

Association of chromosome 12 locus with antihypertensive response to hydrochlorothiazide may involve differential *YEATS4* expression

JD Duarte^{1,2}, ST Turner³,
B Tran¹, AB Chapman⁴,
KR Bailey⁵, Y Gong¹, JG Gums^{1,6},
TY Langaee¹, AL Beitelshees⁷,
RM Cooper-Dehoff^{1,8},
E Boerwinkle⁹ and JA Johnson^{1,8}

¹Department of Pharmacotherapy and Translational Research, Center for Pharmacogenomics, University of Florida, Gainesville, FL, USA; ²Department of Pharmacy Practice, University of Illinois at Chicago, Chicago, IL, USA; ³Division of Nephrology and Hypertension, Mayo Clinic, Rochester, MN, USA; ⁴Renal Division, Department of Medicine, Emory University, Atlanta, GA, USA; ⁵Division of Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN, USA; ⁶Department of Community Health and Family Medicine, University of Florida, Gainesville, FL, USA; ⁷Division of Endocrinology, Diabetes and Nutrition, Department of Medicine, University of Maryland, Baltimore, MD, USA; ⁸Division of Cardiovascular Medicine, University of Florida, Gainesville, FL, USA and ⁹Human Genetics Center and Institute of Molecular Medicine, University of Texas Health Science Center, Houston, TX, USA

Correspondence:

Dr JA Johnson, Department of Pharmacotherapy and Translational Research, Center for Pharmacogenomics, Personalized Medicine Program (UF and Shands), University of Florida, Box 100486, Gainesville, FL 32610-0486, USA.
E-mail: johnson@cop.ufl.edu

Received 3 November 2011; revised 9 January 2012; accepted 10 January 2012;
published online 21 February 2012

A recent genome-wide analysis discovered an association between a haplotype (from rs317689/rs315135/rs7297610) on Chromosome 12q15 and blood pressure response to hydrochlorothiazide (HCTZ) in African-Americans. Our aim was to replicate this association and investigate possible functional mechanisms. We observed similar associations between this haplotype and HCTZ response in an independent sample of 746 Caucasians and African-Americans randomized to HCTZ or atenolol treatment. The haplotype association was driven by variation at rs7297610, where C/C genotypes were associated with greater mean (systolic: 3.4 mmHg, $P=0.0275$; diastolic: 2.5 mmHg, $P=0.0196$) responses to HCTZ vs T-allele carriers. Such an association was absent in atenolol-treated participants, supporting this as HCTZ specific. Expression analyses in HCTZ-treated African-Americans showed differential pre-treatment leukocyte *YEATS4* expression between rs7297610 genotype groups ($P=0.024$), and reduced post-treatment expression in C/C genotypes ($P=0.009$), but not in T-carriers. Our data confirm previous genome-wide findings at 12q15 and suggest differential *YEATS4* expression could underpin rs7297610-associated HCTZ response variability, which may have future implications for guiding thiazide treatment.

The Pharmacogenomics Journal (2013) 13, 257–263; doi:10.1038/tpj.2012.4; published online 21 February 2012

Keywords: blood pressure; diuretics; hydrochlorothiazide; hypertension; *YEATS4*

Introduction

Hydrochlorothiazide (HCTZ) is the most commonly prescribed antihypertensive in the United States, with nearly 118 million prescriptions dispensed in 2010, either alone or combined with another antihypertensive.^{1,2} Current hypertension treatment guidelines also recommend thiazide diuretics as first-line treatment for most patients with uncomplicated essential hypertension, and strongly recommend these agents for all patients requiring two or greater antihypertensives for blood pressure control.³ Although thiazide and thiazide-like diuretics have been used in hypertension treatment for over 50 years, the mechanism by which they chronically lower blood pressure is incompletely understood.^{4,5} In addition, patient response to thiazides varies widely, with differential responses between races.^{6,7} However, even within race, variability of response exists.⁸ Due to the limited mechanistic understanding and wide

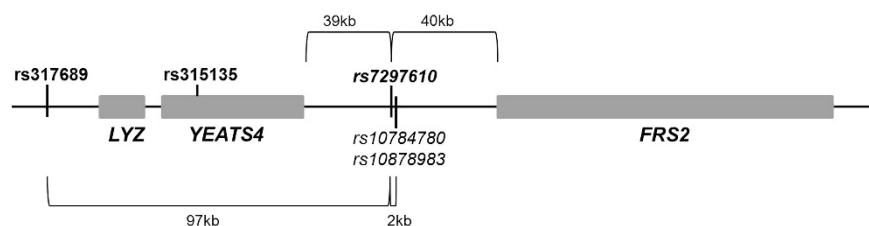


Figure 1 Locus on Chromosome 12 containing SNPs with evidence of association with HCTZ blood pressure response in African-Americans. SNPs in bold composed a haplotype that associated in Turner, *et al.*⁹ SNPs in italics associated with response in PEAR. Total region represented is ~260 kb.

variability of response, clinicians have difficulty predicting which patients will achieve satisfactory blood pressure response following thiazide treatment.

Recently, a genome-wide association study (GWAS) by Turner *et al.*⁹ showed an association between a locus at Chromosome 12q15 and blood pressure response to HCTZ in hypertensive African-Americans. A haplotype made from three single-nucleotide polymorphisms (SNPs), rs317689/rs315135/rs7297610 (Figure 1), was the most strongly associated, with the ATC haplotype correlating with greater HCTZ response, and the ACT and ATT haplotypes correlating with a smaller blood pressure response.⁹ When single SNP analyses were done, the strongest single SNP association was with rs7297610.⁹

As this GWAS was the first to implicate 12q15 in thiazide response, and a plausible mechanistic association with blood pressure response was not readily apparent, replication is needed to confirm these associations before exploring their mechanistic underpinnings. Thus, our aim was to replicate these associations in a separate, but similar clinical population and explore the functional role of this variation.

Methods

Study population

The Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) was a multi-center clinical trial examining the role of genetic variability on blood pressure response to HCTZ and/or atenolol (clinicaltrials.gov no. NCT00246519).¹⁰ The study has been approved by the institutional review boards at each study site, and all subjects provided voluntary, written informed consent before screening. Hypertensive participants (clinic DBP from 90 mmHg to 110 mmHg) were enrolled from the University of Florida (Gainesville, FL, USA), Mayo Clinic (Rochester, MN, USA) and Emory University (Atlanta, GA, USA). Males and females of any race between the ages of 17 and 65 with essential hypertension were recruited to participate and randomized to either HCTZ or atenolol. After a 4-week antihypertensive washout, participants were initially given HCTZ 12.5 mg daily or atenolol 50 mg daily, with most receiving a dose escalation to 25 mg and 100 mg, respectively, for blood pressure greater than 120/70 mmHg after 4 weeks. After 9 weeks, blood pressure response was assessed and, if blood pressure remained above 120/70 mmHg, the other study drug was added for another 9 weeks. To assure a

4-week washout sufficiently erased any blood pressure effects of previous antihypertensive treatments, we confirmed that post-washout blood pressure levels in participants who had previously received antihypertensives, were nearly identical to those who were had never received antihypertensive medication. Participants were not given sodium restrictions, but were counseled to maintain consistent dietary intakes. The primary response phenotype of PEAR was home blood pressure, which participants were required to take seated, in triplicate upon rising and before retiring with a provided blood pressure monitoring device (BP 3AC1-AP Monitor, Microlife USA, Dunedin, FL, USA) at least 5 of 7 days before their blood pressure assessment visit. Blood was drawn before monotherapy, after monotherapy and after treatment with both study drugs. The 746 Caucasian and African-American participants who completed at least the monotherapy stage of the study were included in this analysis.

Genotyping

Genomic DNA from the PEAR study was isolated from blood using the FlexiGene DNA Kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. Three SNPs (rs317689, rs315135 and rs7297610) composing the most strongly associated haplotype in the original GWAS⁹ were genotyped. Genotypes were determined by pyrosequencing using the PSQ HS 96 genotyping platform (Biotage AB, Uppsala, Sweden). Approximately 40 ng of genomic DNA was used for PCR under the following conditions: a strand separation at 95 °C for 15 min, then 40 cycles of denaturation at 95 °C for 30 s, annealing at 59 °C for 30 s and extension at 72 °C for 1 min, then one final extension at 72 °C for 7 min. Genotypes were automatically called by PSQ HS 96 SNP software (Biotage AB). Approximately 5% of participants were genotyped in duplicate to confirm call reliability, with an agreement of 98%. Genotypes for 48 539 other SNPs across Chromosome 12 were later determined in PEAR by HumanOmni1-Quad BeadChip (Illumina, San Diego, CA, USA). Also to confirm call reliability, 0.7% of samples were run in on the BeadChip in duplicate, with a genome-wide agreement of greater than 99.9%.

Bioinformatics and linkage analysis

The UCSC Genome Browser (<http://genome.ucsc.edu/>) was used to visualize the chromosomal region around rs7297610 and view species conservation, as well as look for possible transcription factor-binding sites that rs7297610

could disrupt. Further *in silico* functional predictions for rs7297610 were calculated using the NIH SNPinfo Web Server (<http://snpinf.niehs.nih.gov/>) and PupaSuite 2 (<http://pupasuite.bioinfo.cipf.es/>). Mechanisms explored involved effects on transcription factor binding sites, miRNA sequences, miRNA binding sites, conserved regions and regulators of splicing. To measure linkage disequilibrium with rs7297610 in PEAR African-Americans, both r^2 and D' were calculated in 48 539 SNPs across Chromosome 12 using PLINK 1.07 (<http://pngu.mgh.harvard.edu/purcell/plink/>).¹¹ SNPs with $r^2 \geq 0.5$ or $D' \geq 0.5$ were analyzed for association with HCTZ blood pressure response using PLINK.

Gene expression measurement

Expression of the nearest genes (*LYZ*, *YEATS4* and *FRS2*) was measured in 24 PEAR African-American variant allele (T) carriers at rs7297610 and 24 race and sex-matched major allele (C) homozygotes. RNA was isolated from whole blood using the PAXgene Blood RNA Kit (PreAnalytiX GmbH, Hombrechtikon, Switzerland) and converted to cDNA. Measurement of gene expression was carried out via quantitative real-time RT-PCR using Taqman Gene Expression Assays and Taqman 7900HT Real Time PCR System (Applied Biosystems, Foster City, CA, USA). Expression levels were normalized using the housekeeping gene β -2-microglobulin. Expression was calculated by the $2^{-\Delta C_t}$ method.¹²

Statistical analysis

Hardy-Weinberg equilibrium was calculated separately by race using a χ^2 goodness-of-fit test with one degree of freedom. In PEAR, we replicated the method used by Turner *et al.*⁹ of comparing three-SNP haplotype frequencies in the 1/3 best and 1/3 worst Caucasian and 1/3 best and 1/3 worst African-American responders to HCTZ. HaploStats (<http://cran.r-project.org/web/packages/haplo.stats/>) was used to estimate haplotype frequencies, as in the paper by Turner *et al.*⁹ To be considered a replication, haplotype associations in PEAR were required to have a $P \leq 0.05$ with effects in the same direction as those found previously.⁹ For single SNP analyses in PEAR, differences in systolic and diastolic blood pressure response across genotype were calculated separately by race using ANOVA, adjusting for baseline blood pressure, age and gender. The eight SNPs from the BeadChip in linkage with rs7297610 were tested for association with HCTZ blood pressure response in PEAR African-Americans using linear regression, adjusting for baseline blood pressure, age and gender. SNP associations with a Bonferroni-corrected $P \leq 0.00625$ (for eight tests) were considered significant. In major allele homozygous African-Americans and variant carriers at rs7297610, a paired *t*-test was used to compare relative changes in *LYZ*, *YEATS4* and *FRS2* gene expression after HCTZ treatment, and an unpaired *t*-test was used to compare expression between genotype groups, both with $P \leq 0.05$ considered significant. Statistical analyses in Chromosome 12 data from genome-wide arrays were conducted using PLINK 1.07 (Shaun Purcell, <http://pngu.mgh.harvard.edu/purcell/plink/>). All other statistical

Table 1 Baseline characteristics of PEAR participants

	PEAR HCTZ	PEAR Atenolol
N	372	374
Age (year)	49.0 \pm 9.3	48.7 \pm 9.1
Male (%)	50.8	43.0
Race (%)		
Caucasian	60.3	60.7
African-American	39.7	39.3
BMI (kg m ⁻²)	30.8 \pm 5.1	30.8 \pm 5.9
Mean Home BP		
Systolic (mmHg)	146.7 \pm 10.7	145.0 \pm 10.0
Diastolic (mmHg)	94.2 \pm 6.0	93.2 \pm 5.9

Abbreviations: BMI, body mass index; BP, blood pressure; HCTZ, hydrochlorothiazide; PEAR, pharmacogenomic evaluation of antihypertensive responses. Values are listed as mean \pm s.d.

analyses were completed using SAS version 9.2 (SAS Institute, Cary, NC, USA).

Results

Characteristics in the PEAR study population (Table 1), including gender and racial distributions, mean age and mean baseline blood pressures, were similar to those found in the original GWAS study population.¹³ All pyrosequencing genotype frequencies were in Hardy-Weinberg equilibrium (P -value range: 0.222–0.954) except rs317689 in African-Americans ($P = 0.0006$). Because of this possible discrepancy, all genotypes for this SNP were replaced with the calls from the genome-wide BeadChip when they later became available. Despite 98% concordance with the pyrosequencing genotypes, rs317689 genotypes from the BeadChip were well within Hardy-Weinberg Equilibrium ($P = 0.770$ in African-Americans), with similar genotype frequencies as those found in the ASW and YRI (African ancestry) and CEU (European ancestry) HapMap populations.

Haplotype analysis

Of the observed rs317689/rs315135/rs7297610 haplotypes in PEAR African-Americans, the ATT haplotype was significantly more frequent in poor responders than good responders to HCTZ (Table 2). This association was consistent with findings reported by Turner *et al.*⁹ Directionally similar to the significant previous finding, the ATC haplotype was more frequent in good responders and the ACT haplotype was more frequent in poor responders, although these differences were not statistically significant in PEAR (Table 2). Also consistent with the previous GWAS, these associations were not observed in Caucasians.

Single SNP analysis

To determine which of the three SNPs were most strongly driving this haplotype association, we individually tested associations between rs317689, rs315135 and rs7297610

genotype and blood pressure response to HCTZ. Only rs7297610 was significantly associated with HCTZ response in African-Americans (Figure 2). Compared with C/C

Table 2 Estimated frequencies of rs317689/rs315135/rs7297610 haplotypes in PEAR African-Americans by good or poor response to HCTZ

Response Category	ACT	ATT	ACC	GTC	ATC	GTT
Overall	4.3	15.1	7.9	14.4	57.3	0.9
Poor responders	4.5	23.0	6.7	13.2	52.5	0
Good responders	4.3	7.8	8.9	16.2	61.6	1.1
P	0.740	0.010	0.610	0.492	0.333	NA

Abbreviations: HCTZ, hydrochlorothiazide; PEAR, pharmacogenomic evaluation of antihypertensive responses.

P-value adjusted for age, sex and baseline blood pressure.

Bold represents significant P-value after correction.

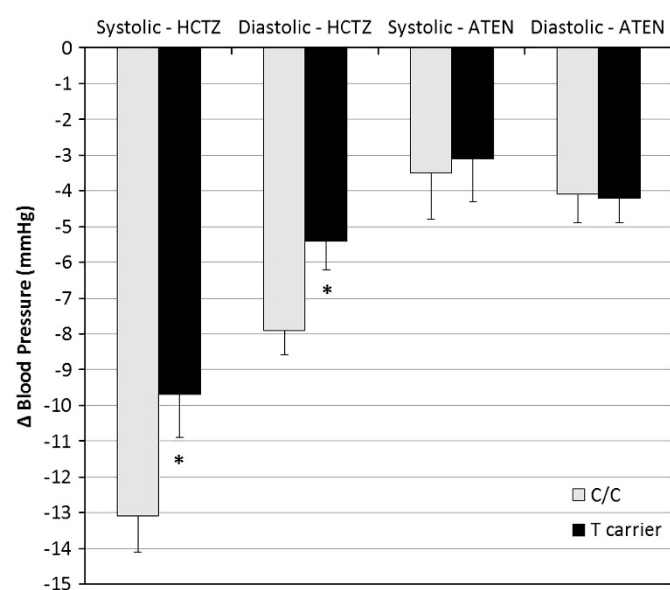


Figure 2 Blood pressure response to HCTZ and atenolol by rs7297610 genotype in PEAR African-Americans. Values are adjusted for age, sex and baseline blood pressure. Error bars indicate s.e. * $P \leq 0.05$ compared with the common C/C genotype.

homozygotes, African-American T-allele carriers treated with HCTZ experienced 3.4 mmHg less systolic ($P = 0.0275$) and 2.5 mmHg less diastolic blood pressure reduction ($P = 0.0196$). Neither rs317689, nor rs315135 possessed any significant associations with HCTZ response (P -value range: 0.331–0.889). To discern whether the association with rs7297610 was specific to HCTZ response, we tested for associations between rs7297610 genotype and blood pressure response to the beta-blocker atenolol in African-Americans, but found no relationship between genotype and atenolol response (Figure 2). Also, consistent with findings in the haplotype analysis, Caucasian HCTZ responses were not associated with rs317689, rs315135 or rs7297610 genotype.

In silico functional analysis

rs7297610 occurs in a large intergenic region between YEATS domain-containing 4 (*YEATS4*) and fibroblast growth factor receptor substrate 2 (*FRS2*), approximately 40 kb from each gene (Figure 1). As the previous GWAS and this study both suggest rs7297610 has the strongest association with HCTZ response in African-Americans, we investigated this SNP *in silico* to develop functional hypotheses for its association. When queried, UCSC Genome Browser, Pupasuite 2 and SNPinfo Web Server software were not able to predict any functional properties for rs7297610. UCSC genome browser did indicate that although the immediate region around rs7297610 is conserved in most primates, humans differ at the rs7297610 locus. The C allele is more common in humans, but the T allele is highly conserved in all other primates and in most mammals (Supplementary Figure 1).

Linkage on Chromosome 12

Because of rs7297610's association with HCTZ response was only observed in African-Americans, its intergenic location, and the fact that no function could be inferred *in silico*, we hypothesized rs7297610 was not a functional SNP. We therefore measured linkage with rs7297610 across Chromosome 12 in PEAR African-Americans in an attempt to locate the functional SNP(s). Of the 48 541 SNPs available across Chromosome 12, only eight SNPs possessed an r^2 or D' > 0.5 with rs7297610, and were all within a region of

Table 3 SNPs genotyped by Omni1-Quad array in moderate to high linkage with rs7297610 (italics) in PEAR African-Americans

SNP	Location on Chromosome 12	Co-inherited allele	Allele frequency	r^2	D'	HCTZ DBP lowering	P
rs7297610	69824024	T	0.284	N/A	N/A	Worse	0.019
rs7959850	69823594	C	0.792	0.358	0.735	Worse	0.270
rs10784780	69826029	A	0.544	0.298	0.947	Worse	0.002
rs10878983	69826093	G	0.611	0.241	0.978	Worse	0.004
rs732214	69815826	C	0.473	0.158	0.601	Worse	0.604
rs12367916	69818080	C	0.914	0.037	1	Worse	0.229
rs17813347	69810152	T	0.007	0.017	1	Better	0.221
rs931070	69809449	T	0.964	0.015	0.999	Worse	0.168
rs17225223	69810208	C	0.964	0.015	0.999	Worse	0.049

Abbreviations: HCTZ, hydrochlorothiazide; PEAR, pharmacogenomic evaluation of antihypertensive responses; SNP, single-nucleotide polymorphism.

Co-inherited allele: allele co-inherited with rs7297610T allele; HCTZ DBP Lowering: directional effect of diastolic blood pressure response to hydrochlorothiazide in patients with allele co-inherited allele, compared with the other allele for that SNP.

Bold represents significant P-value after correction.

approximately 17kb (Table 3). When these eight SNPs were viewed using UCSC Genome Browser, a total of three (rs17813347, rs931070 and rs17225223) occur in an area of high transcription factor binding and histone acetylation, suggesting they occur in a potential gene-regulating region. We then tested for associations between these eight SNPs and blood pressure response to HCTZ. None of the three SNPs in potential regulatory regions were significantly associated ($P \leq 0.00625$) with HCTZ response in PEAR African-Americans (Table 3). Of the eight total SNPs, only rs10784780 and rs10878983 had statistically significant associations, with similar magnitude and directional effect as rs7297610 (Table 3). In fact, the associations between rs10784780 ($P = 0.002$) and rs10878983 ($P = 0.004$) and HCTZ response were stronger in PEAR than observed with rs7297610 ($P = 0.019$). These two additional SNPs also have strong linkage disequilibrium between each other ($r^2 = 0.585$, $D' = 0.878$).

Gene expression

To provide mechanistic insight into the pharmacogenomic effect we observed in this gene region, we measured expression of the three nearest genes (*LYZ*, *YEATS4* and *FRS2*) in 24 PEAR African-Americans with the common C/C

genotype at rs7297610 and 23 T allele carriers (one sample failed RT-PCR). Compared with T-carriers, those with the C/C genotype (good responders to HCTZ) had an approximately 22% higher pre-treatment *YEATS4* expression at baseline ($P = 0.024$, Figure 3a). After treatment with HCTZ, expression of *YEATS4* decreased by 15% in those with the C/C genotype ($P = 0.0088$), but did not significantly change in T-carriers (Figure 3a). After HCTZ treatment, there were no differences in expression by rs7297610 genotype. In addition, no significant differences were observed with *FRS2* and *LYZ* expression at baseline or following HCTZ treatment (Figures 3b and c). When *LYZ*, *YEATS4* and *FRS2* expression was compared in the 47 HCTZ-treated African-Americans by rs10784780 and rs10878983 genotype, no significant differences were observed (data not shown).

Discussion

Herein, we provide the first known independent replication of a hypertension pharmacogenomic GWAS discovery, highlighting the utility of the GWAS to identify novel variants for antihypertensive response. Our findings also represent one of the few replicated GWAS examples in the pharmacogenomics literature for a gene not involved

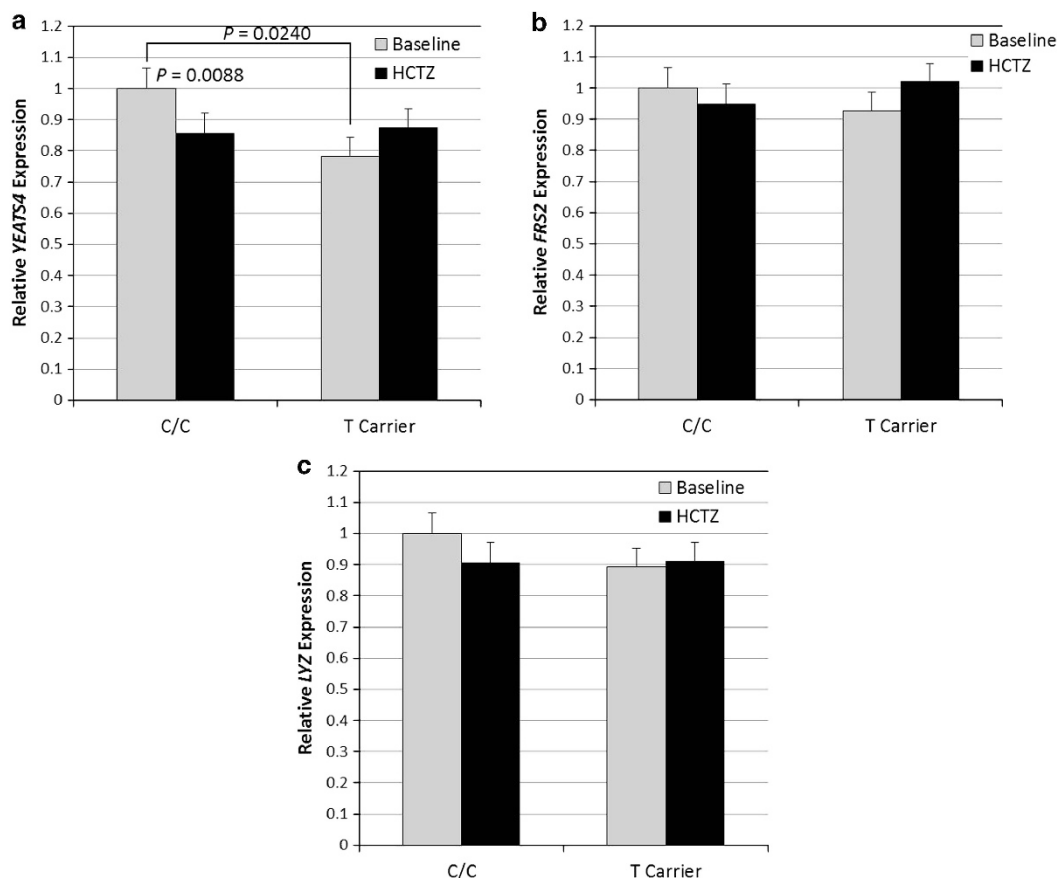


Figure 3 Relative gene expression of *YEATS4* (a), *FRS2* (b), and *LYZ* (c) by rs7297610 genotype in 47 African-American PEAR participants, normalized to the housekeeping gene β -2-microglobulin. Error bars indicate s.e.

in drug metabolism or drug transport. Specifically, we replicated the associations between rs317689/rs315135/rs7297610 haplotypes and HCTZ response in African-Americans. These data demonstrate that African-Americans with the ATT haplotype are more likely to have a smaller blood pressure response to HCTZ compared with those without this haplotype. Haplotype associations seem to be driven by the third SNP in the haplotype, rs7297610. Our data show clinically significant differences in blood pressure response to HCTZ based on rs7297610 genotype, with a 3.4 mmHg difference in mean systolic and a 2.5 mmHg difference in mean diastolic blood pressure reduction between C/C and T-carrier genotype groups. We also present evidence demonstrating rs7297610 is not a universal predictor of blood pressure response to all antihypertensives, but rather a thiazide-specific response locus, as we found no association of this genotype in the response of African-Americans randomized to the beta-blocker atenolol. Further, we found *YEATS4* expression significantly differs by rs7297610 genotype at baseline and HCTZ treatment significantly decreases expression only in African-Americans with the C/C genotype.

rs7297610 occurs in an intergenic region between *YEATS4* and *FRS2*. In humans, the common C allele at rs7297610 varies from other mammals, which almost exclusively possess the T allele, indicating variation evolved recently in the human lineage. Also, because it is highly conserved in other species, this locus could have some yet unknown functional property. To develop a functional hypothesis for this SNP's association with HCTZ response, we investigated possible functional mechanisms *in silico*, but found no explanation for this association. As no functional prediction could be made and linkage analysis produced no functional candidates, we measured associations between rs7297610 genotype and expression of the three nearby genes (*LYZ*, *YEATS4* and *FRS2*) in African-Americans treated with HCTZ. Interestingly, we found that baseline *YEATS4* expression was associated rs7297610 genotype. Upon HCTZ treatment, *YEATS4* expression significantly decreased in those with the C/C genotype, but T-carriers experience no significant change in expression. This association raises the possibility that differential expression of *YEATS4* may be involved in the greater blood pressure response to HCTZ seen in the rs7297610 C/C genotype. However, further research is needed to verify if this is the case, as the mechanism by which rs7297610 could affect *YEATS4* expression and HCTZ blood pressure response remains unclear at this time.

Our strongest hypothesis on why this SNP associates with variability in response to HCTZ is that rs7297610 tags another (or many rare), functional SNP(s). The two major reasons for this hypothesis are: (1) the blood pressure response to HCTZ was only observed in African-Americans and not Caucasians, and (2) the intergenic location of rs7297610 is not in any known coding or regulatory region of the genome. The original GWAS conducted by Turner *et al.*⁹ genotyped ~100 000 SNPs across the genome. Further, fine mapping analyses done by Turner *et al.*⁹ were only completed within the *LYZ*, *YEATS4* and *FRS* genes. So

when nearly 1.1 million genotypes became available on each PEAR participant, a comprehensive linkage analysis could be completed in hopes of finding the functional SNP(s) in linkage disequilibrium with rs7297610. When we measured linkage across Chromosome 12, we found two SNPs (rs10784780 and rs10878983) that were in high linkage disequilibrium with rs7297610 and more strongly associated with HCTZ response. However, we found no association between rs10784780 or rs10878983 genotype and *LYZ*, *YEATS4* or *FRS2* expression changes after HCTZ treatment. These two SNPs also exist in the same intergenic region as the nearby rs7297610, with no known mechanism for gene regulation. Thus, rs10784780 and rs10878983 simply appear to be better tag SNPs for some still undiscovered polymorphism(s). Further sequencing of the Chromosome 12q15 region in a large number of HCTZ-treated hypertensives may discover the functional SNP(s) responsible for our observed associations with HCTZ response.

YEATS4 encodes the protein GAS41, which knock-out studies have shown is essential for RNA transcription and cell viability.¹⁴ As with other YEATS domain-containing proteins, GAS41 has a role in chromatin-modification and transcription, which may involve recognition of chromatin targets and recruiting modification complexes to affect transcription.¹⁵ Although no reference was found in the literature connecting GAS41 with cardiovascular function, this protein has been implicated in mixed lineage leukemia (MLL), and is required for repressing the p53 repressor pathway during normal cellular proliferation.¹⁶ Whether GAS41's effect on proliferation could have a role in hypertension-associated renal cell proliferation is unknown. However, this could be a potential mechanism where *YEATS4* could affect response to the diuretic HCTZ, which acts in the renal tubules. Further studies are needed to clarify if and how GAS41 impacts blood pressure homeostasis and HCTZ response.

The similarity in size and patient characteristics between PEAR and the previous GWAS population was a major strength in our validation effort, and thus, we predicted we would detect similar associations to those found previously, if the associations were real. A limitation to this study is that RNA expression profiles taken from whole blood may not be relevant to assess HCTZ response at the site of action. However, cells from the two primary hypothesized sites of HCTZ action, the kidney and the vasculature, are difficult to obtain from humans, and HCTZ was previously shown to be pharmacologically active in peripheral blood mononuclear cells.^{17,18}

To conclude, we report the first known validation of an antihypertensive pharmacogenomic GWAS, confirming a pharmacogenomic effect of rs7297610 on HCTZ blood pressure response in African-Americans. Further, our data demonstrate this is an HCTZ-specific effect, and suggest variation at rs7297610 is associated with differential *YEATS4* expression. Whether these expression differences have a role in the diminished blood pressure response we observed with HCTZ treatment remains unknown. If they do, this pathway could explain some amount of HCTZ response variability. However, *YEATS4* has not previously been linked to cardiovascular function or thiazide effect, so additional

research is needed to elucidate the functional role of rs7297610, as well as the potential role of *YEATS4* in blood pressure response to HCTZ.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This work was supported by a Grant from the National Institutes of Health (Bethesda, MD, USA), U01 GM074492, funded as part of the Pharmacogenomics Research Network. This work is also supported by the following Grants from the NIH National Center for Research Resources: Grant M01 RR00082 and UL1 RR029890 to the University of Florida, Grants UL1 RR025008 and M01 RR00039 to Emory University and UL1 RR024150 to Mayo Clinic. Support was also provided by K23 HL086558 (RMC), K23 HL091120 (ALB) and T32 DK007518 (JDD). The authors are thankful for the technical assistance of Zhiying Wang and Lynda Stauffer. The authors acknowledge and thank the valuable contributions of the study participants, support staff and study physicians.

References

- 2010 Top 200 generic drugs by total prescriptions. Drug Topics; <http://drugtopics.modernmedicine.com/drugtopics/data/articlestandard//drugtopics/252011/727243/article.pdf>; accessed on 19 July 2011.
- 2010 Top 200 branded drugs by total prescriptions. Drug Topics; <http://drugtopics.modernmedicine.com/drugtopics/data/articlestandard//drugtopics/252011/727256/article.pdf>; accessed on 19 July 2011.
- Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo Jr JL *et al*. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension* 2003; **42**: 1206–1252.
- Duarte JD, Cooper-DeHoff RM. Mechanisms for blood pressure lowering and metabolic effects of thiazide and thiazide-like diuretics. *Expert Rev Cardiovasc Ther* 2010; **8**: 793–802.
- Carter BL, Einhorn PT, Brands M, He J, Cutler JA, Whelton PK *et al*. Thiazide-induced dysglycemia: call for research from a working group from the national heart, lung, and blood institute. *Hypertension* 2008; **52**: 30–36.
- Materson BJ. Variability in response to antihypertensive drugs. *Am J Med* 2007; **120**(4 Suppl 1): S10–S20.
- Johnson JA, Gong Y, Bailey KR, Cooper-DeHoff RM, Chapman AB, Turner ST *et al*. Hydrochlorothiazide and atenolol combination antihypertensive therapy: effects of drug initiation order. *Clin Pharmacol Ther* 2009; **86**: 533–539.
- Comparison of propranolol and hydrochlorothiazide for the initial treatment of hypertension. II. Results of long-term therapy. [Veterans Administration Cooperative Study Group on Antihypertensive Agents.] *JAMA* 1982; **248**: 2004–2011.
- Turner ST, Bailey KR, Fridley BL, Chapman AB, Schwartz GL, Chai HS *et al*. Genomic association analysis suggests chromosome 12 locus influencing antihypertensive response to thiazide diuretic. *Hypertension* 2008; **52**: 359–365.
- Johnson JA, Boerwinkle E, Zineh I, Chapman AB, Bailey K, Cooper-DeHoff RM *et al*. Pharmacogenomics of antihypertensive drugs: rationale and design of the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) study. *Am Heart J* 2009; **157**: 442–449.
- Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D *et al*. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007; **81**: 559–575.
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻(Delta Delta C(T)) Method. *Methods* 2001; **25**: 402–408.
- 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.
- Zimmermann K, Ahrens K, Matthes S, Buerstedde JM, Stratling WH, Phi-van L. Targeted disruption of the GAS41 gene encoding a putative transcription factor indicates that GAS41 is essential for cell viability. *J Biol Chem* 2002; **277**: 18626–18631.
- Schulze JM, Wang AY, Kobor MS. YEATS domain proteins: a diverse family with many links to chromatin modification and transcription. *Biochem cell biol* 2009; **87**: 65–75.
- Park JH, Roeder RG. GAS41 is required for repression of the p53 tumor suppressor pathway during normal cellular proliferation. *Mol cell biol* 2006; **26**: 4006–4016.
- Abuladze N, Yanagawa N, Lee I, Jo OD, Newman D, Hwang J *et al*. Peripheral blood mononuclear cells express mutated NCCT mRNA in Gitelman's syndrome: evidence for abnormal thiazide-sensitive NaCl cotransport. *J Am Soc Nephrol* 1998; **9**: 819–826.
- Abraham AS, Brooks BA, Grafstein Y, Barchilon E, Nubani N, Eylath U *et al*. Effects of hydrochlorothiazide, diltiazem and enalapril on mononuclear cell sodium and magnesium levels in systemic hypertension. *Am j cardiol* 1991; **68**: 1357–1361.

Supplementary Information accompanies the paper on the The Pharmacogenomics Journal website (<http://www.nature.com/tpj>)