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IMPACT OF *TCF7L2* SINGLE NUCLEOTIDE POLYMORPHISMS ON HYDROCHLOROTHIAZIDE-INDUCED DIABETES

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Abstract

OBJECTIVE—Thiazide diuretics have been associated with increased risk for new onset diabetes (NOD), but pharmacogenetic markers of thiazide-induced NOD are not well studied. Single nucleotide polymorphisms (SNPs) in the Transcription Factor 7-Like 2 gene (*TCF7L2*) represent the strongest and most reproducible genetic associations with diabetes. We investigated the association of tag SNPs in *TCF7L2* with thiazide-induced NOD.

METHODS—We identified cases that developed NOD and age, gender, and race/ethnicity-matched controls from the INternational VERapamil SR Trandolapril Study (INVEST). INVEST compared cardiovascular outcomes between two antihypertensive treatment strategies in ethnically diverse patients with hypertension and coronary artery disease. We genotyped 101 *TCF7L2* tag SNPs and used logistic regression to test for pharmacogenetic (SNP*hydrochlorothiazide treatment) interactions. Permuted interaction p values were corrected with the P_{ACT} test and adjusted for diabetes-related variables.

RESULTS—In INVEST whites, we observed three *TCF7L2* SNPs with significant SNP*treatment interactions for NOD. The strongest pharmacogenetic interaction was observed for rs7917983 (synergy index 3.37 [95%CI 1.72–6.59], $p=5.0 \times 10^{-4}$, $P_{ACT}=0.03$), which was associated with increased NOD risk in hydrochlorothiazide-treated patients (OR 1.53 [1.04–2.25], $p=0.03$) and decreased NOD risk in non hydrochlorothiazide-treated patients (OR 0.48 [0.27–0.86], $p=0.02$). The *TCF7L2* SNP rs4506565, previously associated with diabetes, showed a similar, significant pharmacogenetic association.

CONCLUSIONS—Our results suggest that hydrochlorothiazide treatment is an environmental risk factor that increases diabetes risk beyond that attributed to *TCF7L2* variation in white, hypertensive patients. Further study and replication of our results is needed to confirm pharmacogenetic influences of *TCF7L2* SNPs on thiazide-induced NOD.

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CONFLICT OF INTEREST

The authors declared no conflicts of interest.

Keywords

pharmacogenetics; *TCF7L2*; diabetes mellitus; hydrochlorothiazide

INTRODUCTION

Hypertension is an independent risk factor for type 2 diabetes (T2D) and a patient with both diabetes and hypertension is at increased risk of an adverse cardiovascular (CV) outcome versus a patient with hypertension alone.(1, 2) Thiazide diuretics are considered first line agents for the treatment of hypertension, but consistent evidence from randomized antihypertensive trials supports that thiazide diuretics are associated with new onset diabetes (NOD).(3–5) Because thiazides contribute to NOD development, their benefit in a hypertensive patient, including reduction in CV risk, may be offset by this adverse metabolic effect.

Genetic influences on T2D are well established with heritability estimated at 50 percent.(6, 7) Single nucleotide polymorphisms (SNPs) have been reproducibly associated with T2D in genome wide association studies (GWAS) and variants in the Transcription Factor 7-Like 2 gene (*TCF7L2*) are the strongest and most reproducible signals identified to date.(8) Many environmental influences have also been consistently associated with T2D development, (9, 10) but interactions between genetic and environmental risk factors such as pharmacotherapy are not well studied with respect to T2D incidence. Gene environment interactions remain a potentially important component of the milieu that predicts T2D development.

Variants in *TCF7L2* are well replicated genetic signals for diabetes, with an odds ratio (OR) of the rs7903146 T allele of 1.56 [95% confidence interval (95%CI) 1.29–1.89], meta-analysis $p=1\times10^{-140}$).(8, 11–13) *TCF7L2* is a transcription factor involved in the WNT signaling pathway. *TCF7L2* has been implicated in incretin signaling pathways since it has been shown to regulate transcription of the glucagon gene, which encodes glucagon-like peptide 1 (GLP1) in the L cells of the gut.(14) The rs7903146 T allele has been associated with increased *TCF7L2* expression and has been implicated as a functional variant, being mapped to open chromatin sites in pancreatic islet cells.(15, 16) Functional impairment of *TCF7L2* by SNPs could cause changes in gene expression and influence diabetes development.

The importance of identifying predictors of thiazide-induced dysglycemia was emphasized by a working group from the National Heart Lung and Blood Institute.(4) *A priori* identification of patients at risk for T2D during thiazide treatment could guide thiazide prescribing to reduce the risk of NOD. We hypothesize that thiazide diuretic treatment in a patient with T2D risk alleles might further increase diabetes risk, constituting a potential pharmacogenetic role for *TCF7L2* SNPs. Although multiple SNPs have been associated with thiazide-induced NOD, (17–19) to our knowledge the pharmacogenetic interaction between *TCF7L2* SNPs and thiazide treatment has not been studied with respect to NOD. Therefore, we investigated the interaction of *TCF7L2* polymorphisms and hydrochlorothiazide (HCTZ) treatment on the development of NOD in hypertensive, coronary artery disease (CAD) patients from the INternational VERapamil SR-Trandolapril STudy (INVEST).

METHODS

Study design

INVEST evaluated adverse CV outcomes and NOD occurring during randomized treatment with either an atenolol-based or a verapamil sustained release (SR)-based antihypertensive strategy in patients with hypertension and CAD. The INVEST design, (20) primary outcome, (21) and NOD (9) results have been previously published in detail.(9, 20, 21) Briefly, patients were eligible if they were aged 50 years or older and had documented CAD, with essential hypertension as defined by the Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC VI) requiring drug therapy.(22) Patients were excluded if they had class IV heart failure, significant renal insufficiency, were taking β -blockers within 2 weeks of randomization, or were taking β -blockers for a myocardial infarction that occurred in the previous 12 months. The verapamil-based strategy consisted of verapamil SR 240 mg daily (Step 1), addition of trandolapril 2 mg daily (Step 2), dose titration to verapamil SR 240 mg/trandolapril 2 mg twice daily (Step 3), and HCTZ 25 mg daily add-on treatment (Step 4) to achieve JNC VI blood pressure (BP) goals. The atenolol-based strategy consisted of atenolol 50 mg daily (Step 1), addition of HCTZ 25 mg daily (Step 2), titration to atenolol 50 mg/HCTZ 25 mg twice daily (Step 3), and trandolapril 2 mg daily add-on treatment (Step 4) for BP control as necessary. (Table S1 in Supplemental Materials, Supplemental Digital Content 1, <http://links.lww.com/FPC/A651>) Therefore, patients in the atenolol-based strategy were prescribed HCTZ at step 2 if BP control was not achieved and patients in the verapamil SR-based strategy were prescribed HCTZ at step 4 if BP control was not achieved. Both strategies were optimized to provide end organ protection with trandolapril in patients with diabetes and/or renal insufficiency. All patients enrolled in INVEST provided written informed consent and the institutional review boards of participating study centers approved the study protocol. INVEST is registered at clinicaltrials.gov (NCT00133692).

The INVEST Genetic Substudy (INVEST-GENES) collected DNA samples from 5,979 INVEST patients at 187 sites in the United States and Puerto Rico, who provided additional written informed consent for genetic studies. We conducted a nested case control study including those who developed NOD during follow-up (cases) and age, race/ethnicity, and gender-matched participants who remained diabetes-free during follow-up (controls). NOD was determined by site investigators from a review of all available patient data, as well as new use of an anti-diabetic medication.(9) Information regarding diabetes status and use of anti-diabetic medications was collected at each follow-up visit, which occurred every 6 weeks for the first 6 months and then every six months until the study ended.(9) Patients with diabetes at baseline, defined by a history of diabetes mellitus and/or use of oral hypoglycemic medication or insulin, were excluded from this analysis.(23, 24)

Genotyping and quality control

Genotyping was accomplished using the HumanCVD BeadChip, (25) which contains approximately 50,000 cosmopolitan tag SNPs for 2,100 CV and metabolic-related genes. (25) The HumanCVD BeadChip includes 101 *TCF7L2* tag SNPs, selected for $r^2 > 0.5$ and minor allele frequency (MAF) > 0.05 . Genotypes were called using BeadStudio software and the GenTrain2 calling algorithm (Illumina, San Diego, CA). The INVEST-GENES NOD case control cohort was genotyped as part of larger INVEST-GENES cohort (n=2,239) and quality control procedures were performed on the entire sample set. Patients were excluded if sample genotype call rates were below 95% and SNPs were excluded if genotype call rates were below 90%. Monomorphic SNPs were removed and SNPs were excluded if GenTrain score was less than 0.3. A total of 87 blind duplicates were included in genotyping and had a concordance rate of 99.997%. Gender was confirmed from X chromosome

genotype data, and those with discordant gender data were excluded (n=14). Cryptic relatedness was estimated by pairwise identity-by-descent (IBD) analysis implemented using PLINK.(14) Forty-one pairs of samples were identified as first degree relatives (π -hat 0.30–0.57) and these individuals were maintained in the analysis. Heterozygosity was assessed using PLINK, by estimating the inbreeding coefficient, F. Six subjects had F values >4 standard deviations from the mean. One of these subjects also had a high missing genotype rate of > 4% and this subject was excluded. Outliers in the by race/ethnic group Principal Component Analysis were also removed (n=4). The final dataset consisted of 2,214 subjects, with 1,435 in the final NOD dataset.

Definition and treatment of race/ethnicity

INVEST race/ethnic groups were determined by patient report with interaction by the study investigator (26) and confirmed through principal components analysis (PCA) with linkage disequilibrium (LD)-pruned HumanCVD BeadChip data. PCA was performed using JMP Genomics version 5.0 (SAS, Cary, NC) in which the EIGENSTRAT method was implemented.(27) To minimize confounding by population stratification, pharmacogenetic analyses were performed separately by race/ethnicity and adjusted for principal components (PCs) one, two, and three. PCs were generated separately for each race/ethnic group. PCs one, two, and three were used since three PCs provided adequate separation of race/ethnic groups, fewer PCs did not provide adequate separation of race/ethnic groups, and additional PCs provided no further precision. LD analysis and pairwise LD (r^2) were performed within race/ethnic groups using Haploview.(28)

Statistical analysis

Baseline characteristics for cases and controls were compared using t tests and chi square tests as appropriate. Multi-variable logistic regression models were used to estimate ORs for NOD, assess *TCF7L2* SNP*HCTZ treatment interactions by race/ethnicity, and calculate synergy indices (SIs) for an estimate of multiplicative SNP*HCTZ interactions.(29) Relative risk estimates of interactions on an additive scale were also calculated using the Relative Excess Risk due to Interaction (RERI) for allele carriers with likelihood-based 95% confidence bounds as previously described.(30) In the absence of an interaction effect, the SI equals 1 and the RERI equals 0. Empirical p values were computed for all logistic regressions using Monte Carlo estimation of the exact p value with 10,000 permutations performed. Interaction p values were adjusted for correlated tests using P_{ACT} , which accounts for linkage disequilibrium of SNPs, attaining the accuracy of permutation or simulation-based tests in much less computation time.(31) Statistical significance was established at $P_{ACT}=0.05$. ORs per allele copy and 95% CIs were calculated in HCTZ-treated patients, non-HCTZ treated patients, and all patients using allelic trend tests adjusted for variables with potential impact on NOD. HCTZ treatment was defined as exposure to HCTZ with any dose or duration before diagnosis of NOD. Similar treatment definitions were used for atenolol and trandolapril, which belong to drug classes also known to affect NOD incidence.(5)

Sensitivity analyses were performed in patients treated with HCTZ 25 mg/day and in patients treated with continuous HCTZ for 6 months. All logistic regression models were adjusted for variables chosen based on potential impact on NOD, which include age, gender, body mass index (BMI), average on-treatment systolic BP, hypercholesterolemia, smoking history, PCs one, two, and three, treatment with trandolapril or atenolol, as well as trandolapril, atenolol, and HCTZ treatment duration. SNPs were fit as numeric variables to model effects and SNP*HCTZ interactions and HCTZ exposure is included in statistical models of interactions. Deviations from Hardy Weinberg Equilibrium (HWE) were assessed using exact statistics for Fisher's Exact Test by race/ethnicity with $\alpha=0.01$. Variants

which deviated from HWE were flagged and are not presented in the results. All statistical analyses were performed using SAS version 9.2 and JMP Genomics version 5.0 (SAS, Cary, NC).

RESULTS

For the NOD case control cohort, over a mean follow-up of 2.8 years, we identified 446 NOD cases who were age and race/ethnicity matched to 1,025 controls, in INVEST-GENES (Table 1). A total of 410 NOD cases were successfully genotyped on the HumanCVD BeadChip (Illumina, San Diego, CA). A total of 98 *TCF7L2* SNPs passed QC and the average genotyping call rate was 99.75%. Three of 101 SNPs departed from HWE in at least one race/ethnic group, but none of these SNPs showed significant pharmacogenetic interactions. HWE p values and minor allele frequencies are presented in Supplementary Materials, Supplemental Digital Content 1, <http://links.lww.com/FPC/A651>. Characteristics for the NOD case control cohort are summarized in Table 1 and characteristics according to race/ethnicity are summarized in Table S2 in the Supplemental Materials, Supplemental Digital Content 1, <http://links.lww.com/FPC/A651>.

A higher mean atenolol dose was observed in NOD cases (72 mg versus 67 mg, $p=0.02$) than controls. Associations between model covariates and NOD are presented in Supplementary Table S3, Supplemental Digital Content 1, <http://links.lww.com/FPC/A651>. At baseline, NOD cases had higher BMI and diastolic BP, and a higher percentage with left ventricular hypertrophy. During follow-up, the proportion of patients who were treated with HCTZ was greater in cases than in controls resulting in a significant association between HCTZ treatment and NOD (OR 1.70 [95%CI 1.31–2.20], $p<0.0001$). The average duration of HCTZ treatment was 87.1 (standard deviation [SD] 73.6) weeks and duration of HCTZ was associated with NOD (OR 1.16 [95%CI 1.10–1.22] per 6 months HCTZ, $p<0.0001$). A total of 409 of the 713 patients (57%) assigned to the verapamil-based strategy were prescribed HCTZ and 532 of the 722 patients (74%) assigned to the atenolol-based strategy were prescribed HCTZ. Overall, HCTZ treated patients had higher metabolic risk compared to non-HCTZ treated patients, since HCTZ treated patients had higher average baseline systolic blood pressure (151 versus 144 mmHg [$p<0.0001$] respectively), higher average on-treatment systolic BP (137 versus 130 mmHg [$p<0.0001$] respectively), and higher BMI (30.1 versus 28.6 kg/m² [$p<0.0001$] respectively). Characteristics for cases and controls by HCTZ treatment status are summarized in Table S4 of the Supplemental Materials, Supplemental Digital Content 1, <http://links.lww.com/FPC/A651>.

In INVEST whites, three *TCF7L2* SNPs out of 101 tested showed a significant pharmacogenetic interaction with HCTZ treatment after P_{ACT} correction. SNPs with significant ($n=3$) or nominally significant ($n=18$) interactions are included in Table 2. All SNPs with significant pharmacogenetic interactions after P_{ACT} correction showed an increased point estimate for NOD in HCTZ treated patients, with ORs ranging from 1.11–2.55. The strongest pharmacogenetic interaction was observed for rs7917983 ($p_{inx}=5.0\times 10^{-4}$, $P_{ACT}=0.03$), which was associated with increased NOD risk in HCTZ-treated patients (OR 1.52 [95%CI 1.04–2.25], $p=0.03$) and decreased NOD risk in non HCTZ-treated patients (OR 0.48 [95%CI 0.27–0.86], $p=0.02$). The interaction resulted in a RERI of 0.99 (95%CI 0.43–1.50), indicating a 99 percent increase in NOD risk due to interaction of HCTZ and rs7917983 T allele carriage relative to the risk without either factor. This interaction resulted in a SI of 3.37 [95%CI 1.72–6.59], indicating a 3-fold risk of NOD per rs7917983 T allele for HCTZ exposure. In HCTZ treated patients, the strongest associations with NOD were for the SNPs rs6585195 (OR 2.55 [95%CI 1.45–4.49], $p=8.0\times 10^{-4}$) and rs12573128 (OR 2.27 [95%CI 1.32–3.92], $p=0.003$), which were both

identified as nominally significant associations in the primary analysis. (Table S5 in Supplemental Materials, Supplemental Digital Content 1, <http://links.lww.com/FPC/A651>)

The *TCF7L2* SNP rs4506565, which has been previously associated with T2D in GWAS, showed a significant pharmacogenetic association in INVEST whites (RERI 1.08 [95%CI 0.36–1.87] and SI 3.58 [95%CI 1.67–7.66], $p_{\text{inx}}=9.0 \times 10^{-4}$, $P_{\text{ACT}}=0.05$). (11, 32) An increased risk for NOD was observed with T2D risk alleles in HCTZ treated patients and decreasing risk for NOD was observed with T2D risk alleles in non-HCTZ treated patients. (Table 2) The most strongly associated SNP from GWAS literature, rs7903146, showed similar trends although with nominal significance (RERI 0.93 [95%CI 0.05–1.84] and SI 2.60 [95%CI 1.22–5.54], $p_{\text{inx}}=0.009$). Another SNP associated with diabetes from GWAS, rs7901695, showed a nominally significant pharmacogenetic interaction (RERI 1.11 [95%CI 0.42–1.88] and SI 3.67 [95%CI 1.71–7.89], $p_{\text{inx}}=0.001$). The SNPs rs12243326 and rs11196213, which have been previously associated with two hour post oral glucose tolerance test glucose and T2D respectively, (33, 34) also showed nominally significant pharmacogenetic interactions. Figure 1 shows ORs, 95% CIs, and p values for significant SNPs and nominally significant SNPs that have been previously associated with T2D or T2D-related traits.

Multiple significant and nominally significant associations were observed for SNPs in high linkage disequilibrium (LD) in INVEST whites. (Figure 2) The SNP rs4074720, for which a nominally significant SNP*HCTZ treatment interaction was observed (RERI 0.91 [95%CI 0.24–1.47] and SI 3.22 [95%CI 1.60–6.48], $p_{\text{inx}}=0.001$), was in high LD with rs6585202 ($r^2=0.87$) and rs7924080 ($r^2=0.87$), which also showed significant interactions. The nominally significant SNP rs7901695 was also in high LD with rs4506565 ($r^2=0.98$), rs4132670 ($r^2=0.95$), and rs7903146 ($r^2=0.85$). The SNP rs7917983, for which the strongest pharmacogenetic interaction was observed, was not in high LD with any other significantly or nominally associated SNP.

In sensitivity analyses, results were similar when the definition of HCTZ treatment was restricted to 6 months or greater and daily HCTZ dose of 25mg or greater. (Table S6 in Supplemental Materials, Supplemental Digital Content 1, <http://links.lww.com/FPC/A651>) For the SNP rs7917983 with the strongest pharmacogenetic interaction, the association strengthened although the SI was similar in patients taking HCTZ for 6 months or more (OR 1.88 [95%CI 1.16–3.04] in HCTZ treated patients and OR 0.49 [95%CI 0.27–0.87] in non-HCTZ treated patients, RERI 1.01 [95%CI 0.43–1.54] and SI 3.34 [95%CI 1.71–6.52], $p_{\text{inx}}=2.0 \times 10^{-4}$). When HCTZ treatment was restricted to 25 mg/day or more, the interaction remained significant (OR 1.89 [95%CI 1.14–3.16] in HCTZ treated patients and OR 0.64 [95%CI 0.38–1.06] in non-HCTZ treated patients, RERI 1.19 [95%CI 0.59–1.87] and SI 3.21 [95%CI 1.61–6.42], $p_{\text{inx}}=0.008$).

No significant pharmacogenetic associations were observed in Hispanics or blacks after P_{ACT} correction. In blacks, a trend toward a significant SNP*HCTZ treatment interaction was observed for the rs290490 G allele ($p_{\text{inx}}=0.04$), with an increased risk for NOD in HCTZ treated patients (OR 1.49 [95%CI 1.06–2.10], $p=0.02$).

DISCUSSION

To our knowledge, the present study is the first to investigate *TCF7L2* SNPs for pharmacogenetic influences on thiazide-induced NOD and the first to observe significant pharmacogenetic interactions between *TCF7L2* SNPs and HCTZ treatment on NOD risk. We have shown that a *TCF7L2* SNP previously associated with T2D in GWAS may also have a pharmacogenetic interaction in whites treated with HCTZ. *TCF7L2* pharmacogenetic

associations were confirmed when HCTZ treatment was limited to higher HCTZ doses and extended treatment duration. The present study is strengthened by our ability to assess *TCF7L2* risk allele point estimates in HCTZ-treated and non HCTZ-treated patients. Our study provides evidence for *TCF7L2* as a candidate gene for the pharmacogenetics of thiazide-induced NOD and suggests the importance of assessing SNP*HCTZ treatment interactions in GWAS of T2D.

Since the initial T2D GWAS in 2006, (35) many T2D GWAS have been published that establish *TCF7L2* SNPs as the strongest and most reproducible genetic risk factors for T2D. (8, 11, 12, 32, 36, 37) We found significant pharmacogenetic associations in whites, which is the race group in which the strongest and most reproducible *TCF7L2* disease associations are found. We observed pharmacogenetic associations with a *TCF7L2* SNP that was previously associated with T2D, namely rs4506565 (OR 1.36 [95%CI 1.20–1.54]).(11, 32) Among the SNPs with nominally significant pharmacogenetic interactions were rs7903146, the strongest SNP from T2D GWAS (OR 1.56 [95%CI 1.29–1.89]), (8, 12), rs7901695 (OR 1.37 [95%CI 1.25–1.49]), (11, 32) and rs12243326, previously associated with two hour glucose after glucose challenge.(33) The strongest pharmacogenetic interaction observed was for the intronic SNP rs7917983, which has not been previously associated with T2D to our knowledge. Although rs7917983 was not in LD with any other significantly or nominally associated SNP, this SNP may be in LD with other *TCF7L2* SNPs not included in this study.

Our results suggest that *TCF7L2* variation affects the influence of HCTZ on risk for NOD. If our results are supported by replication and functional studies, *TCF7L2* SNPs might be used to guide HCTZ treatment to avoid new onset diabetes. In addition, these results suggest the importance of considering antihypertensive treatment when identifying and utilizing genetic predictors for T2D. HCTZ might affect expression of *TCF7L2* or the ability of the transcription factor to bind to promoters, further increasing an individual's risk for T2D. The majority of *TCF7L2* SNPs associated with NOD during HCTZ treatment were primarily intronic with few predicted functional consequences. Furthermore, rs7903146 did not show the strongest pharmacogenetic interaction, suggesting that other SNPs may be responsible for the observed pharmacogenetic association.

We observed differences in associations between race/ethnic groups, which might suggest differences in LD and the need to identify functional variants. Such differences in LD between race/ethnic groups are observable in the INVEST population in LD analyses. Our observation of SNP effects only in whites might also suggest that *TCF7L2* SNPs have pharmacogenetic influences only in individuals with European ancestry. Although GWAS have identified SNPs in *TCF7L2* as consistent and reliable risk factors for T2D in European populations, *TCF7L2* SNPs identified in whites may not be genetic risk factors for T2D in black and Hispanic populations.(35, 36, 38) A relatively small number of studies in populations with African descent and Hispanic ethnicity have inconsistently observed genetic associations.(12, 37, 39–45) These differences in observed associations between *TCF7L2* SNPs and diabetes might explain the differences in results observed between race/ethnic groups in the present study.

Although we observed several *TCF7L2* SNPs with an increased risk of NOD in HCTZ treated patients and a decreased NOD risk in non HCTZ treated patients, many of the ORs for NOD in HCTZ treated patients were not significant. In sensitivity analyses, many of these ORs were significant when HCTZ treatment was more stringently defined (e.g. longer duration or higher dose). In addition, a number of the observed interactions appeared to be driven largely by decreased odds ratios in non HCTZ treated patients. These inverse associations might suggest that lack of HCTZ treatment is protective for diabetes when

TCF7L2 risk SNPs are present. The possibility also exists that HCTZ treatment is collinear with another unknown factor affecting diabetes risk. These results are difficult to interpret since GWAS studies do not generally consider HCTZ treatment as a covariate. These data underscore the need for replication and additional study of these associations and might suggest the importance of considering antihypertensive treatment when assessing *TCF7L2* SNPs as predictors of diabetes risk.

We observed a significant difference in baseline systolic BP, average on-treatment systolic BP, and BMI between HCTZ treated and non-HCTZ treated patients, suggesting that HCTZ treated patients were at higher metabolic risk. Because HCTZ was not randomly assigned and was part of stepped care as an add-on treatment to achieve BP goals, patients receiving HCTZ during INVEST were more likely to have uncontrolled hypertension. Since increased BP and BMI have been identified as risk factors for diabetes, (9) BP control and an increased BMI may be partially responsible for the strength of the observed association between *TCF7L2* SNPs and NOD in HCTZ treated patients and the inverse association in non-HCTZ-treated patients. In addition, concomitant antihypertensive treatments used in INVEST treatment strategies, which can affect NOD development, may have contributed to the inverse NOD association in non HCTZ treated patients. HCTZ treatment was not uniform within INVEST treatment strategies and our results may be applicable only when HCTZ is used in antihypertensive strategies similar to INVEST. We attempted to reduce confounding of these variables by including on-treatment systolic BP, BMI, and additional drug exposure and duration as covariates in all models.

Since the pathophysiologic role *TCF7L2* plays in T2D development remains unclear, speculation regarding the physiology of a pharmacogenetic interaction is difficult. *TCF7L2* is primarily thought of as a T2D genetic risk factor. However, HCTZ treatment may represent an environmental risk factor that precipitates T2D in a patient who is otherwise at risk for T2D development based on their *TCF7L2* genotype. Our observation of multiple significant SNP*HCTZ interactions and significant interaction point estimates using both RERIs and SIs supports this hypothesis. In a different population, we have shown that SNPs identified in GWAS studies of hypertension also affect variability in response to antihypertensive drugs.(46) If such associations are replicated and prospectively evaluated, SNPs from GWAS might be used to tailor antihypertensive treatment.

Our study has several limitations. First, our sample size by race/ethnicity is small relative to most T2D disease genetics studies. However, *TCF7L2* is the strongest known T2D risk factor and we observed a number of significant pharmacogenetic interactions, suggesting that we had sufficient power to detect associations in whites. In INVEST, NOD diagnosis was based on investigator reports or new use of anti-diabetes drugs, rather than collection of glucose data. However, accuracy of investigator-reported diabetes compared to laboratory data has been verified by others, (47) and has been used in other trials.(48–50) Furthermore, we have shown previously that NOD and baseline diabetes in INVEST are associated with clinical characteristics indicative of type 2 diabetes, including increased BMI, Hispanic ethnicity, left ventricular hypertrophy, and hypercholesterolemia, increasing our confidence in the validity of the NOD outcome used in INVEST.(9, 21, 23, 24) Although antihypertensive treatment during INVEST might have confounded our associations, statistical models were adjusted for exposure to drugs associated with diabetes. We also did not observe consistent *TCF7L2* pharmacogenetic associations in Hispanic and African race/ethnic groups. Our observations may reflect differences in LD structure or may represent type 2 error. In whites, significant pharmacogenetic associations after P_{ACT} correction, multiple pharmacogenetic associations, and consistency of these associations with different HCTZ treatment definitions lend credence to the validity of pharmacogenetic findings. We

do recognize the potential for false positive results in pharmacogenetic analyses and our observations need to be independently replicated.

In summary, our results suggest that genetic variation in *TCF7L2*, including SNPs associated with T2D from GWAS, may influence the risk of HCTZ-associated NOD. Our observations suggest that an environmental risk factor such as HCTZ treatment further increases diabetes risk in *TCF7L2* risk allele carriers. Our observation of SNP effects only in whites suggests differences in LD, or that *TCF7L2* SNPs have pharmacogenetic influences only in individuals with European ancestry. Functional studies and replication of pharmacogenetic associations are needed to confirm our observed association and define the potential role of *TCF7L2* SNPs in predicting NOD during HCTZ treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. *CF7L2* SNP*HCTZ treatment interactions in INVEST whites, including significant SNPs after P_{ACT} correction and nominally significant SNPs that have been previously associated with T2D or T2D-related traits. Odds ratios, 95% confidence intervals, and interaction p values adjusted for age, gender, body mass index, average on treatment systolic blood pressure, hypercholesterolemia, history of smoking, principal components one, two, and three, trandolapril and atenolol treatment, and trandolapril, atenolol, and HCTZ treatment duration. Superscript letters indicate that SNPs are in linkage disequilibrium ($r^2 > 0.80$); *Allele previously associated with T2D or T2D-related traits. HCTZ indicates hydrochlorothiazide; NOD, new onset diabetes; SNP, single nucleotide polymorphism.

Figure 2. Haploview-generated linkage disequilibrium (LD) plot of *TCF7L2* SNPs, including significant SNPs after P_{ACT} correction and nominally significant SNPs in INVEST whites. Regions of higher LD are shaded darker according to higher r^2 values. The number within each box indicates the r^2 value.

Table 1

Characteristics of new onset diabetes cases and controls at baseline and during INVEST

Characteristic ¹	NOD Cases (n=446)	Controls (n=1,025)	p value ²
At baseline			
Age (years)	65 (10)	65 (9)	0.73
Female, n (%)	250 (56)	573 (56)	0.96
BMI (kg/m ²)	31 (6)	29 (5)	<0.0001
Race/ethnicity, n (%)			0.66
White	176 (40)	409 (40)	
African American	51 (11)	121 (12)	
Hispanic	217 (49)	492 (48)	
Blood pressure (mm Hg)			
Systolic	149 (19)	148 (18)	0.26
Diastolic	87 (10)	86 (10)	0.04
Verapamil SR strategy, n (%)	208 (47)	519 (51)	0.16
Hypercholesterolemia, n (%) ³	242 (54)	548 (54)	0.78
History of LVH, n (%)	77 (17)	128 (13)	0.02
History of MI, n (%)	99 (22)	194 (19)	0.15
History of smoking, n (%)	184 (41)	405 (40)	0.53
During INVEST			
Blood pressure (mm Hg) ⁴			
Systolic	135 (11)	134 (11)	0.07
Diastolic	79 (6)	79 (6)	0.49
HCTZ treatment, n (%)	328 (74)	640 (62)	<0.0001
HCTZ (< 6 months), n (%)	320 (72)	627 (61)	<0.0001
HCTZ dose (mg)	28 (12)	26 (12)	0.17
Atenolol treatment, n (%)	229 (51)	479 (47)	0.10
Atenolol dose (mg)	72 (32)	67 (31)	0.02
Trandolapril treatment, n (%)	267 (60)	664 (65)	0.07
Trandolapril dose (mg)	3.3 (2.6)	3.4 (2.6)	0.49
Verapamil SR treatment, n (%)	208 (47)	519 (51)	0.16
Verapamil SR dose (mg)	234 (75)	238 (75)	0.71

INVEST indicates International Verapamil SR and Trandolapril Study; NOD, new onset diabetes; BMI body mass index; SR, sustained release; LVH, left ventricular hypertrophy; HCTZ, hydrochlorothiazide

¹ Values are mean (standard deviation) unless otherwise noted.

² P values represent t-tests and chi square tests where appropriate.

³ History of or currently taking lipid-lowering medications

⁴ Average of clinic blood pressure measurements during study

Table 2

Significant and nominally significant pharmacogenetic interactions for *TCF7L2* SNPs and hydrochlorothiazide treatment on new onset diabetes in INVEST whites

SNP	Allele	Freq.	HWE ¹	OR (95%CI) (HCTZ Treated, n=941) ²	OR (95%CI) (Not HCTZ Treated, n=494) ²	p _{max} ³	P _{ACT} ⁴
rs7917983	T	0.53	0.20	1.53 (1.04–2.25)	0.48 (0.27–0.86)	5.0×10 ⁻⁴	0.03
rs4132670	A	0.32	0.73	1.44 (0.94–2.21)	0.52 (0.28–0.98)	8.0×10 ⁻⁴	0.05
rs4506565*	T	0.31	0.91	1.50 (0.98–2.29)	0.53 (0.28–1.00)	9.0×10 ⁻⁴	0.05
rs4074720	T	0.54	0.49	1.31 (0.89–1.94)	0.45 (0.25–0.82)	0.001	-
rs7901695*	C	0.32	0.82	1.50 (0.98–2.29)	0.52 (0.28–0.99)	0.001	-
rs7924080	T	0.53	0.62	1.26 (0.84–1.88)	0.46 (0.26–0.83)	0.002	-
rs6585202	T	0.53	0.69	1.24 (0.83–1.85)	0.46 (0.26–0.83)	0.002	-
rs11196174	G	0.26	1	1.11 (0.73–1.68)	0.42 (0.20–0.88)	0.004	-
rs6585195	C	0.13	0.63	2.55 (1.45–4.49)	0.87 (0.39–1.95)	0.005	-
rs10885399	A	0.21	0.16	1.87 (1.18–2.97)	0.74 (0.37–1.51)	0.009	-
rs7903146*	T	0.28	0.54	1.38 (0.90–2.12)	0.62 (0.33–1.16)	0.009	-
rs4918789	T	0.55	0.01	0.87 (0.59–1.27)	0.36 (0.19–0.68)	0.01	-
rs12243326*	C	0.28	0.46	1.30 (0.84–2.00)	0.60 (0.33–1.11)	0.01	-
rs7087006	A	0.55	0.02	0.86 (0.59–1.26)	0.36 (0.19–0.68)	0.01	-
rs11196213	T	0.45	0.01	0.87 (0.59–1.27)	0.36 (0.19–0.68)	0.01	-
rs12266632	G	0.06	0.62	2.24 (0.97–5.16)	0.68 (0.21–2.24)	0.02	-
rs12573128	G	0.14	0.37	2.27 (1.32–3.92)	1.01 (0.47–2.17)	0.02	-
rs7079711	A	0.20	1	1.11 (0.70–1.77)	0.48 (0.21–1.09)	0.03	-
rs11594681	A	0.37	0.20	1.14 (0.78–1.68)	0.58 (0.31–1.09)	0.03	-
rs17130192	T	0.08	0.26	1.39 (0.69–2.77)	0.39 (0.11–1.38)	0.04	-
rs4081699	G	0.21	0.42	1.81 (1.15–2.84)	0.84 (0.44–1.61)	0.04	-

SNP indicates single nucleotide polymorphism; Freq, frequency of allele in INVEST whites; HWE, Hardy Weinberg Equilibrium p value; OR, odds ratio; 95%CI, 95% confidence interval; HCTZ, hydrochlorothiazide

¹ Hardy Weinberg equilibrium p value using Fisher's Exact test in whites

² HCTZ treatment defined as exposure to HCTZ with any dose or duration before diagnosis of NOD. Odds ratios and 95% confidence intervals adjusted for age, gender, body mass index, average on treatment systolic blood pressure, hypercholesterolemia, history of smoking, principal components one, two, and three, trandolapril and atenolol treatment, and trandolapril, atenolol, and HCTZ treatment duration.

³ p value for interaction of HCTZ treatment and SNP after adjustment but before P_{ACT} correction

⁴ p value for interaction of HCTZ treatment and SNP after adjustment and correction for multiple testing using P_{ACT}

* SNPs previously associated with type 2 diabetes or diabetes-related traits in genome wide association studies