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ORIGINAL ARTICLE

# Interactions between five candidate genes and antihypertensive drug therapy on blood pressure

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Despite the availability of effective antihypertensive drugs, there is a large variation in response to these drugs. This study investigates whether polymorphisms in the angiotensin converting enzyme (I/D), angiotensinogen (M235T), α-adducin (G460W), angiotensin II type 1 receptor (1166A/C), or G protein $\beta_3$-subunit (825C/T) gene modify the mean difference in blood pressure levels among diuretics, β-blockers, or ACE-inhibitors users. Data were used from the Doetinchem Cohort Study, and blood pressure data were collected from GPs (1987–1997). A marginal generalized linear model (GEE) was used to assess the gene–drug interaction on the mean difference in systolic/diastolic blood pressure. In total, 625 hypertensive individuals were included with a total of 5262 measurements of blood pressure. Only the interaction between diuretic use and the GNB3 825C/T polymorphism was significant (C allele versus TT systolic blood pressure (SBP): 4.33 mmHg [95% CI: 0.14–8.54]). Thus, the mean SBP level among diuretic users may be modified by the GNB3 825C/T polymorphism.
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## Introduction

Hypertension is an important public health problem. Evidence from randomized trials has shown that drug treatment reduces the risk of cardiovascular morbidity and mortality.[1,2] Despite the availability of a variety of effective drugs, inadequate control of blood pressure is still common in hypertensive patients.[3] Among the possible causes are, besides environmental, certain genetic characteristics that could have modified the response to antihypertensive drugs.

Blood pressure levels are homeostatically maintained through complex interactions between environmental and genetic factors. Antihypertensive drugs lower blood pressure by acting on specific targets within this system. Obvious candidate genes for antihypertensive drug–gene interactions are those that code for components of a system, which is pharmacologically influenced by an antihypertensive drug. Other candidates are genes that code for components of the counter-regulatory system.

Examples of candidate genes for blood pressure lowering drugs are those in the renin–angiotensin system, for example: angiotensinogen (AGT), angiotensin converting enzyme (ACE), and angiotensin II receptor type 1 (AGTR1). Plasma AGT is significantly elevated in patients with the AGT T235 allele,[4] and serum ACE is significantly higher in subjects with the ACE D allele.[5] Candidate genes related to other blood pressure regulating systems are α-adducin (ADD1) and

$\beta$3-subunit of G-protein (GNB3). ADD1 may affect blood pressure by modulating renal tubular reabsorption of sodium through the activation of $Na^+$, $K^+$-ATPase (adenosine triphosphatase) with the 460W allele exhibiting higher affinity for the $Na^+$, $K^+$-ATPase pump.[6] The 825T allele of GNB3 gene is associated with a shortened splice variant of the GNB3 protein that gives rise to enhanced signal transduction via pertussis toxin-sensitive G-proteins.[7]

Several nonrandomized trials have studied the influence of these genes on the response to antihypertensive medication,[8] but with conflicting results and as far as we known, the effect of these genes in daily practice has never been evaluated. Therefore, the purpose of the present study was to evaluate the relationship between the I/D (ACE), M235T (AGT), G460W (ADD1), 1166A/C (AGTR1), and 825C/T (GNB3) polymorphisms on the mean difference in blood pressure levels among subjects using diuretics, $\beta$-blockers, or ACE inhibitors in daily practice.

## Results

Between 1987 and 1997, 5262 blood pressure measurements of 625 individuals were included. During follow-up, 106 subjects used diuretics (743 measurements), 229 used $\beta$-blockers (1480 measurements) and 77 used ACE-inhibitors (495 measurements). In 99.4% of the hypertensive individuals, genotypes were assessed for the ACE gene, 99.9% for the ADD1 gene, 99.9% for the AGTR1 gene, 99.3% for the GNB3 gene, and 99.9% for the AGT gene. Characteristics for the 625 subjects stratified by treatment or no treatment during the first examination are presented in Table 1. Subjects in the treated group are older and the percentage of diabetics, female subjects, subjects receiving a low-salt diet, or a cholesterol diet is higher compared to the nontreated group.

Owing to the small sample size, two genotype groups were combined in the analysis for some genes, namely the TT (6.0% of the individuals) and GT genotype of ADD1 gene, the CC (10.2% of the individuals) and AC genotype of AGTR1 gene, the CC (4.6% of the individuals) and CT genotype of GNB3 gene, and the TT (1.0% of the individuals) and GT genotype of AGT gene. The unadjusted difference in systolic blood pressure (SBP) and diastolic blood pressure (DBP) for diuretics users was −3.15 mmHg (95% CI: −4.70 to −1.60) and −3.92 mmHg (95% CI: −4.75 to −3.10), for $\beta$-blockers users −2.53 mmHg (95% CI: −3.78 to −1.27) and −2.13 mmHg (95% CI: −2.80 to −1.46), for ACE inhibitors users 0.35 mmHg (95% CI: −1.62 to 2.32) and 0.48 mmHg (95% CI: −0.55 to 1.52).

The mean difference in defined daily doses (DDDs) between genotypes for users of diuretics, $\beta$-blockers, and ACE inhibitors adjusted for potential confounders is presented in Table 2. There was no statistically significant difference in DDDs between the different genotypes.

After adjustment for potential confounders, the mean difference in SBP and DBP for users of diuretics, $\beta$-blockers, and ACE inhibitors was compared between the genotype groups (Figure 1a–c). The only statistically significant drug–gene interaction was between diuretic users and GNB3 on SBP level (4.33 mmHg [95% CI: 0.14–8.54]). This interaction was not found for the mean difference in DBP (0.51 mmHg [95% CI: −1.13 to 2.15]). In addition, we also adjusted for persons who used another antihypertensive drug prior to the use of a diuretic ($n = 243$ switchers). The reduction for SBP after this adjustment was 4.74 mmHg (95% CI: 0.69–8.78) and for DBP 0.51 mmHg (95% CI: −1.69 to 2.70).

**Table 1 Baseline characteristics of all subjects at the first examination stratified by treatment**

| *Variable* | *Untreated (N = 490)* | *Treated (N = 135)* |
|---|---|---|
| Gender (M) | 279 (56.9%) | 57 (42.2%)* |
| Age (years) | 47.7 ± 9.1 | 52.1 ± 7.6* |
| SBP (mmHg) | 151.4 ± 18.3 | 144.2 ± 20.4* |
| DBP (mmHg) | 96.2 ± 10.0 | 90.5 ± 10.1* |
| BMI (kg/m$^2$) | 28.0 ± 4.5 | 27.5 ± 3.9 |
| Totaal/HDL cholesterol ratio | 5.5 ± 1.9 | 5.8 ± 2.2 |
| Diabetes | 15 (3.1%) | 14 (10.4%)* |
| Myocardial infarction | 7 (1.4%) | 1 (0.7%) |
| Diet for high BP | 56 (11.5%) | 33 (24.4%)* |
| Diet for high cholesterol | 35 (7.1%) | 24 (17.8%)* |
| Ethnicity, caucasian | 477 (97.3%) | 131 (97.8%) |
| *Smoking* | | |
| Current | 167 (34.1%) | 48 (35.6%) |
| Past | 151 (30.8%) | 33 (24.4%) |
| Never | 172 (35.1%) | 54 (40.0%) |
| ACE: DD/ID/II | 160/212/114 | 36/72/27 |
| ADD1: W-allele/GG[a] | 312/92 | 92/43 |
| AGTR1: C-allele/AA[b] | 237/66 | 66/69 |
| GNB3: C-allele/TT[c] | 220/63 | 63/71 |
| AGT: T-allele/MM[d] | 164/325 | 51/84 |
| Diuretic | | 39 (33.9%) |
| $\beta$-Blocker | | 86 (63.7%) |
| ACE inhibitor | | 10 (7.4%) |

*$P$-value < 0.001.
[a]W allele: WW+GW genotype.
[b]C allele: CC+CA genotype.
[c]C allele: CC+CT genotype.
[d]T allele: TT+MT genotype.

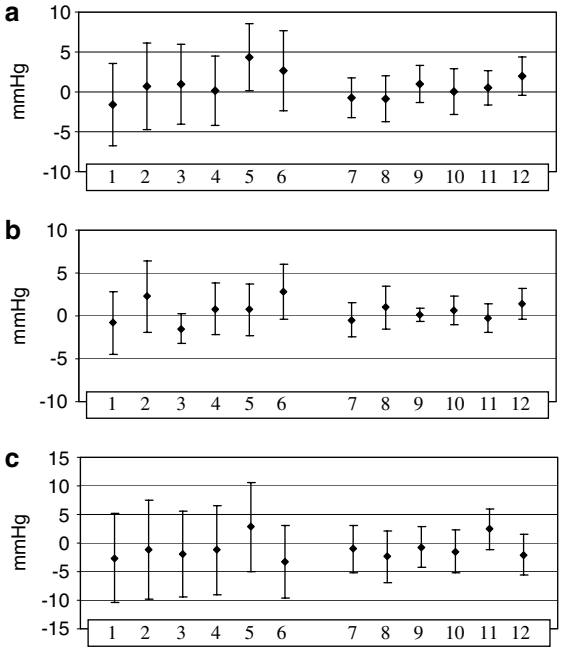
*Gene–gene–drug interactions*

To assess gene–gene–drug interactions, the mean difference in blood pressure was compared between combinations of two of the five genes. Owing to the small sample size, the genotype of the ID and II of the ACE gene were combined and it was impossible to combine more then two genes together.

In total, 36 gene–gene–drug interactions were possible for SBP and DBP. Of these interactions, four were associated with a significant difference in blood pressure (Figure 2).

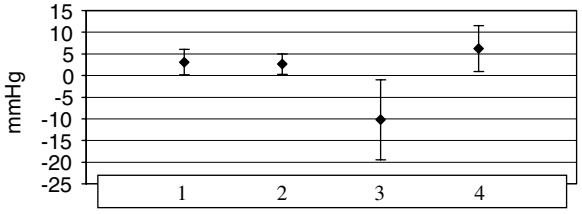
**Table 2 Adjusted DDDs for antihypertensive drug users**

| | *Diuretic* | *β-Blocker* | *ACE inhibitor* |
|---|---|---|---|
| ACE: ID versus DD | −0.04 (−0.32–0.24) | 0.06 (−0.04–0.15) | 0.00 (−0.38–0.37) |
| ACE: II versus DD | 0.09 (−0.26–0.44) | 0.08 (−0.02–0.19) | 0.01 (−0.42–0.44) |
| ADD1: W allele versus GG | −0.10 (−0.33–0.13) | 0.00 (−0.08–0.09) | 0.05 (−0.23–0.33) |
| ATR1: C allele versus AA | 0.12 (−0.13–0.37) | 0.01 (−0.07–0.08) | 0.05 (−0.24–0.37) |
| GNB3: C allele versus TT | 0.01 (−0.21–0.24) | −0.08 (−0.15–0.00) | 0.17 (−0.12–0.44) |
| AGT: T allele versus MM | −0.08 (−0.34–0.18) | −0.01 (−0.08–0.07) | 0.20 (−0.13–0.53) |




1 ID versus DD (ACE) **SBP**
2 II versus DD (ACE) **SBP**
3 W allele versus GG (ADD1) **SBP**
4 C allele versus AA (AGTR1) **SBP**
5 C allele versus TT (GNB3) **SBP**
6 T allele versus MM (AGT) **SBP**
7 ID versus DD (ACE) **DBP**
8 II versus DD (ACE) **DBP**
9 W allele versus GG (ADD1) **DBP**
10 C allele versus AA (AGTR1) **DBP**
11 C allele versus TT (GNB3) **DBP**
12 T allele versus MM (AGT) **DBP**

**Figure 1** (**a**) Adjusted mean difference in systolic or diastolic blood pressure among diuretic users. The diamonds with bars depict the mean difference in blood pressure ± 95% CI. (**b**) Adjusted mean difference in systolic or diastolic blood pressure among *β*-blocker users. The diamonds with bars depict the mean difference in blood pressure ± 95% CI. (**c**) Adjusted mean difference in systolic or diastolic blood pressure among ACE inhibitor users. The diamonds with bars depict the mean difference in blood pressure ± 95% CI.




| | | | |
|---|---|---|---|
| 1= AGT+ADD1 | T-allele+W-allele versus MM+GG | **Diuretic** | **DBP** |
| 2= AGT+ATR1 | T-allele+C-allele versus MM+AA | **β-blocker** | **DBP** |
| 3= AGT+ACE | T-allele+I-allele versus MM+DD | **ACE-inhibitor** | **SBP** |
| 4= ACE+GNB3 | I-allele+TT versus DD+C-allele | **ACE-inhibitor** | **DBP** |

**Figure 2** Adjusted interactions between the five candidate genes resulting in a significant difference in systolic and/or diastolic blood pressure among antihypertensive drug users. The diamonds with bars depict the mean difference in blood pressure ± 95% CI.

These were the interaction between diuretic use and ADD1 W allele + AGT T allele versus GG + MM on DBP (3.09 mmHg [95% CI: 0.16–2.93] 169 versus 149 measurements), *β*-blocker use and ATR1 C allele + AGT T allele versus AA + MM on DBP (2.63 mmHg [95% CI: 0.30–2.33] 436 versus 223 measurements), ACE inhibitor use and ACE I allele + GNB TT versus DD + C allele on DBP (6.22 mmHg [95% CI: 0.93–5.29] 180 versus 18 measurements), and ACE inhibitor use and ACE I allele + AGT T allele versus DD + MM on SBP (−10.21 mmHg [95% CI: −19.47 to −0.95] 239 versus 29 measurements).

## Discussion

The data presented here provide evidence that subjects with the GNB3 TT polymorphism had lower SBP levels, while on treatment with a diuretic. None of the other examined genes had a significant influence on blood pressure. Of the 36 possible gene–gene–drug interactions on blood pressure, four results were significant, namely diuretic use and ADD1 W allele + AGT T allele versus GG + MM (DBP), *β*-blocker use and AGTR1 C allele + AGT T allele versus AA + MM (DBP), ACE inhibitor use and ACE I allele + GNB TT versus DD + C-allele (DBP), and ACE inhibitor use and ACE I allele + AGT T allele versus DD + MM (SBP).

Our results concur with that of a nonrandomized trial that investigated the role of GNB3 in diuretic users.[9] In this study, a significantly greater decline for both SBP and DBP was found in subjects with the TT allele.[9] To confirm these data, additional studies (trials and observational studies) are warranted to confirm this potential drug–gene interaction. Especially, because no interactive effect was found in this

study on DBP and the effect found on DBP was smaller than for example with AGT. In addition, none of the gene–gene–drug interactions was significant with GNB3 in diuretic users.

There are some explanations why our results might be false-positive. First, we have tested multiple genes on multiple outcomes and if we had adjusted for multiple testing, the interaction between GNB3 and diuretic use would not have been significant. A popular correction method for multiple testing is the Bonferroni correction $(1-(1-0.05) \times$ the number of markers); however, this correction would overcorrect the false-positive rate and thereby might disregard valid information. Second, the medication taken during the blood pressure measurement may not have been the first antihypertensive drug, but one that through a process of trail and error was found to be the most effective. With an over-representation of 'good responders', the chance to find a drug–gene interaction is higher. However, after adjustment for switchers, the result remained significant and therefore channeling of diuretics does not seem to be the explanation. Third, observational studies compared to trials may be vulnerable for confounding. Confounding is also unlikely since we adjusted for potential confounders, like dose, duration of therapy, age, gender, and comorbidities. Race could be an additional confounder, but less than 1% of the subjects had a different ethnic background. In addition, confounding by indication might have occurred in our study. As a physician was free to choose whether a patient receives an antihypertensive drug, and which specific patients' characteristics might have influenced this decision. However, the drug–gene interaction between subjects using the same antihypertensive class is most likely not influenced by this bias, since users of the same antihypertensive drug class have most likely the same characteristics and the physician is unaware of a subjects' genotype. There are other variables, for example, exercise and alcohol, which have an impact on blood pressure. Therefore, it is possible that we overestimated or underestimated the blood pressure lowering effect of the antihypertensive drug classes. However, since this is most likely the same for the different genotype groups, it would not have influenced our drug–gene interaction results. Fourth, an advantage of a trial is the possibility to assess the response to an antihypertensive drug, by measuring the blood pressure before treatment and during treatment. Owing to the small number of persons with a baseline measurement just preceding the start of an antihypertensive drug therapy, the mean difference in blood pressure was calculated. If the result is not false-positive, the observed difference of 4.33 mmHg systolic could result in a relative risk reduction of about 10% of cardiovascular disease in 10 years, according to the Framingham Risk function.

Regarding the significant gene–gene–drug interactions, the chance of false-positives is even higher due to the smaller sample size. However, of the 36 possible combinations (SBP, DBP), four were found to be significant. The observed interactions were only found with either SBP or DBP. Thus, further investigations are needed before definitive conclusions can be made. It is, however, apparent that the effects of the investigated single nucleotide polymorphisms are probably small and that this is the same for the gene–gene–drug interactions.

Notwithstanding these caveats, the study suggests that some gene–gene–drug interactions were found on blood pressure levels in daily practice. In addition, the GNB3 polymorphism of the GNB3 gene may influence the mean difference in SBP among users of low-ceiling diuretics.

## Materials and methods

### *Setting*

Data from the Doetinchem Cohort Study were used; a population-based prospective study on cardiovascular disease risk factor conducted in the Netherlands.[10] The baseline examination was carried out from 1987 to 1992 in men and women aged 20–59 years, living in Doetinchem, a Dutch town with circa 40 000 inhabitants.

### *Data collection*

At the start of the Doetinchem Cohort Study, the respondents completed a questionnaire that contained questions on demographic variables, cardiovascular diseases, and risk factors. In addition, weight and height were measured and blood was drawn for total and high-density lipoprotein (HDL) cholesterol determination and DNA extraction. The design of this study has been described elsewhere.[10] In addition, blood pressure data was collected from general practitioners from 1987 to 1997.

Pharmacy records were available for approximately 76% of the Doetinchem cohort as of 1 January 1987. These records include the name of the drug, the day of dispensing, the dosage form, the number of units dispensed, the prescribed daily dose, and the Anatomical Therapeutic Chemical code of the drug.[11]

### *Cohort and outcome definition*

Hypertensive patients were only included if the genotypes could be assessed, additional blood pressure measurements from the GPs were available, and pharmacy data were available. In addition, during follow-up, individuals had to have $\geqslant 1$ blood pressure measurement which met one of the following criteria: SBP $\geqslant 160$ mmHg, and/or diastolic blood pressure (DBP) $\geqslant 95$ mmHg, and/or the use of 1 antihypertensive drug class at the time of a blood pressure measurement (monotherapy). Only subjects using low-ceiling diuretics, $\beta$-blockers, and ACE inhibitors were included in the analysis, because of the small numbers for the other antihypertensive drug classes. Measurements were excluded when a combination of antihypertensive drugs was used. The end of the study period was set at 31 December 1997.

### *Potential confounders and effect modifiers*

As potential confounders, we considered age, sex, body mass index, defined daily dose, smoking at baseline, history of myocardial infarction, diabetes mellitus at baseline, use of nitrates, use of statins, use of NSAIDs, total/hdl cholesterol

level, low-salt diet, low-cholesterol diet, the use of another antihypertensive drug class 2 weeks prior to the blood pressure measurement, the use of an antihypertensive drug 6 of the 8 weeks prior to the blood pressure measurement, and the date of the measurement. To compare dosages of different antihypertensive drugs in our analysis, we used the prescribed daily dose (PDD), expressed as the number of DDDs per day. The DDD is defined as the recommended dose for the main indication in an adult of 70 kg.[12]

*Genotype*

Genomic DNA was isolated from peripheral blood according to standard procedures. The genotyping procedure of ADD1 G460W,[13] ACE I/D,[5] AGT M235T,[14] GNB3 825C/T,[15] and AGTR1 1166A/C[16] was previously described.

*Analysis*

We used ANOVA (continuous variables) and $\chi^2$ testing (categorical variables) to compare baseline characteristics of people with different genotypes. A marginal generalized linear model (GEE) was used to study the potential interaction between the genetic polymorphisms of interest and response to antihypertensive treatment for two outcomes: mean difference in SBP and DBP. We compared the mean SBP and DBP levels between the different genotype groups for subjects using the same antihypertensive drug class. To test for the interaction between the polymorphism in question (e.g. ACE I/D polymorphism) and the use of an antihypertensive drug class in question (e.g. ACE-inhibitors), two dummy variables were added to the model: ACE genotype (ID and II) $\times$ the use of ACE inhibitors during the blood pressure measurement (0/1). The reference group consisted of subjects with the DD genotype, who had a prescription of the antihypertensive drug class in question. The mean blood pressure of treated subjects was defined as the mean blood pressure of subjects who used the antihypertensive drug class in question minus the mean blood pressure in untreated subjects with the same genotype. For the gene–gene–drug interaction, we combined the genotypes of two of the five polymorphisms. For this analysis, we added three dummy variables to the model, that is, the drug–gene combinations (e.g. ACE + ADD1: I allele + T allele, I allele + MM, and D allele + T allele) $\times$ the use of the antihypertensive drug class in question (0/1). The GEE was used to account for intraperson correlations among repeated measurements. To compare the difference in DDD, the model was used stratified for the different genotypes. The covariance matrix of the repeated dependent measurements was exchangeable and data were analysed using SAS statistical software and adjusted for potential confounders.

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