatinine concentration > 1.5 mg/dl); diabetes mellitus (fasting blood glucose level > 140 mg/dl) or hypoglycemic medications. Women taking oral contraceptives were disqualified; however, those receiving post-menopausal hormone replacement were allowed. The institutional review boards at all participating institutions approved the study protocol and all subjects reviewed and signed a written consent form.

Details of the Genetic Epidemiology of Responses to Antihypertensives (GERA) study have been described previously [13]. Briefly, previous blood pressure lowering medication was discontinued for at least 4 weeks. This was followed by 4 weeks of therapy with hydrochlorothiazide, 25 mg orally once daily. Subjects were counseled to maintain a dietary sodium intake of 2 mmol/kg/day. Dietary compliance was monitored by measurement of 24-h urine sodium excretion and food recall diaries. Subjects were withdrawn from the study if blood pressure was $> 180/110$ mmHg during the washout period, or diastolic blood pressure was < 90 mmHg at the end of antihypertensive therapy. A physical examination was performed at the consent visit. Blood pressure was measured, and blood for biochemical measurements was obtained at the end of the washout period (baseline) and at the end of the 4-week diuretic treatment period. Blood pressure measurements were taken with subjects in the seated position after 5 min of rest by trained nurses using a mercury sphygmomanometer. Response to hydrochlorothiazide was defined as the difference between the blood pressure readings taken after 4 weeks of drug therapy and the blood pressure readings taken at the end of the washout period.

Within the GERA cohort, a nested case–control substudy was conducted. Cases were subjects from each race–gender subgroup with a diastolic blood pressure response in the highest tertile (responders), and controls were subjects from each race–gender subgroup with a diastolic blood pressure reponse in the lowest tertile (non-responders). Blood pressure responses were first adjusted for age and baseline diastolic blood pressure level. The mean change in diastolic blood pressure in the highest tertile was -15.8 ± 5.0 mm Hg and the mean change in diastolic blood pressure in the lowest tertile was 0.0 ± 5.0 mmHg. The African-American men ($n = 83$), African-American women ($n = 88$), white men ($n = 103$) and white women ($n = 78$) were each equally distributed between both the responders and non-responders.

Laboratory and genetic analyses

Genotyping was carried out by primer extension and mass spectrometry following polymerase chain reaction amplification of the target region. Genotypes were called directly from the spectra using semi-automated software (Sequenom, San Diego, CA, USA). Primers for the amplification and extension reactions are available upon request.

Statistical analysis

Baseline characteristics of cases and controls were compared using chi-square tests for categorical variables and student's *t*-tests for continuous variables. For each SNP, goodness-of-fit to Hardy–Weinberg expectations was assessed for each race separately. Any SNP with a Hardy–Weinberg chi-square greater than 6.6 (i.e., 99th percentile) in one of the races was excluded because of suspected genotyping errors. In addition, markers that were not polymorphic ($q < 0.01$) were also excluded from further analyses. We adjusted for multiple comparisons using the set association method proposed by Hoh *et al.* [14]. For each SNP marker, a 3×2 contingency chi-square statistic testing the association of genotype with response status was calculated. These chi-square statistics were then ranked from largest to smallest. Progressively larger sums (S_j) were then calculated over the j largest chi-square statistics. For example, S_1 is the largest chi-square statistic of association. S_2 is the sum of the largest and second largest. S_3 is the sum of the largest, second largest and third largest, etc. The empirical significance level (P_j) for each S_j was evaluated by permutation methods carried-out under the null hypothesis of no association of genotype with diuretic response. For this part of the analysis, 20 000 permutations were used for each S_j. According to the method of Hoh *et al.* [14], the smallest of the empirical significance levels (i.e. $P_{j\min}$) identifies the best and most parsimonious model predicting response status. As will be seen in the results section below, $P_{j\min}$ for the data presented here occurred at S_3, the sum of the three largest chi-square tests of association. The overall significance of S_3 adjusting for multiple testing was assessed by permutation testing. Software to execute this algorithm can be obtained from http://linkage.rockefeller.edu/ott/sumstat.pdf [14].

Haplotypes were formed if polymorphisms in the same gene were found statistically significant in the Hoh *et al.* analyses. The PHASE II program was used to infer haplotypes [15,16] (http://www.stat.washington.edu/stephens/software.html). Logistic regression was performed to calculate odds ratios of the polymorphisms/haplotypes predicting responder status after adjusting for possible confounders. First, the most common haplotype/polymorphism was used as the reference. For the tables presented here, subjects with the smallest blood pressure response to hydrochlorothiazide (non-responders) were used as the reference group. The regression model was adjusted for all covariates that were previously found to be predictors of diastolic blood pressure response in the parent cohort (race, gender, age, antihypertensive drug treatment duration, baseline diastolic blood pressure, seated plasma renin activity, 24-h urinary aldosterone excretion, difference [baseline-after treatment] in 24-h urinary excretion of sodium) [13].

Results

The GERA study cohort consisted of 291 African-Americans (150 women and 141 men) and 294 non-Hispanic whites (126 women and 168 men). In this nested case–control sample, 195 responders and 195 non-responders were included. Responders and non-responders were significantly different for baseline systolic blood pressure, body mass index, duration of antihypertensive medication use before the GERA study, 24-h urinary excretion of aldosterone, and for difference in 24-h urinary excretion of sodium (i.e., baseline-final) (see Table 1).

The polymorphisms AGT_rs699, AGT_rs5051, NR3C2_rs3846306, PRKWNKI_rs1159744, and NR3C2_rs2232920 were excluded from further analysis because the HWE goodness-of-fit chi square was > 6.6. Markers AGT_rs1805090, ACE_rs4314, NR3C2_rs5527, SLC12A3_hcv9609124, SCNN1G_hcv11894753 and SLC12A1_rs1552311 were excluded because the frequency of the rare allele was less than 0.01.

Table 2 shows the rare allele frequencies (q), and single marker tests of association for each polymorphism.

Table 1 Baseline characteristics of responders and non-responders to hydrochlorothiazide

Characteristics	Responders Bp lowering in highest tertile	Non-responders BP lowering in lowest tertile	*P*-value of student's *t*-test
N	195	195	
Age, years	47.7	48.1	0.58
Gender (m)	103 (52.8%)	103 (52.8%)	–
Race (white)	98 (50.3%)	98 (50.3%)	–
Baseline SBP (mmHg)	144.9	148.6	0.01
Baseline DBP (mmHg)	96.3	96.5	0.75
BMI	30.7	32.0	0.04
Waist–hip ratio	0.89	0.90	0.30
Duration of antihypertensive drug treatment (years)	4.48	6.81	0.0004
Seated plasma renin activity (ng/ml/h)	0.98	1.17	0.06
24-h urinary excretion of aldosterone (μg/24 h)	8.42	10.89	<0.0001
Difference (baseline-after treatment) in 24-h urinary excretion of sodium (mmol/24 h)	−5.60	13.98	0.012

Means are given for quantitative traits and percentages for categorical traits.

Table 2 Studied polymorphisms and chi square tests of association with DBP response

Gene name and function	Polymorphism ID*	*q* (rare allele frequency)	Contingency chi-square
α-Adducin increase renal tubular reabsorption of Na through activation of Na^+, K^+-ATPase [11]	ADD1_rs4963	0.17 (G)	1.06
	ADD1A_rs4961	0.13 (T)	0.09
Adrenergic, beta-2-, receptor modulation of cardiac inotropy and chronotropy [25]	ADRB2_hcv8950495	0.42 (A)	2.37
	ADRB2_rs2400707	0.43 (A)	2.12
	ADRB2_rs1042714	0.28 (G)	1.13
	ADRB2_rs1042713	0.47 (A)	0.07
Adrenergic, beta-1-, receptor	ADRB1_rs1801253	0.32 (G)	0.54
Aldosterone synthase stimulates the adrenal cortex to synthesize and secrete aldosterone which increases sodium reabsorption	CYP11B2_rs4544	0.19 (G)	3.51
	CYP11B2_rs1799998	0.34 (C)	2.38
Angiotensin II receptor, type 1 mediates vasoconstriction, sodium retention and aldosterone synthase	AT1RN_rs5186	0.22 (C)	0.36
Angiotensinogen precursor of angiotensin-I	AGT_rs699	0.37 (A)	1.45
	AGT_rs7079	0.21 (T)	1.27
	AGT_rs4762	0.08 (A)	0.58
	AGT_rs5051	0.32 (G)	0.33
	AGT_rs1805090	0.002 (T)	2.07
Angiotensin-I converting enzyme generates the active peptide angiotensin II	ACE_rs4339	0.05 (T)	1.00
	ACE_rs4298	0.19 (T)	3.09
	ACE_rs4314	0.001 (T)	0.99
	ACE_rs4364	0.06 (A)	0.16
Chloride channel Kb transepithelial transport of chloride along the loop of Henle and distal tubule [26]	CLCNKB_hcv11647074	0.02 (T)	1.60
	CLCNKB_rs2015352	0.34 (C)	1.55
Cytochrome P450, family 17 catalyzes 17-hydroxylase and 17,20-lyase reactions. This steroid is a key enzyme required for the production of cortisol [27]	CYP17_rs284849	0.10 (T)	1.98
Endothelial nitric oxide synthase amount of NO in blood [8]	ENOSA_rs1799983	0.20 (T)	7.75
G protein, β3 mediates signal transduction across cell membranes [28]	GNB3_rs6489738	0.47 (C)	2.89
Lipoprotein lipase important regulator of lipid and lipoprotein metabolism [29]	LPL_rs328	0.08 (G)	3.13
Nuclear receptor subfamily 3, group C, member 2 ligand-activated mineral corticoid receptor	NR3C2_rs2232920	0.17 (G)	2.13
	NR3C2_rs5527	0.004 (C)	0.36
	NR3C2_rs3846306	0.17 (G)	0.06
Potassium inwardly-rectifying channel transepithelial transport of K^+	KCNJ1_hcv632615	0.01 (G)	0.21
Protein kinase lysine-deficient 1 negatively regulates surface expression of the Na-Cl cotransporter [30]	PRKWNK4_rs2277052	0.11 (A)	2.21
	PRKWNK1_rs2286007	0.05 (T)	1.03
	PRKWNK1_rs2107614	0.40 (T)	0.48
	PRKWNK1_rs1159744	0.25 (C)	0.18
	PRKWNK1_rs2277869	0.18 (C)	0.13
Renin generates angiotensin-I from angiotensinogen	REN_rs5706	0.02 (G)	2.36
	REN_rs5705	0.21 (G)	1.98
	REN_rs5707	0.25 (C)	0.76
	REN_hcv116.22760	0.18 (A)	0.76
Sodium channel, γ-subunit promotor transepithelial Na^+ transport [31]	SCNN1G_rs5729	0.23 (A)	12.41
	SCNN1G_rs5723	0.23 (G)	11.06
	SCNN1G_rs7200183	0.45 (C)	0.65
	SCNN1G_hcv11894753	0.004 (A)	0.34
Sodium channel, nonvoltage-gated 1, beta transepithelial Na^+ transport [31]	SCNN1B_rs250563	0.15 (T)	1.29
Solute carrier family 12 Na^+/Cl^- transporters	SLC12A3_hcv9609124	0.001 (A)	1.03
	SLC12A1_rs1552311	0.004 (T)	0.32

rs: NCBI SNP database (http://www.ncbi.nlm.nih.gov/locuslink); ihcv: Celera discovery system SNP database (http://www.celeradiscoverysystem.com).

In the multilocus analyses based on the method of Hoh *et al.*, set association models including two polymorphisms in the sodium channel γ-subunit promotor gene (SCNN1G_rs5729 ($P = 0.047$) and SCNN1G_rs5723 ($P = 0.018$)), a polymorphism in the endothelial nitric oxide synthase gene (ENOSA_rs1799983 ($P = 0.017$)), and a polymorphism in the aldosterone synthase gene (CYP11B2_rs1042714 ($P = 0.035$)) were significantly associated with blood pressure response to a diuretic (see Table 3). The minimum value of P ($P = 0.017$) was reached in a model that included the first three SNPs (SCNN1G_rs5729, SCNN1G_rs5723 and ENOSA_rs1799983). When more SNPs were added, the *P*-value increased again. The final experiment wise *P*-value for the model was 0.038. Therefore, we rejected the null hypothesis that none of the SNPs was associated with a difference in diastolic blood pressure response to hydrochlorothiazide.

Haplotypes were constructed for the two polymorphisms in the sodium channel γ-subunit promotor gene. The most common haplotype was CT (76.4%), followed by GA (23.3%) and the rare CA (0.14%) or GT (0.14%) haplotype. The observed frequencies of the diploid combinations of haplotypes were 58.6% for CT/CT, 35.5 % for CT/GA and 5.4% for GA/GA. All other combinations

were very rare and were excluded from further analyses. In the logistic regression analyses, shown in Table 4, subjects with the CT/CT haplotype for the sodium channel γ-subunit promotor gene had the lowest probability of being responders to hydrochlorothiazide and were set as the reference group. Subjects with the CT/GA haplotype had a greater probability of being responders (OR 1.50 [95% CI 0.90–2.49], $P = 0.12$) although not statistically significant. Subjects with the GA/GA haplotype had the greatest probability of being responders to hydrochlorothiazide (OR 5.21 [95% CI 1.65–16.47], $P = 0.005$).

Subjects with the GT genotype for the endothelial nitric oxide polymorphism had the lowest probability of being responder to hydrochlorothiazide. Subjects with the TT genotype had a greater probability of being responders (OR 2.12 [95% CI 0.65–6.96], $P = 0.22$) although not statistically significant. Compared to subjects with the GT genotype, subjects with the GG genotype had a significantly greater probability of being responders (OR 2.19 [95% CI 1.27–3.77], $P = 0.005$).

Even though the CYP11B2_rs1042714 polymorphism was not in the most parsimonious model, it was a statistically significant predictor of response category and, thus, we continued to analyze the variant using logistic regression. Subjects with the CC genotype for the aldosterone synthase polymorphism had the lowest probability of being responders. Compared with the CC genotype, subjects with the CT genotype had a higher probability of being responders (OR 5.46 [95% CI 1.26–23.58], $P = 0.02$), and subjects with the TT genotype had the highest probability of being responders (OR 9.06 [95% CI 2.00–41.09], $P = 0.004$).

Table 3 Results of set association approach (corrected for multiple comparisons)

Polymorphism ID	Chi square statistic	Sum	*P*-value
SCNN1G_rs5729	12.16	12.16	0.047
SCNN1G_rs5723	10.79	22.95	0.018
ENOSA_rs1799983*	7.65	30.61	0.017
CYP11B2_rs4544	3.35	33.96	0.035
ACE_rs4298	3.04	37.00	0.058
GNB3_rs6489738	2.87	39.86	0.080
LPL_rs328	2.45	42.31	0.105
CYP11B2_rs1799998	2.36	44.67	0.124
ADRB2_hcv8950495	2.34	47.02	0.141
PRKWNK4_rs2286007	2.17	49.19	0.156
ADRB2_rs2400707	2.09	51.29	0.165
REN_rs5705	1.91	53.20	0.174
REN_rs5706	1.76	54.96	0.183
CYP17_rs284849	1.76	56.71	0.190
CLCNKB_rs2015352	1.54	58.25	0.197

*Final experiment wise *P*-value for the model with the lowest *P*-value = 0.038.

Discussion

The data presented here provide additional evidence that genetic variation influences blood pressure response to hydrochlorothiazide. We conclude that three polymorphisms in two genes (the sodium channel γ subunit promotor gene and the endothelial nitric oxide synthase) are associated with differences in diastolic blood pressure response to hydrochlorothiazide. As the number of polymorphisms in the set association model of Hoh *et al.*'s prediction model increased, the *P*-values decreased to 0.017 when SCNN1G_rs5729, SCNN1G_rs5723, and ENOSA_rs1799983 were included in the model. The CYP11B2_rs1042714 polymorphism was also statistically significant, but not in the most parsimonious model. When more polymorphisms were added to the model, *P*-values increased.

Polymorphisms in the sodium channel γ subunit promotor have not been previously associated with blood pressure response to thiazide diuretics, but variation in this gene has been associated with predisposition to essential hypertension [17]. Mutations in the epithelial sodium channel γ subunit gene may result in inappropriate sodium reclamation in the distal nephron and can cause a salt-sensitive form of human hypertension (Liddle's syndrome) [18]. In a large Japanese cohort, variation in the sodium channel γ subunit promotor had significant effects on blood pressure levels [17]. In our study, subjects with the GA/GA haplotype had the greatest probability of being responders to the diuretic. Between the CT/CT genotype and CT/ GA haplotypes there was no statistically significant difference in probability of response. The SCNN1G_rs5729 polymorphism is

Table 4 Results from logistic regression analyses predicting DBP response

Genotype		*N* responders	*N* non-responders	Odds ratio	Odds ratio*	*P*-value
Sodium channel α-subunit promotor	CT/CT	92 (51.7%)	114 (67.1%)	1	1	
	CT/GA	71 (39.9%)	51 (30.0%)	1.70 (1.07–2.71)	1.50 (0.90–2.49)	0.12
	GA/GA	15 (8.4%)	5 (2.9%)	3.73 (1.28–10.84)	5.21 (1.65–16.47)	0.005
Endothelial nitric oxide synthase	GT	42 (23.6%)	62 (36.5%)	1	1	
	TT	10 (5.6%)	6 (3.5%)	2.28 (0.75–6.89)	2.12 (0.65–6.96)	0.22
	GG	126 (70.8%)	102 (60.0%)	1.87 (1.15–3.05)	2.19 (1.27–3.77)	0.005
Aldosterone synthase	CC	5 (2.8%)	12 (7.1%)	1	1	
	CT	48 (27.0%)	46 (27.1%)	2.89 (0.92–9.09)	5.46 (1.26–23.58)	0.02
	TT	125 (70.2%)	112 (65.9%)	3.45 (1.14–10.43)	9.06 (2.00–41.09)	0.004

*These analyses were adjusted for race, gender, age, antihypertensive drug treatment duration, baseline diastolic blood pressure, seated plasma renin activity, 24-hour urinary aldosterone excretion, and difference in 24-hour urinary excretion of sodium (baseline-after treatment).

located in an untranslated region (UTR3) and the SCNN1G_rs5723 is a synonymous substitution (silent mutation) in the exon. It is our working hypothesis that the association observed for the sodium channel promotor haplotype is due to linkage disequilibrium between the measured haplotype and an unknown functional mutation. The endothelial nitric oxide synthase ENOSA_rs1799983 polymorphism is a missense variation at codon 298 leading to a Glu to Asp substitution. The endothelial nitric oxide synthase Glu298Asp polymorphism was previously shown to be significantly associated with diastolic blood pressure response in the full GERA cohort [8]. This polymorphism was a logical candidate because the T allele (Asp298 allele) has been associated with decreased production of nitric oxide due to decreased nitric oxide synthase activity [8]. Therefore, a lower probability of being responders was expected in subjects with the T allele compared to the G allele [8]. In this study, we observed this effect in the GT group, but not in the TT group, most likely because of the small number of TT subjects ($n = 15$) in this case–control sub sample from the GERA cohort.

The Hoh *et al.* model was still statistically significant when the aldosterone synthase CYP11B2_rs4544 was added to the model. Mutations in the aldosterone synthase gene may be associated with increased aldosterone activity in the distal nephron resulting in enhanced sodium reabsorption and increased blood pressure levels (glucocorticoid remedial aldosteroneism) [19,20]. This gene has not been previously associated with blood pressure response to hydrochlorothiazide, but it has been associated with predisposition to essential hypertension [21]. CYP11B2_rs4544 is a missense variation at codon 399 of the aldosterone synthase gene leading to a Thr (ACC) to Ile (ATC) substitution. Subjects with the TT genotype for the CYP11B2_rs4544 polymorphism had a 9.06 times higher probability of being responders to hydrochlorothiazide compared to the subjects with the CC genotype (only 4.6% of the population). In the CT subjects, this probability was 5.46 times greater than for those with the CC genotype.

Findings in our study are only in partial agreement with findings from previous studies. For example, we did not find a difference in probability of blood pressure response for the α-adducin gene, either in this multi-locus analysis or in an earlier single locus analysis [8]. This might be due to the fact that our sample was more ethnically diverse and included African-Americans and non-Hispanic whites [8], while most previous studies used a sample of only white Europeans [10–12]. In addition, we did not find a significant relationship between the GNB3_rs6489738 (= C825 T polymorphism) in the gene encoding the β3-subunit of the G-protein, and the decline in diastolic blood pressure, as we showed in an earlier publication [7]. This may be due to the differences in the subsample or the study design. In this study we applied a case–control design for investigating drug response, as opposed to linear regression analyses considering the full distribution of drug response.

A strength of our study is that we investigated a large number of polymorphisms in a group of candidate genes. We studied all measured polymorphisms in the same model and took multiple comparisons into account. There might be several reasons for the observed differences from previous studies. First, the results of any association study depend on the outcome, and not all studies used the probability of diastolic blood pressure response as their primary outcome. Other outcomes used in antihypertensive pharmacogenetic studies included change in left ventricular mass [22], systolic blood pressure [23], mean arterial pressure [12], and risk of myocardial infarction and stroke [24]. Second, the results may depend on the populations sampled. Our sample included both African-Americans and non-Hispanic whites from the United States. Because analyzing these groups separately would lead to small sample sizes and loss of power, we analyzed them together, and we used balanced stratification to make sure both races were present in the same proportions among responders and non-responders. Different results may also be found because of differences in allele frequencies in different populations, study design, and methodologies.

In conclusion, this study provides further evidence of a role for genetic factors influencing inter-individual variation in response to hydrochlorothiazide. Accumulating evidence indicates that there is a role for genes in influencing response to other antihypertensive agents. In this study we have moved beyond single gene–single site association studies and considered a large number of polymorphisms in *a priori* biologic candidate genes. This study portends future genome-wide association studies for identifying novel genes (i.e., not *a priori* candidates) influencing drug response.

References

1 Brown MJ, Haydock S. Pathoaetiology, epidemiology and diagnosis of hypertension. *Drugs* 2000; **59(suppl 2)**:1–12; discussion 39–40.
2 Collins R, MacMahon S. Blood pressure, antihypertensive drug treatment and the risks of stroke and of coronary heart disease. *Br Med Bull* 1994; **50**:272–298.
3 Materson BJ, Reda DJ, Cushman WC, Massie BM, Freis ED, Kochar MS, *et al.* Single-drug therapy for hypertension in men. A comparison of six antihypertensive agents with placebo. The Department of Veterans Affairs Cooperative Study Group on Antihypertensive Agents. *N Engl J Med* 1993; **328**:914–21.
4 Sander C. Genomic medicine and the future of health care. *Science* 2000; **287**:1977–1978.
5 Vesell ES. Therapeutic lessons from pharmacogenetics. *Ann Intern Med* 1997; **126**:653–655.
6 Nebert DW. Pharmacogenetics and pharmacogenomics: why is this relevant to the clinical geneticist? *Clin Genet* 1999; **56**:247–258.

7 Turner ST, Schwartz GL, Chapman AB, Boerwinkle E. C825 T polymorphism of the G protein beta(3)-subunit and antihypertensive response to a thiazide diuretic. *Hypertension* 2001; **37**:739–743.

8 Turner ST, Schwartz GL, Chapman AB, Boerwinkle E. Effects of endothelial nitric oxide synthase, alpha-adducin, and other candidate gene polymorphisms on blood pressure response to hydrochlorothiazide. *Am J Hypertens* 2003; **16**:834–839.

9 Schwartz GL, Turner ST, Chapman AB, Boerwinkle E. Interacting effects of gender and genotype on blood pressure response to hydrochlorothiazide. *Kidney Int* 2002; **62**:1718–1723.

10 Glorioso N, Manunta P, Filigheddu F, Troffa C, Stella P, Barlassina C, *et al.* The role of alpha-adducin polymorphism in blood pressure and sodium handling regulation may not be excluded by a negative association study. *Hypertension* 1999; **34**:649–654.

11 Cusi D, Barlassina C, Azzani T, Casari G, Citterio L, Devoto M, *et al.* Polymorphisms of alpha-adducin and salt sensitivity in patients with essential hypertension. *Lancet* 1997; **349**:1353–1357.

12 Sciarrone MT, Stella P, Barlassina C, Manunta P, Lanzani C, Bianchi G, *et al.* ACE and alpha-adducin polymorphism as markers of individual response to diuretic therapy. *Hypertension* 2003; **41**:398–403.

13 Chapman AB, Schwartz GL, Boerwinkle E, Turner ST. Predictors of antihypertensive response to a standard dose of hydrochlorothiazide for essential hypertension. *Kidney Int* 2002; **61**:1047–1055.

14 Hoh J, Wille A, Ott J. Trimming, weighting, and grouping SNPs in human case-control association studies. *Genome Res* 2001; **11**: 2115–2119.

15 Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 2001; **68**:978–989.

16 Stephens M, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. *Am J Hum Genet* 2003; **73**:1162–1169.

17 Iwai N, Baba S, Mannami T, Katsuya T, Higaki J, Ogihara T, *et al.* Association of sodium channel gamma-subunit promoter variant with blood pressure. *Hypertension* 2001; **38**:86–89.

18 Tsukada K, Ishimitsu T, Teranishi M, Saitoh M, Yoshii M, Inada H, *et al.* Positive association of CYP11B2 gene polymorphism with genetic predisposition to essential hypertension. *J Hum Hypertens* 2002; **16**: 789–793.

19 Lifton RP, Dluhy RG, Powers M, Rich GM, Cook S, Ulick S, *et al.* A chimaeric 11 beta-hydroxylase/aldosterone synthase gene causes glucocorticoid-remediable aldosteronism and human hypertension. *Nature* 1992; **355**:262–265.

20 Lifton RP, Dluhy RG, Powers M, Rich GM, Gutkin M, Fallo F, *et al.* Hereditary hypertension caused by chimaeric gene duplications and ectopic expression of aldosterone synthase. *Nat Genet* 1992; **2**:66–74.

21 Hansson JH, Nelson-Williams C, Suzuki H, Schild L, Shimkets R, Lu Y *et al.* Hypertension caused by a truncated epithelial sodium channel gamma subunit: genetic heterogeneity of Liddle syndrome. *Nat Genet* 1995; **11**: 76–82.

22 Kurland L, Melhus H, Karlsson J, Kahan T, Malmqvist K, Ohman P, *et al.* Polymorphisms in the angiotensinogen and angiotensin II type 1 receptor gene are related to change in left ventricular mass during antihypertensive treatment: results from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation versus Atenolol (SILVHIA) trial. *J Hypertens* 2002; **20**: 657–663.

23 Kurland L, Liljedahl U, Karlsson J, Kahan T, Malmqvist K, Melhus H, *et al.* Angiotensinogen gene polymorphisms: relationship to blood pressure response to antihypertensive treatment. Results from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation vs Atenolol (SILVHIA) trial. *Am J Hypertens* 2004; **17**:8–13.

24 Psaty BM, Smith NL, Heckbert SR, Vos HL, Lemaitre RN, Reiner AP *et al.* Diuretic therapy, the alpha-adducin gene variant, and the risk of myocardial infarction or stroke in persons with treated hypertension. *JAMA* 2002; **287**:1680–1689.

25 del Monte F, Kaumann AJ, Poole-Wilson PA, Wynne DG, Pepper J, Harding SE. Coexistence of functioning beta 1- and beta 2-adrenoceptors in single myocytes from human ventricle. *Circulation* 1993; **88**:854–863.

26 Matsumura Y, Uchida S, Kondo Y, Miyazaki H, Ko SB, Hayama A, *et al.* Overt nephrogenic diabetes insipidus in mice lacking the CLC-K1 chloride channel. *Nat Genet* 1999; **21**:95–98.

27 Di Cerbo A, Biason-Lauber A, Savino M, Piemontese MR, Di Giorgio A, Perona M, *et al.* Combined 17α-hydroxylase/17,0-lyase deficiency caused by Phe93Cys mutation in the CYP17 gene. *J Clin Endocrinol Metab* 2002; **87**:898–905.

28 Neer EJ. Heterotrimeric G proteins: organizers of transmembrane signals. *Cell* 1995; **80**:249–257.

29 Eckel RH. Lipoprotein lipase. A multifunctional enzyme relevant to common metabolic diseases. *N Engl J Med* 1989; **320**:1060–1068.

30 Wilson FH, Kahle KT, Sabath E, Lalioti MD, Rapson AK, Hoover RS, *et al.* Molecular pathogenesis of inherited hypertension with hyperkalemia: the Na-Cl cotransporter is inhibited by wild-type but not mutant WNK4. *Proc Natl Acad Sci USA* 2003; **100**:680–684.

31 Horisberger JD, Chraibi A. Epithelial sodium channel: a ligand-gated channel? *Nephron Physiol* 2004; **96**:37–41.