Published in final edited form as:

Pharmacogenomics J. 2015 April ; 15(2): 153–157. doi:10.1038/tpj.2014.46.

Transethnic meta-analysis suggests genetic variation in the HEME pathway influences potassium response in patients treated with hydrochlorothiazide

Jorge L. Del-Aguila¹, Rhonda M. Cooper-DeHoff², Arlene B. Chapman³, John G. Gums², Amber L. Beitelshes⁵, Kent Bailey⁴, Stephen T. Turner⁴, Julie A. Johnson², and Eric Boerwinkle^{1,6}

¹Human Genetics Center, University of Texas Health Science Center at Houston, Houston, TX, USA

²Department of Pharmacotherapy and Translational Research and Division of Cardiovascular Medicine and Center for Pharmacogenomics, University of Florida, Gainesville, FL, USA

³Department of Medicine, Emory University School of Medicine, Atlanta, GA, USA

⁴Division of Nephrology and Hypertension, Mayo Clinic, Rochester, MN, USA

⁵Department of Medicine, University of Maryland School of Medicine, Baltimore, MD, USA

⁶Human Genome Sequencing Center at Baylor College of Medicine, Houston, TX, USA

Abstract

Hypokalemia is a recognized adverse effect of thiazide diuretic treatment. This phenomenon, which may impair insulin secretion, has been suggested to be a reason for the adverse effects on glucose metabolism associated with thiazide diuretic treatment of hypertension. However, the mechanisms underlying thiazide diuretic induced hypokalemia are not well-understood. In an effort to identify, genes or genomic regions associated with potassium response to hydrochlorothiazide, without *a priori* knowledge of biologic effects; we performed a genome-wide association study and a Multi-Ethnic Meta-Analysis in 718 European- and African-American hypertensive participants from two different pharmacogenetic studies. SNPs rs10845697 (Bayes Factor=5.560) on chromosome 12, near to the HEME binding protein 1 gene, and rs11135740 (Bayes Factor= 5.258) on chromosome 8 near the Mitoferrin-1 gene reached GWAS significance (Bayes Factor > 5). These results, if replicated, suggest a novel mechanism involving effects of genes in the HEME pathway influencing hydrochlorothiazide-induced renal potassium loss.

Keywords

Pharmacogenomics; hydrochlorothiazide; hypokalemia; HEME

Correspondence to: Dr. Eric Boerwinkle, Human Genetics Center, University of Texas Health Science, Center at Houston, Houston, TX, USA., Tel: 713-500-9816; Fax: 713-500-0900; eric.boerwinkle@uth.tmc.

Conflict of Interest

The authors declare no conflict of interest

Supplementary information is available at *The Pharmacogenomics Journal's* website

INTRODUCTION

Hypokalemia is a well-known consequence of thiazide diuretic treatment of patients with hypertension[1, 2]. Hypokalemic-impaired insulin secretion has been suggested to be a mechanism for the adverse metabolic effects accompanying diuretic treatment[3, 4], although this hypothesis is not universally supported[5, 6]. The mechanism by which diuretics lead to potassium wasting and hypokalemia in some patients but not others is not well-understood. In an effort to identify genes or pathways associated with hydrochlorothiazide (HCTZ)-induced hypokalemia, we performed a genome-wide association study (GWAS) and a Multi-Ethnic Meta-Analysis (MANTRA)[7] in European- and African-American hypertensive patients from two different pharmacogenetic studies. The data presented here are the first GWAS to our knowledge of diuretic-induced potassium loss, and the analysis was conducted in four different study samples (European and African Americans each from two different studies). The results implicate the HEME pathway in affecting diuretic treatment-induced hypokalemia.

METHODS

Study population

Details of the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR, clinicaltrials.gov identifier NCT00246519) study and The Genetic Epidemiology of Responses to Antihypertensive (GERA, clinicaltrials.gov identifier NCT00005520) study have been previously published[8, 9]. PEAR and GERA study participants were African-Americans and European-Americans with mild to moderate essential hypertension without a history of heart disease or diabetes mellitus. In PEAR, after a wash out period of approximately 4 weeks, blood and urine were collected in a fasting state. After the baseline evaluations were completed, participants were randomized to HCTZ (thiazide diuretic 12.5mg orally once daily) or atenolol (β -blocker 50mg orally once daily) for three weeks, with dose doubling (to 25 mg HCTZ) for those with BP > 120/70 mmHg for an additional 6 weeks. More than 90% of PEAR participants received the higher 25 mg dose of HCTZ. For this analysis of potassium (K⁺) response, we used only those randomized to the HCTZ arm, referred to hereafter as PEAR HCTZ monotherapy.

In GERA, after a wash-out period of at least 4 weeks, blood samples were obtained for baseline biochemical measurement, and then qualifying participants were treated with HCTZ (25 mg orally once daily) for 4 weeks. At the end of the 4 week diuretic treatment period, blood pressure was measured and blood samples were again obtained for biochemical measurements. All blood collections were done in the morning after 8 hours of fasting.

All participants enrolled in PEAR and GERA provided written informed consent, and the institutional review boards of participating study centers approved the study protocols.

Phenotype and Genotype data

Serum potassium concentrations, in PEAR and GERA studies, were measured by flame photometer (Instrumentation Laboratories model 943, Lexington, MA). Plasma insulin, which was used as a covariate during statistical analyses, was measured in both studies with the Access Ultrasensitive Insulin immunoassay system.

In GERA, serum potassium concentration was determined after 2 weeks of HCTZ therapy. In PEAR, serum potassium concentrations were measured at the end of the wash-out period (baseline), after three weeks and at the end of the HCTZ monotherapy. In both studies, there were protocol-mandated potassium supplementation requirements based on serum potassium levels. Potassium supplementation was required for values < 3.6 mEq/L in GERA and < 3.2 mEq/L in PEAR. Potassium supplementation was also available for use by the study physician, at their discretion, at higher potassium levels. To avoid confounding from prescribed potassium supplementation and to promote patient safety, serum potassium response to HCTZ was defined as the difference between the levels measured after the first visit (two weeks in GERA and three weeks in PEAR) of treatment and the baseline visit, prior to initiation of potassium supplementation. Thirteen participants with potassium response values under or over 3 standard deviations from the mean response were removed from the analysis.

Participants were genotyped using the Illumina HumanOmni1-Quad (Illumina, San Diego, California, USA) in the case of PEAR and the GeneChip Human Mapping 500k Array, (Affymetrics, Santa Clara California, USA) in the case of GERA. Standard quality control steps were applied to the raw genotype data in both studies: single nucleotide polymorphisms (SNPs) with minor allele frequency (MAF) $< 1\%$, call rates $< 95\%$, Hardy-Weinberg equilibrium p-values 10^{-5} and participants with more than 10% missing genotypes were removed from the analysis. The software MACH[10] (www.sph.umich.edu/csg/abecasis/MACH/download) was used to impute the approximate 2.5 million HapMap SNPs using the Phase II CEU as the reference panel for European-Americans and a cosmopolitan sample of CEU and YRI for African-Americans. Quality control filters for the imputed results were $RSQ\_HAT < 0.3$ and $MAF \geq 0.05$. MACH generated a file with the highest posterior probabilities for each imputed SNP (i.e. dosage) which was used in the analysis. After quality control and imputation, there were more than 2 million SNPs used for the genotype-phenotype association studies in each race-study group.

Statistical analysis

Additive genetic association analyses of potassium response to HCTZ were performed by self-reported race within each study using ProbABEL[11], while adjusting for sex, age, waist circumference, the baseline values of potassium and insulin, and the two first principal component scores (PCS), to avoid the possibility of spurious association as the result of population substructure[12, 13]. The principal components were calculated using the EIGENSTRAT[12] software. The European-American and African-American GWAS analyses were combined via transethnic meta-analysis using MANTRA (Meta-Analysis of Transethnic Association studies)[7]. MANTRA clusters populations by the mean allele frequency difference. If populations are in the same cluster, MANTRA assumes that they

have the same underlying allelic effects and a fixed effect model is performed. On the other hand, if populations are in different cluster, the assumption will be that they do not have the same underlying allelic effect and a random effect analysis is performed. MANTRA provides a combined association signal called a Bayes Factor (BF) instead of a traditional p-value to compare the null and alternative hypotheses. BF compares of how well two hypotheses agree with the observed data. In the present case, we compare the agreement of the data under a null model of no association of the variant with the trait in any population, $\beta=0$ with agreement of the data with, the alternative model that there is an association in at least one population. The hypothesis that agrees with the observed data better is the one that is said to have more evidence supporting it. A BF of 10^5 , roughly corresponding to a pvalue of 7.9×10^{-7} , and was considered significant evidence of association[14] after accounting for genome-wide multiple comparisons[15].

RESULTS

Tables 1A and 1B summarize the baseline characteristics of the European-American and African-American study participants, respectively. Both studies had a higher percentage of European-Americans male and African American female study participants. The average age and waist circumference were nearly identical between PEAR and GERA. Baseline potassium was lower in African-Americans from GERA compared to the other groups. The mean response in European Americans was lower compared to African Americans. Figure 1 shows the distribution of potassium response in both populations after removing 13 outlier individuals.

No genome-wide significant association was found in each separate study and race group (supplementary information). After the multi-ethnic meta-analysis, the top seven SNPs from MANTRA are shown in Table 2, and four of them, located in three different loci, reached genome-wide significant significance evidence of association. A Manhattan plot of the BFs is shown in Figure 2. The SNPs with significance evidence of association are: 1) rs10845697 (BF=5.560) on chromosome 12, near to the heme binding protein 1 gene (HEBP1), 2) rs1007869 and rs12596186 (LD: $r^2 > 0.99$, BF=5.347 and 5.114 respectively) on chromosome 16, near the junctophilin 3 gene (JPH3) and 3) rs11135740 (BF=5.258) on chromosome 8, near to the Mitoferrin-1 gene (SLC25A37 also known as MFRN1). The effects of rs10845697 were not consistent among study samples (Table 2). Whether this inconsistency is due to chance sampling variation, differences in linkage disequilibrium, or differences in allelic effects among the study samples is not known.

Because HEBP1 and SLC25A37 are related to HEME synthesis, we carried out a targeted analysis of other genes and SNPs related to HEME synthesis. Although rs12095896 on chromosome 1 did not reach GWAS significance (BF=4.980), it was found in region of the genome previously related to bilirubin levels (<http://www.genome.gov/gwastudies/index.cfm?pageid=26525384#searchForm>). Bilirubin is a bile pigment that is a degradation product of HEME. rs10845697 near HEBP1, rs11135740 near SLC25A37, and rs12095896 in the bilirubin locus combined accounted for 5% and 7% of the variance of potassium response in PEAR and GERA European Americans, respectively. In African Americans,

these values were 16% for PEAR and 12% for GERA. The effect size estimates of the top signals from the HEME synthesis pathway are shown in Table 3.

Discussion

Hypokalemia is a well-known adverse effect in patients taking HCTZ. The purpose of this study was to identify genes or genomic regions associated with potassium response after HCTZ treatment. We conducted a GWAS and a Multi-Ethnic Meta-Analysis (i.e. MANTRA) from two studies of hypertensive European and African American individuals. Four SNPs achieved a log 10 Bayes Factor greater than 5 which was defined *a priori* to be GWAS significance evidence of association. Confirmation of these results will be required in an independent sample of hypertensive patients treated with HCTZ. Among the top SNPs, rs10845697 near HEBP1 and rs11135740, near Mitoferrin-1 (SLC25A37) have a role in heme biosynthesis[16–19]. Heme has been identified as a modulator of the large-conductance Ca^{2+} -activated K^+ channels (BK, or maxi K) channels[20]. BK channels are located in the medullary, cortical thick ascending limbs[21–23] and the distal convoluted tubule[24] in the kidney. They are sensitive to change in both voltage and calcium concentration and they work as volume regulatory channels and secrete potassium[25]. However they have not previously been considered important contributors to renal potassium secretion because their low open probabilities (P_o) as well as their low concentration on the principal cell in the collecting ducts of the kidney [25]. The main route for potassium secretion is considered to be the inwardly-rectifying channel, subfamily J, member 1 (ROMK1) encoded by the gene KCNJ1 which has higher P_o and higher density on principal cells than BK channels[26]. Although KCNJ1 has shown significant associations with change in glucose in multiple studies[27, 28], SNPs in and around KCNJ1 were not associated with potassium response to HCTZ (data not shown) in this study.

We hypothesize that HCTZ affects serum K in part by influencing heme levels; the bulk of the effect would be inhibition of Na-Cl cotransporter. Heme has been shown to increase the P_o of the BK channels at negative voltages and decrease it at more positive voltages[29]. As a result, the secretion of potassium by BK channels would be positively associated with heme levels.

Herein, we report the first GWAS to our knowledge of potassium response to HCTZ and provide evidence for a novel mechanism of HCTZ-induced hypokalemia. Our study is unique in that it included a washout of all antihypertensive agents prior to initiation of HCTZ and collected detailed data regarding potassium supplementation and levels. However, there are weaknesses that must be acknowledged, for instance, although MANTRA can provide an overall Bayes factor as the combined association signal, it fails in estimate a proper combined effect size from the joint analysis of all the studies. Another weakness is the absence of replication sample. During this study, we contacted multiple potential collaborators in the United States and Europe, but none had appropriate potassium response data to serve as a replication for this study. This experience underscores the need for an international pharmacogenetics of antihypertensives consortium.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

We acknowledge and thank the valuable contributions of the study participants, study physicians, support staff and the technical assistance: Zhiying Wang, Megan Grove, Jodie Van De Rostyne, Jeremy Palbicki, Robert Tarrell, and Prabin Thapa.

SOURCES OF FUNDING

GERA: was supported by NIH grants HL74735, HL53335, and the Mayo Foundation.

PEAR: is supported by an NIH, grant 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.

References

1. Kaplan, NM. Clinical Hypertension. seventh edition. Williams & Wilkins; 1998.
2. Sica DA. Diuretic-related side effects: development and treatment. *J Clin Hypertens (Greenwich)*. 2004; 6:532–540. [PubMed: 15365284]
3. Shafi T, Appel LJ, Miller ER III, Klag MJ, Parekh RS. Changes in serum potassium mediate thiazide-induced diabetes. *Hypertension*. 2008; 52:1022–1029. [PubMed: 18981326]
4. Zillich AJ, Garg J, Basu S, Bakris GL, Carter BL. Thiazide diuretics, potassium, and the development of diabetes: a quantitative review. *Hypertension*. 2006; 48:219–224. [PubMed: 16801488]
5. Eriksson JW, Jansson PA, Carlberg B, Hagg A, Kurland L, Svensson MK, et al. Hydrochlorothiazide, but not Candesartan, aggravates insulin resistance and causes visceral and hepatic fat accumulation: the mechanisms for the diabetes preventing effect of Candesartan (MEDICA) Study. *Hypertension*. 2008; 52:1030–1037. [PubMed: 18981327]
6. Smith SM, Anderson SD, Wen S, Gong Y, Turner ST, Cooper-Dehoff RM, et al. Lack of correlation between thiazide-induced hyperglycemia and hypokalemia: subgroup analysis of results from the pharmacogenomic evaluation of antihypertensive responses (PEAR) study. *Pharmacotherapy*. 2009; 29:1157–1165. [PubMed: 19792989]
7. Morris AP. Transethnic meta-analysis of genomewide association studies. *Genet Epidemiol*. 2011; 35:809–822. [PubMed: 22125221]
8. 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. [PubMed: 19249413]
9. 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. [PubMed: 11849460]
10. Li Y, Willer C, Sanna S, Abecasis G. Genotype imputation. *Annu Rev Genomics Hum Genet*. 2009; 10:387–406. [PubMed: 19715440]
11. Aulchenko YS, Struchalin MV, van Duijn CM. ProbABEL package for genome-wide association analysis of imputed data. *BMC Bioinformatics*. 2010; 11:134. [PubMed: 20233392]
12. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet*. 2006; 38:904–909. [PubMed: 16862161]

13. Campbell CD, Ogburn EL, Lunetta KL, Lyon HN, Freedman ML, Groop LC, et al. Demonstrating stratification in a European American population. *Nat Genet.* 2005; 37:868–872. [PubMed: 16041375]
14. Wang X, Chua HX, Chen P, Ong RT, Sim X, Zhang W, et al. Comparing methods for performing trans-ethnic meta-analysis of genome-wide association studies. *Hum Mol Genet.* 2013; 22:2303–2311. [PubMed: 23406875]
15. Stephens M, Balding DJ. Bayesian statistical methods for genetic association studies. *Nat Rev Genet.* 2009; 10:681–690. [PubMed: 19763151]
16. Chen W, Paradkar PN, Li L, Pierce EL, Langer NB, Takahashi-Makise N, et al. Abcb10 physically interacts with mitoferrin-1 (Slc25a37) to enhance its stability and function in the erythroid mitochondria. *Proc Natl Acad Sci U S A.* 2009; 106:16263–16268. [PubMed: 19805291]
17. Micaelo NM, Macedo AL, Goodfellow BJ, Felix V. Tetrapyrrole binding affinity of the murine and human p22HBP heme-binding proteins. *J Mol Graph Model.* 2010; 29:396–405. [PubMed: 20800521]
18. Shaw GC, Cope JJ, Li L, Corson K, Hersey C, Ackermann GE, et al. Mitoferrin is essential for erythroid iron assimilation. *Nature.* 2006; 440:96–100. [PubMed: 16511496]
19. Yuan X, Fleming MD, Hamza I. Heme transport and erythropoiesis. *Curr Opin Chem Biol.* 2013; 17:204–211. [PubMed: 23415705]
20. Tang XD, Xu R, Reynolds MF, Garcia ML, Heinemann SH, Hoshi T. Haem can bind to and inhibit mammalian calcium-dependent Slo1 BK channels. *Nature.* 2003; 425:531–535. [PubMed: 14523450]
21. Taniguchi J, Guggino WB. Membrane stretch: a physiological stimulator of Ca²⁺-activated K⁺ channels in thick ascending limb. *Am J Physiol.* 1989; 257:F347–F352. [PubMed: 2782419]
22. Guggino SE, Guggino WB, Green N, Sacktor B. Ca²⁺-activated K⁺ channels in cultured medullary thick ascending limb cells. *Am J Physiol.* 1987; 252:C121–C127. [PubMed: 2435160]
23. Merot J, Poncet V, Bidet M, Tauc M, Poujeol P. Apical membrane ionic channels in the rabbit cortical thick ascending limb in primary culture. *Biochim Biophys Acta.* 1991; 1070:387–400. [PubMed: 1684913]
24. Belfodil R, Barriere H, Rubera I, Tauc M, Poujeol C, Bidet M, et al. CFTR-dependent and -independent swelling-activated K⁺ currents in primary cultures of mouse nephron. *Am J Physiol Renal Physiol.* 2003; 284:F812–F828. [PubMed: 12475745]
25. Pluznick JL, Sansom SC. BK channels in the kidney: role in K(+) secretion and localization of molecular components. *Am J Physiol Renal Physiol.* 2006; 291:F517–F529. [PubMed: 16774904]
26. Gray DA, Frindt G, Palmer LG. Quantification of K⁺ secretion through apical lowconductance K channels in the CCD. *Am J Physiol Renal Physiol.* 2005; 289:F117–F126. [PubMed: 15727988]
27. Karnes JH, McDonough CW, Gong Y, Vo TT, Langaee TY, Chapman AB, et al. Association of KCNJ1 variation with change in fasting glucose and new onset diabetes during HCTZ treatment. *Pharmacogenomics J.* 2012
28. Maitland-van der Zee AH, Turner ST, Schwartz GL, Chapman AB, Klungel OH, Boerwinkle E. A multilocus approach to the antihypertensive pharmacogenetics of hydrochlorothiazide. *Pharmacogenet Genomics.* 2005; 15:287–293. [PubMed: 15864129]
29. Horrigan FT, Heinemann SH, Hoshi T. Heme regulates allosteric activation of the Slo1 BK channel. *J Gen Physiol.* 2005; 126:7–21. [PubMed: 15955873]

Figure 1. The distribution of potassium response due to Hydrochlorothiazide in both populations. The mean in the distribution was -0.03738(sd = 0.459) in European Americans (A), in the case of African Americans (B), it was 0.0483 (sd = 0.475).

Figure 2.

MANTRA manhattan plot showing association of potassium response to HCTZ treatment in African Americans and European Americans in the PEAR and GERA studies. Three SNPs on chromosome 12, 8 and 16 showed genome-wide significance ($BF > 5.00$). Evidence of heterogeneity in allelic effects (posterior probability greater than 0.5) is shown in chromosome 12, 16 and 1.

Table 1

PEAR and GERA sample characteristics measured at baseline

(A)

Variables	PEAR: European Americans	GERA: European Americans
Total	229	196
Gender(%men)	59.8	57.1
Age(y)	49.9±9.4	48.5±7.3
Waist Circumference (cm)	94.8±13.6	103.7±14.7
Insulin ((μIU/ml)	9.4±8.7	8.5±4.9
Plasma Glucose(mg/dL)	92.9±12.2	95.1±13.2
Serum Potassium(mEq/L)	4.39±0.441	4.06±0.246
Systolic blood pressure(mmHg)	151.8±13.5	142.6±12.6
Diastolic blood pressure(mmHg)	97.9±5.8	95±5.7

(B)

Variables	PEAR: African Americans	GERA: African-Americans
Total	148	194
Gender(%men)	37.84	48.5
Age(y)	47.4±8.9	47.4±6.3
Waist Circumference (cm)	97.3±13.5	96.8±14.4
Insulin ((μIU/ml)	9.9±9.6	12.9±10.1
Plasma Glucose(mg/dL)	90.8±12.7	101.12±15.6
Serum Potassium (mEq/L)	4.13±0.48	3.76±0.307
Systolic blood pressure(mmHg)	151.8±13.14	150.8±14.9
Diastolic blood pressure(mmHg)	97.9±5.8	97.2±5.5

Table 2

Top Seven SNPs from Multi-Ethnic Meta-Analysis.

SNP	CHR	Position	EA	Nearby Gene	Bayes Factor	Direction (AA,AA,EU, EU)	Phet ^{\$\$}
rs10845697	12	13076153	G	HEBP1	5.560	---+	1
rs1007869	16	86174420	T	JPH3	5.347	---+	1
rs11135740	8	23423702	A	SLC25A37	5.258	++++	0.11
rs12596186	16	86174068	A	JPH3	5.114	---+	1
rs13250356	8	23415776	T	SLC25A37	4.984	++++	0.12
rs721183	8	23441779	G	SLC25A37	4.982	++++	0.15
rs12095896	1	81723330	C	--	4.980	+---	1

^{\$\$}The posterior probability of heterogeneity between studies

* Log10 Bayes factor (BF) from the MANTRA analysis. A log10 BF of 5 and higher was considered as a conservative threshold for GWAS.

EA=effect allele, AA=African American, EU=European American

Table 3

The effect size estimates from each separate GWAS for each of the top signals from trans-ethnic Meta-analysis related to HEME synthesis.

Effect size (se)		rs10845697	rs11135740	rs12095896
African Americans	GERA	0.14218 (0.0454)	0.13685 (0.02667)	−0.09491 (0.05482)
	PEAR	−0.12882 (.0539)	0.13461 (0.02809)	0.21906 (0.05015)
European Americans	GERA	−0.05039 (0.0401)	0.13683 (0.02741)	−0.05655 (0.03434)
	PEAR	0.18161 (0.0459)	0.13410 (0.02835)	−0.06223 (0.03491)