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Lack of association between polymorphisms in *STK39*, a putative thiazide response gene, and blood pressure response to hydrochlorothiazide

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Abstract

STK39 was previously implicated as a hypertension susceptibility gene and is thought to be involved in control of Na+-Cl− cotransporter (NCC) activity. *STK39* has been implicated as a putative thiazide diuretic response gene, as NCC activity is inhibited by thiazides. Thus, we aimed to determine whether *STK39* is a thiazide response gene. 195 “good” and 194 “poor” responders to hydrochlorothiazide (HCTZ) were genotyped for approximately 100 single nucleotide polymorphisms (SNPs) within 5000 bases of *STK39*. SNPs meeting criteria for advancement to replication analysis (P<0.01), along with those previously associated with hypertension, were then analyzed in a second population of 201 HCTZ-treated hypertensives. Two SNPs met these criteria and were analyzed for replication. However, neither these, nor previously implicated SNPs significantly associated with blood pressure response to HCTZ. These data suggest common variants in *STK39* likely do not have a clinically relevant role in blood pressure response to HCTZ in hypertensives.

Keywords

STK39; SPAK; hypertension; diuretics; hydrochlorothiazide; blood pressure; pharmacogenetics; thiazides; single nucleotide polymorphisms

Hydrochlorothiazide (HCTZ), the second most commonly prescribed antihypertensive in the United States, inhibits Na^+–Cl^- cotransporter (NCC) activity in the renal distal convoluted tubule. NCC activation can occur by direct phosphorylation from ste20/SPS1-related

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proline/alanine rich kinase (SPAK).[1] SPAK, encoded by the gene *STK39*, is regulated by the "with no lysine" (WNK) kinases. WNK1 has been shown to cause an increase in NCC activity, and WNK4, a decrease in activity.[2,3] Recently, a genome-wide association study published by Wang, et. al. implicated *STK39* as a hypertension susceptibility gene in Caucasians.[4] Two SNPs (rs6749447 and 3754777) were initially associated with blood pressure (BP), and these associations were also found in three other Caucasian replication cohorts.[4] Functional studies revealed a third SNP (rs35929607), hypothesized to be a better functional candidate SNP than rs6749447 or rs3754777.[4] Importantly, Wang and colleagues postulate *STK39* is a thiazide response gene.[4] Based on the proposed mechanism by which SPAK may affect blood pressure via modulation of NCC activity, sequence variation in *STK39* could affect patient response to HCTZ and other thiazide diuretics. Moreover, the WNK-SPAK-NCC pathway has been previously implicated in thiazide response, as variations in WNK1 were associated with differential BP response to HCTZ.[5] The objective of this study was to test the association between *STK39* variation and BP response to HCTZ in hypertensive participants.

The discovery population was the Genetic Epidemiology of Responses to Antihypertensives (GERA), a two-center clinical trial designed to determine whether polymorphisms in reninangiotensin-aldosterone system genes are predictive of BP response to HCTZ.[6] Samples (N=585) consisted of non-Hispanic Caucasian and African-American (AA) hypertensive participants (blood pressure >140/90 mmHg or a previous diagnosis of essential hypertension and current antihypertensive prescription) between the ages of 30 and 59. All study participants had a BP ≥140/90 or had been diagnosed as hypertensive by their primary care physician. After a minimum four-week antihypertensive drug washout period, participants were included if diastolic BP was ≥90 mmHg. After baseline BP assessment, participants received four weeks of treatment with HCTZ 25 mg daily, followed by a final BP assessment. The difference in treated and untreated BP represented their BP response. Participants were counseled to maintain sodium intake at approximately 2 mmol/kg/day, which was confirmed by periodic sodium measurement in 24-hour urine samples or 24-hour dietary recall diaries. All BP assessments were measured in duplicate in the clinic setting.

The replication population was the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR), an ongoing, multi-center clinical trial examining the role of genetic variability on blood pressure response to HCTZ and/or atenolol.[7] Like GERA, all PEAR participants had been previous diagnosed as hypertensive and were between 17 and 65 years of age. After a mean 4 week washout, participants were required to have an average home DBP ≥85 mmHg (based on at least 30 readings in the previous week) and office DBP ≥90 mmHg for inclusion. Included participants were randomized to either HCTZ 12.5 mg daily or atenolol 50 mg daily, with most receiving a dose escalation to 25 mg and 100 mg, respectively for BP >120/70 mmHg. After nine weeks, both clinic and home BP responses were assessed. While participants were given no specific sodium intake instructions, they were instructed to avoid changes to their diet during the study. The first 201 AA and Caucasian participants randomized to HCTZ monotherapy (approximately 98% of which received dose escalation to 25 mg) are included in this report.

Genomic DNA was obtained via blood samples. In GERA, top and bottom tertiles, defined as "good" (N=195; mean DBP response: −16.0 mmHg) and "poor" (N=194; mean DBP response: −0.7 mmHg) responders, each group with approximately equal numbers of AA and Caucasian were genotyped as described previously.[8] Briefly, these subjects were genotyped using the GeneChip Human Mapping 100K and 500K Array Sets, (Affymetrix, Santa Clara, CA, USA). Genotypes for SNPs within 5kb of *STK39* were investigated herein. In PEAR, genotypes for rs6749447 and rs3754777 were determined using the Human CVD BeadChip (Illumina, San Diego, CA, USA).[9] Both rs35929607 and rs10497338 were

genotyped in PEAR and the middle tertile of GERA by pyrosequencing using the PSQ HS 96 genotyping platform (Biotage AB, Uppsala, Sweden). Hardy-Weinberg equilibrium was calculated in both populations separately by race using a chi-squared goodness-of-fit test.

Our study design used GERA as the discovery population and PEAR as the replication population (Figure). Because of the design of the GERA genome-wide association study (i.e. genotyping was limited to the tails of the DBP distribution), logistic regression, assuming an additive model, was used to analyze the association between *STK39* polymorphisms relative to DBP "responder" status. Analyses were done within race and sex, adjusting for age and baseline BP. Power calculations indicated using all 294 Caucasian participants in GERA and α=0.01, we had 80% to detect a 3.4 mmHg difference in DBP response (3.5 mmHg in 291 AAs) between genotype groups in SNPs with a minor allele frequency of 0.15, which 81% of the screened SNPs possessed (70% in AAs). Those SNPs which associated (P<0.01) in screening were confirmed in the total GERA population by analysis of variance (ANOVA) within race, adjusting for age, gender, and baseline BP. Any SNP associations confirmed in GERA by maintaining P<0.01 were moved to PEAR for replication. Because of their previously recognized putative function, SNPs implicated in hypertension by Wang, et. al. were also analyzed in PEAR regardless of association in GERA. In PEAR, influences of *STK39* genotype on home BP response, adjusted for age, gender, and baseline BP were tested by ANOVA. To be considered a replication, SNP associations in PEAR were required to have a $P \leq 0.05$ and be in the same direction as those found in GERA. Statistical analyses were conducted using SAS version 9.2 (SAS Institute, Cary, NC, USA).

Demographics were similar between the GERA population and PEAR HCTZ monotherapy population (Supplementary Table). All genotype frequencies were in Hardy-Weinberg equilibrium. In GERA, 111 SNPs were available for screening in AA participants (N=194) and 100 SNPs in Caucasian participants (N=195). SNPs implicated by Wang, et. al. (rs6749447, rs3754777, and rs35929607) did not have any significant association with BP response to HCTZ in GERA. While no SNPs in AAs met initial screening criteria allowing analysis in the total GERA population, two SNPs in Caucasians were identified: rs10497338 (P=0.0089) and rs1356373 (P=0.0089). For rs10497338, odds ratios indicated increased likelihood for being a "good" responder with each copy of T allele, compared to CC homozygous (Table 1a). In both AAs and Caucasians, rs10497338 and rs1356373 were in perfect linkage disequilibrium (r^2=1), so only rs10497338 was analyzed in the entire GERA population. The associations in SBP and DBP response achieved nominal P<0.05, but not the reduced P<0.01 pre-specified to move forward to PEAR (Table 1b). However, to ensure this SNP's status with BP response, we also tested in PEAR, where no association was observed (Table 1b). In PEAR, 116 Caucasian and 85 AA subjects were genotyped. Those SNPs implicated by Wang, et. al. (rs6749447, rs3754777, and rs35929607) were analyzed, but none significantly associated with home BP response to HCTZ (P-values >0.20). As with home BP response, no significant association was found with clinic BP response in PEAR.

To our knowledge, this is the first study investigating the pharmacogenetic role of *STK39* polymorphisms in thiazide BP response. Our data provide no evidence for clinically significant associations between SNPs in this gene region and BP response to a thiazide diuretic. While two SNPs (rs1356373 and rs10497338) met screening criteria for advancement into the total GERA population, these SNPs did not sustain the predefined threshold level for significance in GERA or PEAR. More importantly, SNPs previously implicated in hypertension by Wang, et. al. (rs6749447, rs3754777, and rs35929607) did not significantly associate with thiazide BP response in GERA or PEAR.

Rather than using a Bonferroni correction, a more liberal *P*-value was used in screening of *STK39* in GERA because the gene was previously associated with hypertension and is a hypothesized thiazide response gene. Small effects could be missed if a more conservative *P*-value would have been used. However, replication in a second independent population was needed to validate any initial findings and guard against false positives. In fact, requiring a *P*-value <0.01 in the total GERA analysis and replication in the same direction in PEAR with a *P*-value ≤0.05 results in a more stringent *P*-value requirement (0.01*0.05*0.5=0.00025) than a single analysis with a Bonferroni correction (0.05/100=0.0005).[10]

In addition, the availability of genotype data on extreme phenotypes in GERA helped filter out the imprecision of BP response. However, one-third of patient data is excluded using this method, so to avoid this data loss, associations were confirmed using the whole GERA population with identical statistical analyses as those used in PEAR. GERA also proved to be a useful discovery population because subjects were asked to maintain a uniform sodium intake, which would help control any possible confounding by the effect of dietary sodium on BP. In fact, Wang and associates noted that association between *STK39* and BP was stronger when sodium intake was standardized.[4] Despite these theoretical benefits, no significant associations in GERA were detected.

One limitation that may have prevented us from observing associations involves effect size. Because BP regulation is polygenic, a possibility exists that small genetic effects went undetected. However, as we reported, we were powered to detect clinically significant effects in SNPs with minor allele frequencies (MAFs) of ≥0.15. Moreover, with MAFs of ≥0.25 in Caucasians, which rs10497338, rs6749447, and rs35929607 possessed, we had 80% power to detect a difference of 2.9 mmHg DBP. Although we may have lacked the power to detect effect sizes smaller than 2.9 mmHg, the clinical use of such a small difference may be difficult to measure in a single patient treated with HCTZ. Additionally, we cannot rule out that rare variants with larger effects could impact BP response to HCTZ, but very large populations would be needed to detect these rare variants. While it could also be possible that SNPs unknown at this point are associated with HCTZ response, the SNPs most likely to be associated – those previously implicated in hypertension – were all genotyped, regardless of their presence on the Affymetrix platforms used for screening. Thus, we conclude it is likely no appreciable clinically relevant associations exist between common *STK39* variants and BP response to HCTZ. However, future studies should be undertaken in larger populations to confirm the absence of smaller pharmacogenetic effects or high-impact rare variants in this gene.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Richardson C, Rafiqi FH, Karlsson HK, Moleleki N, Vandewalle A, Campbell DG, et al. Activation of the thiazide-sensitive Na+-Cl− cotransporter by the WNK-regulated kinases SPAK and OSR1. J Cell Sci 2008;121:675–84. [PubMed: 18270262]
2. Moriguchi T, Urushiyama S, Hisamoto N, Iemura S, Uchida S, Natsume T, et al. WNK1 regulates phosphorylation of cation-chloride-coupled cotransporters via the STE20-related kinases, SPAK and OSR1. J Biol Chem 2005;280:42685–93. [PubMed: 16263722]
3. Kahle KT, Ring AM, Lifton RP. Molecular physiology of the WNK kinases. Annu Rev Physiol 2008;70:329–55. [PubMed: 17961084]
4. Wang Y, O'Connell JR, McArdle PF, Wade JB, Dorff SE, Shah SJ, et al. From the Cover: Whole-genome association study identifies STK39 as a hypertension susceptibility gene. Proc Natl Acad Sci U S A 2009;106:226–31. [PubMed: 19114657]
5. Turner ST, Schwartz GL, Chapman AB, Boerwinkle E. WNK1 kinase polymorphism and blood pressure response to a thiazide diuretic. Hypertension 2005;46:758–65. [PubMed: 16172412]
6. Chapman AB, Schwartz GL, Boerwinkle E, Turner ST. Predictors of antihypertensive response to a standard dose of hydrochlorothiazide for essential hypertension. Kidney Int 2002;61:1047–55. [PubMed: 11849460]
7. 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;157:442–9. [PubMed: 19249413]
8. 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;52:359–65. [PubMed: 18591461]
9. Keating BJ, Tischfield S, Murray SS, Bhangale T, Price TS, Glessner JT, et al. Concept, design and implementation of a cardiovascular gene-centric 50 k SNP array for largescale genomic association studies. PLoS ONE 2008;3:e3583. [PubMed: 18974833]
10. McPherson R, Pertsemlidis A, Kavaslar N, Stewart A, Roberts R, Cox DR, et al. A common allele on chromosome 9 associated with coronary heart disease. Science 2007;316:1488–91. [PubMed: 17478681]

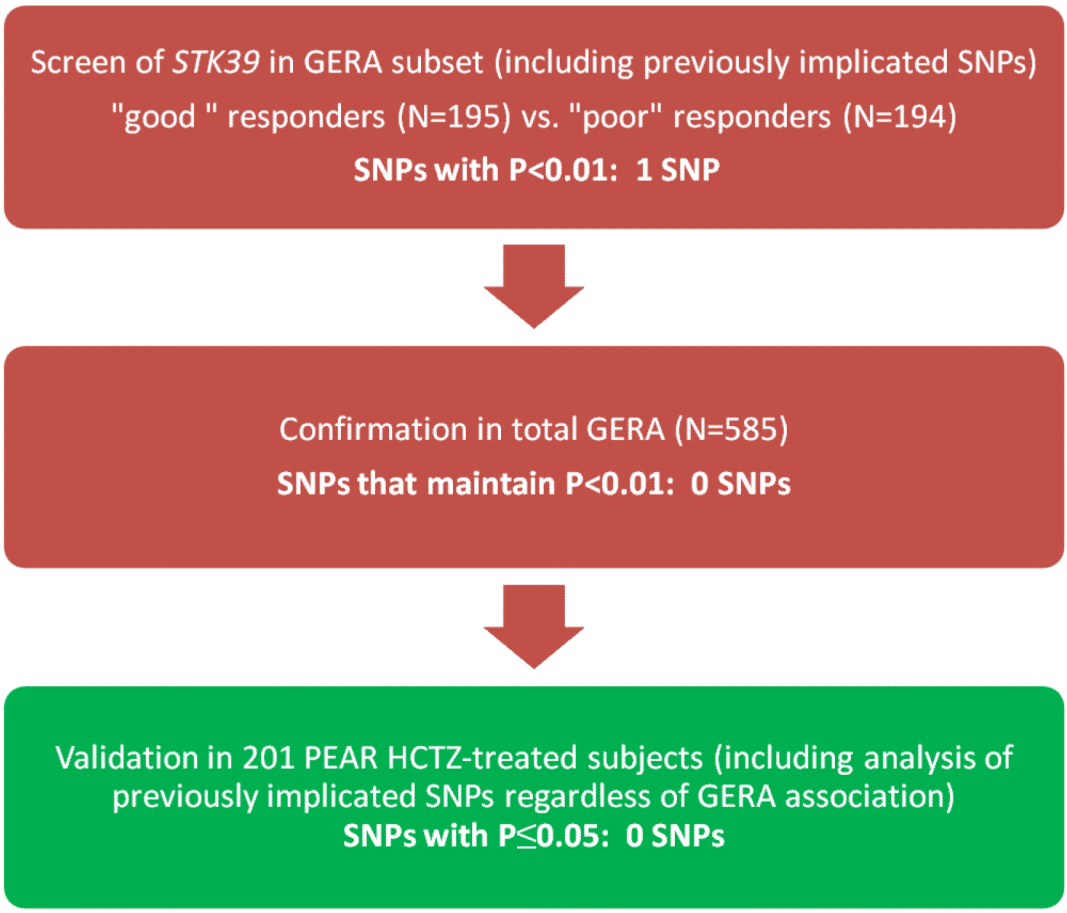

Figure 1.
Study design for identification and replication of *STK39* variants associated with BP response to HCTZ.

Table 1a

Associations between rs10497338 and good (vs. poor) DBP responder status in GERA.

	N	MAF	Odds Ratio (95% Confidence Interval)	P
Caucasian	195	0.483 (T)	1.83 (1.16 – 2.88)	**0.009**
AA	194	0.260 (C)	0.76 (0.46-1.25)	0.280

Table 1b

Mean BP response in total GERA and PEAR populations by rs10497338 genotype.

	Genotype	GERA				PEAR				
		N	Δ SBP	*P*	Δ DBP	*P*	N	Δ SBP	*P*	Δ DBP	*P*
Caucasian	C/C	58	−7.6 ± 10.6	0.028	−5.0 ± 7.4	0.016	28	−8.2 ± 6.7	0.482	−5.6 ± 4.4	0.074
	C/T	154	−11.8 ± 12.2		−6.4 ± 7.6		49	−8.7 ± 9.3		−4.8 ±5.1	
	T/T	61	−10.9 ± 12.7		−7.8 ± 7.5		36	−8.9 ± 7.7		−3.7 ± 5.0	
African American	C/C	12	−17.3 ± 11.7	0.891	−10.3 ± 8.9	0.326	1	−2.20 ± *	0.677	−4.10 ± *	0.737
	C/T	104	−17.6 ± 13.1		−10.0 ± 8.0		38	−11.9 ± 9.3		−7.40 ± 7.25	
	T/T	158	−17.8 ± 11.7		−9.1 ± 9.1		42	−13.3 ± 10.6		−7.84 ± 6.82	

Odds ratios are reported as ratio of responder vs. non-responder. Values are listed as mean ± SD. Models are adjusted for baseline BP, age, and gender.

* - No SD available (N=1).