Genome-wide association analyses suggest NELL1 influences adverse metabolic response to HCTZ in African Americans

J L Del-Aguila, University of Texas
A L Beitelshees, University of Maryland
R M Cooper-DeHoff, University of Florida
Arlene Chapman, Emory University
J G Gums, University of Florida
K Bailey, Mayo Clinic
Y Gong, University of Florida
S T Turner, Mayo Clinic
J A Johnson, University of Florida
E Boerwinkle, University of Texas

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INTRODUCTION

Hypertension is major risk factor for stroke, cardiovascular and kidney diseases. Hydrochlorothiazide (HCTZ) is a widely prescribed thiazide diuretic for initial and combination drug treatment of hypertension with over 110 million prescriptions annually in the United States, but its ability to produce a variety of adverse metabolic effects (AMEs), such as hyperglycemia and dyslipidemia, is well known. Despite being recognized for decades, the mechanisms of these thiazide-induced AMEs are not well understood. The ability of thiazides to increase glucose has primarily been attributed to their ability to cause hypokalemia, thereby impairing insulin secretion. However, the associations between glucose increase and potassium lowering are mostly based on study-level associations of average changes, and we previously did not observe a relationship between patient-level changes in glucose and potassium, raising questions about this mechanism. More recently, additional mechanisms have been suggested including their ability to increase visceral and hepatic fat accumulation, which could promote insulin resistance. The mechanisms underlying thiazide-induced increases in triglycerides are even less well understood. It has been suggested that thiazides’ ability to cause volume depletion produces hemoconcentration of lipoproteins. Volume contraction stimulates the renin–angiotensin–aldosterone system thereby stimulating catecholamine release and subsequent adipose tissue lipolysis. However, various studies have found that the degree of hemoconcentration is inconsistent. Another possible mechanism is an incremental reduction of lipoprotein lipase activity due to interference in the production, release or action of insulin by HCTZ. One way to help identify underlying mechanisms of these adverse effects and to distinguish among these competing hypotheses is to identify genetic variations that are predictive of inter-individual variation in AME after HCTZ treatment. Using a genome-wide association study (GWAS) it is possible to identify genes or genomic regions associated with a phenotype without a priori knowledge of a biologic effect of the gene on the trait of interest. In this study, we report the results of the first GWAS of HCTZ-induced AMEs in European- and African-American hypertensive patients from two different pharmacogenetic studies.

MATERIALS AND METHODS

Study population

Phenotype and genotype data were collected from 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. In both studies, participants had mild-to-moderate primary hypertension without a history of heart disease or diabetes mellitus. Primary hypertension was defined as blood pressure (BP) levels >140/90 mm Hg or current use of prescription antihypertensive medications in the absence of a known cause for elevated BP. If at any time during the study protocols described below the average diastolic BP (DBP) rose to >110 mm Hg or the systolic BP to >180 mm Hg, participants were withdrawn from further study participation and prescribed effective antihypertensive drug therapy. Details about the study designs and exclusion and inclusion criteria have
been described previously. Briefly, in the PEAR study, individuals of any race-ethnicity and gender combination from age 17 to 65 years old with mild-to-moderate primary hypertension were recruited. Participants were enrolled in Gainesville, FL, USA; Atlanta, GA, USA; and Rochester, MN, USA. All participants were newly diagnosed hypertensives, untreated hypertensives or treated hypertensives taking less than three antihypertensive drugs. The protocol of the study was as follows: a wash out period of at least 4 weeks was done in order to remove the effects of previous BP medication if any from the participants. If at the end of the wash out period the average seated home DBP was >85 mm Hg, office DBP was >90 mm Hg and the home and office systolic BP was <180 mm Hg the individuals were enrolled into the randomized phage (plasmid) biological samples (blood and urine) were collected in the fasting state. As soon as the baseline evaluations were completed, the individuals were randomized to HCTZ (thiazide diuretic 12.5 mg orally once daily) or atenolol (β-blocker 50 mg orally once daily) for 3 weeks, with dose doubling (25 mg) for those with BP >120/70 mm Hg for an additional 6 weeks. More than 90% of PEAR participants received the higher 25 mg dose of HCTZ. For this AME GWAS analysis, we only used the patients randomized to the HCTZ from the PEAR study, referred to hereafter as PEAR HCTZ monotherapy.

In the GERA study, African Americans and European Americans with primary hypertension were recruited at Emory University in Atlanta, GA, USA and at the Mayo Clinic in Rochester, MN, USA, respectively. The protocol for the GERA study was as follows: a wash out period of at least 4 weeks was done in order to remove the effects of previous BP medication. If at the end of the wash out period the average office DBP was >90 mm Hg, qualifying individuals were treated with HCTZ (25 mg orally once daily) for 4 weeks. BP was measured in the seated position using a mercury sphygmomanometer and blood samples were obtained for baseline biochemical measurements. At the end of the 4-week diuretic treatment period, BP was measured and blood samples were again obtained for biochemical measurements. All blood collections were done in the morning after 8 h of fasting.

All patients 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
All biomedical measurements were made in a central laboratory at the Mayo Clinic. In the PEAR study, these methods were implemented on a Hitachi 911 Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN, USA). In the GERA study, plasma glucose concentrations were determined by automated spectrophotometric methods and implemented on an IL 1100 Chemistry system 760 (Instrumentation Laboratories, Lexington, MA, USA). Triglyceride concentrations were determined spectrophotometrically using Roche reagents on a Cobas Mira analyzer (Roche Diagnostics, Somerville, NJ, USA). In both studies, plasma glucose and triglyceride concentrations were measured at the end of the wash out period (baseline) and at the end of the 4-week phage (plasmid) period. Glucose and triglyceride responses to HCTZ were defined as the difference between the levels at the final and the baseline visits. Individuals with response values under or over 3 standard errors from the mean response were removed from the analysis. Plasma insulin, which was used as a covariate during statistical analyses, was measured using the Access Ultrasensitive Insulin immunoassay system (Beckman Instruments, Chaska, MN, USA).

In the PEAR study, individuals were genotyped on the Illumina HumanOmni1-Quad (Illumina, San Diego, CA, USA). GERA participants from the opposite extremes of the DBP response distribution were genotyped using the GeneChip Human Mapping 500k Array (Affymetrics, Santa Clara, CA, USA) using standard procedures. As part of routine quality control steps, single-nucleotide polymorphisms (SNPs) with minor allele frequency <1%, call rate <95%, Hardy–Weinberg equilibrium P-values >10^−5 and individuals with >10% missing genotypes were removed from the analysis. The software MACH 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 for the imputed results was done using standard procedures (RSQ_HAT <0.3 and minor allele frequency 0.05). MACH generated a file with the highest posterior probabilities for each imputed SNP, which was used in the analysis. After quality control and imputation, there were >2 million SNPs used for the genotype-phenotype association studies in each race-study group.

RESULTS
Baseline characteristics of the European-American and African-American study participants are shown in Tables 1a and 1b, which shows the relative similarity in participant characteristics between the two studies. In both PEAR and GERA, there were a higher percentage of European-American male participants, while for African Americans there were a higher percentage of females. Average age and body mass index were nearly identical in PEAR and GERA. Baseline triglycerides were higher in European Americans from GERA compared with the other groups. Response to HCTZ treatment are shown in Table 2, and document that HCTZ significantly increased glucose and triglyceride in both race groups in both studies.

Table 1. PEAR and GERA sample characteristics measured at baseline

<table>
<thead>
<tr>
<th>Variables</th>
<th>PEAR: European Americans</th>
<th>GERA: European Americans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>229</td>
<td>196</td>
</tr>
<tr>
<td>Gender (% men)</td>
<td>59.8</td>
<td>57.1</td>
</tr>
<tr>
<td>Age (years)</td>
<td>49.9 ± 9.4</td>
<td>48.5 ± 7.3</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>94.9 ± 13.6</td>
<td>103.7 ± 14.7</td>
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<tr>
<td>Insulin (μU ml⁻¹)</td>
<td>9.4 ± 8.7</td>
<td>8.5 ± 4.9</td>
</tr>
<tr>
<td>Plasma glucose (mg dl⁻¹)</td>
<td>92.9 ± 12.2</td>
<td>92.4 ± 12.5</td>
</tr>
<tr>
<td>Plasma triglyceride (mg dl⁻¹)</td>
<td>149.6 ± 100.9</td>
<td>184.3 ± 97.55</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>151.8 ± 13.5</td>
<td>142.6 ± 12.6</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>97.3 ± 5.8</td>
<td>95 ± 5.7</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Variables</th>
<th>PEAR: African Americans</th>
<th>GERA: African Americans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>148</td>
<td>194</td>
</tr>
<tr>
<td>Gender (% men)</td>
<td>37.84</td>
<td>48.5</td>
</tr>
<tr>
<td>Age (years)</td>
<td>47.4 ± 8.9</td>
<td>47.4 ± 6.3</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>97.3 ± 13.5</td>
<td>96.8 ± 14.4</td>
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<tr>
<td>Insulin (μU ml⁻¹)</td>
<td>9.0 ± 9.6</td>
<td>12.9 ± 10.1</td>
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<tr>
<td>Plasma glucose (mg dl⁻¹)</td>
<td>90.8 ± 12.7</td>
<td>97.4 ± 14.4</td>
</tr>
<tr>
<td>Plasma triglyceride (mg dl⁻¹)</td>
<td>105.84 ± 93.98</td>
<td>122.42 ± 57.52</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>151.8 ± 13.4</td>
<td>150.8 ± 14.9</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>97.9 ± 5.8</td>
<td>97.2 ± 5.5</td>
</tr>
</tbody>
</table>

Abbreviations: GERA, Genetic Epidemiology of Responses to Antihypertensive; PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses.
GWAS of adverse effect of HCTZ on glucose

The meta-analysis of glucose change during HCTZ treatment revealed no SNPs that achieved the a priori definition of genome-wide significance in either European Americans or African Americans. The Manhattan plots and QQ plots for these analyses are shown in the online Supplementary Figures S1 and S3 (Supplementary Information) However, in European Americans 48 SNPs from 8 genomic regions achieved our definition of suggestive significance with $P < 10^{-5}$ having effects in the same direction in both studies. Similarly, in African Americans 61 SNPs in 12 genomic regions met these criteria. Each of these SNPs is summarized in Supplementary Tables S1.1, S1.2 and S3.1, S3.2 (Supplementary Information).

GWAS of adverse effect of HCTZ on triglycerides

Among African Americans, two SNPs (rs12279250 and rs4319515 ($r^2 = 0.73$)) on chromosome 11, reached genome-wide significance ($\beta = 28\text{ mg dl}^{-1}, P = 6.6 \times 10^{-9}$ and $\beta = 27\text{ mg dl}^{-1}, P = 4.05 \times 10^{-8}$, respectively). The Manhattan plot is shown in Figure 1, and Figure 2 displays the triglyceride response by genotype in both PEAR and GERA. These SNPs are in the NELL1 gene, whose encoded protein is involved in adipose cell differentiation.25 No SNPs achieved genome-wide significance for triglyceride response among European Americans.

As was the case with the HCTZ-induced change in glucose, the triglyceride response also had many SNPs that met our suggestive significance threshold. In European Americans, 25 SNPs in 10 genomic regions met the threshold of $P < 10^{-5}$ with a same direction effect, with 77 SNPs in 27 genomic regions meeting this threshold in African Americans. Many of these SNPs were genes previously associated with metabolic syndrome, diabetes, insulin and other metabolic traits. These SNPs are described in detail in Supplementary Tables S2.1, S2.2 and S4.1, S4.2 (Supplementary Information).

**DISCUSSION**

We conducted a GWAS and meta-analysis of adverse metabolic responses to HCTZ from two studies of hypertensive patients. We focused on change in glucose and triglycerides, which are known adverse effects of thiazide diuretics and may partially offset the antihypertensive benefit of HCTZ.3 Among African Americans, two SNPs in NELL1 achieved genome-wide significance for their association with triglyceride response. NELL1 protein has osteoinductive properties26 and has been found to repress adipogenic differentiation in both unipotent preadipocytes and in multipotent adipose-derived stromal cells.25 The regulation of adipose stores through differentiation is tightly controlled, with adverse metabolic consequences of disordered fat storage.

<table>
<thead>
<tr>
<th>Variables</th>
<th>PEAR: European Americans</th>
<th>GERA: European Americans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose response (mg dl$^{-1}$)</td>
<td>1.44 ± 8.9 ($P = 0.01$)</td>
<td>3.03 ± 7.6 ($P &lt; 0.0001$)</td>
</tr>
<tr>
<td>Plasma triglyceride response (mg dl$^{-1}$)</td>
<td>8.21 ± 58.7 ($P = 0.2$)</td>
<td>14.57 ± 59.9 ($P &lt; 0.0001$)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variables</th>
<th>PEAR: African Americans</th>
<th>GERA: African Americans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose response (mg dl$^{-1}$)</td>
<td>2.55 ± 10.8 ($P &lt; 0.005$)</td>
<td>4.3 ± 9.5 ($P &lt; 0.0001$)</td>
</tr>
<tr>
<td>Plasma triglyceride response (mg dl$^{-1}$)</td>
<td>7.95 ± 40.6 ($P = 0.02$)</td>
<td>9.56 ± 40.3 ($P &lt; 0.0001$)</td>
</tr>
</tbody>
</table>

Abbreviations: GERA, Genetic Epidemiology of Responses to Antihypertensive; PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses.
Herein, we report the first GWAS to our knowledge of HCTZ-induced AMEs. The strengths of this study include the population-based design, the high quality of genotyping and phenotyping, the wash out period of all antihypertensives before initiation of HCTZ, as well as detailed follow-up of study participants. At the same time, we are aware of the limitation posed by small sample sizes, especially to detect true effects size. We are also aware of possible biases resulting from inclusion of the baseline value as a covariate. Although under-powered, it is interesting to note that many of our suggestive regions have been identified through previous GWAS results as being related to type 2 diabetes, obesity, fatty acid metabolism, or body mass index or to other a priori identified biologic candidate genes (please see http://hyper.ahajournals.org). However, the associations we have identified are novel in that they have not previously been related to drug-induced adverse metabolic side effects. These findings may help provide insight into the mechanisms of antihypertensive-induced AMEs. It will be important to replicate these findings identified through meta-analysis in independent populations of individuals treated with thiazide diuretics.

CONFLICT OF INTEREST

The authors declare no conflict of interest.
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