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Targeted Sequencing Identifies Missense variant in the *BEST3* gene associated with Antihypertensive Response to Hydrochlorothiazide

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Abstract

Chromosome 12q15 was identified in Genetic Epidemiology of Response Assessment (GERA) and replicated in Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) for its association with blood pressure (BP) response to hydrochlorothiazide (HCTZ). However, the functional variant is unknown and we aimed to identify the likely functional variants through targeted sequencing. The chromosome 12q15 region was sequenced in 397 best and worst responders to HCTZ in PEAR (N=199) and GERA (N=198) hypertensive study participants. Logistic regression was used for the association analysis adjusting for age, gender, race and principal components 1 and 2. For validation, the significant SNP was tested for association with the change in systolic (SBP) and diastolic BP (DBP) post treatment in the entire PEAR (N=370) and GERA (N=570) cohorts. A novel missense polymorphism (G>A, Pro383Leu) in *BEST3*, rs61747221, was significantly associated with better HCTZ response ($p=0.0021$, OR=2.05). It was validated in the entire cohort of PEAR (SBP: $p=0.021$, $\beta=-1.60$, DBP:

$p=0.023$, $\beta=-1.08$) and GERA (SBP: $p=0.028$, $\beta=-1.95$, DBP: $p=0.032$, $\beta=-1.28$). *BEST3* encodes the calcium sensitive chloride channel in the vascular smooth muscle implicated in regulation of BP, especially in response to vasoconstrictors like angiotensin II. These results suggest that *BEST3* is involved in the chronic BP lowering mechanism of thiazides and highlight its importance as a genetic predictor of the BP response to thiazide diuretics.

Introduction:

Hydrochlorothiazide(HCTZ) is the most commonly prescribed thiazide in the US. However, there is inter-individual variability in blood pressure(BP) response to HCTZ, and genetics may contribute to this variability^{1, 2}

Two pharmacogenomic studies from our laboratories – The Genetic Epidemiology of Response Assessment(GERA) and Pharmacogenomic Evaluation of Anti-hypertensive Response(PEAR) have investigated the genetic markers associated with differential BP response to HCTZ treatment. GERA identified a three-nucleotide haplotype(rs317689/rs315135/rs7297610) present on chromosome12q15 that was significantly associated with better BP response to HCTZ treatment³ and was further replicated in the PEAR⁴. Even though these studies suggested the potential involvement of this novel region in the HCTZ mediated BP response, they did not identify any functional polymorphisms that could explain the mechanism underlying the association. Moreover, the mechanism of the novel genes identified in this region in BP regulation is not fully understood. With the goal of identifying the functional polymorphism associated with HCTZ drug response in the chromosome12 locus we performed targeted sequencing to capture all the polymorphisms in this region.

Methods:

Study design:

PEAR and GERA(clinicaltrials.gov identifier:NCT00246519 and NCT00005520) studies^{5, 6} were aimed at assessing the genomic variability attributed to BP following treatment with atenolol or HCTZ and the combination in PEAR and HCTZ in GERA. For both, participants underwent a washout period to re-establish hypertensive BPs before starting the drug treatment. Blood samples were collected, along with BP measurement at the end of washout(baseline) and following treatment with HCTZ (PEAR:9 weeks, GERA:4 weeks). The difference in the BP from baseline to post treatment was defined as delta systolic BP(SBP) and delta diastolic BP(DBP).

Phasel:

A total of 361 participants (119 European Americans(EA) and 242 African Americans(AA), from PEAR(n=196) and GERA(n=165), were selected based on the top and bottom quartiles of the distribution of the DBP response to HCTZ such that half of the participants in PhaseI comprised of the best responders to HCTZ(cases) and the other half comprised of poor responders to HCTZ(controls). These participants were sequenced for chr12q15 locus (hg19 coordinates:69357185–70047375) that included *CPFS6*, *LYZ*, *YEATS4*, *FRS2*, *CCT2*,

LRRC10 and 3' end of *BEST3*. Case-control association analysis was conducted in PLINK1.07^{7,8} using logistic regression adjusting for age, gender, race and principal components 1 and 2.

PhaseII:

The candidate polymorphism identified through PhaseI sequencing was in the tail end of the targeted region, in *BEST3*. This gene had not been annotated at the time PhaseI sequencing was planned and only the 3' distal part of this gene was captured. The PhaseII sequencing effort was undertaken to capture the entire *BEST3* and adjacent *RAB3IP* (hg19 coordinates: 70,047,349–70216949) to insure no additional SNPs with stronger associations in this region were present. PhaseII included 397 participants (355 participants were in PhaseI with an additional 42 participants in PhaseII). Similar to Phase I, the 397 participants (154 EA and 243 AA) came from both PEAR(n=200) and GERA(n=197) consisting of responders (n=202) and non-responders (n=195). All the participants of PhaseII were genotyped for the candidate polymorphism identified in Phase I, for inclusion in the PhaseII analysis. Case-control association analysis like PhaseI was performed.

Detailed sequencing methods have been outlined in the Supplementary Methods.

Validation:

The candidate polymorphism identified from PhaseI and PhaseII was further validated by genotyping the entire population of GERA(N=571) and PEAR(N=370) for the variant and testing for association with SBP and DBP. Analysis was performed in SAS9.4 using linear regression for SBP and DBP adjusting for the same covariates as before.

In-silico analysis:

In-silico analysis that predicts the effect of variants on protein function using two bioinformatics tools – PROVEAN and SIFT(<http://provean.jcvi.org/index.php>), was used to assess rs61747221.

Results:

The clinical characteristics of PhaseI and PhaseII participants are described in Table1. Association analysis for PhaseI revealed several SNPs to be associated with BP response to HCTZ(Table S1). To investigate functional variants, we further focused only on coding region variants(Table S2). No new functional variants were identified in LD with the originally discovered haplotype (rs317689/rs315135/rs7297610). However we did identify rs61747221, a missense variation in *BEST3*, which was the most significant potentially functional SNP associated with BP response to HCTZ in the PhaseI analysis (p-value=0.0021, OR=2.056, 95% confidence interval=1.298,3.286)(Figure1). Post PhaseII sequencing, which captured the entire *BEST3* and *RAB3IP*, a total of 108,853 variants were detected of which 3,349 were novel and classified as: stop gain/stop loss-1, frameshift-17, missense-133, synonymous-682, intronic-84,178, intergenic-9,574 and others including UTRs and ncRNAs-14,268.

In PhaseII, rs61747221 remained the most significant SNP in the region associated with HCTZ BP response (p-value=0.010, OR=1.76, confidence interval=1.131,2.752) (Figure 1). For further validation, we tested this association in the entire cohort of PEAR(N=370) and GERA (N=570) treated with HCTZ. Rs61747221 remained associated with BP response in PEAR (SBP:p=0.021,β=-1.60mmHg, DBP:p=0.023,β=-1.08mmHg) and GERA (SBP:p=0.028,β=-1.95mmHg, DBP:p=0.032,β=-1.28 mmHg). For assessing the magnitude of effect of rs61747221 on BP response, we grouped the carriers of the variant(AA+AG) together in a dominant model due to the low sample size of the homozygote variant group(Minor allele frequency=0.13). Rs61747221 was significantly associated with BP response in a dominant model as well (PEAR- SBP: β=-1.96mmHg, DBP: β=-1.39mmHg and GERA- SBP: β=-2.24mmHg, DBP: β=-1.46mmHg). A-allele carriers of rs61747221 had better BP response to HCTZ compared to the non-carriers in all analyses(Figure 2). Furthermore, though PROVEAN predicted this variant as “tolerant” SIFT predicted this missense variant, rs61747221 to have an “intolerant/damaging” effect on BEST3 protein(SIFT prediction score=0.019).

Discussion:

With the aim of identifying the functional polymorphism in the previously identified chromosome12 locus, we undertook targeted resequencing of this region. We identified a novel missense SNP, rs61747221 in the *BEST3* that was significantly associated with better BP response to HCTZ treatment. Rs61747221 was not in LD with the original 3-SNP haplotype that was previously identified, thus the functional variation or mechanism underlying the association with this haplotype(rs317689/rs315135/rs7297610) remains unclear.

However, through the resequencing effort we did identify a novel missense variant rs61747221 in *BEST3*, which was the most significant association among the coding variants, associated with increased BP response in the PhaseI sequencing of the chr12q15 locus. Since only the distal 3' region of *BEST3* was captured in PhaseI sequencing, PhaseII sequencing with an extended cohort of participants was undertaken, which captured *BEST3* and *RAB3IP* completely. Upon PhaseII analysis, rs61747221 was still the most significantly associated coding variant with HCTZ BP response. Further validation came with the successful documentation of the association between BP response and rs61747221 in the entire cohort of PEAR and GERA, when genotyped for this variant and analyzed for association with SBP and DBP. In each analysis the variant carriers had better BP response to the HCTZ versus the non-carriers.

Rs61747221(G>A) is a missense polymorphism in *BEST3* resulting in proline to leucine substitution at position 383(c.1148C>T and p.Pro383Leu). Bestrophin-3(BEST3) is a transmembrane protein that acts as a calcium-activated chloride channel^{11, 12} and is widely expressed in the heart, smooth muscle cells, endothelium, and kidney^{13–16}. Studies have shown the involvement of calcium-activated chloride channels in the regulation of smooth muscle contraction^{17–20}. Additionally, downregulation of *BEST3* is associated with a significant reduction in cyclic GMP-dependent protein kinase¹⁴ which is associated with vascular smooth muscle relaxation via its effect on calcium-activated potassium

channels^{21, 22}. Interestingly, previous studies have hypothesized that thiazide diuretics chronic BP lowering mechanism might be mediated via the opening of the calcium-activated potassium channels²³. Taken together, these data suggest that *BEST3* might be involved in the chronic BP lowering mechanism underlying thiazide diuretics via their effect on the vascular smooth muscle regulation. Rs61747221 is eQTL for *RAB3IP*(Heart_Atrial_Appendage,adrenal glands GTEx_v7)²⁴ and may have an plausible role to play in the BP response to HCTZ. RAB3IP is a guanylyl exchange factor and has been reported to be downregulated in the prefrontal cortex of hypercholesteremic plus hypertensive rabbits²⁵ and in the peripheral blood mononuclear cells of high altitude pulmonary hypertension susceptible cattle²⁶. However no specific studies have so far implicated the involvement of this gene in BP regulation or related pathways.

We acknowledge that our study has limitations, primarily the lack of an independent replication cohort. However, rs61747221 is present in 1000G reference panel and to our knowledge all the previous GWAS studies aimed at determining genetic determinants of HCTZ BP response have used HAPMAPII reference panel which does not include this SNP^{27–29}. This is possibly the reason why this SNP has not been reported by other GWAS's and we were able to discover this association only through our deep sequencing efforts. We have, however, attempted to provide additional evidence in support of rs61747221 by validating this association in two larger cohorts albeit with some overlap in the patient population between discovery and validation. Furthermore, SIFT pathogenicity prediction predicts rs61747221 to have deleterious effect on BEST3 protein, further strengthening this association. However, this association though validated in a larger cohort needs to be tested for replication in an independent cohort. In conclusion, these results highlight the importance of *BEST3* and rs61747221 as a potential genetic marker associated with BP response to HCTZ and elucidation of the exact mechanism of *BEST3* and the underlying functional role of rs61747221 can help advance our current understanding of HCTZ mediated long term BP control.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References:

1. Butler MG, Genetics of hypertension. Current status. J Med Liban 2010, 58 (3), 175–8. [PubMed: 21462849]
2. Cooper-DeHoff RM; Johnson JA, Hypertension pharmacogenomics: in search of personalized treatment approaches. Nat Rev Nephrol 2015.
3. Turner ST; Bailey KR; Fridley BL; Chapman AB; Schwartz GL; Chai HS; Sicotte H; Kocher JP; Rodin AS; Boerwinkle E, Genomic association analysis suggests chromosome 12 locus influencing antihypertensive response to thiazide diuretic. Hypertension 2008, 52 (2), 359–65. [PubMed: 18591461]

4. Duarte JD; Turner ST; Tran B; Chapman AB; Bailey KR; Gong Y; Gums JG; Langaee TY; Beitelshes AL; Cooper-Dehoff RM; Boerwinkle E; Johnson JA, Association of chromosome 12 locus with antihypertensive response to hydrochlorothiazide may involve differential YEATS4 expression. *Pharmacogenomics J* 2013, 13 (3), 257–63. [PubMed: 22350108]
5. Johnson JA; Boerwinkle E; Zineh I; Chapman AB; Bailey K; Cooper-DeHoff RM; Gums J; Curry RW; Gong Y; Beitelshes AL; Schwartz G; Turner ST, Pharmacogenomics of antihypertensive drugs: rationale and design of the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) study. *Am Heart J* 2009, 157 (3), 442–9. [PubMed: 19249413]
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 (3), 1047–55. [PubMed: 11849460]
7. Purcell S; Neale B; Todd-Brown K; Thomas L; Ferreira MA; Bender D; Maller J; Sklar P; de Bakker PI; Daly MJ; Sham PC, PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007, 81 (3), 559–75. [PubMed: 17701901]
8. Chang CC; Chow CC; Tellier LC; Vattikuti S; Purcell SM; Lee JJ, Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience* 2015, 4, 7. [PubMed: 25722852]
9. Challis D; Yu J; Evani US; Jackson AR; Paithankar S; Coarfa C; Milosavljevic A; Gibbs RA; Yu F, An integrative variant analysis suite for whole exome next-generation sequencing data. *BMC Bioinformatics* 2012, 13, 8. [PubMed: 22239737]
10. Li H; Durbin R, Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 2009, 25 (14), 1754–60. [PubMed: 19451168]
11. Huang F; Wong X; Jan LY, International Union of Basic and Clinical Pharmacology. LXXXV: calcium-activated chloride channels. *Pharmacol Rev* 2012, 64 (1), 1–15. [PubMed: 22090471]
12. Tsunenari T; Sun H; Williams J; Cahill H; Smallwood P; Yau KW; Nathans J, Structure-function analysis of the bestrophin family of anion channels. *J Biol Chem* 2003, 278 (42), 41114–25. [PubMed: 12907679]
13. O'Driscoll KE; Hatton WJ; Burkin HR; Leblanc N; Britton FC, Expression, localization, and functional properties of Bestrophin 3 channel isolated from mouse heart. *Am J Physiol Cell Physiol* 2008, 295 (6), C1610–24. [PubMed: 18945938]
14. Matchkov VV; Larsen P; Bouzinova EV; Rojek A; Boedtker DM; Golubinskaya V; Pedersen FS; Aalkjaer C; Nilsson H, Bestrophin-3 (vitelliform macular dystrophy 2-like 3 protein) is essential for the cGMP-dependent calcium-activated chloride conductance in vascular smooth muscle cells. *Circ Res* 2008, 103 (8), 864–72. [PubMed: 18776041]
15. Song W; Yang Z; He B, Bestrophin 3 ameliorates TNF α -induced inflammation by inhibiting NF- κ B activation in endothelial cells. *PLoS One* 2014, 9 (10), e111093. [PubMed: 25329324]
16. Golubinskaya V; Elvin J; Ebefors K; Gustafsson H; Mallard C; Nystrom J; Nilsson H, Bestrophin-3 is differently expressed in normal and injured mouse glomerular podocytes. *Acta Physiol (Oxf)* 2015, 214 (4), 481–96. [PubMed: 25912364]
17. Dam VS; Boedtker DM; Nyvad J; Aalkjaer C; Matchkov V, TMEM16A knockdown abrogates two different Ca(2+)-activated Cl⁻ currents and contractility of smooth muscle in rat mesenteric small arteries. *Pflugers Arch* 2014, 466 (7), 1391–409. [PubMed: 24162234]
18. Broegger T; Jacobsen JC; Secher Dam V; Boedtker DM; Kold-Petersen H; Pedersen FS; Aalkjaer C; Matchkov VV, Bestrophin is important for the rhythmic but not the tonic contraction in rat mesenteric small arteries. *Cardiovasc Res* 2011, 91 (4), 685–93. [PubMed: 21498420]
19. Davis AJ; Shi J; Pritchard HA; Chadha PS; Leblanc N; Vasilikostas G; Yao Z; Verkman AS; Albert AP; Greenwood IA, Potent vasorelaxant activity of the TMEM16A inhibitor T16A(inh)-A01. *Br J Pharmacol* 2013, 168 (3), 773–84. [PubMed: 22946562]
20. Bulley S; Neeb ZP; Burris SK; Bannister JP; Thomas-Gatewood CM; Jangsangthong W; Jaggar JH, TMEM16A/ANO1 channels contribute to the myogenic response in cerebral arteries. *Circ Res* 2012, 111 (8), 1027–36. [PubMed: 22872152]
21. Archer SL; Huang JM; Hampl V; Nelson DP; Shultz PJ; Weir EK, Nitric oxide and cGMP cause vasorelaxation by activation of a charybdotoxin-sensitive K channel by cGMP-dependent protein kinase. *Proc Natl Acad Sci U S A* 1994, 91 (16), 7583–7. [PubMed: 7519783]

22. Fukao M; Mason HS; Britton FC; Kenyon JL; Horowitz B; Keef KD, Cyclic GMP-dependent protein kinase activates cloned BKCa channels expressed in mammalian cells by direct phosphorylation at serine 1072. *J Biol Chem* 1999, 274 (16), 10927–35. [PubMed: 10196172]
23. 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 (6), 793–802. [PubMed: 20528637]
24. The Genotype-Tissue Expression (GTEx) Project was supported by the Common Fund of the Office of the Director of the National Institutes of Health, a. b. N., NHGRI, NHLBI, NIDA, NIMH, and NINDS. The data used for the analyses described in this manuscript were obtained from the GTEx Portal on 07/02/2018.
25. Loke S-Y; Wong PT-H; Ong W-Y, Global gene expression changes in the prefrontal cortex of rabbits with hypercholesterolemia and/or hypertension. *Neurochemistry International* 2017, 102, 33–56. [PubMed: 27890723]
26. Newman JH; Holt TN; Hedges LK; Womack B; Memon SS; Willers ED; Wheeler L; Phillips JA; Hamid R, High-altitude pulmonary hypertension in cattle (brisket disease): Candidate genes and gene expression profiling of peripheral blood mononuclear cells. *Pulm Circ* 2011, 1 (4), 462–9. [PubMed: 22530101]
27. Hiltunen TP; Donner KM; Sarin AP; Saarela J; Ripatti S; Chapman AB; Gums JG; Gong Y; Cooper-DeHoff RM; Frau F; Glorioso V; Zaninello R; Salvi E; Glorioso N; Boerwinkle E; Turner ST; Johnson JA; Kontula KK, Pharmacogenomics of hypertension: a genome-wide, placebo-controlled cross-over study, using four classes of antihypertensive drugs. *J Am Heart Assoc* 2015, 4 (1), e001521. [PubMed: 25622599]
28. Salvi E; Wang Z; Rizzi F; Gong Y; McDonough CW; Padmanabhan S; Hiltunen TP; Lanzani C; Zaninello R; Chittani M; Bailey KR; Sarin AP; Barcella M; Melander O; Chapman AB; Manunta P; Kontula KK; Glorioso N; Cusi D; Dominiczak AF; Johnson JA; Barlassina C; Boerwinkle E; Cooper-DeHoff RM; Turner ST, Genome-Wide and Gene-Based Meta-Analyses Identify Novel Loci Influencing Blood Pressure Response to Hydrochlorothiazide. *Hypertension* 2017, 69 (1), 51–59. [PubMed: 27802415]
29. Turner ST; Boerwinkle E; O'Connell JR; Bailey KR; Gong Y; Chapman AB; McDonough CW; Beitelshes AL; Schwartz GL; Gums JG; Padmanabhan S; Hiltunen TP; Citterio L; Donner KM; Hedner T; Lanzani C; Melander O; Saarela J; Ripatti S; Wahlstrand B; Manunta P; Kontula K; Dominiczak AF; Cooper-DeHoff RM; Johnson JA, Genomic association analysis of common variants influencing antihypertensive response to hydrochlorothiazide. *Hypertension* 2013, 62 (2), 391–7. [PubMed: 23753411]

Figure 1. Adjusted odds ratio and 95% CI for rs61747221 in PhaseI and PhaseII analysis. OR- Odds Ratio, CI- Confidence Interval

Figure 2.

Plot of blood pressure response to hydrochlorothiazide by rs61747221 genotype of participants from the four analyses. The blood pressure responses are least square adjusted means that have been adjusted for baseline blood pressure levels, age, sex, race and principal components 1 and 2. The p-value represents the difference in the adjusted blood pressure means by genotype.

Table 1.

Demographics of participants of PhaseI and PhaseII

Characteristics	PHASEI (361)		PHASEII (397)	
	Responder (%)	Non-Responder (%)	Responder (%)	Non-Responder (%)
N	181(50.01)	180(49.86)	202(50.81)	195(49.11)
PEAR participants	97(27.14)	99(27.70)	103(26.19)	97(24.43)
GERA participants	84(23.26)	81(22.43)	99(24.93)	98(24.93)
African Americans	122(33.79)	120(33.24)	126(31.73)	117(29.47)
Baseline SBP (mmHg)	146.66±12.19	150.05±12.94	146.43±12.64	148.91±13.01
Baseline DBP (mmHg)	95.76±5.81	95.64±6.19	96.14±5.86	95.49±6.08
Post treatment SBP (mmHg)	125.06±10.54	145.67±13.53	124.87±10.57	145.40±13.37
Post treatment DBP (mmHg)	80.62±6.52	96.87±7.66	80.70±6.31	97.47±6.79
SBP (mmHg)	-21.78±9.72	-3.75±10.24	-21.79±8.69	-2.80±9.04
DBP (mmHg)	-15.04±6.04	1.14±5.59	-15.32±4.88	2.06±4.07

Data are presented as mean±SD; SBP – Delta systolic blood pressure, DBP – Delta diastolic blood pressure, SD – standard deviation.