Antihypertensive Drug-Gene Interactions and Cardiovascular Outcomes

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Manuscripts based on studies presented in this thesis

Chapter 2.1

Schelleman H, Klungel OH, Kromhout D, de Boer A, Stricker BHCh, Verschuren WMM. Prevalence and determinants of undertreatment of hypertension in the Netherlands. J Hum Hypertens 2004; 18:317-24.

Chapter 3.1

Schelleman H, Stricker BHCh, de Boer A, Kroon AA, Verschuren WMM, van Duijn CM, Psaty BM, Klungel OH. Drug-gene interactions between genetic polymorphisms and antihypertensive therapy. Drugs 2004; 64(16):1801-16. *(modified version)*

Chapter 4.1

Schelleman H, Klungel OH, van Duijn CM, Witteman JCM, Hofman A, de Boer A, Stricker BHCh. Insertion/deletion polymorphism of the ACE gene and adherence to ACE-inhibitors. Br J Clin Pharmacol 2005; 4: 483-5.

Chapter 4.2

Schelleman H, Klungel OH, van Duijn CM, Witteman JCM, Hofman A, de Boer A, Stricker BHCh. Drug-gene interaction between the insertion/deletion polymorphism of the angiotensin converting enzyme gene and antihypertensive therapy on blood pressure. Ann Pharmacother; *accepted*

Chapter 4.3

Schelleman H, Klungel OH, Witteman JCM, Hofman A, van Duijn CM, de Boer A, Stricker BHCh. The influence of alpha-adducin G460W and angiotensinogen M235T polymorphism on the association between antihypertensive medication and blood pressure. *submitted*

Chapter 4.4

Schelleman H, Stricker BHCh, Verschuren WMM, de Boer A, Kroon AA, de Leeuw PW, Kromhout D, Klungel OH. Interactions between five candidate genes and antihypertensive therapy on blood pressure. Pharmacogenomics J; *accepted*

Chapter 5.1

Schelleman H, Klungel OH, van Duijn CM, Witteman JCM, Hofman A, de Boer A, Stricker BHCh. Pharmacogenetic interactions of three candidate gene polymorphisms with ACE-inhibitors or β-blockers therapy and the risk of atherosclerosis. *submitted*

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Schelleman H, Klungel OH, van Duijn CM, Breteler MMB, Danser AHJ, Hofman A, de Boer A, Stricker BHCh. Interaction between the angiotensinogen M235T polymorphism and ACE-inhibitors or β-blockers therapy and the risk of myocardial infarction and stroke. *submitted*

# Contents

Chapter 1

Introduction

Introduction

Hypertension is a complex disease with a high prevalence. It is defined as a systolic blood pressure (SBP) ≥ 140 mmHg and a diastolic blood pressure (DBP) ≥ 90 mmHg for persons up to 60 years of age and for subjects with diabetes mellitus or familiar hypercholesterolemia and as a SBP ≥ 160 mmHg and a DBP ≥ 90 mmHg for persons of 60 years and older without diabetes mellitus or familiar hypercholesterolemia.[1] Hypertension is a risk factor for myocardial infarction, stroke, congestive heart failure, end-stage renal disease, and peripheral vascular disease.[2-5] The World Health Organization reported that suboptimal blood pressure (SBP > 115 mmHg) is responsible for 62% of all cerebrovascular diseases and 49% of all ischemic heart diseases. In addition, suboptimal blood pressure is the number one cause of death throughout the Western world.[6]

Many physiological, biochemical, and anatomical traits contribute to an individual's blood pressure level, which is homeostatically maintained through complex interactions of interrelated systems that exert redundant and counterbalancing pressor and depressor effects.[7] In normotensive and hypertensive individuals, cardiac output (heart rate x stroke volume) and peripheral resistance are controlled by overlapping control mechanisms i.e. the baroreflexes mediated by the sympathetic nervous system, the parasympatic nervous system, and the renin-angiotensin system. Antihypertensive drugs lower blood pressure by acting on specific targets within these systems.

Antihypertensive treatment can be divided into four main classes: diuretics, β-blockers, calcium channel blockers, and renin-angiotensin system inhibitors (angiotensin converting enzyme (ACE)-inhibitors and angiotensin II type 1 receptor antagonists). Diuretics are currently recommended as the first-line treatment for hypertension.[1, 8] Diuretics lower blood pressure, initially by increasing sodium and water excretion and with long-term treatment by decreasing peripheral resistance (see figure 1). The impairment in sodium excretion may be one of the first changes in the development of hypertension.[9] Beta-blockers decrease the cardiac output by acting on β1-receptors and by inhibiting the release of renin. Calcium channel blockers block the inward movement of calcium by binding to L-type calcium channels in the heart and in smooth muscle of the coronary and peripheral vasculature. This causes a relaxation of smooth muscle cells, although, there are differences between calcium channel blockers in their affinity. ACE-inhibitors inhibit the conversion of angiotensin I to angiotensin II resulting in a reduction of the peripheral vascular resistance. In addition, ACE-inhibitors decrease the secretion of aldosteron, which results in a decreased sodium and water retention. It also reduces the breakdown of the vasodilator bradykinin. Angiotensin II type 1 receptor antagonists block the action of angiotensin II at the angiotensin (AT)1 receptors. In clinical trials, antihypertensive therapy has been associated with a 35% to 40% risk reduction in stroke incidence, a 20% to 25% reduction in myocardial infarction incidence, and a more than 50%

reduction in heart failure incidence.[10]

Figure 1. Diagram showing the main mechanisms involved in arterial blood pressure regulation and the sites of action of antihypertensive drugs.[11]
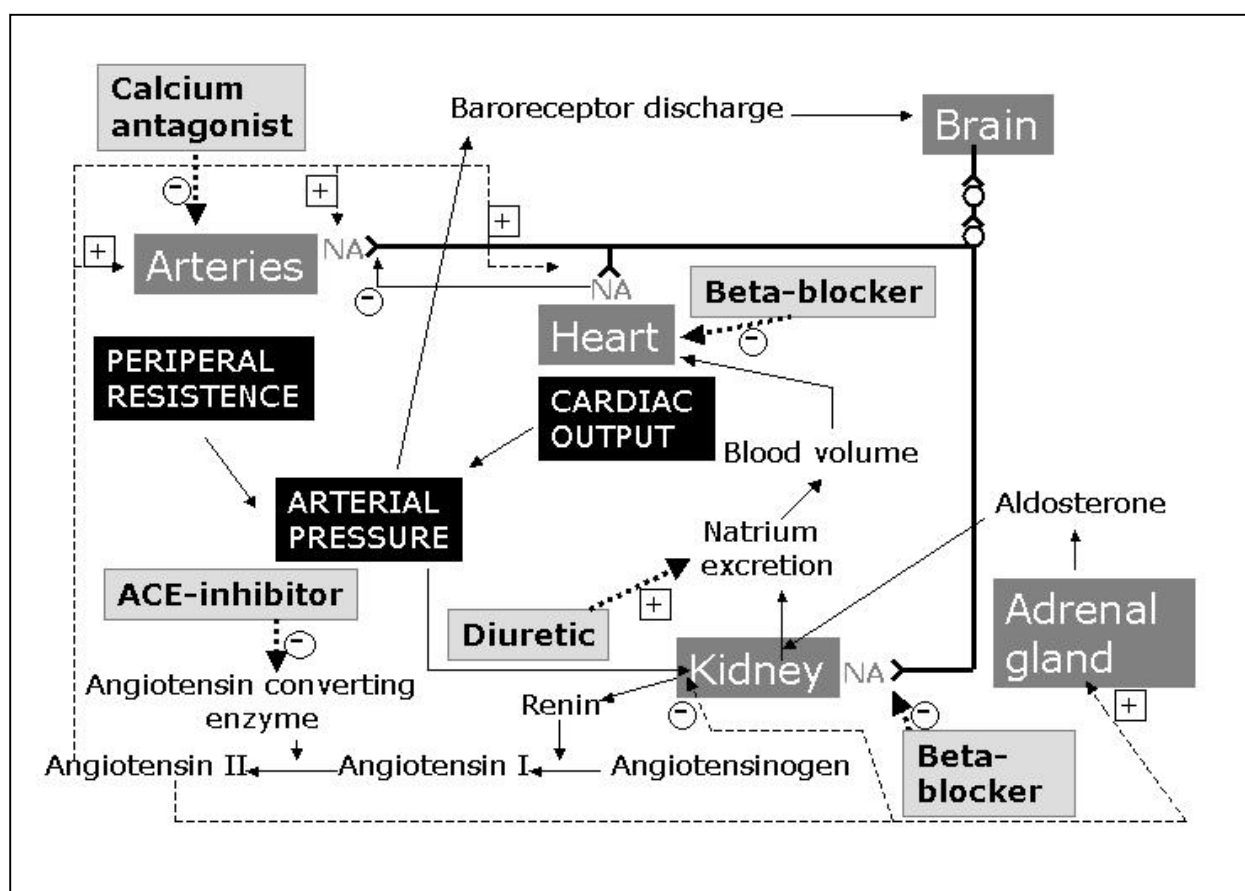
Finding the most appropriate pharmacological treatment for an individual patient is difficult as the response can not be predicted with patient characteristics such as age, gender, or body mass index.[12, 13] Pharmacogenetics aims to understand how genetic variations contribute to variation in response to medication. Polymorphisms in genes that code for drug-metabolizing enzymes, drug transporters, drug receptors, and ion channels can alter the response of drug treatment in an individual. Targeting treatment to the genetic components may enhance treatment efficacy and improve overall benefit, resulting in more effective blood pressure control and a lower incidence of hypertension-related morbidity.

Aim and outline of this thesis

This thesis consists of a number of studies, which are aimed at gaining more insight into the variation in response to antihypertensive drugs by investigating the interaction between antihypertensive drugs and genetic polymorphisms on short and long-term cardiovascular outcomes.

In **Chapter 2**, population-based estimates of the prevalence of undertreatment of hypertension were determined. Furthermore, this chapter presents determinants associated with non-use and uncontrolled blood pressure levels. **Chapter 3** gives an overview of studies that investigated the influence of genetic variants on the response to antihypertensive drugs. **Chapter 4** contains pharmacogenetic studies and short-term outcomes. The first study (**Chapter 4.1**) describes the influence of the ACE insertion/deletion (I/D) polymorphism on the adherence to ACE-inhibitors in the Rotterdam Study.[14] The second (**Chapter 4.2**) and third study (**Chapter 4.3**) examine the influence of the ACE I/D, α-adducin G460W, and angiotensinogen M235T polymorphism on blood pressure response in the Rotterdam Study. In **Chapter 4.4** the interaction between antihypertensive drugs and the ACE I/D, α-adducin G460W, angiotensinogen M235T, the β3-subunit of the G-protein 825C/T polymorphism, and angiotensin II type 1 receptor 1166A/C polymorphism on blood pressure were evaluated in the Doetinchem Cohort Study.[15] The long-term outcomes are described in **Chapter 5**. In the first study the ACE I/D, angiotensinogen M235T, and angiotensin II type 1 receptor 573C/T polymorphism modify the risk of atherosclerosis associated with ACE-inhibitors or β-blockers therapy (**Chapter 5.1**). **Chapter 5.2** describes pharmacogenetic studies with the long-term outcomes myocardial infarction and stroke. For both outcomes the interaction between the angiotensinogen M235T polymorphism and the use of ACE-inhibitors on the risk of myocardial infarction and stroke was investigated in the Rotterdam Study. In chapter 6 the main findings and possible clinical implications are discussed and suggestions for further research are given.

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Chapter 2

Hypertension

Chapter 2.1

Prevalence and determinants of undertreatment of hypertension in the Netherlands

Abstract

The objective of this study was to determine the prevalence, treatment, and control of hypertension and the determinants of undertreatment in the Dutch population. The study-design was cross-sectional. A population-based survey on cardiovascular disease risk factors in the Netherlands from 1996 to 2002 was the setting of the study. A total of 10,820 man and women, aged 30-59 years, were included in the study. The mean outcome measures of the study were: prevalence of hypertension, treatment, and control of hypertension and determinants of undertreatment of hypertension. Hypertension was defined as: systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg, and/or the use of antihypertensive medication. Treated and controlled hypertension was defined as SBP < 140 mmHg and DBP < 90 mmHg. Multivariate logistic regression was used to assess the determinants of undertreatment. The prevalence of hypertension in men was 21.4% and in women 14.9%. About 18% of the hypertensive men and 39% of the hypertensive women were receiving antihypertensive medication. Of the untreated hypertensives, 21.9% of the men and 13.6% of the women were eligible for treatment with antihypertensive medication according to Dutch guidelines. Female gender and use of cholesterol-lowering medication were associated with an increased chance of being treated. Subjects who were physically active, on a low salt diet, and current smokers had an increased chance of being untreated. Using cholesterol-lowering medication and no asthma or allergy were factors associated with better control of blood pressure. In conclusion, a considerable proportion of hypertensives were untreated and uncontrolled. Therefore, the detection and control of hypertension in the Netherlands needs to improve. Several groups of hypertensives were identified that need additional care and attention.

Introduction

Hypertension is a major public health hazard because of its high prevalence[1] and its strong positive association with cardiovascular diseases.[2-6] The overall beneficial effect of treatment of hypertension has been demonstrated.[7-9] Therefore, the detection and adequate treatment of hypertension is important to reduce the incidence of cardiovascular diseases. Knowledge of factors that are associated with undertreatment of hypertension may help to identify subgroups that need additional care and attention. Previously, it was reported that the prevalence of hypertension (systolic blood pressure (SBP) $\geq$ 140 mmHg and/or diastolic blood pressure (DBP) $\geq$ 90 mmHg and/or use of antihypertensive medication) in the Netherlands in the period 1993 to 1997 was approximately 20.2% of the population in the age-group from 40 to 59 years.[10] Studies from other countries suggest that the proportion of hypertensives treated and/or controlled has been stable in recent years or has even decreased.[11, 12]

Therefore, we performed the present study to assess the prevalence and determinants of undertreatment of hypertension in the Netherlands during 1996-2002.

Methods

Data

Data were obtained from population-based surveys on cardiovascular disease risk factors conducted in The Netherlands. The Monitoring Project on Cardiovascular Disease Risk Factors (MPCDRF) was carried out from 1987 to 1992 in men and women aged 20-59 years. Each year, a new random sample was collected in basic health services in Amsterdam, the capital in the west with about 700,000 inhabitants, Doetinchem a small town with circa 40,000 inhabitants in a rural area in the east, and Maastricht in the south with roughly 100,000 inhabitants at that time.

The overall response rate in Amsterdam, Maastricht, and Doetinchem was 45%, 58%, and 62%, respectively. The average response rate for men was 50% and for women 57%. This project was continued from 1993 to 1997 as the "Monitoring risk factors and health in The Netherlands" (MORGEN) project. In Amsterdam and Maastricht, new random subjects were collected for those aged 20-59 years, whereas in Doetinchem the study population consisted of individuals who had participated in the previous study. So, patients in Doetinchem were re-examined after 5 years. The response rate in Amsterdam was 30% for men and 37% for women, in Maastricht it was 42% and 49%, and in Doetinchem it was 57% and 60%, respectively. From 1998 to 2002, data were only collected from the Doetinchem cohort, which was the second re-examination of the participants of the PCDRF (aged 30-69 years). The overall response rate was 68% for men and 63% for women.

All respondents completed a questionnaire that contained questions on demographic variables, cardiovascular risk factors, and current use of medication. After this, blood pressure, weight, and height were measured and blood was drawn for total and high-density lipoprotein cholesterol determination. The design of this study has been described in detail elsewhere.[10, 12] A nonresponse survey was conducted in order to assess possible selection bias. Of all nonrespondents (n=1,620) 61% agreed to participate, 23% could not be reached, and 16% refused to participate. The results suggested that no selection bias with respect to educational level has occurred. Educational level is a main determinant of nonresponse and is associated with blood pressure.[13, 14] Therefore, no substantial differences are expected in blood pressure between respondents and nonrespondents.[10]

A random zero sphygmomanometer was used to measure blood pressure with the subject in a sitting position using a cuff of proper size for arm circumference. After the first measurement, the heart rate was measured for 30 s and after 5 min by a second blood pressure measurement.

We selected patients group aged 30-59, because persons aged 20-29 were not included in the re-examination of the Doetinchem cohort (1998-2002) and in Amsterdam and Maastricht (1996-1997) persons aged 60-69 were not included.

Definitions

Hypertension was defined according to the WHO-ISH criteria as SBP $\geq$ 140 mmHg and/or DBP $\geq$ 90 mmHg and/or the use of antihypertensive medication (irrespective of the level of blood pressure). Participants with hypertension were further classified as: treated and adequately controlled, treated but uncontrolled, and untreated. Uncontrolled persons were treated but had their SBP $\geq$ 140 mmHg and/or DBP $\geq$ 90 mmHg. For the analysis of undertreatment, we used Dutch guidelines of 2000 for the treatment of hypertension.[15] If SBP $\geq$ 180 mmHg and/or DBP $\geq$ 100 mmHg treatment is always necessary. In addition, when the 10-year cardiovascular risk (estimated with the multifactorial Framingham risk equation) exceeds 20%, hypertension should also be pharmacologically treated. Persons aged 40-59 years with a cardiovascular disease, subjects with diabetes aged 50-59 years, persons with diabetes and smoking aged 40-49 years, and smoking males aged 50-59 years with SBP between 140 and 180 mmHg and/or DBP between 90 and 100 mmHg should be treated because their 10-year cardiovascular risk exceeds 20%. Among treated hypertensives, blood pressure is considered controlled if SBP < 140 mmHg and DBP < 90 mmHg.

For the analysis of the determinants or treatment and control, besides a cut-off value of 140/90 mmHg, also a higher SBP cut-off ($\geq$ 160 mmHg) and/or DBP cut-off ($\geq$ 95 mmHg) was chosen, in order to minimize misclassification of subjects as untreated or uncontrolled hypertensives on the basis of two blood pressure measurements on the same day.

Correction for within-person variability

Repeated measurements of blood pressure and total cholesterol were available from a sample of the population screened from 1987 to 1992.[9] Among 924 subjects who were examined in 1989 and 1990, in each year two blood pressure measurements (in duplicate) and a total cholesterol determination were performed. These measurements were used to calculate blood pressure, serum total cholesterol, and HDL-cholesterol levels adjusted for within-person variability.

The adjustment for SBP and DBP was performed separately for persons untreated for hypertension with no other risk factor present, respondents who were untreated for hypertension with one or more risk factors, and drug-treated persons after stratification by gender and 10-year age category.[16] This adjustment was performed within these strata because each stratum can be considered a separate subpopulation with a specific distribution of blood pressure values. By this approach, each blood pressure value was corrected towards the mean of the stratum to which that individual belonged. This will correct for the possibility to classify a person with normal blood pressure as hypertensive. For total cholesterol values, this correction was performed after stratification for gender and 10-year age category.[16]

Statistical analysis

The prevalence of treatment and undertreatment of hypertension was estimated and standardized to the age and gender distribution of the general population in 1999. Multivariate logistic regression analysis was used to assess the association between demographic variables, cardiovascular disease risk factors, medication use as independent variables and treatment and control of hypertension as dependent variables. The Chi-square statistics for trend was used for the time trends.

Results

Prevalence of hypertension, treatment and control of hypertension

After exclusion of pregnant women (n=125) and subjects with missing blood pressure data (n=12), 10,820 subjects remained available for analysis. Using the WHO-ISH guidelines,[19] 20.1% (2,176/10,820) of the study population was classified as hypertensive (see table 1). Of the hypertensives, 70% (1,530/2,176) were not receive any antihypertensive medication, while among those treated 54% (347/646) had blood pressure levels ≥ 140/90 mmHg. Table 2 lists the prevalence of hypertension by age and gender. Among men, 21.4% had hypertension, 17.9% was treated, and in 67.6% of those treated blood pressure was not controlled. According to the current Dutch guidelines, 21.9% of the untreated hypertensive men were eligible for treatment.

Table 1. Prevalence of hypertension according to the WHO-ISH classification, adjusted for within-person variability.

WHO-ISH grade	SBP (mmHg)	DBP (mmHg)	Total study population (n (%))	Treated (n (%))	Untreated (n (%))
Normotensive					
Optimal	< 120	< 80	4,247 (39.3)	32 (0.3)	4,215 (40.0)
Normal	120-129	80-84	2,713 (25.1)	93 (0.9)	2,620 (24.2)
High normal	130-139	85-89	1,983 (18.3)	174 (1.6)	1,809 (16.7)
Hypertensive					
Grade 1	140-159	90-99	1,509 (13.9)	257 (2.4)	1,252 (11.6)
Grade 2	160-179	100-109	311 (2.9)	77 (0.7)	234 (2.2)
Grade 3	≥ 180	≥ 110	57 (0.5)	13 (0.1)	44 (0.4)
All			10,820 (100)	646 (6.0)	1,530 (14.2)[1]

[1] Only the sum of the percentages of grade 1,2, and 3
All percentages refer to the total population

Table 2. Prevalence of hypertension (≥ 140/90 mmHg), treated and undertreated hypertension in men and women by 10-year category and adjusted for within-person variability.

	Respondents	Hypertension (n (%))[1]	Patients treated (n (%))[2]	Patients treated but uncontrolled[5] (n (%))[3]	Patients untreated (n (%))[2]	Patients untreated but should be treated[6] (n (%))[4]
Men						
30-59[7]	5,004	1,201 (21.4)	285 (17.9)	181 (67.6)	916 (82.1)	271 (21.9)
30-39	1,237	130 (10.5)	11 (8.5)	8 (72.7)	119 (91.5)	14 (11.8)
40-49	1,958	419 (21.4)	75 (17.9)	50 (67.7)	344 (82.1)	65 (16.3)
50-59	1,809	652 (36.0)	199 (30.5)	123 (61.8)	453 (69.5)	192 (42.4)
Women						
30-59[7]	5,816	975 (14.9)	361 (38.5)	166 (51.9)	614 (61.5)	94 (13.6)
30-39	1,593	59 (3.7)	24 (40.7)	16 (66.7)	35 (59.3)	4 (11.4)
40-49	2,306	312 (13.5)	119 (38.1)	46 (38.7)	193 (61.9)	26 (13.5)
50-59	1,917	604 (31.5)	218 (36.1)	104 (47.7)	386 (63.9)	64 (16.6)

[1] All percentages refer to the total population
[2] All percentages refer to those hypertensive
[3] All percentages refer to those treated
[4] All percentages refer to those untreated
[5] DBP ≥ 140 mmHg and/or SBP ≥ 90 mmHg
[6] Untreated should be treated refers to the subject not treated for hypertension, who should be treated according to the CBO consensus Hypertension because their risk for developing a cardiovascular disease is more then 20% based on their age, gender, blood pressure, smoking status, and presence of diabetes, or cardiovascular diseases
[7] Weighed by age and gender distribution of the general Dutch population in 1999

Among women, 14.9% had hypertension, 38.5% was treated, of whom 51.9% had their blood pressure uncontrolled. About 14% of the untreated hypertensive women were eligible for treatment. The prevalence of hypertension increased with age for both men and women. In each age category, the treatment with antihypertensive medication was more prevalent in women compared to men. Among the 2,176 subjects with hypertension, a total of 365 untreated subjects were eligible for

treatment and of those treated (n=646) 347 persons had their blood pressure uncontrolled.

Time trends in the treatment and control of hypertension

The prevalence of hypertension decreased significantly from 1996 to 2002 in Doetinchem (trend test p=0.04). An increasing trend was observed for the percentage of treated hypertensives (trend test p=0.02).

   The percentage of controlled persons fluctuated, with the worst situation between 1998 and 1999 (see table 3). During this period, the percentage of untreated hypertensives who were eligible for treatment was highest. The proportion of treated hypertensives with uncontrolled blood pressure and the proportion of untreated hypertensives who were eligible for treatment was lowest from 2000 to 2002. However, none of these differences were statistically significant.

Table 3. Prevalence of hypertension (≥ 140/90 mmHg), treated and undertreated hypertension for different time periods, weighted by age and gender distribution of the general Dutch population in 1999 and adjusted for within-person variability.

	Respondents	Hypertension (n (%))[1]	Patients treated (n (%))[2]	Patients treated but uncontrolled[5] (n (%))[3]	Patients untreated (n (%))[2]	Patients untreated but should be treated[6] (n (%))[4]
1996-1997						
Amsterdam	2,421	389 (15.8)	133 (32.3)	58 (54.2)	256 (67.6)	62 (14.5)
Maastricht	2,089	440 (16.7)	157 (38.0)	82 (62.8)	283 (62.0)	68 (25.3)
Doetinchem	2,518	427 (21.4)	91 (20.7)	63 (65.2)	336 (79.3)	77 (23.0)
1998-1999						
Doetinchem	1,446	349 (21.3)	94 (27.2)	55 (67.6)	255 (72.8)	78 (23.0)
2000-2002						
Doetinchem	2,346	571 (20.6)	171 (28.5)	89 (56.3)	400 (71.5)	80 (14.9)

[1] All percentages refer to the total population
[2] All percentages refer to those hypertensive
[3] All percentages refer to those treated
[4] All percentages refer to those untreated
[5] DBP ≥ 140 mmHg and/or SBP ≥ 90 mmHg
[6] Untreated should be treated refers to the subject not treated for hypertension, who should be treated according to the CBO consensus Hypertension because their risk for developing a cardiovascular disease is more then 20% based on their age, gender, BP, smoking status, and presence of diabetes, or cardiovascular diseases

Determinants of untreated and treated but uncontrolled hypertension

Determinants of undertreatment, defined as eligible for treatment but not receiving antihypertensive medication, are reported in figure 1a. Subjects who used cholesterol-lowering medication and subjects screened during the years 1998 and 1999 were less likely to be untreated (Odds ratio (OR) < 1). Males, current smokers, subjects on a low salt diet, and physically active hypertensives were more likely to be untreated (OR > 1).

Figure 1. Determinants of untreated and uncontrolled hypertension according to the CBO consensus Hypertension. All odds ratio are adjusted for demographic variables, cardiovascular risk factors, and medication use.

Untreated versus Treated (Odds ratio)

Less likely to be treated ← ┊ → More likely to be treated

0,98	Older age vs. younger age
3,72	Male vs. female
0,42	Maastricht vs Doetinchem
0,34	Amsterdam vs. Doetinchem
1,03	Living without a partner vs. with
0,94	Education low vs. high
1,06	Education middle vs. high
2,24	Physically active vs. not
2,38	BMI = 30 kg/m² vs. < 30 kg/m²
1,03	Past alcohol user vs. current
0,85	Never used alcohol vs. current
2,18	Current smoker vs. no
1,30	Past smoker vs. no
0,34	Cholesterol medication yes vs. no
0,58	Evidence for cardiovascular disease yes vs. no
0,61	Diabetes yes vs. no
3,06	Asthma or allergy yes vs. no
1,76	Diabetes mother or father yes vs. no
2,51	MI mother or father yes vs. no
0,41	1998-1999 vs. 1996-1997
0,63	2000-2002 vs. 1996-1997

0,01   0,1   1   10   100

Uncontrolled versus Controlled (Odds ratio)

Less likely to be uncontrolled ← ┊ → More likely to be uncontrolled

0,97	Older age vs. younger age
2,07	Male vs. female
0,25	Maastricht vs Doetinchem
0,64	Amsterdam vs. Doetinchem
1,12	Living without a partner vs. with
2,75	Education low vs. high
1,06	Education middle vs. high
1,68	Physically active vs. not
2,38	BMI = 30 kg/m² vs. < 30 kg/m²
0,41	Past alcohol user vs. current
0,34	Never used alcohol vs. current
2,18	Current smoker vs. no
1,30	Past smoker vs. no
0,34	Cholesterol medication yes vs. no
0,58	Evidence for cardiovascular disease yes vs. no
0,61	Diabetes yes vs. no
3,06	Asthma or allergy yes vs. no
1,76	Diabetes mother or father yes vs. no
2,51	MI mother or father yes vs. no
0,41	1998-1999 vs. 1996-1997
0,52	2000-2002 vs. 1996-1997

0,01   0,1   1   10   100

Determinants of treated but uncontrolled hypertension are reported in figure 1b. The use of cholesterol-lowering medication was significantly associated with a lower probability of uncontrolled hypertension (OR <1) and having asthma or allergy with a higher probability of uncontrolled hypertension.

We also performed an analysis with a higher SBP cut-off (≥ 160 mmHg) and/or DBP cut-off (≥ 95 mmHg). The association between determinants and treatment status was similar compared to the analysis with lower blood pressure cut-offs. Although the following factors showed the same trend, they were no longer significant: year of screening (1998-1999 vs. 1996-1997; OR=0.43; 95% CI: 0.16-1.16) and being physically active (OR=1.96; 95%CI: 0.96-3.99). The place of residence became significant (Maastricht vs. Doetinchem; OR=0.28; 95%CI: 0.08-0.78, Amsterdam vs. Doetinchem; OR=0.25; 95%CI: 0.09-0.84). The association between determinants and uncontrolled blood pressure was also similar compared to the analysis with a lower blood pressure cut-off level. However, year of screening (2000-2002 vs. 1996-1997; OR=0.20; 95%CI: 0.05-0.82) and older age were significantly associated (OR=0.93; 95%CI: 0.86-0.99). Asthma or allergy was no longer significant (OR=1.69; 95%CI: 0.62-4.57).

Discussion

Approximately 21% of the men and 15% of the women aged 30-59 years were hypertensive. Approximately 18% of the hypertensive men and 39% of the hypertensive women were receiving antihypertensive medication. According to the Dutch guidelines, only 21.9% of the untreated hypertensive men and 13.6% of the untreated hypertensive women were eligible for pharmacological treatment. Of the treated persons, 67.6% of the men and 51.9% of the women had uncontrolled blood pressure levels despite pharmacological treatment. A possible explanation for the differences found in men and women could be the higher rate of patient-physician contact of women and the higher compliance of women in our study population. Unfortunately, we did not have information on these variables and were therefore unable to investigate these factors. The prevalence of hypertension decreased and the prevalence of treatment increased between 1996 and 2002 in Doetinchem.

The prevalence of hypertension in the Netherlands is similar compared to other Western-European studies. In England, the prevalence is approximately 24% for men and 22% for women (aged 30-59 years);[17] in France, it is 16% for men and 9% for women (aged 18-50 years);[18] and in Germany 39% for men and 25% for women (aged 25-64 years).[19] The percentage of treated hypertensives in West-Europe is around 60% and the percentage of uncontrolled hypertensives around 20%. Wolf-Maier et al.[20] published that the prevalence of hypertension was 44% in six European countries and that only 8% of the hypertensive persons had their blood pressure controlled (aged 35-64 years). However, because of differences in study design, such

as age range, years of screening, and method for blood pressure measurement, it is difficult to compare the results. In most studies, treatment and control of hypertension is better in women than in men.[19]

The results from the multivariate analysis show that females, not being physically active, not having been screened at the beginning of the follow-up, low intake of salt, and use of cholesterol-lowering medication are positively associated with treatment of hypertension. Also, in other European studies, females and non-smoking are positively associated with treatment of hypertension, while results for age and evidence for cardiovascular diseases are not the same.[21-23] This is probably caused by the difference in age range. In our study, subjects who are physically active or having a low salt diet are less likely to receive treatment. This might have occurred because these subjects were borderline hypertensive and were advised by their doctors to be more active in order to reduce their blood pressure or eat a low salt diet. The use of cholesterol-lowering medication and having asthma or allergy are factors associated with a better control of blood pressure. A possible explanation is that patients who already use medication besides blood pressure-lowering drugs have a higher compliance. In other European studies, female gender and evidence for cardiovascular disease are associated with a better control of blood pressure.[21-23] The results of our study are similar, although, the differences in study designs make it difficult to compare the results.

Treatment considerations

At the moment, two different treatment guidelines are used in the Netherlands.[22, 31] The CBO consensus is the most recent guideline and is the result of a consensus between various health-care professionals, whereas the NHG guideline is less recent and is an advice from the Dutch General Practitioners Association. The NHG guideline still uses blood pressure $\geq$ 160/95 mmHg as a definition for hypertension, and recommends treatment goals < 160/90 mmHg. This may explain the poor control of blood pressure since 1996 and improvement during the most recent years. Nonetheless, even in the most recent years treatment and control rates of hypertension were far from optimal. Recently, the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood pressure even recommended starting antihypertensive drug treatment in patients with blood pressure $\geq$ 140/90 mmHg, irrespective of their cardiovascular risk factor profile.[25]

One possible explanation for lack of control of blood pressure among treated hypertensives might be the lack of aggressiveness in treating persons. Another reason for not achieving the target blood pressure is poor patient compliance with the antihypertensive medication. According to several studies, about 50% to 60% of hypertensives adhere well to antihypertensive medication.[26, 27] Lack of treatment among those eligible for drug treatment may be caused by a lack of detection of hypertension, physician noncompliance with treatment guidelines,[28-32] or reluctance of persons to receive drug treatment.[33, 34]

Unfortunately, despite various intervention strategies of different aspects in the management of hypertension, only a few of these interventions have been effective in achieving improved control of blood pressure.[35] Multiple interventions at the level of patients, health-care providers are probably more effective than a single interaction by a health-care provider alone.[36]

The WHO reported in 1999 that there are worrying signs that the control rates had stabilized or even declined in some cases.[11] This study demonstrates that the prevalence of hypertension has decreased and that the number of hypertensives treated has increased between 1996 and 2002 in Doetinchem. It is, however, difficult to compare the results from Doetinchem with the general Dutch population. The results from the multivariate analysis show that hypertensives living in Doetinchem were less likely to be treated and have their blood pressure controlled compared to hypertensives living in Amsterdam or Maastricht between 1996 and 1997. So, even in a small country as the Netherlands there are regional differences in treatment probability, which may be related to differences in lifestyle.

Strengths and limitations

A potential bias is that patients are classified as hypertensives based on measurements, which were obtained on a single occasion, although, averaged over two readings. However, we adjusted for within-person variability in blood pressure and total cholesterol. Ignoring this variability would have lead to incorrect classification of persons with normal blood pressure as hypertensives and therefore influence prevalence estimates. Also, a higher blood pressure ($\geq$ 95/160 mmHg) cut-off was used for the analysis of determinants of hypertension, since this could minimize the number of falsely assignment hypertensives. The results are, however, similar.

Another limitation is that we used self-reported data and did not include an examination of subjects' medical records. The influence of misclassification is difficult to assess because over and under-reporting of cardiovascular risk factors and diseases occur.[37] The use of information from self-reported medication has most likely not influenced our prevalence estimates, because agreement between self-reported antihypertensive drug use in this survey and the pharmacy records of antihypertensive drug dispensing is excellent.[38]

The Doetinchem cohort was a re-examination. It is possible that persons who participate in the re-examination are more conscious of their health (e.g. better compliance with drugs, better lifestyle, and/or more visits to a physician). If this is the case we underestimated the number treated and controlled hypertensives in the general population.

We decided to consider only subjects whose risk of developing a cardiovascular disease within the next 10 years exceeding 20% as "untreated but should be treated". However, according to the CBO guidelines[13] when the cardiovascular risk is between 10% and 20%, drug treatment is cost-effective and may therefore be considered. So,

the eligible group for treatment is most likely larger than considered in this study. We did not include this group in our analysis because the guidelines leave this to the individual choice of the physician and patient.

Conclusions

Overall, the results suggest that approximately 14% of the Dutch population aged 30-59 years has hypertension (blood pressure ≥ 140/90 mmHg). The situation is better for women than for men. There remains a considerable proportion of hypertensives who are eligible for treatment but are untreated (18%) and treated patients whose blood pressure is not controlled (46%). Although treatment improved slightly during the study period in Doetinchem, control of hypertension in our study is far from optimal. Owing to the strong association between blood pressure and cardiovascular disease, it is necessary to improve treatment and control rates of hypertension in the Netherlands. To improve the management of hypertension, physicians may focus on the subgroups, which are identified in this study.

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Chapter 3

Pharmacogenetics and antihypertensive drugs

Chapter 3.1

Drug-gene interactions between genetic polymorphisms and antihypertensive therapy

Abstract

Genetic factors may influence the response to antihypertensive medication. A number of studies have investigated genetic polymorphisms as determinants of cardiovascular response to antihypertensive drug therapy. In most candidate gene studies, no such drug-gene interactions were found. However, there is observational evidence that hypertensive patients with the 460W-allele of the α-adducin gene have a lower risk of myocardial infarction and stroke when treated with diuretics compared with other antihypertensive therapies. With regard to blood pressure response, interactions were found between genetic polymorphisms in the endothelial nitric oxide synthase gene and diuretics, the α-adducin gene and diuretics, the α-subunit of G protein and β-blockers, and the angiotensin converting enzyme (ACE) gene and angiotensin receptor II type 1 (AT1) receptor antagonists. Other studies found an interaction between ACE-inhibitors and the ACE insertion/deletion (I/D) polymorphism, which resulted in difference in AT1 receptor mRNA expression, left ventricular hypertrophy, and arterial stiffness between different genetic variants. Also, drug-gene interactions between calcium channel blockers and ACE I/D polymorphism regarding arterial stiffness have been reported. Unfortunately, the quality of these studies is quite variable. Given the methodological problems, the results from the candidate gene studies are still inconclusive and further research is necessary.

Introduction

Hypertension is a major public health hazard because of its high prevalence, which is approximately 20% of the adult population in most developed countries[1] and its high risk of cardiovascular diseases. Despite the availability of a variety of effective antihypertensive drugs, inadequate control of blood pressure is common in hypertensive patients, and is responsible for a large proportion of cardiovascular diseases in the population.[2-4] The average response to antihypertensive drugs is similar across different classes of antihypertensives. For example, in the Veterans Affairs study,[5] a randomized placebo-controlled clinical trail in which patients were randomly allocated to six different drugs or placebo, 31.7% of all hypertensive patients who were allocated to monotherapy with an antihypertensive drugs failed to achieve a normal diastolic blood pressure. When the initial treatment failed, 85.9% of these patients were randomized to another antihypertensive drugs which failed in 37.8% of these patients.[5] Although, the currently used 'trial and error' approach to antihypertensive drug therapy can be efficient in treating high blood pressure, it is not feasible with regards to long-term effects, such as myocardial infarction (MI) and stroke. Important factors in interpreting the variability in outcome of drug therapy include the patient's general health, prognosis, disease severity, quality of drug prescribing and dispensing, compliance with prescribed pharmacotherapy, and the genetic profile of the patient.[6, 7]

Multiple susceptibility genes and the environment explain the phenotype of essential hypertension. From family, twin, and adoption studies it has been estimated that 30% to 60% of the variation in blood pressure between individuals is caused by genetic factors.[8] However, there is a small proportion of familial forms of hypertension that have a single gene (Mendelian) inheritance pattern. These include: apparent mineralocorticoid excess, glucocorticoid remediable aldosteronism, hypertensive forms of congenital adrenal hyperplasia, Liddle's syndrome, pseudohypoaldosteronism type II/Gordon's syndrome, early-onset, autosomal dominant hypertension with severe exacerbation in pregnancy, and Bardet-Biedl syndrome types 2 and 4.[9-11]

Pharmacogenetics focuses on the extent to which variability in genetic make-up is responsible for the observed differences in therapeutic response and adverse reactions between patients.[6, 7] In other words, pharmacogenetics studies the interaction between drugs and genes, where interaction is defined as being present if the joint effect of a drug and genetic polymorphism is greater than the sum (additive scale) or product (multiplicative scale) of the individual effects of the drug and the genetic polymorphism. The purpose of pharmacogenetics is to understand the effects of genetic diversity on human response to drugs and other foreign substances and to use this information to avoid the occurrence of therapeutic failure and adverse drug reactions in susceptible persons.[12] Drugs that are more specific for functional characteristics associated with an individual patient's polymorphism may contribute to a better response and reduced toxicity of pharmacotherapy.

Genetic polymorphisms may influence drug response in three ways.[13]
1. Through variation in pharmacokinetics that may pertain to absorption, but is mostly explained by altered metabolic clearance. Many pharmacokinetic drug-gene interactions are related to the cytochrome P450 (CYP) enzyme system.[14] The majority of these enzymes are located in the endoplasmatic reticulum of the hepatocytes. This enzyme system can be modulated by genetic polymorphisms, causing some individuals to be poor (slow) metabolizers and others to be extensive (rapid) metabolizers. This is the case for many calcium channel blockers that are metabolized by CYP3A4, many lipophilic β-blockers by CYP2D6, and losartan and irbesartan by CYP2C9.[15] However, it is unlikely that pharmacokinetic effects cause most of the antihypertensive drug-gene interactions.
2. Through altered pharmacodynamic drug-gene interactions. These involve, for example, gene products expressed as drug targets such as receptors and signal transduction molecules, which are relevant to the pharmacodynamics of drugs.
3. Through genes that are in the causal pathway of the disease and are able to modify the effects of drugs.

It is important to realize that most of the variation in blood pressure can be explained by a pharmacodynamic, rather than by pharmacokinetic, mechanism.[16] This is most apparent in studies in which pharmacokinetic and pharmacodynamic assessments are available in the same subjects, and in which inter-subject variability can be expressed as a coefficient of variation.

Six previous review articles presented pharmacogenetic concepts relevant to antihypertensive drug therapy. These articles included a brief overview of candidate genes studies with respect to blood pressure response and other cardiovascular responses to antihypertensive drug therapy.[17-22] Our review extends the previous overviews and discusses some of the reasons for inconsistent results regarding drug-gene interactions between genetic polymorphisms and antihypertensive drug therapy. Studies were identified in the Medline database from 1966 to October 2003 by combinations of the keywords: 'antihypertensive drug', 'genetics', and 'polymorphism' and by checking the references of all identified papers. All studies that reported data on genetic polymorphisms and response to antihypertensive drug therapy were included. Response to antihypertensive drugs was not pre-specified and could include blood pressure response, change in left ventricular mass, risk of MI and stroke, and other cardiovascular effects.

Candidate gene studies and antihypertensive drugs

Forty-one studies were found on the potential gene-drug interaction between genetic polymorphisms and antihypertensive drugs. Details of these studies are given in table 1 and 2.

Table 1. The influence of genetic polymorphisms on the effect of antihypertensive medicine in patients with essential hypertension.

Sample	Duration of therapy	Gene (location)	Polymorphism	Allelic association[1]	References
Thiazide diuretic					
n=143 Caucasian	8wk	ADD1 (4p16.3)	G460W	460W-allele associated with greater BP response	23, 24
n=1038 mixed	4y		G460W	460W-allele associated with a lower risk of (combined) MI and stroke in comparison to other antihypertensive therapies (observational study)	30
n=87 Caucasian	2mo		G460W	460W-allele associated with greater BP reduction	29
n=585 mixed	4wk		G460W	No association (BP)	34
n=87 Caucasian	2mo	ACE (17q23)	I/D	I-allele associated with a greater BP response	29
n=376-585 mixed	4wk		I/D	No association (BP)	34, 35
n=387-585 mixed	4wk	GNB3 (12p13)	825C/T	T-allele associated with greater BP response	33
n=585 mixed	4wk	ADRB1 (10q24-q26)	R389G	No association (BP)	34
n=585 mixed	4wk	ADRB2 (5q31-q32)	R16G	No association (BP)	34
n=585 mixed	4wk	LPL (8p22)	S477Stop	No association (BP)	34
n=585 mixed	4wk	NOS2A (17p11-q12)	E298D	E-allele associated with greater DBP response	34
β-blocker					
n=63-91 Caucasian	4wk	AGT (1q42-q42)	M235T	No association (BP)	44
n=84-86 Caucasian	1-3mo		M235T	No association (BP and LVM)	45, 46
n=84-86 Caucasian	1-3mo		T174M	No association (BP and LVM)	45, 46
n=63-91 Caucasian	4wk	ACE (17q23)	I/D	No association (BP)	44
n=50 Caucasian	15d		I/D	No association (AT1R mRNA expression)	47
n=84-86 Caucasian	1-3mo		I/D	No association (BP and LVM)	45, 46
n=84-86 Caucasian	1-3mo	AGTR1 (13q21-q25)	1166A/C	No association (BP and LVM)	45, 46
n=147 Caucasian	4wk	ADRB1 (10q24-q26)	G389R	No association (BP and heart rate)	48
n=40 mixed	>4wk		G389R	R-allele associated with greater DBP reduction	51
n=40 mixed	>4wk		S49G	S-allele associated with a trend towards DBP reduction	51
n=84-86 Caucasian	3mo	CYP11B2 (8q21-q22)	-344C/T	No association (BP and LVM)	46, 49
n=97 Caucasian	3mo	74 SNPs[2] ADRA2A		Association with: SBP (278G/T) and DBP	59

Sample	Duration	Gene (locus)	Polymorphism	Association	Ref
		(10q24-q26)		(1309G/A)	
		ADRB2 (5q31-q32)		SBP (1342G/C) and DBP (1817G/A)	
		AGT (1q42-q43)		SBP (1015C/T)	
		ADNRB3 (13q22)		SBP and DBP (40G/A)	
		NOS3 (7q36)		SBP (498G/A) and DBP (2996A/G)	
		LIPC (15q21-q23)		DBP (110A/G)	
n=90 Caucasian	48wk	BDKRB2 (14q32)	+9/-9	No association (LVM)	[50]
n=66 Caucasian	4wk	GNAS (20q13)	FokI (+/-)	FokI +allele associated with greater BP reduction	[43]
n=90 Caucasian	48wk	TGFB1 (19p13.2)	915G/C	No association (LVM)	[103]

ACE-inhibitor

Sample	Duration	Gene (locus)	Polymorphism	Association	Ref
n=63-91 Caucasian	4wk	AGT (1q42-q42)	M235T	No association (BP)	[44]
n=125 Caucasian	4wk		M235T	235T-allele associated with greater BP reduction	[61]
n=1041 mixed			M235T	235T-allele associated with a lower risk of stroke (observational study)	[62]
n=63-91 Caucasian	4wk	ACE (17q23)	I/D	No association (BP)	[44]
n=125 Caucasian	4wk		I/D	No association (BP)	[61]
n=104 Caucasian	6mo		I/D	D-allele associated with greater BP reduction	[64]
n=50 Japanese	15d		I/D	D-allele associated with greater AT1R mRNA expression	[47]
n=54 Japanese	>2y		I/D	I-allele associated with greater regression of LVH	[65]
n=75 Japanese	6mo		I/D	I-allele associated with greater regression of LVH	[67]
n=60 Japanese	1y		I/D	D-allele associated with positive effect on LVH and reduced diastolic filling	[63]
n=57 Japanese	6wk		I/D	I-allele associated with trend towards greater DBP reduction	[66]
n=517 Chinese	6wk		I/D	No association (BP)	[75]
n=82 Japanese	1h		I/D	No association (BP and plasma renin activity)	[74]
n=40 Caucasian	2mo	AGTR1 (13q21-q25)	1166A/C	1166C-allele associated with greater BP reduction and arterial stiffness; no association heart rate	[68]
n=125 Caucasian	4wk		1166A/C	No association (BP)	[61]

AT1R antagonist

Sample	Duration	Gene (locus)	Polymorphism	Association	Ref
n=84-86 Caucasian	1-3mo	AGT (1q42-q42)	M235T	No association (BP and LVM)	[45, 46]

n=84-86 Caucasian	1-3mo		T174M	174M-allele associated with positive effect on LVM; no association BP	[45, 46]
n=84-86 Caucasian	1-3mo	ACE (17q23)	I/D	I-allele associated with greater DBP reduction; no association LVM	[45, 46]
n=42 Japanese	12w		I/D	I-allele associated with greater DBP reduction	[104]
n=84-86 Caucasian	1-3mo	AGTR1 (13q21-q25)	1166A/C	1166A-allele associated with a trend towards greater SBP reduction; no association LVM	[45, 46]
n=84 Caucasian	3mo	CYP11B2 (8q21-q22)	-344C/T	-344T-allele associated with greater SBP reduction; no association LVM	[46, 49]
n=84 Caucasian	12wk	CYP2C9 (10q24)	*1 and *2	*1/*1 compared with *1/*2 associated with greater DBP reduction	[105]
n=97 Caucasian	3mo	74 SNPs[2] APOA1 (11q23-q24) CYP11B2 (8q21-q22) EDNRB (13q22) NOS3 (7q36) ACE (17q23) AGT (1q24-q43) LIPC (15q21-q23)		Association with: SBP and DBP (1449 A/G) SBP (267T/C) SBP (40G/A) SBP (498G/A) DBP (12257A/G) DBP (1198 C/T) DBP (110A/G)	[59]
n=90 Caucasian	48wk	BDKRB2 (14q32)	+9/-9	No association (LVM)	[50]
n=90 Caucasian	48wk	TGFB1 (19q13.1)	915G/C	C-allele associated with greater reduction LVM	[103]
Calcium channel blocker					
n=63-91 Caucasian	4wk	AGT (1q42-q42)	M235T	No association (BP)	[44]
n=50 Caucasian	15d	ACE (17q23)	I/D	I-allele associated with reduced AT1R mRNA expression	[47]
n=40 Caucasian	2mo	AGTR1 (13q21-q25)	1166A/C	1166A-allele associated with greater reduction arterial stiffness; no association BP and heart rate	[77]

[1] Comparisons are versus untreated or placebo, unless otherwise specified.
[2] 74 single nucleotide polymorphisms in 25 genes involved in BP regulation.
d=days; h=hours; wk=weeks; y=years
AT1=angiotensin II type 1; BP=blood pressure; DBP=diastolic blood pressure; I/D= insertion/deletion; LVH=left ventricular hypertrophy; LVM=left ventricular mass; MI=myocardial infarction; mRNA=messenger RNA; SBP=systolic blood pressure

Diuretics

One of the first polymorphisms examined for blood pressure response in patients treated with diuretics was the G460W (a Gly to a Trp substitution at residue 460) α-adducin polymorphism.[23, 24] The human α-adducin 460W-allele can be considered as a candidate gene for hypertension, because it may affect blood pressure by increasing renal tubular reabsorption of sodium through the activation of Na^+,K^+-ATPase (adenosine triphosphatase). Linkage and association studies, performed with markers mapping in the region (loci) of the α-adducin locus and with the α-adducin G460W polymorphism, respectively, yielded positive associations.[23] Compared with hypertensive patients who are homozygous for the 460G wild-type allele, hypertensive patients carrying at least one 460W-allele have a less steep pressure natriuresis slope. This means that they need a higher arterial pressure to excrete the same amount of sodium after saline infusion.[25] Moreover, they have lower plasma renin activity,[23] enhanced proximal tubular reabsorption,[26] and a more pronounced blood pressure decrease after acute sodium depletion or long-term diuretic treatment.[23] The 460W-allele of the α-adducin gene is associated with a higher affinity for the Na^+,K^+-ATPase pump than the 460G-allele.[27] This last finding is particularly relevant because the same functional protein alteration has been demonstrated in both the rat and human 'hypertensive' α-adducin variant, suggesting that the protein plays a crucial role in Na^+/K^+ metabolism.[27, 28] In two trials, the 460W-allele was associated with a greater blood pressure reduction in response to treatment with diuretics. In heterozygous (G/W) hypertensive patients a mean blood pressure decrease of 14.7 ± 2.2 mmHg was found versus 6.8 ± 1.4 mmHg in homozygous (G/G) hypertensive patients.[23, 24] Recently, a second group of researchers also found an interaction between mean arterial pressure (diastolic blood pressure + (systolic blood pressure-diastolic blood pressure)/3) reduction and the G460W polymorphism. Homozygous (G/G) hypertensive patients had a reduction of 6 mmHg and patients with at least one 460W-allele had a reduction of 12 mmHg in mean blood pressure.[29] In another study the 460W-allele was associated with a lower risk (Odds ratio (OR)=0.49; 95%CI: 0.32-0.77) of MI and stroke in diuretic users compared with users of other antihypertensive drug therapies.[30]

Recently, the ACE insertion/deletion (I/D) polymorphism was investigated for its role in blood pressure response to a diuretic (hydrochloorthiazide 25 mg). In this study a significant association was found between the ACE I/D polymorphism and response to hydrochlorothiazide. Hypertensive patients with the II genotype had a mean arterial pressure reduction of approximately 10 mmHg and those with the DD genotype a reduction of 3.8 mmHg.[29]

A third polymorphism that may influence the effect of a diuretic is the 825C/T (cytosine into a thymine) polymorphism (exon 10) of the gene encoding for the β3-subunit of the G-protein. The G-protein mediate signal transduction across cell membranes.[31] The 825T-allele of the β3-subunit of the G-protein polymorphism has been related to an RNA splice variant that results in the deletion of nucleotides 498-

Table 2. The influence of genetic polymorphisms on the effect of antihypertensive medicine in non-hypertensive patients, but related diseases.

Sample	Duration of therapy	Gene (location)	Polymorphism	Allelic association[1]	References
β-blocker					
Nondiabetic nephropathy; n=81 Caucasian	3-4y	ACE (17q23)	I/D	D-allele associated with reduced glomerular filtration	52
Chronic heart failure; n=328 Caucasian	2y		I/D	D-allele associated with decreased chance of needing a heart transplantation	53
ACE-inhibitor					
Proteinuric renal disease; n=36 Caucasian	12wk	ACE (17q23)	I/D	No association (proteinuria)	69
Diabetic nephropaty; n=35 Caucasian	7y		I/D	D associated with reduced glomerular filtration	68
Chronic heart failure; n=34 Caucasian	6wk		I/D	I-allele associated with greater BP reduction (captopril); no association (lisinopril)	70
Post-PTCA; n=126 Japanese	3-6mo		I/D	I-allele associated with reduced chance of restenosis	71
Nondiabetic nephropathy; n=81 Caucasian	3-4y		I/D	D-allele associated with reduced glomerular filtration	52
Nondiabetic nephropathy; n=88 Caucasian	4-12wk		I/D	D-allele associated with reduced proteinuria (when there is a high salt excration)	58
Post-coronary stents; n=345, Caucasian	6mo		I/D	I-allele associated with increased chance of restenosis	72
Cerebrovascular disease (stroke/TIA); n=5688 mixed	4wk		I/D	No association (predicting cardiovascular risk of effect treatment)	73
AT1R antagonist					
Diabetic nephropathy; n=45 Caucasian	4mo	ACE (17q23)	I/D	No association (BP and albuminuria)	106

[1] Comparisons are versus untreated or placebo, unless otherwise specified.
d=days; wk=weeks; y=years
AT1=angiotensin II type 1; BP=blood pressure; I/D=insertion/deletion; PTCA=percutaneous transluminal coronary angioplasty; TIA=transient ischaemic attack

620 of exon 9 and structural changes in the β-subunit.[32] Moreover, an enhanced signal was observed in lymphoblast lines from hypertensive individuals carrying the

825T-allele,[31] which suggests that this genetic variation may indeed affect signal transduction. In one trial, a positive association was found between the 825T-allele and the effect of hydrochlorothiazide on blood pressure. Mean declines in systolic and diastolic blood pressures were 6 ± 2 and 5 ± 1 mmHg greater in TT than in CC homozygous patients, respectively.[33] In the same study an association was found between thiazides and the 298E-allele of nitric oxide synthase gene on diastolic blood pressure with a larger sample size.[34] Hypertensive patients homozygous for E-allele had a diastolic blood pressure reduction of 8.6 ± 0.4 mmHg compared to 7.1 ± 0.6 mmHg for the other genotype groups. Their previously reported interaction with ACE gene[35] could not be replicated with this larger sample size.[34]

Recently, an alternatively spliced transcript of the β3-subunit of the G-protein referred to as Gβ3S2 was identified. Transcripts of the Gβ3S2 lack a 129 base pair of coding sequence of the β3-subunit of the G-protein. A close association between Gβ3S2 expression and T-allele status of the 825 C/T polymorphism of the β3-subunit of the G-protein was found. The data suggest that Gβ3S2 is a biologically active variant of the β-subunit of the G-protein, which may play a role in the manifestation of the complex phenotype associated with the 825C/T polymorphism.[36]

A polymorphism of the α-adducin gene may be used to identify patients with hypertension who are salt sensitive and respond relatively well to treatment with a diuretic. Nevertheless, two studies did not find an association between salt sensitive and this gene in young men[37] or the general population.[38] The β3-subunit of the G-protein was also proposed as an candidate gene, but Ciechanowicz et al.[39] could not find an association between polymorphism of the α-adducin gene and salt sensitivity of blood pressure. Furthermore, it has been suggested that polymorphisms of the angiotensin II type 1 receptor gene (AGTR1) and the γ-subunit of the epithelial Na^+-channel are also associated with salt sensitivity.[40, 41] However, Giner et al. could not find an association.[42] Whether genetic polymorphisms that are associated with salt-sensitivity also modify the response to diuretics remains to be investigated.

β-Blockers

In only two of 11 studies was a drug-gene interaction found on blood pressure response to a β-blocker.[43-51] This reduced response was attributed to a FokI +/- polymorphism encoding for the α-subunit of the G-protein. Good responders (62.5% had a FokI +allele) had a mean arterial blood pressure decrease of > 15 mmHg and poor responders (41.7% had a FokI +allele) had a decrease of < 11 mmHg.[43] The α-subunit of each heterotrimeric G-protein contains the guanine nucleotide-binding site, which has intrinsic guanosine triphosphatase activity and confers the functional specificity on each G-protein that allows it to discriminate among multiple receptors and effectors. In the cardiovascular system, the α-subunit of the G-protein couples β1- and β2-adrenoceptors in order to stimulate the cyclic adenosine monophosphate (cAMP) production. Johnson et al.[51] found an interaction between β1-adrenergic receptor and metoprolol. Patients homozygygous for arginine at codon 389 had a

nearly 3-fold greater reduction in daytime diastolic blood pressure compared with those who carried the variant allele.

In patients with nondiabetic nephropathy, the presence of the ACE D-allele was associated with a reduction of glomerular filtration.[52] Another drug-gene interaction with the ACE D-allele was observed in patients with chronic heart failure. In this study,[53] treatment with a β-blocker was associated with a decreased need for a heart transplantation.

The A (adenine) into a C (cytosine) transversion at nucleotide position 1166 is located in the 3' untranslated region of the AGTR1 gene on chromosome 3q21-q25. Some studies have shown that it was associated with hypertension,[54, 55] left ventricular hypertrophy (LVH),[56] coronary heart disease, MI,[57] and progression of diabetic nephropathy.[58] The 1166A/C polymorphism of the AGTR1 gene was, however, not related to variation in blood pressure response or degree of LVH during β-blocker therapy in small groups of hypertensive patients.[44, 45]

Recently, Liljedahl et al.[59] tested a microarray-based minisequencing system on DNA samples of 97 hypertensive patients, of whom 49 were treated with atenolol. This group of researchers genotyped 74 Single Nucleotide Polymorphisms (SNPs) in 25 genes that were involved in blood pressure regulation using stepwise multiple regression. Their results indicated drug-gene interactions between atenolol and several genes which resulted in a change in blood pressure, including: the 40G/A polymorphism of the endothelin receptor type B gene, the 278G/T and 1817G/A polymorphism of the adrenergic α1a-receptor gene, the 1342G/C and 1309G/A polymorphism of the adrenergic β2-receptor gene, the 498G/A and 2996A/G polymorphism of the endothelial nitric oxide synthase gene, and the 110A/G polymorphism of the lipase hepatic gene.[59] However, this study included only a small number of patients and was focused on the applicability of the minisequencing system rather than on finding drug-gene interactions. Because of the large number of SNPs tested without adjustment for multiple testing and the small number of patients, this study could have resulted in a large number of false-positives and false-negative associations. The minisequencing method and study population was also used for the analysis of 30 SNPs in seven candidate genes of the renin-angiotensin system. In this study the researchers found that the -6A/G and M235T polymorphism of the angiotensinogen gene were associated with systolic blood pressure response.[60]

ACE-inhibitors

Most studies on interactions between ACE-inhibitors and genetic polymorphisms have concentrated on the renin-angiotensin system (RAS). One of the steps of the RAS system is the expression of angiotensinogen precursor in the liver. In response to lowered blood pressure it is cleaved by the enzyme renin. The resulting product, angiotensin I, is then cleaved by ACE to generate the physiologically active enzyme angiotensin II. This protein is involved in maintaining long-term blood pressure and in the pathogenesis of essential hypertension.

In one study, no drug-gene interaction was found between ACE-inhibitors and the M235T (methionine into a threonine) polymorphism of the angiotensinogen gene.[44] However, in a larger study an interaction was found.[61] In this study, the reduction in systolic blood pressure was 20 ± 3 mmHg in patients with the TT genotype compared with 22 ± 2 mmHg in patients with MT genotype and 13 ± 4 mmHg in patients with the MM genotype. The reduction in diastolic blood pressure in patients carrying the TT genotype was 11 ± 3 mmHg compared with 14 ± 2 mmHg in patients with the MT genotype, and 8 ± 2.5 mmHg in patients with the MM genotype.[61] Another group of researchers found that ACE-inhibitor use compared with other antihypertensive drugs was associated with a lower risk of stroke among the TT genotype (OR=0.37; 95%CI: 0.14-0.99) than among MT or MM genotype (OR=1.2; 95%CI: 0.88-2.40). No drug-gene interaction on the risk of MI was found.[62]

Most studies have concentrated on investigating the association between the I/D polymorphism (intron 16) of the ACE gene and the response to an ACE-inhibitor. In 12 out of 18 studies, an association could be found.[47, 63-75] In one study, a greater blood pressure reduction with ACE-inhibitor therapy was observed in subjects with at least one copy of the D-allele. In this study, the reduction in systolic blood pressure in patients with the DD genotype was 5.6 ± 3.1 mmHg compared with 3.1 ± 1.1 mmHg with the II genotype and 3.6 ± 2.2 mmHg with the ID genotype. The reduction in diastolic blood pressure in patients with the DD genotype was 8.9 ± 6.0 mmHg compared with 5.5 ± 3.4 mmHg with the II genotype and 5.8 ± 4.0 mmHg with the ID genotype.[64] In the other studies, the I-allele was associated with a reduced regression of LVH in patients with hypertension[65, 66] and increased chance on restenosis in patients with coronair stents.[72] The D-allele was associated with reduced AT1 receptor mRNA expression,[47] left ventricular hypertrophy, reduced diastolic filling,[63] greater reduction of glomerular filtration in diabetic and non-diabetic patients,[52, 68] and less reduction of proteinuria in patients with nondiabetic nephropathy (primarily combined with a high excretion of salt).[76]

In two studies, the interaction between the 1166A/C polymorphism of the AGTR1 gene and the response to ACE-inhibitors was investigated. In a small study, no interaction was found.[61] In a larger study, the C-allele was associated with greater reduction of arterial stiffness and blood pressure by ACE inhibition. Patients with the AA genotype had a blood pressure reduction of approximately 6 mmHg and those with AC/CC genotype had a reduction of approximately 14 mmHg.[77]

Angiotensin II type 1 receptor antagonists

Only one group has investigated the role of genetic polymorphisms and the response to an angiotensin II type 1 (AT1) receptor antagonist (irbesartan).[44, 45, 48, 49, 58] The 174M-allele of the angiotensinogen gene was associated with a positive effect of irbesartan on LVH. No interactions were found between the M235T polymorphism of the angiotensinogen gene or the −344C/T (cytosine to a thymine) polymorphism of the aldosterone synthase gene (CYP11B2) and irbesartan on reduction of LVH.[45, 46, 49]

However, the -344T-allele of the aldosterone synthase gene was associated with an increased reduction of systolic blood pressure after treatment with the AT1 receptor antagonist. Patients with TT genotype had a mean reduction in blood pressure of 21 ± 19 mmHg compared with 14 ± 18 mmHg in patients with TC genotype and 0 ± 17 mmHg in patients with CC genotype.[49] Also the I-allele of the ACE gene was associated with a reduction of diastolic blood pressure. Patients with the II genotype had a reduction of 18 ± 12 mmHg compared with 8 ± 11 mmHg in patients with ID genotype and 6 ± 9 mmHg in patients with DD genotype.[45] Aldosterone synthase is a key rate-limiting enzyme for the biosynthesis of aldosterone. The −344C/T polymorphism is associated with elevated plasma aldosterone concentration through increased aldosterone synthesis.[78] These results suggest that the −344T-allele is functionally associated with increased sodium reabsorption and thereby maintains blood pressure at a higher level due to volume expansion.

Liljedahl et al.[59] tested a microarray-based minisequencing system on DNA samples of 97 hypertensive patients of whom 48 were treated with irbesartan. They found that lowering of blood pressure by irbesartan was modified by several polymorphisms, including: the 1449A/G polymorphism of the apolipoprotein A, the 267C/T polymorphism of the cytochrome P450, family 11, subfamily B, polypeptide 2 gene (CYP11B2), the 40G/A polymorphism of the endothelin receptor type B gene, the 498G/A polymorphism of the endothelial nitric oxide synthase gene, the 1015C/T polymorphism of the angiotensinogen gene, the 12257G/A polymorphism of the ACE gene, and the 110A/G polymorphism of the lipase hepatic gene.[59]

Calcium channel blocker

The influence of genetic polymorphisms on calcium channel blockers had been examined in three studies. In one study, an interaction was observed between calcium channel blockers and the ACE gene: patients with the I-allele had a reduced expression of AT1 receptor mRNA.[47] Another study found a drug-gene interaction with the A-allele of the 1166A/C polymorphism of the AGTR1 gene, which lead to greater reduction of arterial stiffness.[77] A drug-gene interaction between the M235T-allele of the angiotensinogen gene and calcium channel blockers could not be demonstrated regarding blood pressure response.[44, 77]

Potential reasons for inconsistent findings

Most of the studies exhibited inconsistent findings and did not yield conclusive results. In one out of four studies, for example, an interaction was found between the ACE gene and ACE-inhibitors regarding blood pressure response. The most promising result was the interaction between the α-adducin G460W polymorphism and blood pressure response and risk of MI and stroke in response to diuretics. However, these results need to be replicated before definitive conclusions can be made.

It is known that it may be problematic to demonstrate linkage or association consistently. For example, some groups were able to confirm an association between hypertension and the M235T polymorphism, while others could not.[79-81] A meta-analysis of 5,500 subjects reported a significant but weak association (OR=1.2; p < 0.0001) between this polymorphism and hypertension.[82] This is most likely the same for studies investigating the role of genetic polymorphisms and the response to antihypertensive treatment. For this reason, pharmacogenetic studies require a large group of patients in order to have sufficient power to detect small genetic effects. This will reduce the likelihood of getting false-positive or false-negative results. For instance, Turner et al.[34] found no interaction between the ACE gene and the effect of hydrochlorothiazide on blood pressure response, in contrast to their previous reported association with a smaller sample size.[33, 35] The sample size in the studies investigating antihypertensive drug-gene interactions in hypertensive patients ranged from 40 to 1,048 persons. However, > 60% of the studies had less than 100 patients which is not sufficient for conclusive results when, besides a genetic factor, also an interaction is investigated. The number of patients needed to detect drug-gene interactions depends on the outcome studied (e.g. continuous vs. categorical), the contrast between responders and nonresponders in different genotype groups (the amount of interaction), and the precision of the measurements.[83, 84]

Genetic diversity between populations

Genetic diversity between populations can hinder replication of results. Gene variants that were selected during evolution to conserve salt, for example, may play a larger role in hypertensive patients with ancestors from Africa.[18] In a study where the disease-causing allele is more prevalent, it might be easier to find an interaction. One examples which was investigated for antihypertensive drug-gene interaction is the frequency of the I-allele of the ACE gene which is different between Asian and Caucasian population, i.e. 62% versus 50%, respectively.[61, 64, 66]

Different study design

There are often different inclusion criteria for different study population. If, for example, one study only includes patients with severe hypertension, it is possible that these patients have a genetic profile which differs from moderate hypertensive patients examined in another study. This might be the case when the study of Stavroulakis et al. (SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg)[52] is compared with Hingorani et al. (SBP > 160 mmHg or DBP > 90 mmHg).[61] Furthermore, differences in treatment regime were found between these studies. Both studies had a 4-week washout period, but in one study patients were given 20 mg fosinopril one daily[64] (defined daily dose [DDD] equivalent=1.33) while in the other study, patients were given 50 mg/day captopril or 10 mg/day enalapril or 10 mg/day lisinopril or 4 mg/day perindopril (all DDD equivalent=1).[61] Another influence may be the variation in duration of therapy in different studies. The duration of therapy ranged from 15 days

to 7 years in studies which focused on antihypertensive drug-gene interactions in hypertensive patients.

Different results may be explained by the use of different study designs, such as experimental (e.g. randomized clinical trail) and observational (e.g. cohort and case-control) studies. In observational study designs for example confounding maybe a problem (e.g. confounding by indication).

Another potential explanation for different results relates to the definition of outcome. For instance, Scarrione et al.[29] used the reduction in mean blood pressure, while Turner et al.[34] used systolic and diastolic blood pressure separately.

Genetic polymorphism and disease-causing factors

Most of the examined polymorphisms are probably not the disease-causing factors.[69] An example is the M235T polymorphism of the angiotensinogen gene. There is now evidence that an A for G nucleotide substitution in the promoter region of the angiotensinogen gene 6 nucleotide upstream from the start site of transcription is the functional mutation.[85, 86] The A substitution alters the binding of a nuclear protein, resulting in increased gene transcription compatible with increased angiotensinogen levels. Fortunately, it has been suggested that the −6G/A polymorphism is nearly in complete linkage disequilibrium (LD) with the M235T polymorphism.[82, 87] The same holds for the I/D polymorphism in the ACE gene. The I/D polymorphism predicts approximately half of the interindividual variability in serum[88, 89] and tissue.[90] Thus, the probability that the ACE gene is not in linkage equilibrium with the functional polymorphism is considered small.[89, 91, 92]

Considerations for the design of a pharmacogenetic study

There are several ways to design studies to investigate interactions between antihypertensive drugs and genetic polymorphisms. All studies performed to date investigated (allelic) polymorphisms. This sort of study provides the most powerful approach to identify genes of small effect in complex traits[93] because the markers that are used are either very close to the susceptibility locus or lie in the gene of interest itself. It is difficult to perform a linkage study, because a high number of patients would be needed and only relatives who use the same antihypertensive drugs can be included. In the future, genome-wide association studies using SNPs will become possible, which will make it available to use unrelated cases and controls to map regions of the genome, and eventually the whole genome.

It is possible to consider different endpoints when investigating hypertension. For example, studies could consider long-term outcomes such as MI and/or stroke, intermediate-term outcomes such as atherosclerosis and/or LVH, or short-term outcomes such as blood pressure. Another option is to investigate adverse effects of antihypertensive drugs or adherence to antihypertensive medication. Moreover, there

are several potential inclusion criteria and there is no clear indication whether it is, for instance, best to choose mild or severe hypertension. It is important to consider the appropriate group of controls because a strong difference in response can results in spurious drug-gene interactions.

The number of markers and the question which markers an investigator wants to test also need to be considered. There are biallelic (SNPs and I/D polymorphisms) and microsatellite markers (tandem repeats). Biallelic markers are relatively less polymorphic, but they are more abundant and accessible. The number of markers depends on the amount of LD in the candidate gene in the study population. LD occurs when two particular alleles at loci on the chromosome go together more often then may be expected from independent segregation in a population. LD can be determined with a small pilot sample. This can help to optimize marker selection and provide information for haplotype analysis.[94] Genotyping more markers gives more information and thus more power. It is, nonetheless, more expensive and time consuming and the sample size has to be increased because more genotypes groups are identified. When more markers are tested, adjustment should be made for multiple testing. It is best to investigate only candidate genes, which can be linked to a biological system. For antihypertensive response, for instance, genes in the renin-angiotensin system are prominent, and other regulatory mechanisms of pressure-natriuresis are important because of their role in blood pressure homeostasis.

A more crucial issue is whether checking for population stratification is needed. Population stratification refers to a form of confounding. The bias from population stratification is the distortion in the association between the genetic variant and the outcome that can occur when the variant is associated with an unknown risk factor which varies by ethnicity. Population stratification may also be important in drug response. For instance, African Americans may react differently to a specific antihypertensive drugs class compared with Caucasians.[95-98] The impact of population stratification is, however, not yet clear. Some conditions must be met before a substantial bias occurs: (I) there must be substantial variation across ethnicities in the allele frequency of the relevant gene; (II) there must be substantial variation in disease rates; (III) allele frequencies must correlate with adjusted disease rates between ethnic groups; and (IV) adjustment for ethnicity must reduce the relevant effect.[99] At present it is still unclear whether population stratification biases results in a substantial way.[94, 100] To minimize the effect of population stratification, a solution could be the typing of additional markers unrelated to the outcome[101] or match for ethnicity.[100, 102]

Future prospects

Although there are many difficulties to overcome, pharmacogenetics may yield successful strategies to optimize drug therapy. Several potential candidate genes are

currently under investigation for their potential to modify response to antihypertensive drugs. Findings from previous studies require conformation in other studies to be able to make definitive conclusions about current positive drug-gene interactions. It is also important that research groups collaborate more it order to facilitate the conduct of a meta-analysis for conclusive results. With the development of efficient methods for analyzing massive amounts of data, pharmacogenetic studies may eventually lead to the optimization of antihypertensive drug therapy based on genetic profiles of patients.

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Chapter 4

Pharmacogenetics and short-term outcomes

Chapter 4.1

Insertion/deletion polymorphism of the ACE gene and adherence to ACE-inhibitors

Abstract

Aim: To investigated whether the insertion/deletion (I/D) polymorphism of the angiotensin converting enzyme (ACE) gene modified the adherence to ACE-inhibitors as measured by the discontinuation of an ACE-inhibitor, or the addition of another antihypertensive drug.

Methods: This was a cohort study among 239 subjects who started ACE-inhibitor therapy. A Cox proportional hazard model was used to calculate relative risk (RR).

Results: During follow-up there was no significant difference between subjects with the DD, ID, or II genotype (DD vs. II; RR=1.17; 95%CI: 0.78-1.77 and ID vs. II; RR=1.06; 95%CI: 0.73-1.55) in adherence.

Conclusion: The I/D polymorphism of the ACE gene does not influence the adherence to ACE-inhibitors.

Introduction

Hypertension is a major public health hazard and despite the availability of a variety of effective antihypertensive drugs inadequate control of blood pressure is common in hypertensive patients. One of the factors in interpreting the variability in outcome of drug therapy includes the genetic profile of a patient.[1]

A candidate gene for the control of blood pressure is the angiotensin converting enzyme (ACE) gene. Individuals with the DD genotype display twice as high serum ACE concentrations as individuals with the II genotype,[2] but without clear correlation to blood pressure.[2] The I/D polymorphism of the ACE gene has been associated with differential blood pressure responses to ACE-inhibitors. However, the results have been controversial.[3-6]

Given the controversial results, we investigated whether the I/D polymorphism of the ACE gene was associated with the response to ACE-inhibitor therapy as measured by the discontinuation of an ACE-inhibitor, or addition of another antihypertensive drug class.

Methods

Setting

The Rotterdam Study started in 1990 as a population-based prospective follow-up study. In total, 7,983 residents of the suburb Ommoord in Rotterdam aged 55 years or over participated. The baseline measurements took place until 1993. The design of this population-based study has been described elsewhere.[7] Pharmacy records were available for approximately 99% of the cohort as of January 1st, 1991.

Cohort and outcome definition

For the analysis, we included patients who had at least 6 months of medication history at the pharmacy before starting with an ACE-inhibitor and who did not use antihypertensive drugs during that period. We excluded persons with only one ACE-inhibitor prescription.

To study the potential interaction between the I/D polymorphism and response to ACE-inhibitors, we used two proxy outcomes. The first outcome was defined as the discontinuation of ACE-inhibitors for $\geq$ 180 days. The second outcome was addition of another antihypertensive drug to the ACE-inhibitor therapy. Subjects were followed until the outcome of interest, death, moving outside of the study area, or the end of the study period, whichever came first.

Genotype

The I and D-allele of the ACE genotype were identified on the basis of polymerase chain reaction (PCR) amplification of the respective fragments from intron 16 of the

ACE gene and size fractionation and visualization by electrophoresis as described before.[8]

Analysis

We used ANOVA (continuous variables) and Chi-square testing (categorical variables) to compare baseline characteristics of people with different genotypes. For the outcome of interest, a Cox proportional hazard model was used to calculate the relative risk (RR) and 95% confidence interval (95%CI) of discontinuation of an ACE-inhibitor or addition of another antihypertensive drug.

Results

Between January 1st, 1991 and December 31st, 1999, 1,488 subjects were identified as ACE-inhibitor users and 239 subjects had not used antihypertensive medication between January 1st and July 1st, 1991 prior to the start of ACE-inhibitor medication.

Cohort study among starters of ACE-inhibitors

In total, 65, 117, and 57 had the DD, ID, and II genotypes, respectively. Different ACE-inhibitors were used as a first prescription including: enalapril (48.3%), lisinopril (16.8%), captopril (9.7%), quinapril (8.8%), perindopril (6.7%), fosinopril (6.3%), ramipril (3.0%), and cilazapril (0.4%). The distribution of age, gender, systolic blood pressure, diastolic blood pressure, smoking, and body mass index (BMI) were similar between the three genotype groups (see table 1).

Table 1. Baseline characteristics. Values are presented as means ($\pm$ SD), or number (%).

Variable	DD (n=65)	ID (n=117)	II (n=57)	P
Gender, F	36 (55.4%)	67 (57.3%)	32 (56.1%)	0.97
Age, y	68.7± 9.7	69.2 ± 7.4	69.3 ± 8.2	0.90
SBP[1], mmHg	148.6 ± 20.9	154.7± 22.4	150.4 ± 22.0	0.21
DBP[1], mmHg	78.7 ± 10.6	80.3 ± 12.9	79.1 ± 9.8	0.66
Smoking				
current	12 (18.5%)	33 (29.2%)	13 (22.8%)	0.53
past	31 (47.7%)	46 (40.7%)	23 (40.4%)	
never	22 (33.8%)	34 (30.1%)	21 (36.8%)	
BMI, kg/m^2	26.5 ± 3.2	26.4 ± 3.7	26.8 ± 4.0	0.96
Diabetes mellitus, yes	11 (16.9%)	21 (17.9%)	7 (12.3%)	0.76
MI, yes	6 (9.2%)	16 (13.7%)	2 (3.5%)	0.11

[1] Only persons with a blood pressure measurement before they started an ACE-inhibitor therapy were included (n=211)

The Kaplan-Meier function showed that there were no significant differences in the rate of discontinuation of ACE-inhibitors or addition of other antihypertensive medication (DD vs. II; RR=1.17; 95%CI: 0.78-1.77 and ID vs. II; RR=1.06; 95%CI:

0.73-1.55) (see figure 1). During the entire follow-up there was no significant difference between the three genotypes. The results were similar when the outcomes of discontinuation and addition of other antihypertensive drugs were analyzed separately (data not shown). Excluding of stoppers (n=38) did not effect our findings. The effect was not caused by a difference in the average last prescribed daily dose before a censoring event (0.76 ± 0.38, 0.83 ± 0.64, and 0.78 ± 0.37 for the DD, ID, and II genotype; p=0.63).

Figure 1. Kaplan-Meier function of addition of other antihypertensive medication and/or stopping of an ACE-inhibitor stratified by ACE genotype (adjusted for gender, body mass index, systolic blood pressure diastolic blood pressure, myocardial infarction, diabetes mellitus, smoking, and death).
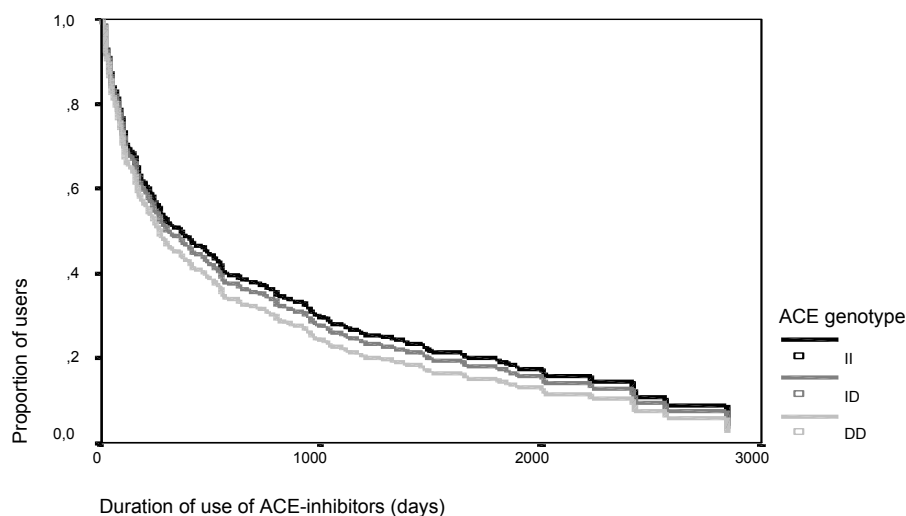
Discussion

Our findings suggest that the ACE I/D polymorphism in starters of ACE-inhibitors does not influence the response when evaluated by discontinuation of ACE-inhibitors and/or addition of other antihypertensive drugs.

Previously, four other studies investigated the role of the ACE gene in the response of blood pressure to ACE-inhibitors, but the results were inconclusive.[3-6] Our study corroborates the results of two of four studies.[5, 6] However, we used a proxy for blood pressure response instead of actual blood pressure measurements. Although, insufficient blood pressure control and side-effects account for most of the treatment switching,[9] our proxy might not be good for measuring the nonsatisfactory response of blood pressure to ACE-inhibitors. For example, our results could be influenced by adverse reactions to ACE-inhibitors, like dry cough. The role of the ACE gene in the occurrence of cough is, however, still unclear.[10, 11] It is also possible, that due to satisfactory blood pressure response a physician advices a patient to stop using

antihypertensive medication. However, this is a rare occurrence and we saw no difference between switchers and stoppers.

This study suggests that the ACE I/D polymorphism of the ACE gene does not influence the adherence to ACE-inhibitors.

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Chapter 4.2

Drug-gene interaction between the insertion/deletion polymorphism of the angiotensin converting enzyme gene and antihypertensive therapy on blood pressure

Abstract

Background: Despite the availability of a variety of effective drugs, inadequate control of blood pressure is common. There have been some indications that the angiotensin converting enzyme (ACE) gene modifies the response to antihypertensive drugs, although, the results were inconclusive.

Aim: To investigate whether the insertion/deletion polymorphism of the ACE gene modifies blood pressure difference among subjects using diuretics, β-blockers, calcium channel blockers, or ACE-inhibitors.

Methods: Data were used from the Rotterdam Study, a population-based prospective cohort study in the Netherlands, which started in 1990 and included 7,983 subjects aged 55 years or older. Data from three subsequent cross-sectional investigations was used. Subjects were included if they had a high blood pressure during ≥ 1 examinations and/or used monotherapy with a diuretic, β-blocker, calcium channel blocker, or ACE-inhibitor. A marginal generalised linear model was used to assess the association between the mean difference in systolic/diastolic blood pressure and antihypertensive classes stratified by the three genotypes.

Results: In total, 3,025 hypertensive individuals were included, totalling 6,500 measurements of blood pressure. Of these, 28.3%, 51.4%, and 20.3% had the DD, ID, and II genotypes, respectively. The mean difference in systolic blood pressure between the II and DD genotype was 0.23 mmHg (95%CI: -5.48-5.94) for diuretic, −2.41 mmHg (95%CI: -6.72-1.90) for β-blocker, 2.12 mmHg (95%CI: -4.64-8.89) for calcium channel blocker, and −2.01 mmHg (95%CI: -9.82-5.79) for ACE-inhibitor users.

Conclusion: The adjusted mean difference in diastolic and systolic blood pressure among diuretic, β-blocker, calcium channel blocker, or ACE-inhibitor users was not modified by the ACE insertion/deletion polymorphism.

Introduction

The renin-angiotensin system (RAS) regulates blood pressure and fluid homeostasis. Angiotensin converting enzyme (ACE), which is one of the enzymes of the RAS, converts angiotensin I to the vasoactive angiotensin II and inactivates bradykinin. An insertion/deletion (I/D) polymorphism of the 187-bp Alu element in intron 16 of the ACE gene predicts approximately half of the inter-individual variability in serum ACE levels.[1] In general, individuals with the DD genotype have serum ACE levels which are twice as high as individuals with the II genotype,[1] although, there is no clear correlation to blood pressure.[2]

Predicting the effect of a particular antihypertensive agent in an individual patient is difficult. To overcome this problem researchers are currently investigating which genes influence the response to various antihypertensive drugs.

Some studies have investigated the effect of the ACE I/D polymorphism on blood pressure response in patients treated with ACE-inhibitors[3-11] and, less so, in patients treated with β-blockers.[4,12] The latter reduces angiotensin II levels by inhibiting the β-adrenergic mediated renin release from the kidneys.[13] With regard to ACE-inhibitor users, three studies indicated that the D-allele had a stronger blood pressure lowering effect,[8-10] while two studies indicated the I-allele,[6,7] and four studies found no drug-gene effect.[3-5,11] No drug-gene interaction was found in studies with β-blocker users.[4,12] Due to conflicting results, it is still unclear whether the I/D polymorphism of the ACE gene influences the response to ACE-inhibitors or β-blockers.

Diuretics and calcium channel blocker are also influenced by the RAS i.e. by a counter regulatory system. For example, diuretic therapy leads to salt loss, which in turn, results in volume depletion, causing an increase in the plasma renin activity.[14] Calcium channel blockers block the inward movement of calcium by binding to L-type calcium channels in the heart and in smooth-muscle of the coronary and peripheral vasculature. This could result in an activation of the RAS.[15]

The purpose of this study was to evaluate the relationship between the I/D polymorphism of the ACE gene on the mean difference in systolic blood pressure (SBP) and diastolic blood pressure (DBP) among diuretics, β-blockers, calcium channel blockers, or ACE-inhibitors users.

Methods

Setting
The Rotterdam Study started in 1990 as a population-based prospective follow-up study. All 10,275 residents of the suburb Ommoord in Rotterdam aged 55 years or older were invited to participate. The aim of the Rotterdam Study is to investigate determinants of disease occurrence and progression in the elderly. Our study was

approved by the Medical Ethics Committee of Erasmus University and conducted in compliance with their requirements. In total, 7,983 (78%) subjects gave written informed consent and of 6,869 (86%) the ACE genotype was assessed. The baseline measurements took place until 1993. The design of this population-based study has been described elsewhere.[16]

The baseline examination included several details, such as an interview on demographics, current health status, medical history, family history of diseases, smoking habits, and current use of medication. During a physical examination, blood pressure, weight, and height were measured and blood was drawn for DNA extraction. Blood pressure was measured in sitting position at the right upper arm with a random-zero sphygmomanometer. The average of the two measurements, separated by a count of pulse rate, was used in the analysis. All participants were subsequently examined in follow-up examination rounds every 2-3 years (1993-1995, 1997-1999). Blood pressure data from all three examinations were used in this study.

Pharmacy records were available for approximately 99% of the cohort as of January 1st, 1991. 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.[17]

Cohort and outcome definition

The study population included all individuals with hypertension in the Rotterdam Study for whom the ACE genotype was assessed and where 1 or more blood pressure measurements were available. We defined hypertension as ≥ 1 blood pressure measurement during follow-up, which met one of the following criteria: SBP ≥ 160 mmHg, and/or DBP ≥ 95 mmHg. Subjects who used, antihypertensive drugs during follow-up were also defined as hypertensive. When a blood pressure measurement occurred, we assessed whether a prescription was filled by the pharmacy on this date. Hereto, the length of each prescription was calculated by dividing the number of dispensed tablets or capsules by the prescribed daily number. When the blood pressure measurement date fell within the usage period, the patient was considered as currently exposed. When > 1 antihypertensive drug class was used at the time of the blood pressure measurement the measurement was excluded. Antihypertensive drug treatment was classified in four groups i.e. diuretics, β-blockers, calcium channel blockers, or ACE-inhibitors. Subjects could switch between no treatment and different antihypertensive drug classes and between different antihypertensive drug classes. Due to the small numbers for the other antihypertensive drug classes, only subjects using diuretics, β-blockers, calcium channel blockers and ACE-inhibitors were included in the analysis. Pharmacy records were available as of January 1st, 1991. Nevertheless, blood pressure measurements from 1990 were included if an individual did not use an antihypertensive drug according to self-reported questionnaire information and did not start antihypertensive therapy before July 1st, 1991 according to the pharmacy dispensing records. The end of the study period was set at December 31st, 1999.

Potential confounders and effect modifiers

The potential confounders considered were age, sex, body mass index (BMI), defined daily dose (DDD), which (re-)examination (1st, 2nd, or 3rd), smoking at baseline, history of myocardial infarction, diabetes mellitus at baseline, use of nitrates, use of statins, use of NSAID's, use of another antihypertensive drug class two weeks prior to the blood pressure measurement, use of an antihypertensive drug for six of the eight weeks prior to the blood pressure measurement, and the cumulative number of days an antihypertensive drug was used. History of myocardial infarction was self-reported and confirmed by a physician or demonstrated on the baseline ECG. 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 average daily dose for the main indication in an adult of 70 kg.[18] DDDs provide a fixed unit of measurement independent of price and formulation, enabling the researcher to assess trends in drug consumption and to perform comparisons between population groups.

Smoking was also considered as an effect-modifier, since smoking and the D-allele have been associated with increased generation of angiotensin II.[19]

Genotype

The I and D-allele of the ACE gene were identified on the basis of polymerase-chain-reaction (PCR) technique in accordance with the method described by Lindpainter et al.,[17] with some modifications. Because the D-allele in heterozygous samples is preferentially amplified, there is a tendency of misclassification for about 4-5% of the ID to DD genotypes. For this reason, a second PCR was performed with a primer pair that recognises an insertion specific sequence (5' TGG GAC CAC AGC GCC CAC TAC 3' and 5' TCG CCA GCC CTC CCA TGC CCA TAA 3'). The reaction yielded a 335-bp amplicon only if the I-allele was present. Two independent investigators read pictures from each gel and all ambiguous samples were analysed a second time.

Analysis

We used ANOVA (continuous variables) and Chi-square testing (categorical variables) to compare baseline characteristics of people with different genotypes. To compare the difference in DDDs for each examination, an ANOVA was used, stratified by genotype. A marginal generalised linear model (GEE) was used to investigate any association between I/D polymorphism of the ACE gene and antihypertensive treatment for two outcomes: mean difference in systolic blood pressure (SBP) and diastolic blood pressure (DBP). A p-value of ≤ 0.05 was considered statistically significant. Since subjects could have one, two, or three measurements, the GEE model was used to account for intraperson correlations among repeated measurements. The covariance matrix of the repeated dependent measurements was unstructured and data were analysed using SAS statistical software and corrected for potential confounders.

We performed two separate analyses. In the first analysis we compared the mean systolic and diastolic blood pressure levels between the different genotype groups (DD, ID, and II) for untreated and treated patients. In this analysis the reference group comprised of untreated subjects with the DD genotype. In the second analysis we focused on the drug-gene interaction. In this analysis we compared the mean systolic and diastolic blood pressure levels between the different genotype groups for subjects using the same antihypertensive drug class. The reference group comprised of subjects with the DD genotype who had a prescription of the antihypertensive drug class in question. The mean SBP and DBP of treated subjects was defined as the mean SBP or DBP of subjects who used the antihypertensive drug class in question minus the mean SBP or DBP in untreated subjects with the same genotype.

Results

Of the 6,869 subjects, who participated in the Rotterdam Study between January 1st, 1990 and December 31st, 1999 3,025 were classified as hypertensive. These 3,025 subjects had a total of 6,500 blood pressure measurements. In total, 28.3%, 51.4%, and 20.3% had the DD, ID, and II genotypes, respectively. Of these 3,025 persons, 431 subjects used diuretics (603 measurements), 745 used β-blockers (1,078 measurements), 306 used calcium channel blockers (400 measurements), and 317 used ACE-inhibitors (420 measurements). A person may switch from one antihypertensive drug class to another. Baseline characteristics at the first examination are presented in table 1. The mean DDD at baseline for diuretics was 0.81 ± 0.44, for β-blockers 0.67 ± 0.17, for calcium channel blockers 0.79 ± 0.43, and for ACE-inhibitors 1.01 ± 0.63, respectively. During the first examination round, 855 subjects were treated with an antihypertensive drug. During the three examination rounds there was no statistically significant difference in the DDDs between the different genotype groups for any of the different antihypertensive drug classes.

In the univariate analysis without correction for potential confounders, none of the antihypertensive drug classes were associated with a significant decrease in the mean difference in SBP or DBP for the three genotype groups (data not shown). After adjustment for potential confounders, the ACE gene did not significantly influence the mean difference in SBP (ID vs. DD=0.42 mmHg; 95%CI: -5.18-6.01 and II vs. DD=-1.67 mmHg; 95%CI: -9.60-6.27) or the mean difference in DBP (ID vs. DD=-0.21 mmHg; 95%CI: -3.24-2.82 and II vs. DD=-0.84 mmHg; 95%CI: -5.19-3.51) when all antihypertensive drugs were combined. The adjusted mean difference in SBP and DBP is shown in figures 1 and 2 with the mean SBP or DBP levels in untreated subjects with the DD genotype as a reference. Diuretics users with the DD genotype had a 5.19 mmHg (95%CI: -10.16-0.78) lower mean SBP and a 0.44 mmHg (95%CI: -3.76-2.88) lower mean DBP compared to untreated subjects with the DD genotype.

Table 1. Baseline characteristics of all patients at the first examination. Values are presented as means (± SD), or number (%).

	Untreated			Treated		
Variable	DD (n=452)	ID (n=896)	II (n=357)	DD (n=265)	ID (n=415)	II (n=175)
Gender, male	172 (38.1%)	370 (41.3%)	139 (38.9%)	93 (35.1%)	168 (40.5%)	61 (34.9%)
Age, years	70.1 ± 8.9	69.8 ± 8.4	69.2 ± 8.5	71.4 ± 9.1	71.3 ± 8.9	70.3 ± 9.35
SBP, mmHg	154.6 ± 21.8	153.4 ± 21.3	152.2 ± 22.8	145.1 ± 22.1	145.3 ± 23.3	142.5 ± 23.3
DBP, mmHg	79.0 ± 11.9	78.7 ± 12.2	79.8 ± 12.4	75.2 ± 10.9	76.1 ± 11.9	74.7 ± 13.2
BMI, kg/m2	26.5 ± 3.5	26.3 ± 3.5	26.3 ± 3.4	27.0 ± 3.9	27.2 ± 3.7	27.3 ± 4.1
Diabetes mellitus	51 (11.6%)	104 (12.0%)	42 (12.1%)	27 (10,3%)	72 (17.4%)	24 (14.0 %) *
Smoking						
Current	74 (16.7%)	195 (22.1%)	73 (20.7%)	39 (15.0%)	113 (43.5%)	108 (41.5%)
Past	196 (44.2%)	358 (40.5%)	157 (44.6%)	70 (17.0 %)	184 (44.7%)	158 (38.3%)
Never	173 (39.1%)	330 (37.4%)	122 (34.7%)	31 (17.9%)	77 (44.5%)	65 (37.6%)
Diuretics				77 (33.6%)	104 (45.4%)	48 (21.0%)
β-blockers				116 (30.3%)	179 (46.9%)	87 (22.8%)
Calcium channel blocker				34 (27.4%)	69 (55.6%)	21 (16.9%)
ACE-inhibitor				38 (31.7%)	63 (52.5%)	19 (15.8%)

* = Significantly different in treated or untreated group (p < 0.05)

In addition, we investigated whether there was an interaction between the ACE I/D polymorphism and diuretics, β-blockers, calcium channel blockers, or ACE-inhibitors users (figure 3). The reference group in this analysis was the mean SBP or DBP of subjects with the DD genotype of the investigated antihypertensive drug class. Diuretic users with the II genotype had a 0.23 mmHg (95%CI: -5.48-5.94) higher mean SBP and a 0.81 mmHg (95%CI: -4.14-2.52) lower mean DBP compared to diuretic users with the DD genotype. After adjustment for the covariates, there was only a trend towards an association with the II genotype versus the DD genotype when treated with a β-blocker (p=0.096). However, there was no dose response relationship with regard to blood pressure with the I-allele.

In addition, because a previous study in the Rotterdam Study found a smoking-dependent effect of the ACE gene on blood pressure in current smokers,[20] we assessed the drug-gene interactions in smokers. No drug-gene interaction was found with any of the antihypertensive drug classes in current smokers (data not shown).

Figure 1. Adjusted mean systolic blood pressure among antihypertensive drug users and subjects who were not treated for the 3 ACE genotypes with as reference untreated subjects with the DD genotype.
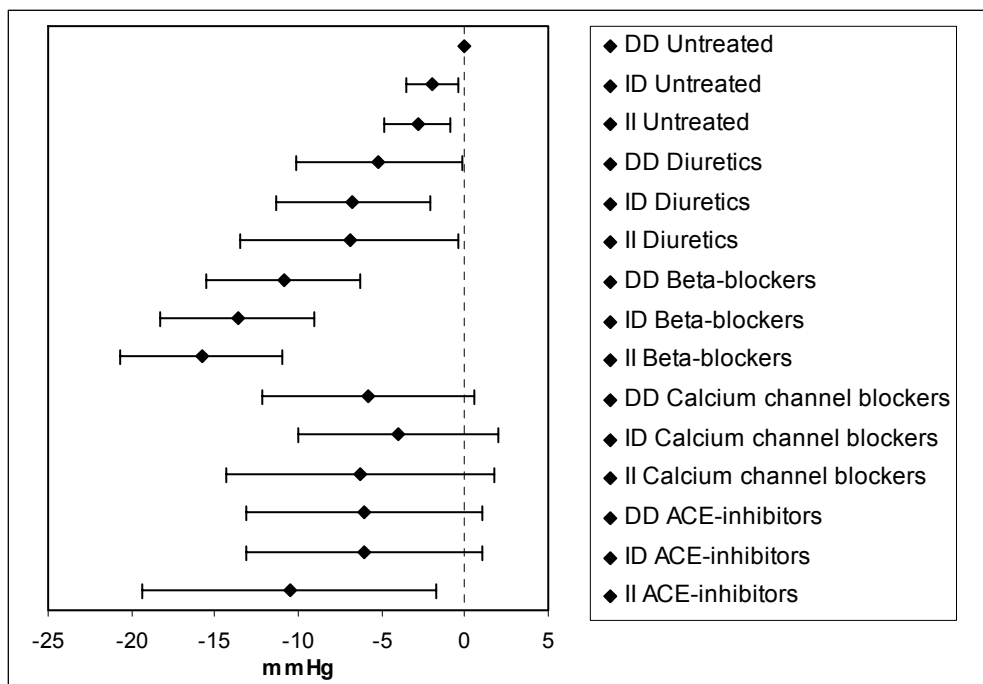

Figure 2. Adjusted mean diastolic blood pressure among antihypertensive drug users and subjects who were not treated for the 3 ACE genotypes with as reference untreated subjects with the DD genotype.
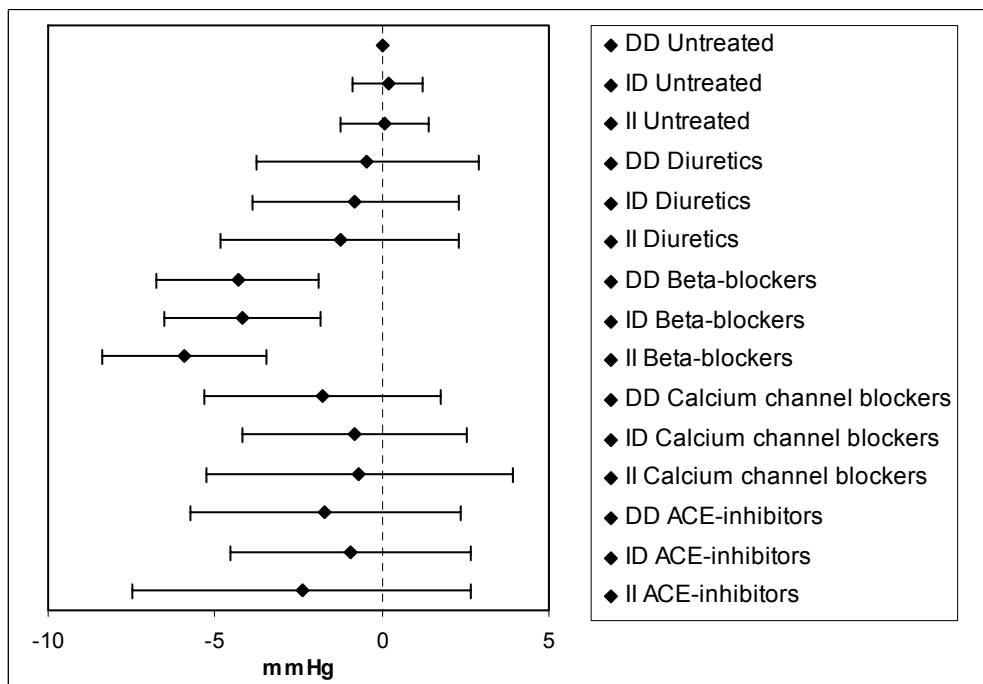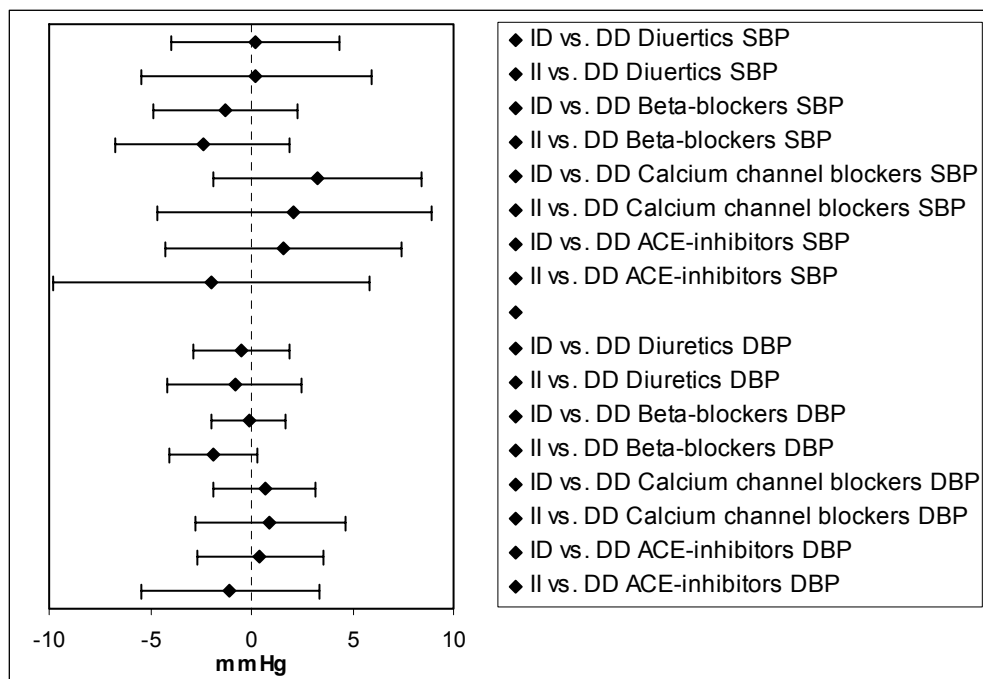
Figure 3. Adjusted mean systolic and diastolic blood pressure among antihypertensive drug users with as reference the DD genotype of the antihypertensive drug classes.

Discussion

Our findings in a Caucasian population suggest that the ACE I/D polymorphism does not influence the mean SBP or DBP difference in users of diuretics, β-blockers, calcium channel blockers, or ACE-inhibitors, even after adjusting for DDDs and other potential confounders.

Previous studies investigating the interaction between the I/D polymorphism and antihypertensives on blood pressure response have been inconclusive.[3-12] Of the eight studies that investigated the interaction between the ACE gene and ACE-inhibitors on SBP and DBP in hypertensive patients, three suggested that the D-allele had a stronger drug effect,[8-10] while two studies indicated the I-allele,[6,7] and four studies found no difference between the two alleles.[3-5,11] Regarding β-blockers, two studies found no drug-gene interaction on blood pressure[4,12] and one study found an interaction with thiazide diuretics.[21] In this study diuretics users with one or two copies of the I-allele of the ACE gene and one copy of the 460Trp-allele of the α-adducin gene showed the largest blood pressure decrease.

The main difference between these studies and the current study was that the latter was an observational study and previous studies were non-randomized trials. In trials, treatment groups can be standardized with respect to dose, medication, duration of therapy, time between blood pressure measurement, and medication intake. In addition, it is possible that, in the current study, the medication taken at the time of the blood pressure measurement was not the initial drug chosen, but rather represents

an alternative drug which, through a process of trial and error, was found to be the most effective. The potential overrepresentation of "good responders" increased the chance of finding a drug-gene interaction.

A strength of an observational study is that it resembles more closely daily clinical practice and the analysis can be adjusted to account for potential confounders like dose and duration of therapy. Another strength of the current study was the large sample size.

A limitation of the current study is that no (pretreatment) baseline measurement immediately preceding the commencement of an antihypertensive drug was available and measurements were only taken every 2-3 years. This made it impossible to calculate the immediate drug effect after administration of an antihypertensive drug. Therefore, short-lived and temporary interactions would be missed in this study. Other limitations were the absence of a clinically confirmed diagnosis of hypertension and the overrepresentation of subjects with isolated systolic hypertension (approximately 50% of the untreated patients). As the mean of the treated patients is above 70 years of age, it is reasonable to assume that also in this group there is an overrepresentation of patients with isolated systolic hypertension. Thus, the results can not be generalized to all patients with hypertension.

Another potential limitation of our study is that we studied only one genetic polymorphism, which is linked to the serum ACE activity, but it is controversial in hypertension. Zhu et al.[22] found two other ACE gene mutations, which were linked with blood pressure and ACE serum levels. Therefore, it might be necessary to type additional markers. Finally, observational studies may be vulnerable to selection, information, and confounding bias. Confounding is very unlikely given that the data were adjusted for potential confounders, but it is impossible to adjust for other unmeasured confounders. Race could have been an additional confounder, however, given that less than 1% of the subjects had a different ethnic background, it is unlikely that this biased our results. Since our study population consisted of > 99% of Caucasians, our results can only be generalized to Caucasians. There are other additional variables, e.g. exercise, which have an impact on blood pressure. Therefore, it is possible that we over or underestimated the blood pressure lowering effect of the antihypertensive drug classes. However, since this is likely the same for the different genotypes this has not influenced the results of the drug-gene interaction. In addition, difference in blood pressure between treated patients and untreated patients could be the result of confounding by indication. As a physician is free to choose a specific antihypertensive drug or no treatment, specific patient characteristics may have influenced this decision. Therefore, we also investigated the mean difference in blood pressure between users of the same antihypertensive drug therapy, as they were most likely to have the same patient characteristics. Information bias is also unlikely, as data on drug exposure were prospectively gathered via computerised pharmacies in a similar and unbiased fashion for all subjects. It is, however, possible that we under- or overestimated baseline characteristics for which we used self-reported data. In

addition, we assumed that all prescribed pills were taken and thereby may have overestimated compliance. As this is likely the same for all three genotypes, this unlikely to have biased our result. Additionally, selection bias is unlikely, because this study was population-based and loss to follow-up was negligible.

Conclusion

Notwithstanding the caveats, the study suggests that the ACE I/D polymorphism of the ACE gene does not influence the mean blood pressure difference among users of low-ceiling diuretics, β-blockers, calcium channel blockers, or ACE-inhibitors. Although, it seems that the ACE I/D polymorphism does not have a clinical relevance in the response to antihypertensive drugs, further investigations with this polymorphism on short and long-term outcomes will be needed to make definitive conclusions.

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Chapter 4.3

The influence of the alpha-adducin G460W polymorphism and angiotensinogen M235T polymorphism on the association between antihypertensive medication and blood pressure

Abstract

Introduction: Despite the availability of a variety of effective antihypertensive drugs, inadequate control of blood pressure is common in hypertensive patients.

Aim: To investigate whether the α-adducin G460W or angiotensinogen M235T polymorphism modifies the mean difference in blood pressure in subjects using antihypertensive drugs.

Methods: Data were used from the Rotterdam Study, a population-based prospective cohort study in the Netherlands. This study started in 1990 and included 7,983 subjects of 55 years or older. Data from three examination rounds were used. Subjects were included if their blood pressure was elevated at one or more examinations and/or a diuretic, β-blocker, calcium channel blocker, or ACE-inhibitor was used. A marginal generalised linear model (GEE) was used to assess the drug-gene interaction.

Results: In total, 3,025 hypertensives were included. No drug-gene interaction on blood pressure levels was found. The mean difference in systolic blood pressure between subjects with the W-allele and GG genotype of the α-adducin gene was for diuretic users 1.25 mmHg (95%CI: -2.86-5.35), for β-blocker users 0.02 mmHg (95%CI: -3.39-3.42), for calcium channel blocker users −0.70 mmHg (95%CI: -5.61-4.21), and for ACE-inhibitors user −3.50 mmHg (95%CI: -9.02-2.02). The mean difference in systolic blood pressure between subjects with the TT and MM genotype was for diuretic users −2.33 mmHg (95%CI: -8.32-3.66), for β-blocker users −0.06 mmHg (95%CI: -4.91-4.79), for calcium channel blocker users 0.59 mmHg (95%CI: -5.95-7.13), and for ACE-inhibitor users -2.33 mmHg (95%CI: -9.66-5.01).

Conclusion: The G460W and the M235T polymorphism did not modify differences in blood pressure levels among subjects who used diuretics, β-blockers, calcium channel blockers, or ACE-inhibitors.

Introduction

Hypertension is the most prevalent cardiovascular risk factor in the industrialized world. Despite the availability of a variety of effective antihypertensive drugs, inadequate control of blood pressure is common in hypertensive patients. This is caused by environmental and genetic factors.

A number of studies have investigated genetic polymorphisms as determinants of cardiovascular response to antihypertensive drug therapy, e.g. the G460W polymorphism of the α-adducin (ADD1) gene and the M235T polymorphism of the angiotensinogen (AGT) gene. In four studies, the interaction between the ADD1 G460W polymorphism and antihypertensive drugs on blood pressure response was evaluated.[1-4] Three studies, with partly the same study population, found a greater blood pressure reduction with the 460W-allele than with the 460G-allele,[1-3] while another study could not replicate this finding[4]. All these studies were non-randomized trials. Three research groups studied the influence of the M235T polymorphism of the AGT on the blood pressure response to antihypertensive medication.[5-7] Two of these studies where non-randomized trials[5, 7] and the other study was a placebo-controlled crossover.[6] Hypertensive subjects with the 235T-allele when treated with ACE-inhibitors had a greater blood pressure reduction in one study,[5] but this could not be reproduced in another study.[6] No drug-gene interactions were found with β-blockers or calcium channel blockers.[6, 7]

The purpose of this study was to evaluate the relationship between the G460W polymorphism of the α-adducin gene and the M235T polymorphism of the angiotensinogen gene on mean systolic and diastolic blood pressure difference in hypertensive patients treated with diuretics, β-blockers, calcium channel blockers, or ACE-inhibitors.

Methods

Setting

The Rotterdam Study started in 1990 as a population-based prospective follow-up study. All 10,275 residents of the suburb Ommoord in Rotterdam aged 55 years or older were invited to participate. In total, 7,983 (78%) subjects gave written informed consent and of 86% of them blood samples were available for genotyping. The baseline measurements took place until 1993 and the design has been described elsewhere.[8] The baseline examination included several details, such as an interview on demographics, current health status, medical history, family history of diseases, smoking habits, and current use of medication. During a physical examination, blood pressure, weight, and height were measured and blood was drawn for DNA extraction. Blood pressure was measured in sitting position at the right upper arm with a random-zero sphygmomanometer. The average of the two measurements, separated by a

count of pulse rate, was used in the analysis. All participants were subsequently examined in follow-up examination rounds every two to three years (1993-1995, 1997-1999).

Pharmacy records were available for approximately 99% of the cohort as of January 1st, 1991. 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.[9]

Cohort and outcome definition

The study population included all hypertensive individuals in the Rotterdam Study for whom the AGT M235T or ADD1 G460W genotype was assessed. Hypertension was defined as one or more blood pressure measurements with a systolic blood pressure (SBP) above 160 mmHg and/or diastolic blood pressure (DBP) above 95 mmHg, and/or use of one antihypertensive drug at the time of a blood pressure measurement (monotherapy). When a blood pressure measurement occurred, we assessed whether a prescription was filled by the pharmacy on this date. When the blood pressure measurement date fell within the usage period, the patient was considered currently exposed. Due to the small numbers for the other antihypertensive drug classes, only subjects using diuretics, β-blockers, calcium channel blockers, and ACE-inhibitors were included in the analysis. In addition, blood pressure measurements were excluded when combinations of different antihypertensive drugs classes were used on the date of the blood pressure measurement. Pharmacy records were available as of January 1st, 1991, nevertheless, blood pressure measurements from 1990 were included if an individual did not use an antihypertensive drug according to self-reported questionnaire information and did not start antihypertensive therapy before July 1st, 1991. The end of the study period was set at December 31st, 1999.

In addition, we distinguished between starters and continuous users. Starters were defined as hypertensives who did not have a prescription before July 1st, 1991 and used their antihypertensive medication, less then six of the eight weeks prior to their blood pressure measurement. In addition, starters had to have at least a 30 days gap between the start date of prescription, which was used at the date of the blood pressure measurement, and the end date of the previous prescription.

Potential confounders

The potential confounders considered were age, sex, body mass index (BMI), defined daily dose (DDD), (re-)examination round, smoking at baseline, salt intake at baseline (g/day), history of myocardial infarction, diabetes mellitus at baseline, use of nitrates, use of statins, use of NSAID's, use of another antihypertensive drug class two weeks prior to the blood pressure measurement, use of an antihypertensive drug for six of the eight weeks prior to the blood pressure measurement, and the cumulative number of days an antihypertensive drug was used.

History of myocardial infarction was self-reported confirmed by a physician or demonstrated on the baseline ECG. 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.[9]

Genotype

Genomic DNA was extracted from whole blood samples using standard methods, described previously.[10] Samples were genotyped with TaqMan allelic discrimination Assays-By-Design (Applied Biosystems, Foster City, CA). Forward and reverse primer sequences were 5' GAG AAG ACA AGA TGG CTG AAC TCT 3' and 5' GTC TTC GAC TTG GGA CTG CTT 3' and the minor groove binding probes were 5' ATT CTG CCA TTC CTC 3' (VIC) and 5' ATT CTG CCA TTC CTC 3' (FAM) for the ADD1 gene. Forward and reverse primer (anti sense strand) sequences were 5' AGG TTT GCC TTA CCT TGG AAG TG 3' and 5' GCT GTG ACA GGA TGG AAG ACT 3' and the minor groove binding probes were 5' CTG GCT CCC ATC AGG 3' (VIC) and 5' CTG GCT CCC GTC AGG 3' (FAM) for the AGT gene. The assays utilized 5 nanograms of genomic DNA and 2 microliter reaction volumes. The amplification and extension protocol was as follows: an initial activation step of 10 min at 95 deg preceded 40 cycles of denaturation at 95 deg for 15 s and annealing and extension at 50 deg for 60 s. allele-specific fluorescence was then analyzed on an ABI Prism 7900HT Sequence Detection System with SDS v 2.1 (Applied Biosystems, Foster City, CA).

Analysis

We used ANOVA (continuous variables) and Chi-square testing (categorical variables) to compare baseline characteristics of people with different genotypes. ANOVA was used to compare, for each examination, the difference in DDD between the genotype groups, stratified by genotype groups. A marginal generalised linear model (GEE) was used to investigate the potential interaction between the α-adducin G460W and angiotensinogen M235T polymorphism 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. The mean SBP and DBP of treated subjects was defined as the mean SBP and DBP of subjects who used the antihypertensive drug class in question minus the mean SBP or DBP in untreated subjects with the same genotype. The GEE model was used to account for intraperson correlations between repeated measurements. The covariance matrix of the repeated dependent measurements was unstructured and data were analysed using SAS statistical software and adjusted for potential confounders (SAS version 8.2).

Results

Alpha-Adducin gene (ADD1) G460W polymorphism

Between January 1st, 1990 and December 31st, 1999, 6,500 blood pressure measurements of 3,025 hypertensive individuals were included. In 91% of the hypertensive individuals the ADD1 genotype could be assessed. In total, 396 individuals used diuretics (559 measurements), 685 β-blockers (997 measurements), 281 calcium channel blockers (366 measurements), and 294 ACE-inhibitors (389 measurements). Baseline characteristics of all subjects stratified by the ADD1 genotype are presented in table 1. There was a significant difference in SBP levels between the genotype groups in treated subjects during the first examination.

Table 1. Baseline characteristics of all patients at the first examination stratified by α-adducin genotype. Values are presented as means (± SD), or number (%)

Variable	Untreated		Treated		
	GG (n=950)	W-allele (n=602)	GG (n=469)	W-allele (n=316)	
Gender, M	389 (40.9%)	246 (40.9%)	178 (38.0%)	132 (41.8%)	
Age, years	69.9 ± 8.6	69.2 ± 8.3	70.7 ± 9.1	71.6 ± 8.8	
SBP, mmHg	153.0 ± 21.9	152.9 ± 21.3	146.0 ± 22.7	142.5 ± 23.4	**
DBP, mmHg	78.9 ± 12.0	78.6 ± 11.9	76.0 ± 11.8	74.9 ± 11.8	
BMI, kg/m2	26.4 ± 3.5	26.2 ± 3.4	27.1 ± 3.8	27.3 ± 3.9	
Diabetes mellitus	109 (11.7%)	72 (12.8%)	67 (14.4%)	44 (14.1%)	
Smoking					
Current	187 (20.0%)	129 (21.8%)	70 (15.0%)	59 (19.0%)	
Past	393 (42.1%)	259 (43.7%)	213 (45.7 %)	134 (43.2%)	
Never	354 (37.9%)	205 (34.6%)	183 (39.3%)	117 (37.7%)	
Diuretic			125 (36.7%)	87 (27.5%)	
β-blocker			203 (43.3%)	147 (46.5%)	
Calcium channel blocker			72 (15.4%)	43 (13.6%)	
ACE-inhibitor			69 (14.7%)	39 (12.3%)	

* = Significantly different in treated or untreated group (p < 0.10)
** = Significantly different in treated or untreated group (p < 0.05)

The mean DDD at baseline for users of diuretics was 0.81 ± 0.46, for β-blockers 0.68 ± 0.35, for calcium channel blockers 0.77 ± 0.34, and for ACE-inhibitors 1.11 ± 0.65. There was no statistically significant difference in DDDs during follow-up between the different genotypes (data not shown).

Due to the small number of subjects with the WW genotype (7.5% of the individuals) this group was combined with the GW genotype group in the analysis. After adjustment for potential confounders, the mean difference in SBP and DBP was compared between the two genotype groups for the different antihypertensive drug classes during the three examination rounds (see figure 1). Among starters of diuretic therapy (n=57) there was no drug-gene interaction on the mean difference in SBP (p=0.81) and DBP (p=0.88). There was also no drug-interaction among starters of β-blocker therapy (n=63) (SBP p=0.95 and DBP p=0.75). However, we could only

include a small number of measurements. The number starters of calcium channel blockers therapy (n=23) and ACE-inhibitors therapy (n=15) were too small to examine.

Figure 1. Adjusted mean difference in systolic and diastolic blood pressure among antihypertensive drug users with the GG genotype of the α-adducin gene compared to subjects with the W-allele of α-adducin gene.
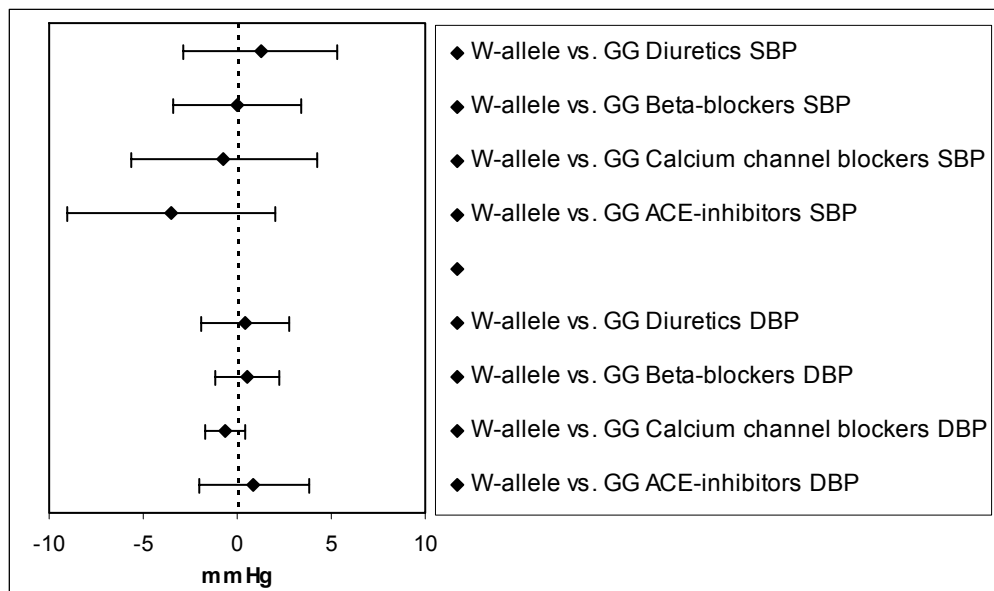
Angiotensinogen gene (AGT) M235T polymorphism

For the AGT gene 91% of the genotypes could be ascertained. In total, 395 individuals used diuretics (559 measurements), 689 β-blockers (1,002 measurements), 282 calcium channel blockers (367 measurements), and 293 ACE-inhibitors (388 measurements), respectively. Baseline characteristics for all subjects stratified by AGT genotypes are presented in table 2. There was no significant difference in baseline characteristics between the different genotype groups during the first examination. Although, there was some indication that untreated subjects with the MT genotype had a higher SBP level compared to the other genotypes. There was no statistically significant difference in DDDs for the different genotypes.

The adjusted difference in blood pressure when treated with diuretics, β-blockers, calcium channel blockers, or ACE-inhibitors is presented in figure 2. Also, with this polymorphism no statistically significant drug-gene interaction was found. There was no drug-gene interaction for starters of diuretic (SBP p=0.88 and DBP p=0.65) or β-blocker therapy (SBP p=0.72 and DBP p=0.41).

Table 2. Baseline characteristics of all patients at the first examination stratified by angiotensinogen genotype. Values are presented as means (± SD), or number (%).

Variable	Untreated			Treated		
	MM (n=541)	MT (n=755)	TT (n=252)	MM (n=280)	MT (n=397)	TT (n=113)
Gender, M	207 (38.3%)	325 (43.0%)	102 (40.5%)	111 (39.6%)	152 (38.3%)	49 (43.4%)
Age, years	69.4 ± 8.3	70.0 ± 8.6	68.8 ± 8.4	71.0 ± 8.9	70.9 ± 8.8	70.5 ± 9.4
SBP, mmHg	151.3 ± 21.9	154.0 ± 21.7	152.8 ± 21.0 *	142.8 ± 22.6	145.7 ± 23.0	144.6 ± 23.3
DBP, mmHg	78.4 ± 12.0	79.0 ± 12.1	78.7 ± 11.8	75.3 ± 11.6	75.3 ± 11.7	77.6 ± 12.7
BMI, kg/m2	26.4 ± 3.6	26.3 ± 3.4	26.4 ± 3.2	27.4 ± 4.1	27.0 ± 3.6	27.0 ± 3.9
Diabetes mellitus	61 (11.5%)	90 (12.3%)	24 (9.7%)	39 (13.9%)	56 (14.3%)	16 (14.4 %)
Smoking						
Current	100 (18.9%)	152 (28.8%)	61 (24.5%)	39 (14.1%)	73 (18.6%)	20 (17.9%)
Past	223 (42.2%)	327 (43.8%)	101 (40.6%)	131 (47.5 %)	171 (43.5%)	47 (42.0%)
Never	205 (37.5%)	267 (35.8%)	87 (34.9%)	106 (38.4%)	149 (37.9%)	45 (40.2%)
Diuretic				84 (30.0%)	106 (26.7%)	23 (20.4%)
β-blocker				118 (42.1%)	185 (46.6%)	49 (43.4%)
Calcium channel blocker				39 (13.9%)	57 (14.4%)	19 (16.8%)
ACE-inhibitor				39 (13.9%)	49 (12.3%)	22 (19.5%)

* = Significantly different in treated or untreated group (p < 0.10)
** = Significantly different in treated or untreated group (p < 0.05)

In addition, we assessed the effect of drug-gene-gene interactions between the ACE I/D polymorphism, AGT M235T polymorphism, and ADD1 G460W polymorphism and antihypertensive drugs on blood pressure. None of the drug-gene-gene combinations (AGT-ADD1 or AGT-ACE or ADD1-ACE) modified the mean difference in SBP or DBP level.

Discussion

This study suggests that there is no interaction between the G460W polymorphism of the α-adducin gene or the M235T polymorphism of the angiotensinogen gene and the use of monotherapy with a diuretic, β-blocker, calcium channel blocker, or ACE-inhibitor on SBP and DBP levels. Furthermore, combinations of these two polymorphisms and the I/D polymorphism of the ACE gene did not result in significant drug-gene-gene interactions.

Figure 2. Adjusted mean difference in systolic and diastolic blood pressure among antihypertensive drug users with the TT and MT genotype of the angiotensinogen gene compared to the MM genotype of the angiotensinogen gene.
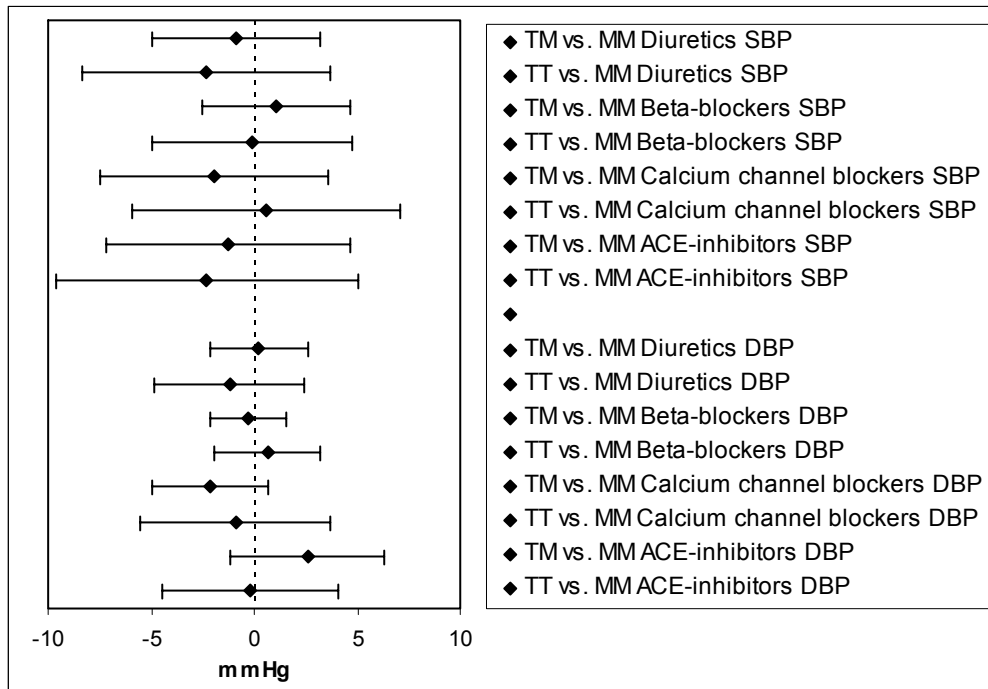
The G460W and M235T polymorphisms were logical candidates to investigate as polymorphisms that might influence antihypertensive drug response. Studies with the Milan hypertensive rat and humans with essential hypertension suggest that genetic alterations in α-adducin may contribute to hypertension.[11-13] The α-adducin gene may affect blood pressure by increasing renal tubular reabsorption of sodium through the activation of Na^+,K^+-ATPase (adenosine triphosphatase) and the 460W-allele of the α-adducin gene is associated with a higher affinity for the Na^+,K^+-ATPase pump than the 460G-allele.[14] Angiotensinogen is the inactive precursor of the potent vasoactive and salt-retaining hormone angiotensin II and thus a major component of the renin-angiotensin system. The M235T polymorphism of the angiotensinogen gene has an effect on plasma angiotensinogen concentration. Subjects with two copies of the 235T-allele have 15% to 40% higher levels compared with subjects with two copies of the 235M-allele.[15] In a meta-analysis the M235T polymorphism was significantly associated with hypertension.[16] Persons with a copy of the T-allele had a higher risk of hypertension. In our study, subjects with the T-allele had a higher SBP level compared to the subjects with MM genotype at baseline.

Previous studies investigating the interaction between the G460W polymorphism of the α-adducin gene or M235T polymorphism of the angiotensinogen gene and antihypertensive drugs on blood pressure response have been inconclusive. In a study of Italian families, there was evidence that the G460W polymorphism predicted a twofold mean difference in blood pressure response to hydrochlorothiazide among hypertensive subjects when treated for 4 weeks,[1-3] although, this could not be

replicated in another and larger study.[4] We did observe a significant difference between the genotype groups in SBP in treated groups during the first examination, but this disappeared when we analyzed the complete data.

In our study, we had only measurements every three years and could not investigate the immediate effect on blood pressure response after administration of an antihypertensive drug and therefore calculated the difference in blood pressure between genotype groups. It is possible that previously reported drug-gene interaction were the results of a temporary difference between the genotype groups, which occurred shortly after administration of an antihypertensive drug and therefore missed in our study. However, no drug-gene interaction was found in our group of "starters".

The main difference between the previous studies and ours, is that we conducted an observational study and the other studies were trials. The advantage of an observational study is that it resembles daily clinical practice. A limitation of observational studies is that they are vulnerable to confounding. For example, confounding by indication might have biased our results. As a physician was free to choose whether a patient receives a 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. In addition, we adjusted for potential confounders, such as dose, BMI, and salt-intake. Another limitation is the overrepresentation of patients with isolated systolic hypertension. In addition, it is possible that the medication taken during the blood pressure measurement was not the initial drug chosen, but rather represents an alternative drug, which through a process of trail and error was found to be the most effective. However, the potential overrepresentation of "good responders" increased our chance of finding a drug-gene interaction.

Notwithstanding these caveats, this study suggests that in daily practice the G460W polymorphism of the ADD1 gene and M235T polymorphism of the AGT gene do not influence differences in blood pressure levels among users of low-ceiling diuretics, β-blockers, calcium channel blocker, or ACE-inhibitors.

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Chapter 4.4

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

Abstract

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 β3-subunit (825C/T) gene modify the mean difference in blood pressure levels among diuretic, β-blocker, or ACE-inhibitor users. Data were used from the Doetinchem Cohort Study, and blood pressure data was collected from GPs (1987-1997). A marginal generalised 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 5,262 measurements of blood pressure. Only the interaction between diuretic use and the GNB3 825C/T polymorphism was significant (C-allele versus TT; difference in systolic blood pressure=4.33 mmHg; 95%CI: 0.14-8.54). Thus, the mean systolic blood pressure level among diuretic users may be modified by the GNB3 825C/T polymorphism.

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 pharmacology influenced by an antihypertensive drug. Other candidates genes are those that code for components of counter-regulatory systems.

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 235T-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 β3-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 non randomized 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) polymorphism on the mean difference in blood pressure levels among subjects using diuretics, β-blockers, or ACE-inhibitors in daily practice.

Materials and Methods

Setting

Data from the Doetinchem Cohort Study was used; a population-based prospective study on cardiovascular disease risk factor conducted in the Netherlands.[9] 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.[9] 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 January 1st, 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.[10]

Cohort and outcome definition

Hypertensive patients were only included if their 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 one or more blood pressure measurements which met one of the following criteria: systolic blood pressure (SBP) ≥ 160 mmHg, and/or diastolic blood pressure (DBP) ≥ 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, β-blockers, or 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 December 31st, 1997.

Potential confounders and effect modifiers

As potential confounders we considered age, gender, body mass index, defined daily dose (DDDs), 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 two weeks prior to the blood pressure measurement, the use of an antihypertensive drug six of the eight 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.[11]

Genotype

Genomic DNA was isolated from peripheral blood according to standard procedures. The genotyping procedure of the ADD1 G460W,[12] ACE I/D,[5] AGT M235T,[13] GNB3 825C/T,[14] and AGTR1 1166A/C,[15] polymorphism were previously described.

Analysis

We used ANOVA (continuous variables) and Chi-square 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) x 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 drug-gene-gene interaction, we combined the genotypes of two of the five polymorphisms. For this analysis, we added three dummy variables to the model i.e. the drug-gene combinations (e.g. ACE+ADD1: I-allele+T-allele, I-allele+MM, and D-allele+T-allele) x 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.

Results

Between 1987 and 1997, 5,262 blood pressure measurements of 625 individuals were included. During follow-up, 106 subjects used diuretics (743 measurements), 229 used β-blockers (1,480 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 were older and the percentage of diabetics, female subjects, subjects receiving a low-salt diet or a low-cholesterol diet was higher compared to the untreated group.

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 mellitus	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%)	**
Smoking			
current	167 (34.1%)	48 (35.6%)	
past	151 (30.8%)	33 (24.4%)	
never	172 (35.1%)	54 (40.0%)	
Ethnicity, caucasian	477 (97.3%)	131 (97.8%)	
ACE: DD/ID/II	160/212/114	36/72/27	
ADD1: W-allele/GG [1]	312/92	92/43	
AGTR1: C-allele/AA [2]	237/66	66/69	
GNB3: C-allele/TT [3]	220/63	63/71	
AGT: T-allele/MM [4]	164/325	51/84	
Diuretic		39 (33.9%)	
Beta-blocker		86 (63.7%)	
ACE-inhibitor		10 (7.4%)	

** P < 0.001
[1] W-allele: WW+GW genotype
[2] C-allele: CC+CA genotype
[3] C-allele: CC+CT genotype
[4] T-allele: TT+MT genotype

Owing to the small sample size, two genotype groups were combined in the analysis for most 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 SBP and DBP was for diuretics users -3.15 mmHg (95%CI: -4.70--1.60) and -3.92 mmHg (95%CI: -4.75--3.10), for β-blockers users -2.53 mmHg (95%CI: -3.78--1.27) and -2.13 mmHg (95%CI: -2.80--1.46), for ACE-inhibitors users 0.35 mmHg (95%CI: -1.62-2.32) and 0.48 mmHg (95%CI: -0.55-1.52).

The mean difference in DDDs between genotypes for users of diuretics, β-blockers, and ACE-inhibitors adjusted for potential confounders is presented in table 2. There was no statistically significant difference in DDDs between the different genotype groups.

Table 2. Adjusted DDDs for antihypertensive drug users.

	Diuretic	Beta-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)
AGTR1: 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)

After adjustment for potential confounders, the mean difference in SBP and DBP for users of diuretics, β-blockers, and ACE-inhibitors was compared between the genotype groups (see 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: -1.13-2.15). 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: -1.69-2.70).

Drug-gene-gene-interactions

To assess drug-gene-gene 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.

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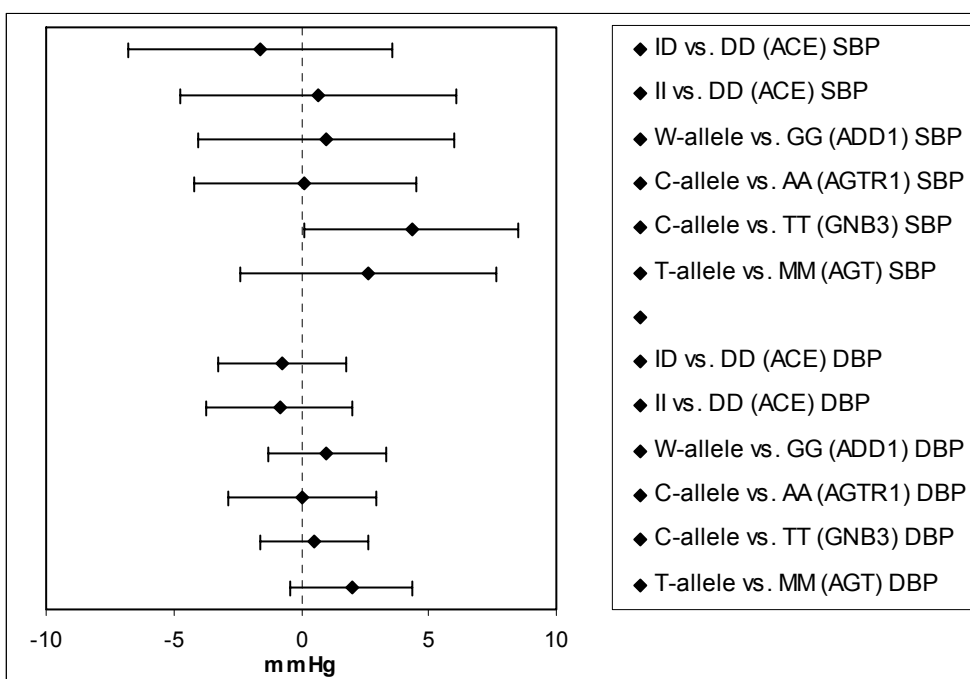
Figure 1b. Adjusted mean difference in systolic or diastolic blood pressure among β-blocker users. The diamonds with bars depict the mean difference in blood pressure ± 95%CI.
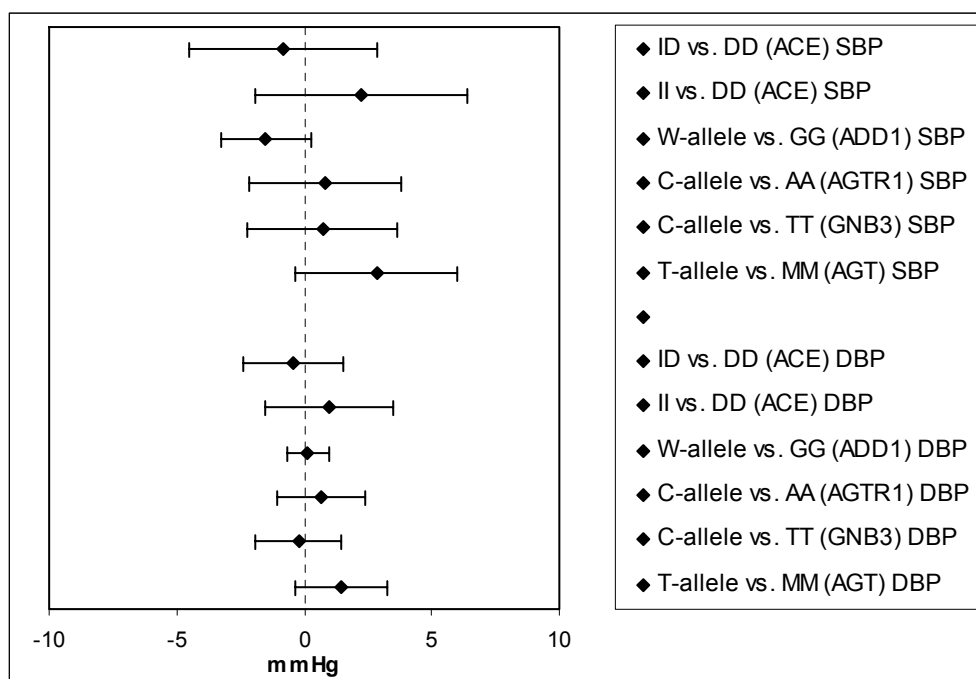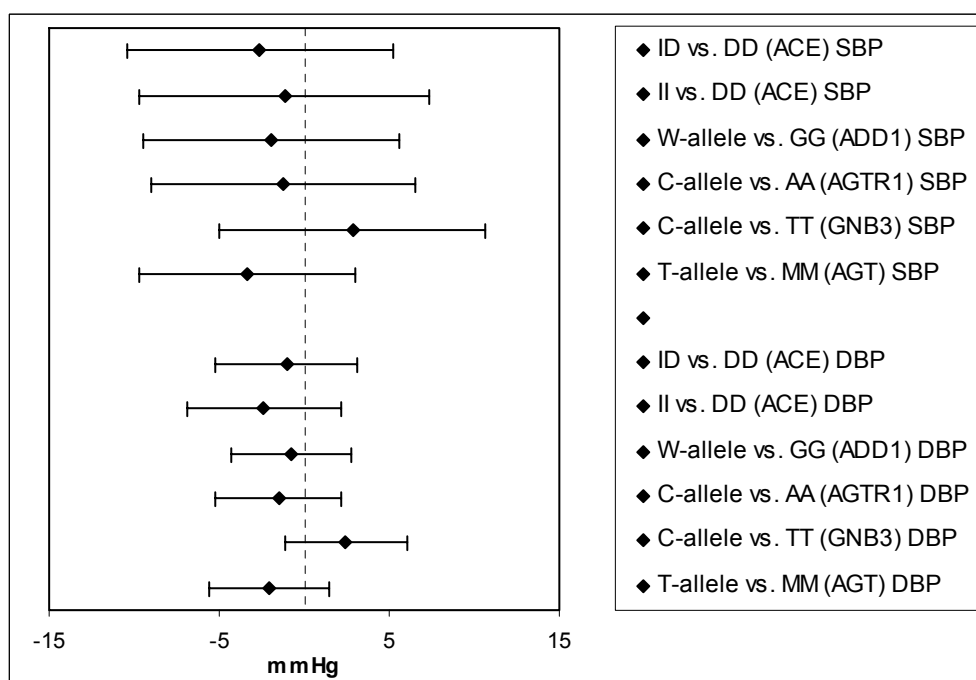

Figure 1c. Adjusted mean difference in systolic or diastolic blood pressure among ACE-inhibitor users. The diamonds with bars depict the mean difference in blood pressure ± 95%CI.

In total, 36 drug-gene-gene interactions were possible for SBP and DBP. Of these interactions, four were associated with a significant difference in blood pressure (see figure 2). These were the interaction between diuretic use and ADD1 W-allele+ AGT T-allele vs. GG+ MM on DBP (3.09 mmHg; 95%CI:0.16-2.93; 169 versus 149 measurements), β-blocker use and AGTR1 C-allele+ AGT T-allele vs. AA+ MM on

DBP (2.63 mmHg; 95%CI: 0.30-2.33; 436 versus 223 measurements), ACE-inhibitor use and ACE I-allele+ GNB3 TT vs. 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 vs. DD+ MM on SBP (-10.21 mmHg; 95%CI: -19.47--0.95; 239 versus 29 measurements).

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.
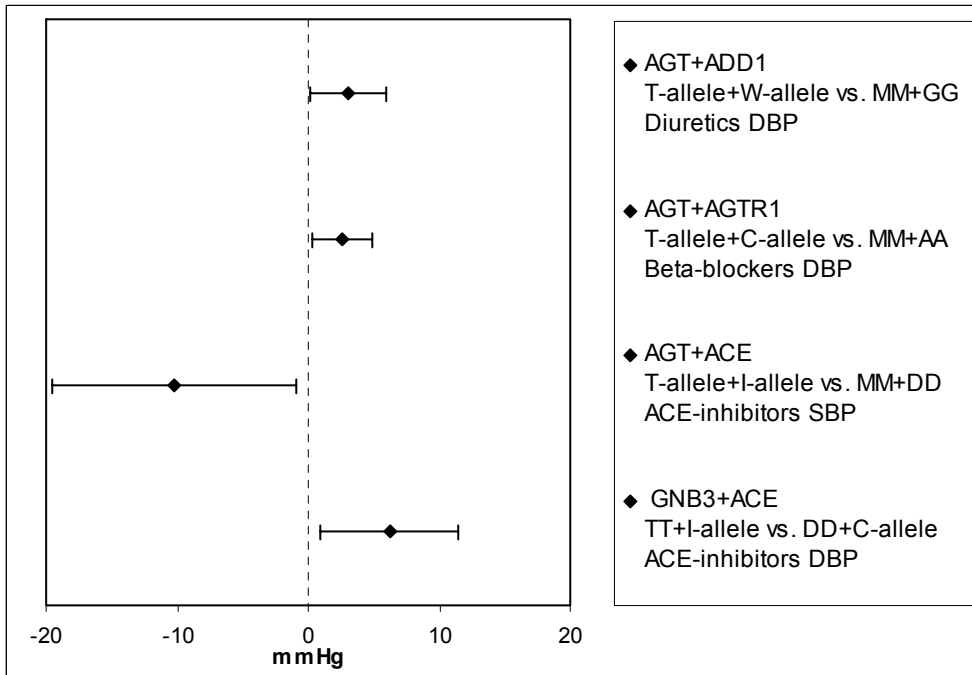
Discussion

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

Our results concur with that of a non-randomized trial that investigated the role of GNB3 in diuretic users.[16] In this study a significantly greater decline for both SBP and DBP was found in subjects with the TT genotype.[16] 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 the AGT M235T

polymorphism. In addition, none of the drug-gene-gene interactions were 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)ˆthe 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 overrepresentation 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 co-morbidities. 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 e.g. 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 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-positice, the observed difference of 4.33 mmHg systolic could result in a relative risk reduction of about 10% of cardiovascular disease in 10 year, according to the Framingham Risk function.

Regarding the significant drug-gene-gene interactions the chance of a false-positive result is even higher due to the smaller sample size. However, of the 36 possible combinations four were found to be significant. The observed interactions were only found either with 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 drug-gene-gene interactions.

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

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Chapter 5

Pharmacogenetics and long-term outcomes

Chapter 5.1

Pharmacogenetic interactions of three candidate gene polymorphisms with ACE-inhibitors or ß-blockers therapy and the risk of atherosclerosis

Abstract

Introduction: Knowledge of pharmacogenetics might optimise drug therapy in patients treated with antihypertensive drugs.

Aim: To investigate whether the angiotensin converting enzyme (ACE) insertion/deletion (I/D), angiotensinogen M235T, or angiotensin II receptor type 1 573C/T polymorphism modify the risk of atherosclerosis associated with β-blocker or ACE-inhibitor therapy.

Methods: Data were used from the Rotterdam Study, a population based prospective cohort study in the Netherlands, which started in 1990 and included 7,983 subjects of 55 years and older. Three sub-clinical measurements were used for atherosclerosis, i.e. peripheral arterial disease, carotid atherosclerosis, and aortic atherosclerosis. This study included 2,216 subjects with hypertension. Pharmacy records were available as of January 1st, 1991. The interaction between antihypertensive drugs and genetic polymorphisms on the risk of atherosclerosis was determined with binary logistic regression analysis.

Results: Of the 2,216 subjects, 1,267 were treated with β-blockers and 727 with ACE-inhibitors. The risk of peripheral arterial disease associated with short-term (0-4 years) use of ACE-inhibitors compared to no use of ACE-inhibitors, was higher among subjects with the II genotype than among subjects with the DD genotype of the ACE gene (interaction Odds ratio (OR)=2.21; 95%CI: 1.01-4.85). The risk of aortic atherosclerosis associated with short-term use of ACE-inhibitors compared to no use of ACE-inhibitors, was lower among subjects with the TT genotype than among subjects with the MM genotype of the AGT gene (interaction OR=0.39; 95%CI: 0.15-0.99). In contrast, the risk of aortic atherosclerosis associated with long-term ($\geq$ 4 years) β-blockers treatment compared to no use of β-blockers, was higher among subjects with the TT genotype compared to subjects with the MM genotype of the AGT gene (interaction OR=3.36; 95%CI: 1.14-9.97). The risk of carotid atherosclerosis associated with long-term ACE-inhibitors treatment compared to no use of ACE-inhibitors, was lower among subjects with the TT genotype than among subjects with the MM genotype of the AGT gene (interaction OR=0.20; 95%CI: 0.04-0.95). The risk of carotid atherosclerosis associated with short-term use of ACE-inhibitors compared to no use of ACE-inhibitors, was higher among subjects with the CT genotype than among subjects with the CC genotype of the AGTR1 gene (interaction OR=2.63; 95%CI: 1.11-6.24).

Conclusion: Overall, we could not consistently demonstrate that the risk of atherosclerosis associated with the use of β-blockers or ACE-inhibitors was strongly modified by any of the three candidate gene polymorphisms.

Introduction

The renin-angiotensin system plays an important role in vascular homeostasis. Sequential cleavage occurs of angiotensinogen (AGT) by renin to angiotensin I, and subsequently by angiotensin converting enzyme (ACE) to the vasoactive peptide angiotensin II. Besides regulating blood pressure, angiotensin II has also various actions that can damage blood vessels. For example, angiotensin II stimulates NADH and NADPH activity and raises thereby the oxidative potential of vascular tissue.[1,2] In addition, it plays a role in the vascular-injury response since it stimulates leukocyte adhesion to the site of the injury. In addition, it favors superoxide and peroxynitrite formation and proliferation and migration of various cell types towards the luminal site of injury.[3] The cascade of events that follows can result in atherosclerotic plaques. Angiotensin II and some of its constituent peptides also stimulate the synthesis of the plasminogen activator inhibitor 1 (PAI1). Therefore, it is thought that activation of the renin-angiotensin system predisposes to atherosclerosis and thromboembolic events, including myocardial infarction (MI) and stroke.[4,5]

Of the four mainly prescribed antihypertensive drug classes (i.e. diuretics, β-blockers, calcium channel blockers, and ACE-inhibitors), only ACE-inhibitors and β-blockers have a direct effect on the renin-angiotensin system, i.e. ACE-inhibitors inhibit the conversion from angiotensin I into angiotensin II and β-blockers inhibit the β-adrenoceptor mediated release of renin from the kidneys. The objective of this study was to determine whether the risk of atherosclerosis varies between ACE-inhibitor or β-blocker users with different genotypes of genes that are involved in the renin-angiotensin system, i.e. ACE, AGT, and angiotensin receptor II type 1 (AGTR1) gene.

Methods

Setting and design
The Rotterdam Study is a prospective, population-based cohort study, which started in 1990 as a population-based prospective follow-up study. All 10,275 residents of the suburb Ommoord in Rotterdam, aged 55 years or older were invited to participate in an extensive home interview and two visits to the research center. In total, 7,983 (78%) subjects gave written informed consent and baseline measurements took place until 1993. Information was collected on age, gender, present health status, and medical history, including previous MI and stroke. All reported MIs or stroke at baseline were verified with medical records. During a physical examination, blood pressure, weight, and height were measured and blood was drawn for DNA extraction. In total, blood samples were available for genotyping of 86% of the cohort. The design of this population-based study has been described elsewhere.[6] A second (1993-1995) and third (1997-1999) cross-sectional assessment were conducted in a similar way. Only atherosclerosis measurements from the third cross-sectional assessment were

included, since a limited number of atherosclerosis measurements were performed in the second assessment and pharmacy records were only available as of January 1st, 1991 which is later than most of the baseline atherosclerosis assessments. Of 3,506 participants' information on at least one measure of atherosclerosis, assessed during the third cross-sectional assessment, was available. Only individuals with hypertension were included in this study. Hypertension was defined as systolic blood pressure ≥ 160 mmHg, and/or diastolic blood pressure ≥ 95 mmHg, and/or the use of a antihypertensive drug during follow-up.

Outcome definition

Atherosclerosis of the lower extremities

Systolic blood pressure at the ankles (posterior tibial artery) was measured in the supine position with a random-zero sphygmomanometer and an 8-MHz continuous wave Doppler probe (Huntleigh 500D, Huntleigh Technology). The ratio of the systolic blood pressure at the ankle to the systolic blood pressure at the arm was calculated to obtain the ankle-arm index (AAI). Peripheral arterial disease was considered present when the ankle-brachial blood pressure index was lower than 0.90 in at least one leg.[7] The sensitivity and the specificity of this cut-off are 90% and 98%, respectively, for an angiographically defined stenosis of 50% or more in a major leg artery.[8]

Aortic atherosclerosis

Aortic atherosclerosis was diagnosed by radiographic detection of calcified deposits in the abdominal aorta on a lateral abdominal film.[9] Calcified deposits were graduated on a graded scale (with scores of zero to five corresponding to 0, ≤1, 1 to 2.5, 2.5 to 4.9, 5.0 to 9.9 and ≥10 cm, respectively). Aortic atherosclerosis was considered present if the score was one or higher. In addition, aortic atherosclerosis was divided in degrees of severity, i.e. score of zero, one to two, and three or higher.

Carotid atherosclerosis

Ultrasonography of both carotid arteries was performed with a 7.5-MHz linear-array transducer and a duplex scanner (ATL Ultra-Mark IV, Advanced Technology Laboratories). The common carotid artery, carotid bifurcation, and internal carotid artery were examined on both the left and right sides for the presence of plaques as described before.[10] A weighted plaque score ranging from zero to six was computed by adding the number of sites at which a plaque was detected, divided by the number of sites for which an ultrasonographic image was available, and multiplied by six (the maximum number of sites). Carotid atherosclerosis was considered present if the plaque score was one or higher, respectively. Carotid atherosclerosis was also divided in degrees of severity, i.e. plaque score of zero, one to two, three, and four or higher.

Exposure definition

Pharmacy records were available for approximately 99% of the cohort as of January 1st, 1991. 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 (ATC) code of the drug.[11] The exposure of interest included ACE-inhibitors and β-blockers, because of their direct effect on the renin-angiotensin system. The use of these antihypertensive drug classes was divided into three categories, i.e. no use, short-term (0-4 years), and long-term (≥ 4 years). These categories were chosen, because of the expected lag-time between drug exposure and the effect on atherosclerosis.

On the date of the atherosclerosis measurement, the cumulative duration of use was calculated for all antihypertensive drug classes for each participant. Hereto, we first calculated each prescription length by dividing the number of dispensed tablets or capsules by the prescribed daily number. Each refill at the pharmacy which occurred within seven days after last intake from the previous prescription was considered as a continuous drug episode. For dose-effect associations, we used the defined daily dose (DDDs) which consists of the recommended daily dose for the indication hypertension in an adult.

Genotyping

Genomic DNA was extracted from whole blood samples using standard methods, described previously.[12] The I and D-allele of the ACE gene were identified on the basis of polymerase-chain-reaction (PCR) technique according to the method of Lindpainter et al.[13] with some modifications. Because the D-allele in heterozygous samples is preferentially amplified, there is a tendency of misclassification for about 4-5% of the ID to DD genotypes. For this reason, a second PCR was performed with a primer pair that recognises an insertion specific sequence (5' TGG GAC CAC AGC GCC CAC TAC 3' and 5' TCG CCA GCC CTC CCA TGC CCA TAA 3'). The reaction yielded a 335-bp amplicon only if the I-allele was present. Two independent investigators read pictures from each gel and all ambiguous samples were analysed a second time.

The AGT M235T and AGTR1 573C/T (exon 5) polymorphism were genotyped with TaqMan allelic discrimination Assays-By-Design (Applied Biosystems, Foster City, CA). Forward and reverse primer (anti sense strand) sequences were 5' AGG TTT GCC TTA CCT TGG AAG TG 3' and 5' GCT GTG ACA GGA TGG AAG ACT 3' and the minor groove binding probes were 5' CTG GCT CCC ATC AGG 3' (VIC) and 5' CTG GCT CCC GTC AGG 3' (FAM) for the AGT M235T polymorphism. Forward and reverse primer sequences were 5' TGT GCT TTC CAT TAT GAG TCC CAA A 3' and 5' CAG AAA AGG AAA CAG GAA ACC CAG TAT A 3' and the minor groove binding probes were 5' CTA TCG GGA GGG TTG 3' (VIC) and 5' CTA TCG GAA GGG TTG 3' (FAM) for the AGTR1 573C/T polymorphism. The assays utilized 5 nanograms of genomic DNA and 2 microliter reaction volumes. The amplification and extension protocol was as follows: an initial activation step of 10 min at 95 deg preceded 40 cycles of denaturation at 95 deg for 15 s and annealing and extension at 50 deg for 60 s. allele-specific fluorescence was then analyzed on an ABI Prism 7900HT Sequence Detection System with SDS v 2.1 (Applied Biosystems, Foster City, CA).

Potential confounders

As potential confounders we consider age, gender, diabetes mellitus, systolic blood pressure, diastolic blood pressure, body mass index (BMI), use of coumarins, angina pectoris, history of stroke, history of coronary heart disease, smoking, cholesterol level (total cholesterol/high density cholesterol), use of statins, follow-up time, cumulative use of other antihypertensive drugs, and DDDs. We adjusted for the combined use of other antihypertensive drugs classes by adding each antihypertensive drug class separately in the model for no use, short-term, and long-term treatment. The same duration of use categories were used for statin therapy. History of angina pectoris was defined as the use of two or more prescriptions of nitrate. History of coronary heart disease was defined as a history of MI, history of percutaneous transluminal coronary angioplasty, and history of coronary artery bypass grafting.

Statistical analysis

Binary logistic regression was used for the endpoints: presence of peripheral arterial disease, presence of aortic atherosclerosis, and presence of carotid atherosclerosis. Cumulative use of antihypertensive drugs was divided into three mutually exclusive groups, i.e. no, short-term (0-4 years), and long-term treatment ($\geq$ 4 years). In a sensitivity analysis also cut-off point of two and three years were used. Multinomial logistic regression was used for the degrees of severity analysis for the outcomes: aortic and carotid atherosclerosis. To study the effect modification by the ACE gene and the use of ACE-inhibitors (or β-blockers) subjects with the DD genotype and those who did not use ACE-inhibitors (or β-blockers) were used as the reference group. For the drug-gene interactions four dummy variables were added to the model, e.g. ACE genotype (ID or II) x ACE-inhibitor (short-term or long-term treatment).

Results

In total, there were 2,305 subjects with hypertension during follow-up. Of 2,216 subjects (96.1%) data on atherosclerosis and blood samples were available. Of these 2,216 subjects, 727 were treated with ACE-inhibitors, 1,267 were treated with β-blockers, and 1,556 were treated with antihypertensive drugs from other classes. A subject could contribute to one or more categories of antihypertensive drug classes during follow-up. Table 1 shows the characteristics of the subjects included in this study at the moment of the third cross-sectional assessment.

The ACE genotype could be assessed in 2,164 subjects. Of these, 26.3%, 52.2%, and 21.4% had the DD, ID, and II genotype, respectively. The AGT genotype could be assessed in 2,056 subjects, of whom 37% had the MM, 46.8% the MT, and 16.2% the TT genotype. With regard to the AGTR1 genotype, 2,032 could be genotyped, of whom 27.5% had the CC, 49% had the CT, and 23.5% had the TT genotype, respectively.

Table 1. Baseline characteristics of the study population

| Characteristics	n=2,216
Age, years	73.74 ± 23.26
Gender, male	915 (41.3%)
SBP, mmHg	153.08 ± 71.13
DBP, mmHg	82.41 ± 74.13
BMI, kg/m^2	73.74 ± 7.02
Total cholesterol/HDL, mmol/l	10.59 ± 23.26
Cardiovascular disease, yes	437 (19.7%)
Stroke, yes	128 (5.8%)
Smoking, current/past/never	329/1,110/758
ACE gene, DD/ID/II	570/1,130/464
AGT gene, MM/MT/TT	760/963/333
AGTR1 gene, CC/CT/TT	559/995/478
Use of ACE-inhibitors, 0/0-4/ ≥ 4 years	1,489/495/232
Use of β-blockers, 0/0-4/ ≥ 4 years	949/773/494
Use of other antihypertensive drugs	1,556 (70.2%)
Use of statins	489 (32.1%)
Use of coumarins	162 (7.3%)

ACE I/D polymorphism

In figure 1 the association between the use of β-blockers or ACE-inhibitors and ACE I/D polymorphism and the risk of peripheral arterial disease, aortic atherosclerosis, and carotid atherosclerosis is presented. Hypertensive subjects not treated with ACE-inhibitors with the ACE II genotype had a similar risk of peripheral arterial disease compared with untreated hypertensive subjects with the DD genotype (Odds ratio (OR)=0.89; 95%CI: 0.58-1.35) (see figure 1a). Individuals treated short-term (0-4 years) with ACE-inhibitors with the DD genotype the risk of peripheral arterial disease was similar compared with untreated subjects with the DD genotype (OR=0.95; 95%CI: 0.45-1.46). Individuals with the II genotype and treated short-term with ACE-inhibitors had an increased risk of peripheral arterial disease (OR=1.87; 95%CI: 1.01-3.46) compared with untreated subjects with the DD genotype. The risk of peripheral arterial disease associated with short-term (0-4 years) use of ACE-inhibitors compared to no use of ACE-inhibitors, was higher among subjects with the II genotype than among subjects with the DD genotype of the ACE gene (interaction (OR)=2.21; 95%CI: 1.01-4.85). In individuals with the DD genotype and those treated long-term (≥ 4 years) with ACE-inhibitors the risk of peripheral arterial disease was increased to 2.75 (95%CI: 1.30-5.81) compared with untreated subjects with the DD genotype. However, there was no significant drug-gene interaction with the ACE I/D polymorphisms in those treated long-term with ACE-inhibitors. In addition, in those treated with β-blockers there was no significant drug-gene interaction with this polymorphism.

Regarding the risk of aortic atherosclerosis, individuals treated short-term with β-blockers with the DD genotype the risk was reduced compared with untreated subjects with the DD genotype (OR=0.51; 95%CI: 0.29-0.91) (see figure 1b). In individuals with the II genotype and those treated short-term with ACE-inhibitors the

risk of aortic atherosclerosis was increased to 2.13 (95%CI: 1.01-4.50) compared with untreated subjects with the DD genotype. In those treated long-term the risk was even higher (OR=4.37; 95%CI: 1.11-17.31). No significant interaction was found between the use of β-blockers or ACE-inhibitors and the ACE I/D polymorphism on the risk of aortic atherosclerosis.

Figure 1. Associations between use of β-blockers or ACE-inhibitors and ACE I/D polymorphisms on the risk of peripheral arterial disease (1st histogram), aortic atherosclerosis (2nd histogram), and carotid atherosclerosis (3rd histogram) (adjusted for all potential confounders).
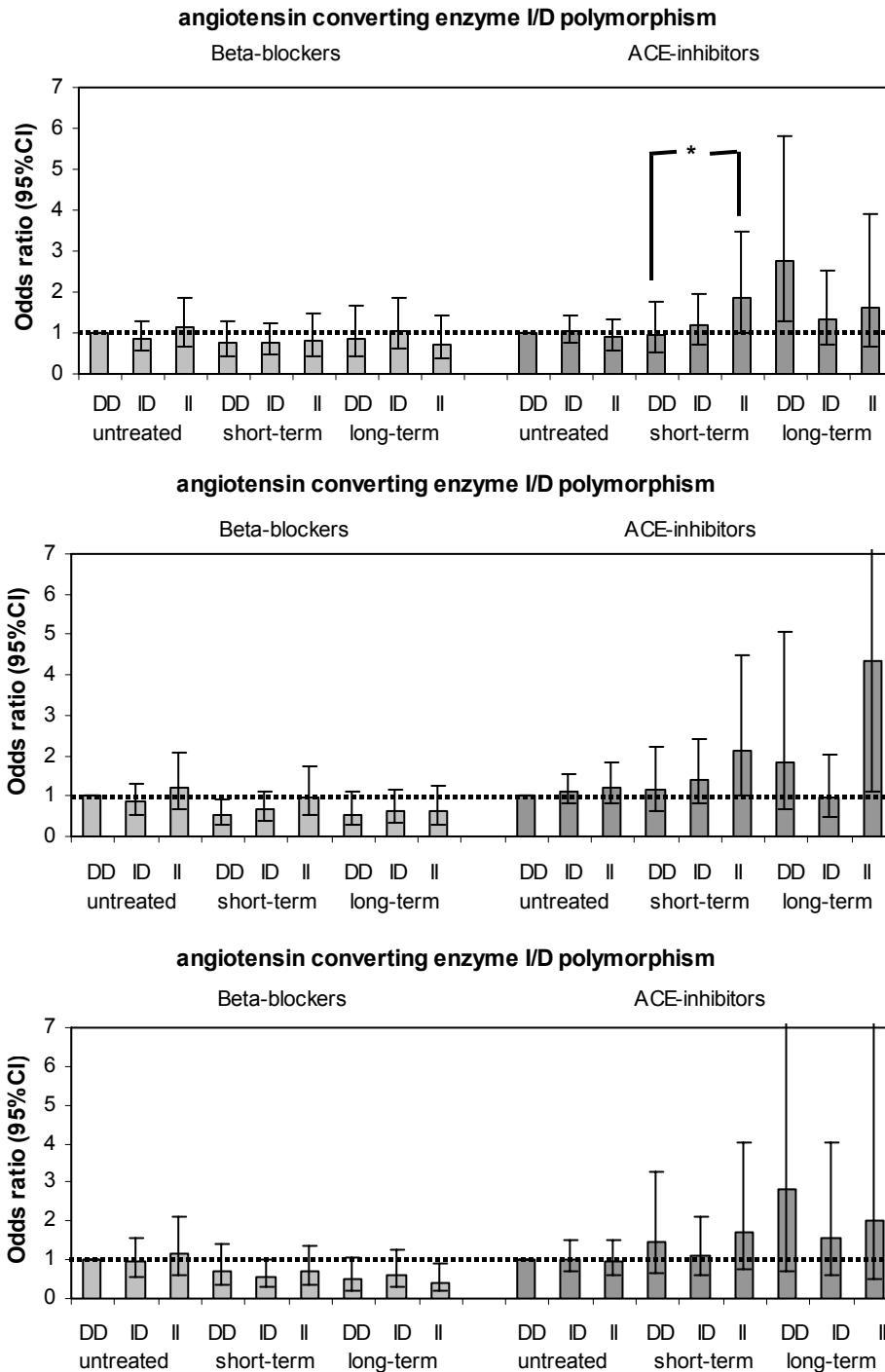
☆ Drug-gene interaction (p < 0.05)

Subjects with the ID genotype and treated short-term with β-blockers, had a lower risk of carotid atherosclerosis was lower compared to subjects with the DD genotype who were not treated with β-blockers (OR=0.55; 95%CI: 0.30-1.00) (see figure 1c) The risk of carotid atherosclerosis was reduced to 0.40 (95%CI: 0.18-0.89) in long-term β-blocker users with the II genotype compared to those untreated with the DD genotype. Again no significant drug-gene interaction was found in those treated with β-blockers or ACE-inhibitors with the ACE I/D polymorphism.

AGT M235T polymorphism

With regard to the AGT M235T polymorphism, the risk of peripheral arterial disease was increased (OR=1.76; 95%CI: 1.03-3.01) in individuals treated short-term with ACE-inhibitors with the MM genotype compared with untreated subjects with the MM genotype (see figure 2a). In addition, the risk was increased to 2.19 (95%CI: 1.11-4.29) for individuals treated long-term with ACE-inhibitors with the MT genotype and to 2.55 (95%CI: 1.09-5.98) for those with the TT genotype compared with untreated subjects with the MM genotype. However, there was no significant interaction between the use of ACE-inhibitors or β-blockers and the AGT M235T polymorphism on the risk of peripheral arterial disease.

Hypertensive subjects with the AGT TT genotype who were not treated with ACE-inhibitors had a non-significant lower risk of aortic atherosclerosis than untreated hypertensive subjects with the MM genotype (OR=0.67; 95%CI: 0.40-1.14). In individuals treated long-term with β-blockers with the MM genotype the risk of aortic atherosclerosis was non-significantly reduced to 0.57 (95%CI: 0.30-1.06) compared with untreated subjects with the MM genotype. In contrast, individuals treated long-term with β-blockers with the TT genotype the risk of aortic atherosclerosis was higher i.e. 1.28 (95%CI: 0.48-3.46) compared with untreated subjects with the MM genotype. The drug-gene interaction was significant (interaction OR=3.36; 95%CI: 1.14-9.97). For those treated short-term with β-blockers there was no significant drug-gene interaction with the AGT M235T polymorphism. The opposite effect was found in those treated with ACE-inhibitors. The risk on aortic atherosclerosis was i.e. reduced, instead of increased, in carriers of the 235T-allele, who were treated short-term with ACE-inhibitors, compared to no use of ACE-inhibitors (MT versus MM; interaction OR=0.42; 95%CI: 0.21-0.86 and TT versus MM; interaction OR=0.39; 95%CI: 0.15-0.99). Compared with the risk of aortic atherosclerosis in subjects with the MM genotype of the AGT polymorphism who were not treated with ACE-inhibitors, the risk was increased in subjects with the MT genotype who were untreated (OR=1.51; 95%CI: 1.10-2.08) and in subjects with the MM genotype who were treated short-term with ACE-inhibitors (OR=2.23; 95%CI: 1.17-4.23). None of the drug-gene interactions showed a trend towards an association when we classified aortic atherosclerosis in more categories of severity. In addition, none of the drug-gene interactions were significant when severe aortic atherosclerosis (score ≥ three)

was compared with no presence of aortic atherosclerosis (score=zero) (data not shown).

Figure 2. Associations between use of β-blockers or ACE-inhibitors and AGT M235T polymorphisms on the risk of peripheral arterial disease (1st histogram), aortic atherosclerosis (2nd histogram), and carotid atherosclerosis (3rd histogram) (adjusted for all potential confounders)
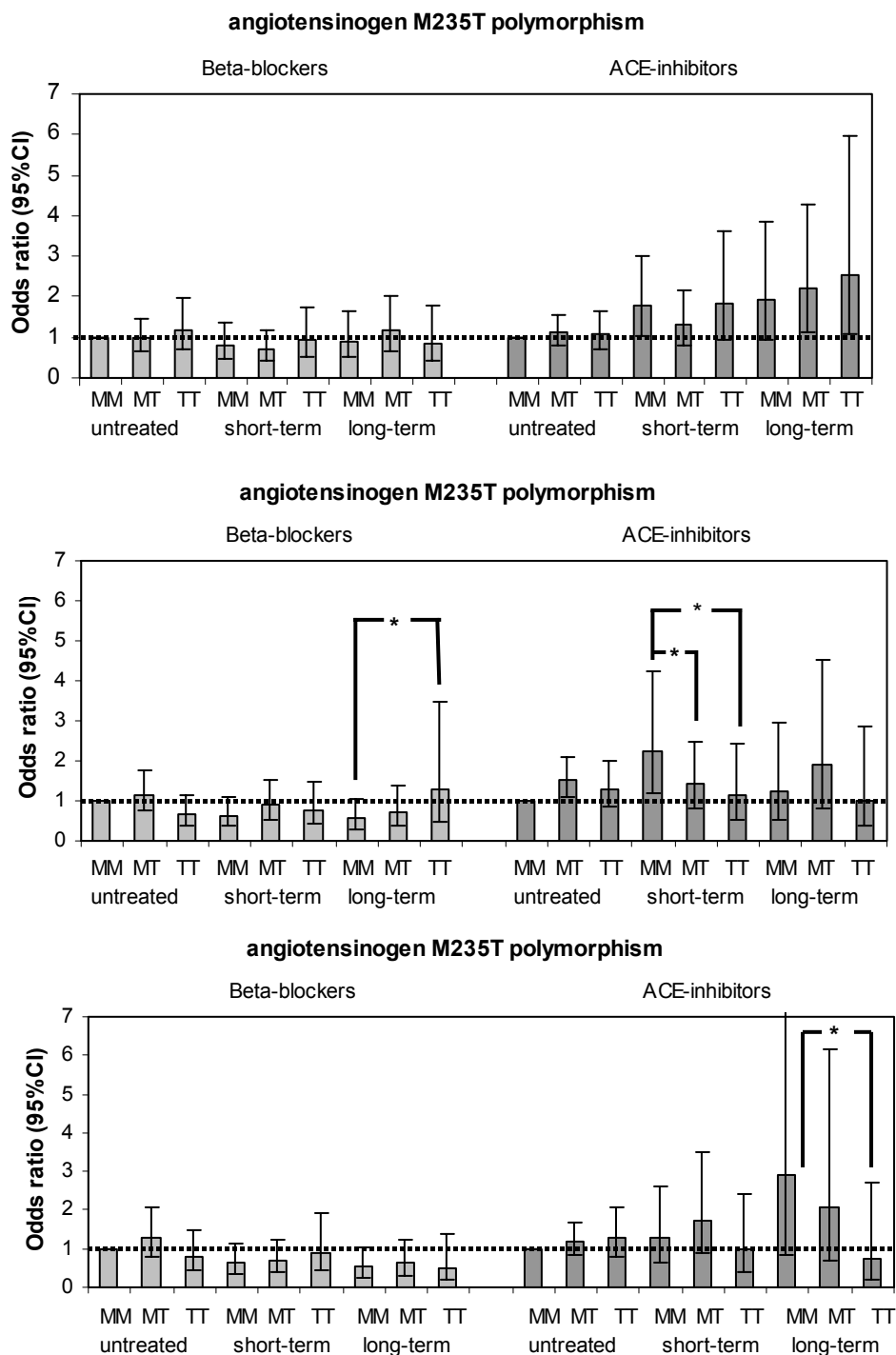
☆ Drug-gene interaction (p < 0.05)

Hypertensive subjects not treated with ACE-inhibitors with the angiotensinogen TT genotype had a similar risk of carotid atherosclerosis compared to subjects with the

MM genotype (OR=1.29; 95%CI: 0.78-2.09). In individuals treated long-term with ACE-inhibitors with the MM genotype, the risk of carotid atherosclerosis was non-significantly increased to 2.89 (95%CI: 0.84-9.09) compared with the reference group. In contrast, individuals treated long-term with the TT genotype had a non-significant lower risk (OR=0.75; 95%CI: 0.21-2.72) compared to the reference group. The interaction between long-term treatment with ACE-inhibitors and the angiotensinogen M235T polymorphism was significant (interaction OR=0.20; 95%CI: 0.04-0.95) (see figure 2c). There was, however, no trend towards a drug-gene interaction when carotid atherosclerosis was classified in more categories of severity. Also, none of the drug-gene interactions were significant when severe carotid atherosclerosis (score ≥ four) was compared with no presence of carotid atherosclerosis (score=zero) (data not shown).

AGTR1 573C/T (exon 5) polymorphism

With regard to AGTR1, no significant drug-gene interaction was found in those treated on the risk of peripheral arterial disease (see figure 3a). Although, there was an increased risk for individuals with the CC genotype who were treated long-term with ACE-inhibitors compared with untreated subjects with the CC genotype (OR=2.93; 95%CI: 1.39-6.17).

Also, no significant drug-gene interaction was found on the risk of aortic atherosclerosis (see figure 3b). Individuals with the CC genotype and treated short-term with β-blockers the risk of aortic atherosclerosis was significantly reduced to 0.51 (95%CI: 0.28-0.93) compared with untreated subjects with the CC genotype.

Long-term β-blockers users with the CC genotype had a reduced risk of carotid atherosclerosis compared to those untreated with the CC genotype (OR=0.32; 95%CI: 0.14-0.71), but no drug-gene interaction in β-blockers users was found. Subjects treated short-term with ACE-inhibitors had a higher risk of carotid atherosclerosis with the CT genotype compared to those treated short-term with the CC genotype (interaction OR=2.63; 95%CI: 1.11-6.24). There was no trend towards a interactive effect between ACE-inhibitors and the AGTR1 573C/T polymorphism when carotid atherosclerosis was classified in more categories of severity.

Discussion

Although some of the individual measurements of atherosclerosis showed a significant drug-gene interaction, there was no consistency between the different atherosclerosis measurements, the different antihypertensive drug classes, or different genotype classes. In addition, there was no trend towards a drug-gene interaction when we classified aortic and carotid atherosclerosis in more categories of degree of severity. It is therefore possible that the drug-gene interactions found were false-positive. In conclusion, the data suggested that there was no strong drug-gene interaction

between ACE I/D, AGT M235T, or AGTR1 573C/T polymorphism and the use of ACE-inhibitors or β-blockers on the risk of atherosclerosis found in daily practice.

Figure 3. Associations between use of β-blockers or ACE-inhibitors and AGTR1 573C/T polymorphisms on the risk of peripheral arterial disease (1st histogram), aortic atherosclerosis (2nd histogram), and carotid atherosclerosis (3rd histogram) (adjusted for all potential confounders).
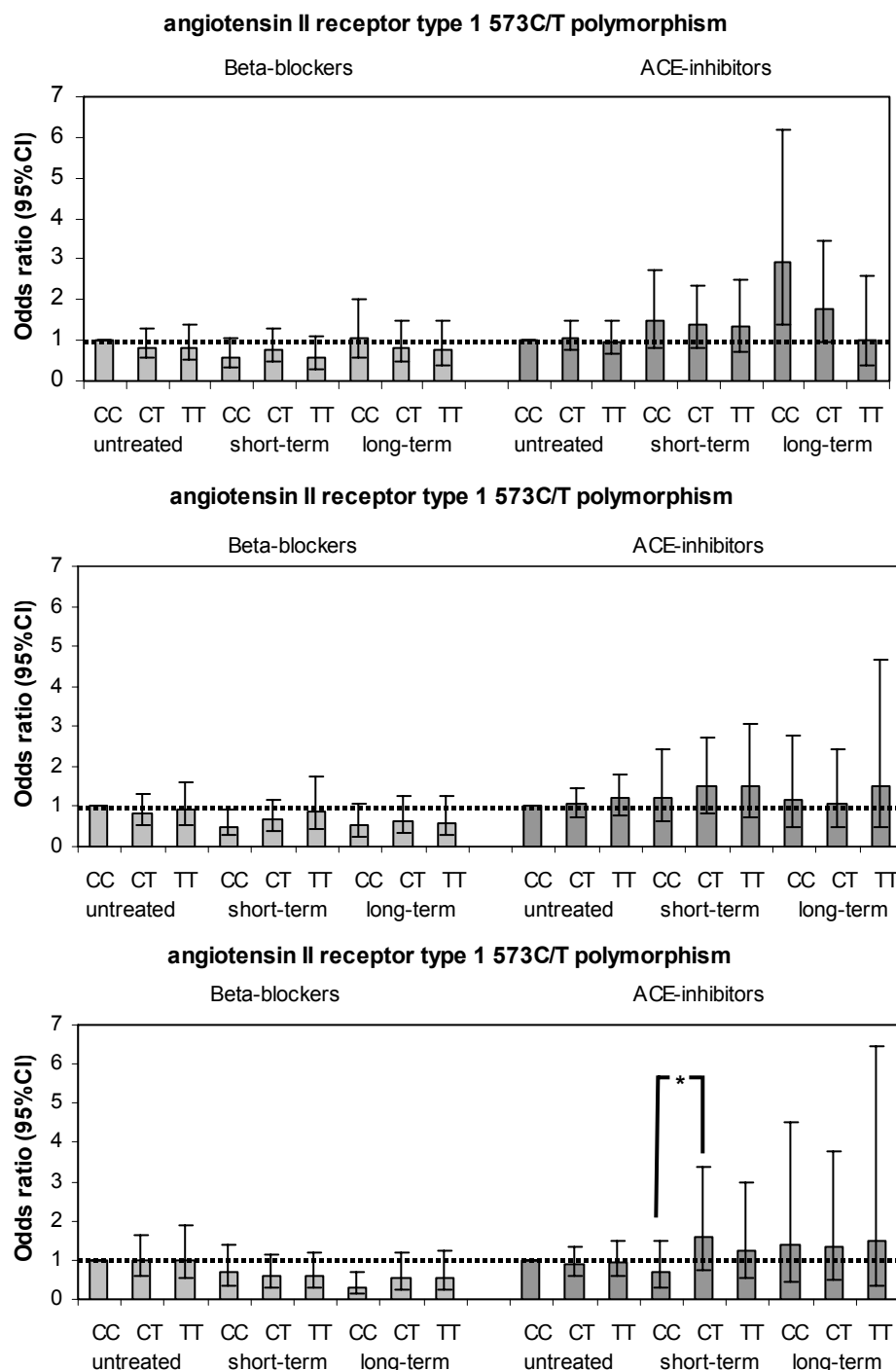
☆ Drug-gene interaction=p < 0.05

In this study, we have used three different sub-clinical measurements for atherosclerosis. All three measurements have been validated before. Carotid atherosclerosis as shown on ultrasound, aortic atherosclerosis on abdominal x-ray,

and lower-extremity atherosclerosis reflected by the AAI are validated measures of atherosclerosis and strongly associated e.g. with the presence of coronary calcification,[14] coronary heart disease,[15,16] and stroke.[17] Although, carotid plaques and ankle-arm index are predictors of stroke they are less strong predictors than aortic calcification.[18]

To our knowledge, there are no prior studies which investigated whether there is a drug-gene interaction between the three candidate gene polymorphisms and ACE-inhibitor therapy or β-blocker therapy with these sub-clinical measurements of atherosclerosis. Two of the three polymorphisms we have investigated have earlier been investigated on other outcomes. For example, the ACE II genotype is associated with lower tissue and plasma levels of ACE compared to the DD genotype.[19,20] In a large trial no drug-gene interaction was found on the risk of cardiovascular disease between the I/D polymorphism and ACE-inhibitor therapy.[21] With regard to the AGT M235T polymorphism, subjects with the TT genotype have in general 10-20% higher plasma AGT levels than individuals with the MM genotype.[22,23] However, Hopkins et al.[24] reported no difference in angiotensin II levels between the genotype groups. Bis et al.[25] reported that subjects carrying one copy of the T-allele who used ACE-inhibitors might have a reduced risk of (non-fatal) stroke compared to users of other antihypertensive drugs, but this interaction was not found on myocardial infarction. Only with regard to the AGTR1 573C/T polymorphism no information is available on plasma levels or on the risk of myocardial infarction or stroke. Benetos et al.[26] found a significantly greater reduction in carotid-femoral pulse wave velocity with the AGTR1 1166A/C (exon 5) polymorphism in 40 patients treated with ACE-inhibitors. The distance between the AGTR1 1166 A/C polymorphism and the 573C/T polymorphism is about 500 base pairs and is most likely in linkage equilibrium with the 1166A/C polymorphism.

A limitation of this study is that we only had a limited number of pre-treatment atherosclerosis measurements. Therefore, we were unable to measure the progression/reduction of atherosclerosis. In addition, subjects included in the Rotterdam Study were 55 years or older when the study started. Younger subject respond better to antihypertensive drug treatment and this might have resulted in greater differences in atherosclerosis levels. Therefore, the results can not be generalized to the general population. Another limitation is that it is unknown how long a patient should be treated with antihypertensive drugs before a change in peripheral, carotid, or aortic atherosclerosis can be achieved. In our analysis, we used four years as a cut-off value. The data was also analyzed with other cut-off values, i.e. two and three years. The results slightly changed, but there was no consistent drug-gene interaction with any of the three candidate gene polymorphisms. Due to the limited number of subjects treated for five years or more, we could not extend our drug treatment window. Another limitation is that only one single nucleotide polymorphism per gene was examined, which may not explain the full variation in plasma or tissue levels. Therefore, we are only able to exclude a candidate gene

polymorphism and not a complete gene as candidate for a drug-gene interaction.

Despite the caveats, the results did not indicate the presence of a strong drug-gene interaction between the use of ACE-inhibitors or β-blockers and the ACE I/D, AGT M235T, or AGTR1 573C/T polymorphism on the overall risk of atherosclerosis. However, these results need to be replicated before definitive conclusions can be made.

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Chapter 5.2

Interaction between the angiotensinogen M235T polymorphism and ACE-inhibitors or ß-blockers therapy and the risk of myocardial infarction and stroke

Abstract

Introduction: Angiotensinogen is an essential component of the renin-angiotensin system. ACE-inhibitors and β-blockers both have a direct influence on this system.

Aim: To investigate whether the association between use of ACE-inhibitors or β-blockers and the risk of stroke or myocardial infarction (MI) is modified by the T-allele of the angiotensinogen M235T polymorphism.

Methods: Data were used from the Rotterdam Study, a population-based prospective cohort study in the Netherlands, which started in 1990 and included 7,983 subjects of 55 years or older. In this study, 4,097 subjects with hypertension were included from July 1st, 1991 onwards. Follow-up ended at the diagnosis of MI or stroke, death, or the end of the study period (January 1st, 2002). The association between the drug-gene interaction and the risk of MI or stroke was determined with a Cox proportional hazard model with adjustment for each drug class as time-dependent covariates.

Results: The interaction between current use of ACE-inhibitors and the angiotensinogen M235T polymorphism was multiplicative on the risk of MI (interaction HR=4.00; 95%CI: 1.32-12.11). Similarly, there was a non-significantly increased risk of stroke (interaction HR: 1.83; 95%CI: 0.95-3.54) in subjects with the MT or TT genotype compared to the MM genotype. No interaction was found between current use of β-blockers and the AGT M235T polymorphism on the risk of MI (interaction HR: 1.30; 95%CI: 0.60-2.83) or stroke (interaction HR: 1.39; 95%CI: 0.81-2.39).

Conclusion: Subjects with at least one copy of the 235T-allele of the AGT gene might have less benefit from ACE-inhibitor therapy.

Introduction

Hypertension is a common disorder affecting approximately 20% of the adult populations of most developed countries[1], and is a major risk factor for cardiovascular disease. In clinical trials, antihypertensive therapy has been associated with a 35% to 40% risk reduction in stroke incidence and a 20% to 25% reduction in the risk of myocardial infarction (MI).[2] Although many effective antihypertensive drugs are available, it remains difficult to predict the effect of a particular antihypertensive agent in an individual patient. Genetic variation in genes may explain differences between individuals in their response to antihypertensive drugs.

One of the genes that might have an influence on the response is the angiotensinogen (AGT) gene. AGT is one of the components of the renin-angiotensin system, which has a central role in the regulation of blood pressure and fluid homeostasis. AGT is cleaved by renin to form angiotensin I, which is converted to the vasoactive angiotensin II by angiotensin converting enzyme (ACE). In 1992, Jeunemaitre et al.[3] reported linkage of the angiotensinogen locus to hypertension in hypertensive sibling pairs. Subsequent screening of the angiotensinogen gene for molecular variants led to the identification of a missense mutation, resulting in the substitution of a threonine (T) for a methionine (M) at codon 235. Further investigations showed that the AGT 235T-allele is in linkage equilibrium with a guanine (G) to adenosine (A) transition 6 base pairs upstream of the initiation site of transcription (-6G/A), which may result in a higher basal transcription rate of this gene.[4] In general, individuals with the TT genotype have plasma AGT levels, which are 10-20% higher than individuals with the MM genotype.[3,5] Two meta-analyses reported a significant association between the M235T polymorphism and hypertension with a combined risk of 1.2 for the 235T-allele in Caucasians.[6,7] However, such an association was not found for MI or stroke.[6,8]

Of the four mainly prescribed antihypertensive drug classes (diuretics, β-blockers, calcium channel blockers, and ACE-inhibitors), only ACE-inhibitors and β-blockers have a direct effect on the renin-angiotensin system. ACE-inhibitors inhibit the conversion from angiotensin I to angiotensin II and β-blockers inhibit the release of renin. Therefore, it is plausible that the response to these antihypertensive drug classes may be modified by the M235T polymorphism of the AGT gene. Bis et al.[9] reported that the 235T-allele was associated with a stronger reduction of the risk of non-fatal stroke in users of ACE-inhibitors than in users of other antihypertensive drugs, whereas there was no difference in the risk of non-fatal MI.

The objective of our study was to determine whether the risk of MI or stroke in hypertensive patients on ACE-inhibitors or β-blockers is modified by the AGT M235T polymorphism.

Methods

Setting

The Rotterdam Study started in 1990 as a population-based prospective follow-up study. All 10,275 residents of the suburb Ommoord in Rotterdam, aged 55 years or older were invited to participate. In total, 7,983 (78%) subjects gave written informed consent. The baseline measurements took place until 1993. The design of this population-based study has been described elsewhere.[10] Information was collected on age, gender, present health status and medical history, including previous MI and stroke. All reported MIs or strokes at baseline were verified with medical records. During a physical examination, blood pressure, weight, and height were measured and blood was drawn for DNA extraction.

Hypertension was defined as use of antihypertensive medication, and/or a systolic blood pressure ≥ 160 mmHg, and/or diastolic blood pressure ≥ 95 mmHg. Since the start of the Rotterdam Study, follow-up examinations have been carried out periodically.

Cohort and outcome definition

Only subjects with hypertension were included in this study. Therefore, follow-up started on the day that an elevated blood pressure was measured and/or the day that a first antihypertensive drug was prescribed, whichever came first. The beginning of the study period was set at July 1st, 1991 because pharmacy records were not available before January 1st, 1991 and this resulted in a drug history of at least six months. The end of the study was set at January 1st, 2002. Follow-up ended on the date of the first MI (or first stroke for the analysis with stroke as primary outcome), or a censoring event (end of study period, moving out of the area, or death), whichever was earlier. All collected events were verified by review of hospital discharge reports and letters from medical specialists, and classified as definitive and possible MI. Two research physicians independently coded events according to the International Classification of Diseases, 10th Revision (ICD-10).[11] MI was defined as ICD codes: I21. A medical expert in cardiovascular disease also reviewed all coded events for final classification.

Stroke was defined as ICD codes: K90. A neurologist reviewed all suspected cerebrovascular cases and classified them into definite, probable, and possible stroke and stroke subtypes.[12] For the analyses, definite and probable cases were included. Sub-classification into hemorrhagic and ischemic stroke was based on neuroimaging, which was available for 64% of all cases.

Exposure definition

Pharmacy records were available for approximately 99% of the cohort as of January 1st, 1991. 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.[13] The exposure of interest included ACE-inhibitors and β-blockers, because of their direct effect on the renin-angiotensin system.

When an MI or stroke occurred, the date was defined as the event date and the cumulative duration of use for current and past exposure of all antihypertensive drug classes on that date was calculated for each participant. Hereto, we first calculated each prescription length by dividing the number of dispensed tablets or capsules by the prescribed daily number. Each refill at the pharmacy which occurred within 7 days after last intake from the previous prescription was considered as a continuous drug episode. Current, past, and non-exposure were defined as mutually exclusive categories. When the event fell within a usage period, the patient was considered as currently exposed, and the cumulative number of days of current use was calculated. Similarly for those who were not current user, but had used a representative of the drug group in the past the number of days since last intake was calculated. Those who had not used the drug during the study period were considered as non-user. For dose-effect associations, we used the defined daily dosages (DDD) which consist of the recommended daily dose for the indication hypertension in an adult.

Genotyping

Genomic DNA was extracted from whole blood samples using standard methods, described previously.[14] Samples were genotyped with TaqMan allelic discrimination Assays-By-Design (Applied Biosystems, Foster City, CA). Forward and reverse primer (anti sense strand) sequences were 5' AGG TTT GCC TTA CCT TGG AAG TG 3' and 5' GCT GTG ACA GGA TGG AAG ACT 3' and the minor groove binding probes were 5' CTG GCT CCC ATC AGG 3' (VIC) and 5' CTG GCT CCC GTC AGG 3' (FAM). The assays utilized 5 nanograms of genomic DNA and 2 microliter reaction volumes. The amplification and extension protocol was as follows: an initial activation step of 10 min at 95 deg preceded 40 cycles of denaturation at 95 deg for 15 s and annealing and extension at 50 deg for 60 s. allele-specific fluorescence was then analyzed on an ABI Prism 7900HT Sequence Detection System with SDS v 2.1 (Applied Biosystems, Foster City, CA).

Potential confounders

For the analysis with MI as an outcome, we adjusted for age, gender, systolic/diastolic blood pressure, body mass index (BMI), current and past smoking, cholesterol level (total cholesterol/ high density cholesterol) at baseline). In addition, adjustments were made for statin use, coumarin use, ASA use, NSAID use, nitrate use, history of stroke, history of MI, history of percutaneous transluminal coronary angioplasty, history of coronary artery bypass grafting, use of anti-diabetic medication, history of angina, past and current use of other antihypertensive drugs, and the defined daily dose as potential confounders (during follow-up). We adjusted for the combined use

of other antihypertensive drug classes by adding each anti-hypertensive drug class separately in the model for past and current users. History of angina was defined as the use of two or more prescriptions of nitrate.

For the analysis with stroke as an endpoint, we considered the same potential confounders, but combined history of MI, history of percutaneous transluminal coronary angioplasty, and history of coronary artery bypass grafting in one variable (coronary heart disease).

Statistical analyses

The outcomes for MI and stroke were analysed separately because of their different aetiology. Both events were evaluated using a Cox proportional hazard model with time-varying exposure for each antihypertensive drug class separately. We created non-cumulative time-dependent categorical variables (yes/no) for current and past use of antihypertensive drugs and follow-up time was the time-axis of the model. Non-use of ACE-inhibitors (in the analysis of the association between ACE-inhibitors and risk of MI or stroke) and non-use of β-blockers (in the analysis of the association between β-blockers and risk of MI or stroke) served as a reference. The associations were expressed as hazard ratios (HR) with 95% confidence limits (CI). To investigate modification by the M235T polymorphism of the effect of ACE-inhibitors (or β-blockers) one dummy variable was added to the model: 235T-allele (0/1) x ACE-inhibitors (or β-blocker) (0/1).

Results

There were 4,097 subjects with hypertension during follow-up. Of these 4,097 persons, 1,642 persons were treated with ACE-inhibitors at any time, 2,387 with β-blockers, and 2,561 with other antihypertensive drugs. A subject may have contributed to one of more categories of antihypertensive drug classes during follow-up.

In our cohort, 35.7% of the subjects had the MM genotype and 64.3% the MT or TT genotype, respectively. Table 1 shows the baseline characteristics of the 4,097 subjects stratified by AGT genotypes.

MI

In total 197 subjects experienced an MI, of whom 30% had an MI before January 1st, 1990. Forty-two subjects had a MI while they were treated with ACE-inhibitors, of whom six had the MM genotype and 36 had the MT or TT genotype. In total, 17 subjects with the MM genotype and 58 subjects with at least one copy of the T-allele had an MI when they were treated with β-blockers (see table 2).

Table 1. Baseline characteristics stratified by AGT genotype.

Characteristics	MM n=1,461	MT or TT n=2,636
Gender, female	901 (61.7%)	1,575 (59.7%)
Age, years	70.6 ± 8.9	70.6 ± 8.9
Stroke at baseline, yes	50 (3.4%)	103 (3.9%)
MI at baseline, yes	226 (15.5%)	406 (15.4%)
Diabetes mellitus, yes	171 (12.5%)	314 (12.7%)
SBP, mmHg	142.8 ± 22.0	144.0 ± 22.6
DBP, mmHg	74.9 ± 11.9	75.3 ± 12.1
BMI, m/kg^2	27.0 ± 3.9	26.8 ± 3.7
Total cholesterol/high density cholesterol, mmol/l	5.3 ± 1.7	5.3 ± 1.6
Smoking		
current, yes	280 (19.7%)	531 (20.7%)
past, yes	609 (42.8%)	733 (42.3%)
Use of ACE-inhibitors	126 (8.6%)	231 (8.7%)
Use of β-blockers	319 (21.8%)	573 (21.7%)
Use of α-blocker	49 (3.4%)	95 (3.6%)
Use of low-ceiling diuretic	267 (18.3%)	440 (16.7%)
Use of high-ceiling diuretic	89 (6.1%)	192 (7.3%)
Use of calcium channel blocker	144 (9.8 %)	236 (9.0%)
Use of statins	45 (3.1%)	69 (2.6%)
Use of coumarins	65 (4.4%)	136 (5.2%)
Use of NSAID	126 (8.6%)	243 (9.2%)
Use of ASA/ salicylate	213 (14.6%)	374 (14.2%)

* Significant difference between MM or MT/TT genotype (p < 0.05)

Table 2. Association of ACE-inhibitor and β-blocker use and AGT M235T polymorphism with MI risk.

AGT M235T genotype	Type of use	MI (N)	HR (95% CI)[1]	HR (95% CI)[2]
ACE-inhibitors				
MM	No	44	1 (reference)	1 (reference)
MM	Current	6	1.06 (0.39-2.85)	0.71 (0.23-2.21)
MT/TT	No	74	0.95 (0.66-1.39)	0.99 (0.67-1.45)
MT/TT	Current	36	3.25 (1.70-6.19)	2.73 (1.42-5.23)
β-blockers				
MM	No	20	1 (reference)	1 (reference)
MM	Current	17	1.69 (0.79-3.61)	1.29 (0.59-2.86)
MT/TT	No	49	1.36 (0.81-2.30)	1.44 (0.84-2.48)
MT/TT	Current	58	3.16 (1.66-6.01)	2.42 (1.23-4.76)

[1] Adjusted for age, gender, other antihypertensive drugs, past exposure to antihypertensive drugs, and DDDs
[2] Adjusted for age, gender, other antihypertensive drugs, past exposure to antihypertensive drugs, DDDs, BMI, cholesterol level, statin use, and history of PTCA, CABG, and MI

Table 3. Gene-drug interaction between ACE-inhibitor and β-blocker use and AGT M235T polymorphism on the risk of MI.

AGT M235T genotype	Type of use	Interaction HR (MT/TT versus MM) (95% CI)[1]	Interaction HR (MT/TT versus MM) (95% CI)[2]
ACE-inhibitors MT/TT versus MM	Current	3.28 (1.28-8.45)	4.00 (1.32-12.11)
β-blockers MT/TT versus MM	Current	1.37 (0.64-2.91)	1.30 (0.60-2.83)

[1] Adjusted for age, gender, other antihypertensive drugs, past exposure to antihypertensive drugs, and DDDs
[2] Adjusted for age, gender, other antihypertensive drugs, past exposure to antihypertensive drugs, DDDs, BMI, cholesterol level, statin use, and history of PTCA, CABG, and MI

In order to investigate the possible gene-drug interaction between ACE-inhibitors or β-blockers users and the AGT M235T polymorphism on the risk of MI, cases were grouped by current use and genotype group (see table 2 and 3). Subjects with the MM genotype who were currently treated with ACE-inhibitors had a non-significantly reduced risk of MI compared to subjects with the MM genotype who never used ACE-inhibitors (HR=0.71; 95%CI: 0.23-2.21). In contrast, subjects with the MT or TT genotype who were currently treated with ACE-inhibitors had a significantly increased risk of MI compared to subjects with the MM genotype who never used ACE-inhibitors (HR=2.73; 95%CI: 1.42-5.23). Among subjects who never used ACE-inhibitors, the MT or TT genotype was not associated with the risk of MI (HR=0.99; 95%CI: 0.67-1.45). The estimate for the risk of MI in subjects with at least one copy of the T-allele (HR=2.73) who were currently treated with ACE-inhibitors was higher than expected from the joint effect of the MT or TT genotype and ACE-inhibitors on a multiplicative scale (0.99x0.71=0.70). This interaction between current use of ACE-inhibitors and the AGT M235T polymorphism was statistically significant (HR=4.00; 95%CI: 1.32-12.11]). There did not seem to be a doses-response effect (MT versus MM genotype HR=3.89; 95%CI: 1.27-11.90 and TT versus MM genotype HR=3.75; 95%CI: 1.04-13.58).

Beta-blocker users who had the MM genotype had a non-significantly increased risk of MI compared to subjects with the MM genotype who never used β-blockers (HR=1.29; 95%CI: 0.59-2.86). Compared to subjects with the MM genotype who had never used β-blockers, β-blocker users with the MT or TT genotype had a significantly increased risk of MI (HR=2.42; 95%CI: 1.23-4.76). The interaction between current use of β-blockers and the AGT M235T polymorphism was non-significant significant (HR=1.30; 95%CI: 0.60-2.83). Additional analyses in which adjustments were made for systolic blood pressure level, diastolic blood pressure level, history of angina, use of ASA, use of coumarins, use of NSAID's, use of anti-diabetic medication, history of stroke, and smoking, yielded similar results and were therefore not shown. After exclusion of all subjects with a history of MI the (adjusted) interaction

between AGT M235T polymorphism and current use of ACE-inhibitors was similar but no longer significant (HR=4.62; 95%CI: 1.00-21.36). In addition, no drug-gene interaction was found between the AGT M235T polymorphism and use of β-blockers after this exclusion (HR=1.19; 95%CI: 0.47-3.02).

When the analysis was repeated with other antihypertensive drug classes (i.e. low-ceiling diuretics or calcium channel blockers) there was no significant drug-gene interaction with any of these antihypertensive drug classes.

Stroke

In total, 349 subjects experienced a stroke during follow-up, of whom 14% had a stroke before baseline. Of the 365 events, 189 (85%) were classified as ischemic and 33 (15%) as hemorrhagic. Sixty-three subjects had a stroke when they were treated with an ACE-inhibitor, of whom 15 had the MM genotype and 48 had at least one copy of the T-allele. During treatment with a β-blocker, 32 subjects with the MM genotype and 71 subjects with had at least one copy of the T-allele had a stroke.

To investigate the possible interaction between ACE-inhibitors or β-blockers users and the AGT M235T polymorphism on the risk of stroke, participants were grouped by current use and genotype group (see table 4 and 5). The drug-gene interaction between current use of ACE-inhibitors and the AGT M235T polymorphism on the risk of stroke was increased but this increase was not statistically significant (HR=1.83; 95%CI: 0.95-3.54). The interaction between current use of β-blockers and the AGT M235T polymorphism on the risk of stroke was non-significant (HR=1.39; 95%CI: 0.81-2.39). Additional analyses in which we adjusted for diastolic blood pressure level, serum cholesterol level, BMI, use of coumarins, use of NSAID's, and smoking, yielded similar results and were therefore not shown.

Table 4. Association of ACE-inhibitor and β-blocker use and AGT M235T polymorphism with stroke risk.

AGT M235T genotype	Type of use	MI (N)	HR (95% CI)[1]	HR (95% CI)[2]
ACE-inhibitors				
MM	No	95	1 (reference)	1 (reference)
MM	Current	15	0.64 (0.33-1.23)	0.58 (0.29-1.14)
MT/TT	No	141	0.84 (0.65-1.09)	0.84 (0.64-1.10)
MT/TT	Current	48	1.04 (0.63-1.71)	0.89 (0.53-1.48)
β-blockers				
MM	No	66	1 (reference)	1 (reference)
MM	Current	32	0.68 (0.40-1.14)	0.73 (0.43-1.23)
MT/TT	No	104	0.85 (0.63-1.16)	0.85 (0.62-1.21)
MT/TT	Current	71	0.82 (0.51-1.30)	0.86 (0.54-1.39)

[1] Adjusted for age, gender, other antihypertensive drugs, past exposure to antihypertensive drugs, and DDDs
[2] Adjusted for age, gender, other antihypertensive drugs, past exposure to antihypertensive drugs, DDDs, BMI, cholesterol level, statin use, and history of PTCA, CABG, and MI

Table 5. Gene-drug interaction between ACE-inhibitor and β-blocker use and AGT M235T polymorphism on the risk of stroke.

AGT M235T genotype	Type of use	Interaction HR (MT/TT versus MM) (95% CI)[1]	Interaction HR (MT/TT versus MM) (95% CI)[2]
ACE-inhibitors MT/TT versus MM	Current	1.93 (1.02-3.65)	1.83 (0.95-3.54)
β-blockers MT/TT versus MM	Current	1.41 (0.84-2.38)	1.39 (0.81-2.39)

[1] Adjusted for age, gender, other antihypertensive drugs, past exposure to antihypertensive drugs, and DDDs
[2] Adjusted for age, gender, other antihypertensive drugs, past exposure to antihypertensive drugs, DDDs, systolic blood pressure level, diabetes mellitus, use of statins, use of ASA, and history of coronary heart disease, stroke, and angina

After exclusion of all subjects with a history of stroke the (adjusted) interaction between AGT M235T polymorphism and current use of ACE-inhibitors was significant (HR=2.14; 95%CI: 1.06-4.33). No drug-gene interaction was found between β-blocker use and the AGT M235T polymorphism after this exclusion (HR=1.59; 95%CI: 0.90-2.78). When we included only ischemic strokes, the interaction was significant for ACE-inhibitors (HR=3.52; 95%CI: 1.27-9.80), but not for β-blockers (HR=1.30; 95%CI: 0.47-3.02). There did not seem to be a doses-response effect in ACE-inhibitor users (MM versus MT genotype HR=1.88; 95%CI: 0.96-3.69 and MM versus TT genotype HR=1.93; 95%CI: 0.80-4.66).

When the analysis was repeated with other antihypertensive drug classes (i.e. low-ceiling diuretics or calcium channel blockers) or all antihypertensive drug classes combined there was no significant drug-gene interaction with current use.

Discussion

In this study, a synergistic interaction between the AGT M235T polymorphism and current use of ACE-inhibitors on the risk of MI was found. Their joint effect was supramultiplicative and approximately four times larger than expected based on the product of their individual effects. On the risk of stroke no significant interaction was found, although the direction of the synergistic effect was similar to that on the risk of MI. No interaction was found between the use of β-blockers and the M235T polymorphism.

Bis et al.[9] reported that users of ACE-inhibitors carrying one copy of the T-allele might have a reduced risk of non-fatal stroke compared to users of other antihypertensive drugs, but there was non-significantly increased risk for non-fatal MI. In our study we found the opposite result, i.e. the MT and TT genotype were associated with an increased risk of MI in ACE-inhibitor users. In addition, the risk of

stroke in β-blockers was higher in subjects carrying a copy of the 235T-allele, albeit not significant. In a non-randomized trial, ACE-inhibitor users with the MT or TT genotype had a greater BP reduction than those with the MM genotype.[15] However, this could not be replicated in a smaller study.[16] With regard to β-blockers, no drug-gene interaction on BP was found.[16,17] The contribution of these findings on blood pressure to cardiovascular risk remains uncertain. For example, no studies have been published investigating whether there is an interaction between M235T polymorphism and antihypertensive drugs on atherosclerosis, which is an important risk factor for MI and stroke besides blood pressure. In addition it is difficult to predict with accuracy, which allele would be associated with an increased risk of stroke or MI during treatment with an ACE-inhibitor or β-blocker. Plasma AGT levels are 10-20% higher in TT homozygotes than in MM homozygotes[3,5], but this did not result in higher angiotensin II levels due to compensation in renin levels[18,19]. There are some differences between our study and Bis et al.[9] which might explain the differences in results. For instance, we included fatal and non-fatal cases of MI and stroke, adjusted for past use, and compared the use of ACE-inhibitor or β-blockers versus non-users instead of users of other antihypertensive drugs.

The main limitation of our study is the relatively small number of events. Although, our analyses consistently showed that current use of ACE-inhibitors among subjects with the T-allele was associated with an increased risk of MI and stroke, the results should still be interpreted with caution. Therefore, these results need to be replicated in other studies before definitive conclusion can be made. In addition, due to limited sample size no subgroup analysis could be made and MT and TT genotype were combined. Also, the risk of MI or stroke was compared in antihypertensive drug users versus non-users and therefore confounding by indication could have biased our results. As a physician was free to choose whether a patient received antihypertensive drug treatment or not and the type of antihypertensive drug, specific patients characteristics my have influenced this decision. However, the interaction between β-blockers or ACE-inhibitors and the AGT M235T polymorphism is probably not influenced by this bias, as the users of the same antihypertensive drug class will have most likely the same characteristics and the prescriber is unaware of a subject's genotype. Furthermore, only one SNP in the AGT gene was investigated. Although the M235T polymorphism is linked with plasma AGT levels and hypertension, it remains controversial in relation to MI and stroke. Since more genes are involved in the renin-angiotensin system and these might have compensated the increased risk of ACE-inhibitors in subjects with the T-allele e.g. by lowering the production of renin. One explanation of our findings might be that the feedback mechanism that normally compensate a rise in the angiotensinogen (e.g. a decrease in renin) are dysfunctional in T-allele carriers. This might be of particular importance during treatment with ACE-inhibitors, since such a treatment is known to be accompanied by a compensatory rise in renin. Future studies, addressing plasma renin levels during ACE-inhibitor treatment in T-allele carriers and controls, should evaluate this possibility.

In conclusion, our results indicate that the T-allele might be associated with an increased risk of MI and stroke in users of ACE-inhibitors.

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Chapter 6

General discussion

Introduction

Despite advances in drug therapy during the past decades, few drugs are effective in all patients, whereas all drugs potentially have adverse effects. Important factors in interpreting the variability in outcomes of drug therapy include environmental factors, a patient's health profile, disease severity, compliance, and the genetic profile of a patient.[1, 2] Clinical observations of genetic differences in drug effects were first documented in the 1950s when researchers described the first genetic polymorphisms that influenced the disposition of succinylcholine, isoniazid, and antimalaria drugs such as primaquine.[3-6] These observations gave rise to the field of pharmacogenetics. Initially pharmacogenetics focused largely on genetic polymorphisms in drug-metabolizing enzymes that influenced drug pharmacokinetics. By the mid 1980s, about 100 of these polymorphisms were identified.[7] Polymorphisms in drug metabolizing enzymes can lead to a variety of outcomes, such as therapeutic failure, adverse effects, and toxicity in selected sub-populations undergoing treatment. For example, CYP2D6 poor metabolizers have a severe impairment of the capacity to eliminate > 30 widely used drugs (e.g. β-blockers and anti-depressants). In contrast, CYP2D6 ultrarapid metabolizers, may fail to respond to drugs which are inactivated by CYP2D6 or may experience exaggerated response and toxicity from exposure to drugs activated by CYP2D6.[7] In the last decade, the scope of pharmacogenetics expanded to transporters and targets that influence the pharmacodynamic response to drugs. For example, cell surface receptors (e.g. ion channels and ion channel transporters) are of interest because of their role in initiation and transmission of cellular responses to hormones, autocoids, neurotransmitters, and environmental chemicals such as drugs.

Patients who are treated with drugs that show large inter-individual differences in response will most likely benefit from pharmacogenetic research. Such an example is the pharmacological treatment of hypertension. For instance, only 50% of the patients have an adequate blood pressure lowering response when treated with a single antihypertensive drug (monotherapy).[8-11] Currently, the selection of the most appropriate drug treatment for an individual patient with hypertension is considered as a "trial and error" approach. Although, there are substantial differences in pharmacokinetic properties the magnitude of the blood pressure lowering effect is similar for most drugs within a class.[10, 11] Therefore, it is unlikely that pharmacokinetic effects are responsible for most of the antihypertensive drug-gene interactions. This may be explained by the withdrawal of antihypertensive drugs from the market that were associated with large interpatient differences in therapeutic or toxic response in the past or the diminished use.[11, 12]

The goal of pharmacogenetics is to identify subjects who are more likely to have an unfavourable response to treatment with a particular drug or drug class prior to drug treatment.[13] Before this goal can be reached the causal genes need to be identified. There are two general approaches to identify these genes i.e. genome wide screens and candidate gene studies. A genome wide screen tests for linkage with anonymous

polymorphic markers, whereas candidate gene studies test for association of specific polymorphic markers within or near the functional gene. This gene is selected based on a priori hypotheses about their aetiological role in drug response. Genome wide screen or candidate gene studies can both be performed as part of a clinical trial and in an observational setting. In chapter 3.1, all studies that investigated interacting effects between antihypertensive drugs and genetic polymorphisms were reviewed. In this thesis, we investigated whether five of these drug-gene interactions modified the effect of antihypertensive drugs on blood pressure, atherosclerosis, and the risk of myocardial infarction (MI) and stroke in daily practise. The five candidate gene polymorphisms were: the angiotensin converting enzyme (ACE) insertion/deletion (I/D), angiotensinogen (AGT) M235T, angiotensin II receptor type 1 (AGTR1) 1166A/C or 573C/T, α-adducin (ADD1) G460W, and β3-subunit of the G-protein (GNB3) 825C/T polymorphism. Since the shortcomings and merits of the individual studies have been discussed in the previous chapters, are the main findings in this chapter placed in a broader perspective. In addition, the clinical implication of these studies will be discussed and recommendations for future research will be given.

Main findings

Despite the awareness of the elevated cardiovascular risk associated with hypertension and the availability of generally effective and well-tolerated antihypertensive drugs, did only a minority of treated hypertensive subjects achieve an adequate blood pressure reduction.[14] In the Netherlands about 60% of those treated (20-59 years) did not have their blood pressure controlled (< 140/90 mmHg) (chapter 2.1). Apart from non-compliance, one of the factors that could have caused the poor response is of genetic origin.

One of the candidate gene polymorphisms that might play a role in the poor response to antihypertensive drugs is the ACE I/D polymorphism. In literature, both the I-allele and the D-allele have been associated with a poor response to ACE-inhibitors (reviewed in chapter 3.1). In the Rotterdam Study and Doetinchem Cohort Study, this polymorphism did not seem to influence the response in ACE-inhibitor users. For example, there was no significant difference in the adherence to ACE-inhibitor therapy between subjects with the II, ID, or DD genotype (chapter 4.1). Neither was an interaction found between the use of ACE-inhibitors, low-ceiling diuretics, β-blockers, or calcium channel blockers and the ACE I/D polymorphism on blood pressure (chapter 4.2 + 4.4) or on atherosclerosis (chapter 5.1). These results corresponded with the largest trial performed.[15] Arnett et al.[15] found no significant difference between the ACE I/D genotype groups and the response to ACE-inhibitors, diuretics, or calcium channel blockers on blood pressure nor on the risk of cardiac heart disease or stroke.

The second polymorphism which was investigated was the AGT M235T polymorphism. Also this polymorphism had no drug-gene interactive effect on blood pressure (chapter 4.3 + 4.4) or on atherosclerosis (chapter 5.1). In a case-control study, Bis et al.[16] found that carriers of the 235M-allele who used ACE-inhibitors had a significantly higher risk of non-fatal stroke but not of non-fatal MI than among ACE-inhibitor users with the TT genotype. Remarkably in our study, we found the opposite. ACE-inhibitor users with at least one copy of the 235T-allele had a higher risk of MI than ACE-inhibitor users with the MM genotype (interaction HR=4.00; 95%CI: 1.32-12.11) (chapter 5.2). In addition, a non-significant increased risk of stroke was found (interaction HR=1.83; 95%CI: 0.95-3.54). Currently it is still difficult to predict with accuracy which allele is the risk allele. It is known that form literature that subjects with the TT genotype have 10% to 20% higher plasma AGT levels (circulating renin-angiotensin system), but the effect on tissue AGT levels is still unknown. The tissue renin-angiotensin system operates in two ways. The first pathway is similar to the circulating i.e. tissue renin generates angiotensin I, subsequently catalyzed by ACE into angiotensin II. The second pathway consists of two alternative enzyme pathways i.e. chymase catalyzes the conversion of angiotensin I to angiotensin II and cathepsin G and the chymostatin-sensitive angiotensin II pathway directly cleave angiotensinogen into angiotensin II.[18] The relative importance of alternative pathways is still uncertain.[19] Since the circulating renin-angiotensin system may be responsible for short-term regulation and the tissue levels serves a role in long-term changes[17] it is impossible to predict with accuracy from plasma levels alone which allele is the risk allele. Since we were unable to detect a drug-gene interaction on atherosclerosis and blood pressure, our results are somewhat contradictory with regard to the long-term outcomes. On the other hand antihypertensive drugs have multiple effects, which may explain why we did find an interaction on the risk of MI and not on blood pressure and atherosclerosis. Furthermore, our studies on blood pressure and atherosclerosis were analyzed cross-sectionally, whereas the risk of MI and stroke were analyzed in a follow-up design.

The third polymorphism which was examined in this thesis was the AGTR1 1166A/C polymorphism. Literature about a possible drug-gene interactive effect on blood pressure is unclear due to small sample sizes and conflicting results.[20, 21] In our study, no drug-gene interactive effect was found with this polymorphism on blood pressure levels (chapter 4.4). In addition, no association was found with the 573C/T polymorphism on atherosclerosis (chapter 5.1). The distance between the 1166A/C polymorphism and the 573C/T polymorphism is approximately 500 base pairs and is probably in linkage equilibrium with the 1166A/C polymorphism.

The fourth polymorphism which was investigated was the ADD1 G460W polymorphism. In our studies, no drug-gene interactive effect was found on blood pressure (chapter 4.3 + 4.4). Trials which investigated this drug-gene interaction on blood pressure were inconclusive due to conflicting results and small sample sizes.[23-26]

Psaty et al.[22] reported that in carriers of at least one copy of the 460W-allele diuretic therapy was associated with a lower risk of combined non-fatal MI or non-fatal stroke than with other antihypertensive therapies. The authors argued that blood pressure did not appear to be affected by the α-adducin gene-diuretic interaction.

The last polymorphism which was examined was the β3-subunit of the G-protein (GNB3) 825C/T polymorphism. Only one trial investigated the role of the 825C/T polymorphism.[27] In this study, subjects with the TT genotype had a greater systolic and diastolic blood pressure reduction when treated with a thiazide diuretic. When the same population was investigated in a nested case-control design (non-responder versus responder) this polymorphism did not significantly influence the chance to become a responder (based on diastolic blood pressure) when treated with hydrochlorothiazide.[28] In our study a drug-gene interactive effect on blood pressure was found (chapter 4.4). Users of low-ceiling diuretics with one copy of the TT genotype had a lower systolic blood pressure than subjects with the CC genotype. Remarkably in the study of Turner et al.[26] and in our study (chapter 4.4), no interaction was found between GNB3 825C/T polymorphism and other candidate polymorphism in diuretic users (drug-gene-gene-interactions).

With regard to drug-gene-gene interactions, none of the possible combinations with three of the candidate gene polymorphisms (ACE, AGT and ADD1) were significant in the Rotterdam Study (chapter 4.2). In the Doetinchem Cohort Study four drug-gene-gene interactions were significant when five of candidate gene polymorphisms (ACE, AGT, AGTR1, GNB3, and ADD1) were combined. There was an interaction between diuretic use and ADD1 W-allele+ AGT T-allele vs. GG+ MM on DBP (3.09 mmHg; 95%CI:0.16-2.93), β-blocker use and AGTR1 C-allele+ AGT T-allele vs. AA+ MM on DBP (2.63 mmHg; 95%CI: 0.30-2.33), ACE-inhibitor use and ACE I-allele+ GNB3 TT vs. DD+ C-allele on DBP (6.22 mmHg; 95%CI: 0.93-5.29), and ACE-inhibitor use and ACE I-allele+ AGT T-allele vs. DD+ MM on SBP (-10.21 mmHg; 95%CI: -19.47--0.95).

Table 1 overview of the results found in this thesis.

Outcome	ACE I/D	AGT M235T	AGTR1 1166A/C or 573C/T	ADD1 G460W	GNB3 825C/T
Blood pressure	-	-	-	-	?[2]
Atherosclerosis	-	-	-		
MI / stroke		?[1]			

ACE I/D=angiotensin converting enzyme insertion/deletion polymorphism; ADD1 =α-adducin; AGT=angiotensinogen; AGTR1=angiotensin II receptor type 1; GNB3=β3-subunit of the G-protein
[1] interaction only found in ACE-inhibitor users on the risk of stroke
[2] interaction only found in diuretic users on systolic blood pressure

Pharmacogenetic study designs

As indicated in the introduction, two different settings can be used to evaluate the effect of polymorphisms/genes on the response to antihypertensive drugs i.e. trials and observational studies. Randomised controlled trials are considered the best method for providing evidence on efficacy. However, due to their highly selected populations and outcomes and expensive nature they have been criticised.[29] Observational studies can be thought of as natural experiments in which outcomes are measured in the "real world" rather than in experimental settings. In these studies it is possible to evaluate large groups of diverse individuals, which can be followed for long periods and provide evidence on a wide range of outcomes. An important advantage of randomised controlled trials is that the random allocation to an intervention enhances the internal validity of a study by minimising confounding.[30] This allocation should results in groups that are comparable in baseline prognosis.[31] In an observational study factors that determined whether a person received a specific drug or not could result in difference between groups in prognostic factors related to the outcome. For example, a physician might think that antihypertensive drugs from one class have fewer side effects than antihypertensive drug from another class, and might therefore prescribe this class to frailer patients. This form of selection bias is referred to as channelling bias or confounding by severity.[32] In observational studies on drug-gene interactive effects, confounding by severity is less likely unless the doctor is aware of the genetic status. Especially in starters of new drugs, however, the doctor is mostly unaware of that which leads to some sort of 'randomization'. For instance, in the Netherlands persons with hypertension are first started on thiazide diuretics. This choice is made without knowledge of the genetic profile and unbiased. Consequently, this makes such observational studies on drug-gene interactions relative resistant to confounding by severity.

Limitations and strengths

All the data that were used for the pharmacogenetic studies described in this thesis were collected in two observational cohorts (Rotterdam Study and Doetinchem Cohort). Most of the pharmacogenetic studies on blood pressure (short-term outcome) were performed in a clinical trial settings and on long-term outcomes in observational studies. To be precise only one study, i.e. GENHAT,[33] used trial data for long-term outcomes.

The use of observational data is a potential limitation for the short-term outcomes presented in this thesis, since well designed trials remain the gold standard. For example, in all clinical trials pre-treatment blood pressure measurements were available, which made it possible to calculate the response after the start of the treatment. In the Rotterdam Study and Doetinchem Study, it was impossible to

perform this analysis. Therefore, we might have missed early responses, which might have diminished over time. Nevertheless, our results corroborated with the results found in most (larger) trials. Another limitation of the use of observational cohort studies is that confounding e.g. residual confounding could have biased our results due to unmeasured or inaccurately measured confounders. This possibility remains even after the adjustment for several potential confounders which were available in the Rotterdam Study and Doetinchem cohort. For example, no adjustments were made for physical activity and therefore we may have over- or underestimated the blood pressure lowering effect. However, even than the drug-gene interaction will remain valid as long as the bias due to the unadjusted variable is similar for the different genotype groups. An additional, difference between observational studies and randomized clinical trials is that all observational studies combined all antihypertensive drugs in classes. Antihypertensive drugs within the same class might have different pharmacokinetic or -dynamic properties, which could have led to differences in responses to antihypertensive drugs of the same antihypertensive drug class. For example, calcium channel blockers such as nifedipine and amlodipine have little effect on the atrioventricular node, but are potent vasodilators and have mild diuretic effects. In contrast, the calcium channel blocker verapamil is an antiarrythmic drug with some vasodilatory action and the related compound diltiazem has some effect on both cardiac conduction and vascular smooth muscle cells. Another example is carvedilol, which is a β1-, β2-, and α-adrenergic blocker which account for its vasodilatory effects in contrast to β1-selective agents.[34] An additional advantage of a trial is that drug treatment can be standardized. This will minimise the number of subjects switching to another drug class or adding another antihypertensive drug class.

A limitation which applies to all pharmacogenetic studies, including ours, is that most studies genotyped only one SNP per gene. Some studies genotyped different polymorphisms in the same gene, but they all failed to fully represent the large variation in genes. Therefore, our results did not rule out involvement of a specific gene but only of a candidate gene polymorphism. A disadvantage of taking only one SNP per gene is that the folding kinetics, stability, or many other physical properties of a protein may depend on the interaction between pairs or combinations of several amino acid sites. APOE is one of the examples of a protein which function is influenced by a pair of polymorphic amino acids. The major alleles ε2, ε3, ε4 differ at two amino acid residues.

One of the main limitations of almost all pharmacogenetic studies is the sample size. This is one of the strengths of our studies with blood pressure as outcome. Most pharmacogenetic trials on short-term outcomes consisted of approximately 100 subjects. This lack of sample size might have increased the chance of missing genuine associations or of reporting spurious results. However, with regard to our study on stroke and MI the number of treated cases was in general small, although, similar as other observational studies. The small number of cases might have lead to biased results, for example, we had to reduce our drug exposure data into a dichotomous

variable (exposed versus non-exposed) and this may have increased the rate of exposure misclassification.[35] An example which shows the merits of large sample size concerns the studies on the role of polymorphisms in the ACE gene and its contribution to the risk of cardiovascular disease. Early publications on this disease association in the ECTIM (Etude Cas-Temoin d'Infarctus du Myocarde) study which involved 610 men who survived an MI and 733 controls, suggested that the ACE gene had a role in the risk of particular subgroups to cardiovascular disease.[36] Confidence intervals for this initial study were large. Subsequent studies often involved fewer patients, and those published produced variable results. When the hypothesis was tested in a very large case-control study (4,629 cases and 5,943 controls), the evidence of an association between ACE and an increased risk of cardiovascular risk was much diminished (RR 1.1, 95%CI 1.00-1.21).[37] This example suggests that for genetic effects of modest magnitude and subgroup analysis, 1,000 to 10,000 of individuals might be required to generate precise estimates. In comparison, to these numbers, many pharmacogenetic studies are too small. Besides false-negative results, also false-positive results could have been reported as most of the pharmacogenetic studies performed multiple tests on the same population. The expected frequency of false-positive results is given by $1-(1-k)^m$ (where m is the number of independent markers and k is the significance level for a single marker).[38] Adjustments for multiple testing can be made, but a disadvantage of these approaches is that genuine associations might be missed. Therefore, some investigators argue that the likelihood of a chance association may be considered in the light of biological plausibility of any observed observation.[39] Therefore, we did not adjust for multiple testing in our analysis.

A strength of our study compared to other pharmacogenetic studies with the outcome blood pressure, is that we evaluated the effect of the five polymorphisms in two study settings in the Netherlands. Replication of the same findings in the same population will give a better prediction of the (non) existence of a drug-gene interaction. The probability that a second association study is also positive in the same population may also vary with sample size, the measurement value, and environmental factors.[40] It may be questioned whether the two cohorts were not too different. Although both cohorts consisted mainly of Caucasians, the mean age difference was approximately 20 years between the hypertensive subjects in both cohorts. The difference resulted in an overrepresentation of subjects with isolated-systolic hypertension in the Rotterdam Study compared to Doetinchem Cohort Study. Furthermore, younger subjects are also more likely to respond to antihypertensive drug therapy then older subjects. In addition, there is also a difference in the standardisation of the blood pressure measurements between both cohorts.

An additional strength of our study is that we were able to evaluate the effect of drug-gene-gene interactions. Namely, the behaviour of antihypertensive drugs are influenced by a range of gene products. Therefore, it might be important to consider groups of potentially interacting genes as a set, such as those that act in common pathways (e.g. renin-angiotensin system), to identify interactions between

polymorphisms in different genes (chapter 4.3 and 4.4). A small number of studies tested for interactions between polymorphisms in different genes. The results for the drug-gene-gene interactions between the Rotterdam Study and Doetinchem Cohort Study were not similar and this could have been the result of differences in study population as mentioned in the previous paragraph. Another possible explanation for the difference in results is the higher chance of reporting false-positive and false-negative results in drug-gene-gene studies. Namely, when more then one variable is studied simultaneously larger patient groups are required to ensure that individual subgroups retain adequate power to detect significant associations with narrow confidence intervals.

Non-replication

Most of the drug-gene interactions in the literature were not replicated by other studies (observational studies and trials). Non-replication can be the result of differences in phenotype definition, lack of statistical power, population stratification, different environmental factors within a population, the effects of other genes, and the varying effects of several causal polymorphisms within the candidate gene.[41, 42] Consistency of association across studies is a useful indicator of a causal association, when present. However, genuine biological differences between study populations could have resulted in non-replication. One of these biological factors is the differences in linkage disequilibrium (LD) between the studied polymorphism and the causal mutation. Difference in LD between populations is the result of a number of contributing factors, including regional variability in recombination patterns, genetic drift, mutation age, ethnic diversity, recent population admixture, local chromosomal composition, and the patterns of mating within a population.[43-47] Due to the variation within populations, markers close to a functional DNA variant might show less or more LD than markers further away. As a result, associations with some markers might not be identified in a region containing a disease-mediating polymorphism, whereas associations at adjacent markers are convincingly detected. Since all of the polymorphisms studied are most likely not the causal mutation this may lead to differences in results. Another factor which could explain the real biological difference between populations is the difference in allele frequencies. For example, the 235T-allele of the AGT gene varies widely in frequency, occurring in 35-45% of whites, 75-80% of Asians, 75-80% of African Americans, and ≥ 90% of Africans.[48, 49] This pattern led to the hypothesis that the 235T-allele, which is associated with a higher angiotensinogen expression (-6G/A) and greater sodium reabsorption, was adaptive in the tropical and sodium-poor environment of Africa. When humans migrated out of Africa into other environments the 235T-allele became neutral or selected against.[50]

Although genuine biological differences are a possible explanation for the discrepancies in results, it most likely explains only a small percentage of the

inconsistencies in results. A more likely explanation for the non-replication is the limited sample sizes as most of the drug-gene interactions were found in studies with small sample sizes. To reduce the number of (potentially) false-positive and false-negative results pharmacogenetic study designs should be standardized, for example, with regard to criteria used for hypertension, the wash-out period, and the minimum duration of treatment. A meta-analysis might provide a quantitative approach for combining the results of various studies on the same topic[51, 52] but due to the variability in inclusion criteria, duration of treatment, and the limited number of studies it is currently not yet possible to perform a meta-analysis. Another solution is to perform multi-centre studies to increase sample size (e.g. GENHAT[33]) or by replication of an interaction in the same population as we did by studying a research hypothesis in two different observational settings, i.e. the Doetinchem Cohort Study and Rotterdam Study.

Clinical implications

Knowledge of polymorphisms or genes that influence the pharmacodynamic response of blood pressure to antihypertensive medication has the potential to provide new insights into the molecular mechanisms that influence drug response. On the basis of previous results, clinical implications are unclear since most of the pharmacogenetic studies so far have fallen short of the ideal approach to study the genetics of drug responses and data most likely have been over interpreted. Also the studies in this thesis do not give conclusive results. However on the basis of our results, it does not seem likely that these five polymorphisms have a big influence on the blood pressure response or on long-term cardiovascular outcomes associated with antihypertensive drug use in daily clinical practise. Although, conclusive answers can not be drawn from the available data this does not mean that pharmacogenetics will never provide the answers to our questions. For example, for genes involved in drug metabolizing enzymes (e.g. CYP2D6 and CYP2C19) diagnostic tools have already been developed.[53] However, in complex causal pathways with multiple interacting risk-enhancing alleles associations tend to be of modest strength. For example, blood pressure levels arecontrolled by a complex combination of processes that influence cardiac output and peripheral resistance.[54, 55] Thus there are many more candidate genes and polymorphisms that could be involved in the response to antihypertensive drugs than the five presented in this thesis for the short and long-term outcomes.

Even after the discovery of the causal polymorphisms (or those in linkage with the causal mutation) there might still be some problems with the implication of genetic tests. For example, the choice which antihypertensive drug to prescribe can be difficult when a subject has risk-enhancing alleles for each class of antihypertensive drug.

Future research

There are still a number of questions that are raised with this thesis that need to be answered. For example, the association between the M235T polymorphism and the risk of stroke and MI warrants further investigation. In addition, the interaction between the 825C/T polymorphism of the GNB3 gene and low-ceiling diuretics is still uncertain. For definitive conclusions larger sample sizes and/or meta-analysis are needed.

In addition, which SNPs and how many to genotype is important to consider. There are approximately 10 million SNP's (minor allele frequencies > 1%) in the human genome.[56] It is not practical, at present, to genotype and test all SNPs in the genome for association with phenotypes. Therefore, it is important to select a limited number. In theory this would be those polymorphisms that affect the function of the protein or its expression, however, in most situations this information is not available. In addition, polymorphisms in non coding regions are still able to affect gene function by altering the stability and splicing of mRNA.[57] Selecting a limited number of markers will result in a loss of power compared to genotyping all SNP's. The loss of power depends on four factors: 1) the strength of the association between a true disease-causing SNP and the disease, 2) linkage disequilibrium (LD) between markers and the causal SNP, 3) the marker allele frequency, 4) the disease allele frequency. In order to create considerable marker redundancy and still capture almost all information the map based strategy was developed. This strategy is greatly influenced by data showing that LD is composed in block like regions of low diversity of haplotypes, and stretches of more rapid breakdown of LD, which correspond to hotspots of meotic recombination.[58, 59] This inspired the idea of haplotype tagging, in which a set of SNPs is identified that tag each of the common haplotypes with in a block of LD (so called tagging SNPs). By some estimates, an average of five to seven SNPs per gene would be required to represent all the common polymorphisms in candidate genes. Tagging SNP's need to be carefully selected and validated, because not all five to seven SNP's will do. A disadvantage of this approach is that it remains unclear whether tagging SNPs will be able to represent variants with lower minor allele frequencies. Although, this frequency bias is likely to be a minor problem, if the response to a drug is caused by common polymorphisms, it will limit the usefulness if it is caused by rare variants.[60, 61]

In the future, it might be possible to perform genome association screens to determine which genes influence the response to antihypertensive drugs. This is not yet feasible, since estimates of the number of SNPs required for a complete genome-wide haplotype map suggest that this is likely to be in the region of 500,000 separate SNPs or fewer if only the genes are genotyped (100,000-200,000). At the moment, studies are using this approach on single chromosome, although, the statistical analysis remains a problem.

To assess whether the intended effects on antihypertensive drug treatment are modified by genetic polymorphisms, randomized controlled trials are preferred. For

short-term outcomes of antihypertensive drug treatment such as blood pressure, it is feasible to design such a trial. However, for long-term outcomes such as MI and stroke, randomized controlled trials may not be feasible due to practical, financial, or ethical reasons. In situations where randomized controlled trials are not feasible, observational studies are an alternative to obtain information on drug-gene interactions in daily clinical practice.

In conclusion, the results of this thesis suggests the presences of two drug-gene interactions i.e. the interaction between the use of ACE-inhibitors and AGT M235T polymorphism on the risk of MI and the interaction between the use of diuretics and the GNB3 825C/T polymorphism on systolic blood pressure.

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Chapter 7

Summary/Samenvatting

Summary

Hypertension is a major public health hazard, because of its high prevalence and strong positive association with cardiovascular diseases. Suboptimal blood pressure control is the number one attributable risk for death in the Western world, despite the possibilities to treat hypertension. Higher pre-treatment blood pressure levels are associated with a greater antihypertensive drug response, but this relation is not specific to a particular antihypertensive drug or drug class nor can it be predicted from patient's characteristics. Therefore, the selection of the most appropriate pharmacological treatment for an individual patient is a matter of 'trial and error'. Pharmacogenetics aims to understand how genetic variations contribute to the variation in response to medication. This thesis contains a number of epidemiological studies that are aimed at gaining more insight into the effect of five candidate gene polymorphisms on the response to antihypertensive medication.

After a general introduction in **chapter 1**, **chapter 2** focuses on the current status of hypertension treatment in the Netherlands. In **chapter 2.1**, estimates are provided on the prevalence, treatment, and control of hypertension and determinants of undertreatment in the Netherlands. Data were obtained from a population-based survey on cardiovascular risk factors in the Netherlands from 1996 through 2002 (MORGEN project). A total of 10,820 men and women were included in this study. The prevalence of hypertension in men was 21.4% and in women 14.9%. Of the hypertensive men 17.9% was treated, of whom 67.6% had a blood pressure level during treatment which was too high according to the guidelines. Of the hypertensive women, 38.5% were receiving antihypertensive medication, of whom 51.9% had a blood pressure level which was too high. One of the factors associated with a better control of blood pressure was the use of cholesterol-lowering medication. Of the untreated patients, 21.9% of the men and 13.6% of the women were eligible for treatment according to the Dutch guidelines for antihypertensive treatment. Subjects who were physically active, on a low salt diet, and current smokers had an increased chance of being untreated.

In **chapter 3**, a general introduction is given on the interaction between antihypertensive drugs and genes. **Chapter 3.1** contains all studies until October 2003 that reported data on genetic polymorphisms and response to antihypertensive drugs. In some candidate gene studies, drug-gene interactions were found. Unfortunately, the quality of these studies is quite variable and initial associations were often difficult to replicate. Therefore, further research is needed to be able to make definitive conclusions.

Chapter 4 consists of four studies that investigated the drug-gene interaction between five candidate gene polymorphisms and four antihypertensive drug classes on short-term outcomes. The first three studies were performed in the Rotterdam Study, a population-based prospective cohort study among 7,983 individuals aged 55 years or over. In **chapter 4.1** we investigated whether the angiotensin converting enzyme

(ACE) insertion/deletion (I/D) polymorphism modified the adherence to ACE-inhibitors as measured by the discontinuation of an ACE-inhibitor or addition of another antihypertensive drug. There was no significant difference between subjects with the DD, ID, or II genotype in adherence (DD versus II; relative risk (RR)=1.17; 95%CI: 0.78-1.77 and ID versus II; RR=1.06; 95%CI: 0.73-1.55). Also, no difference in blood pressure levels was found in ACE-inhibitor users, as demonstrated in **chapter 4.2** (systolic blood pressure II versus DD; -2.01 mmHg; 95%CI: -9.82-5.79 and diastolic blood pressure II versus DD; -1.04 mmHg; 95%CI: -5.45-3.37). nor did it modify blood pressure levels in users of low-ceiling diuretics, β-blockers, or calcium channel blockers. Also, the angiotensinogen (AGT) M235T and α-adducin (ADD1) G460W polymorphisms did not modify blood pressure levels in antihypertensive drug users as described in **chapter 4.3**. **Chapter 4.4** contains data from the Doetinchem Cohort Study. This cohort is part of a population-based prospective study on cardiovascular risk factors conducted in the Netherlands (MORGEN project). In total, 625 hypertensive subjects had complete information on blood pressure, medication use and genotypes. No drug-gene interaction was found with the ACE I/D, AGT M235T, angiotensin receptor II type 1 (AGTR1) 1166A/C, or ADD1 G460W polymorphism. Only the β_3-subunit of G-protein (GNB3) 825C/T polymorphism modified the systolic blood pressure levels in diuretic users (C-allele versus TT 4.33 mmHg; 95%CI: 0.14-8.54). Thus subjects with one or two copies of the 825C-allele might have less benefit from the use of low-ceiling diuretics. In addition, four significant drug-gene-gene interactions were found which were associated with an increased systolic or diastolic blood pressure.

Chapter 5 provides the data of two studies investigating drug-gene interactions on long-term outcomes. These studies were performed in the Rotterdam Study. In **chapter 5.1**, we investigated whether the ACE I/D, AGT M235T, or AGTR1 573C/T polymorphism modifies atherosclerosis levels in patients treated with ACE-inhibitors or β-blockers. We used three different sub-clinical measurements of atherosclerosis, i.e. peripheral arterial disease, carotid atherosclerosis, and aortic atherosclerosis. No consistent drug-gene interaction was found in association with these three measurements. **Chapter 5.2**, presents the data on the risk of myocardial infarction (MI) and stroke and the interaction between the AGT M235T polymorphisms and the use of ACE-inhibitors and β-blockers. The interaction between current use of ACE-inhibitor users was multiplicative on the risk of MI (hazard ratio (HR)=4.00; 95%CI: 1.32-12.11) in subjects with the MT or TT genotype compared to subjects with the MM genotype. Similarly, there was a non-significantly increased risk of stroke (HR=1.83; 95%CI: 0.95-3.54). No significant interaction was found between the current use of β-blockers and the AGT M235T polymorphism on the risk of MI or stroke. Subjects with at least one copy of the 235T-allele of the AGT gene might have less benefit from ACE-inhibitor therapy.

Chapter 6 contains the main findings of the studies in this thesis and the main limitations/strengths of these studies. In addition, the clinical relevance of the findings and recommendations for further research are given.

Samenvatting

Hypertensie is een belangrijk probleem voor de volksgezondheid, vanwege de hoge prevalentie en sterke associatie met cardiovasculaire ziektes. Suboptimale bloeddruk controle is de belangrijkste doodsoorzaak in de Westerse wereld, ondanks de ontwikkelingen in de behandeling van hypertensie. Ofschoon een hogere bloeddruk voor behandeling geassocieerd is met een betere respons op antihypertensiva is de relatie niet specifiek voor een bepaald antihypertensivum of gerelateerd aan speciale patiëntkarakteristieken. De selectie van de beste farmacologische behandeling voor een individu is daarom moeilijk. Farmacogenetica heeft als doel om meer inzicht te geven in de bijdrage van genetische variatie in de response op geneesmiddelen. Dit proefschrift bevat een aantal epidemiologische studies, die als doel hadden de effecten van vijf polymorfimen in kandidaat-genen op de response van antihypertensiva te onderzoeken.

Na een algemene introductie in **hoofdstuk 1** wordt in **hoofdstuk 2** de actuele status van de behandeling van hypertensie in Nederland besproken. In **hoofdstuk 2.1** worden schattingen gegeven over de prevalentie, behandeling en controle van hypertensie. Daarbij worden de determinanten van onderbehandeling van hypertensie in Nederland onderzocht. Gegevens waren afkomstig van het bevolkingsonderzoek naar risicofactoren voor hart- en vaatziekten in Nederland van 1996 tot en met 2002 (MORGEN project). In totaal werden 10.820 mannen en vrouwen in deze studie opgenomen. De prevalentie van hypertensie was 21,4% voor mannen en 14,9% voor vrouwen. Ongeveer 18% van de mannen met hypertensie werd behandeld. Hiervan had 67,6% een te hoge bloeddruk tijdens de behandeling volgens de richtlijnen. Van de vrouwen met hypertensie kreeg 38,5% antihypertensiva. Hiervan had 51,9% een te hoge bloeddruk tijdens behandeling. Een van de factoren die geassocieerd was met een betere controle van de bloeddruk is het gebruik van cholesterolverlagende geneesmiddelen. Van de onbehandelde patiënten zou 21,9% van de mannen en 13,6% van de vrouwen volgens de richtlijnen in aanmerking komen voor behandeling. Mensen die lichamelijk actief waren, een zoutarm dieet hadden of rookten hadden een verhoogde kans om niet behandeld te worden.

In **hoofdstuk 3** wordt een algemene introductie gegeven over interacties tussen antihypertensiva en genen. **Hoofdstuk 3.1** geeft een overzicht van alle studies (tot oktober 2003), die gegevens bevatten over genetische polymorfismen en de respons op antihypertensiva. In sommige studies met kandidaatgenen werden geneesmiddel-gen interacties gevonden. Helaas was de kwaliteit van deze studies variabel and was het over het algemeen lastig om eerder beschreven associaties te reproduceren. Daarom is extra onderzoek nodig om definitieve conclusies te trekken.

Hoofdstuk 4 bestaat uit vier studies, waarin wij onderzochten of er een geneesmiddel-gen interactie aantoonbaar was tussen vijf polymorfismen in kandidaat-genen en vier verschillende groepen antihypertensiva op korte termijn uitkomsten. De eerste drie studies werden uitgevoerd met gegevens van het Erasmus Rotterdam

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Gezondheid en Ouderen (ERGO) onderzoek, een bevolkingsonderzoek van 7.983 personen van 55 jaar en ouder. In **hoofdstuk 4.1** hebben we onderzocht of het ACE I/D polymorfisme het gebruik van ACE-remmers verandert. Een verandering werd gedefinieerd als het stoppen van het gebruik van een ACE-remmer of de additie van een ander antihypertensivum. Er was geen significant verschil tussen mensen met het DD, ID en II genotype in het gebruik van ACE-remmers (DD versus II; relatieve risico (RR)=1.17; 95%BI: 0.78-1.77 en ID versus DD; RR=1.06; 95%BI: 0.73-1.55). Daarnaast werd er bij gebruikers van ACE-remmers geen verschil gevonden in de hoogte van de bloeddruk, zoals aangetoond in **hoofdstuk 4.2** (systolische bloeddruk II versus DD; -2,01 mmHg; 95%BI: -9,82-5,79 en diastolische bloeddruk II versus DD -1,04 mmHg; 95%BI: -5,45-3,37). Ook veranderde het ACE I/D polymorfisme de bloeddruk niveau's niet in mensen die diuretica (exclusief lisdiuretica), β-blokkers of calciumantagonisten gebruikten. Ook de AGT M235T en ADD1 G460W polymorfismen veranderde bloeddruk niveau's niet, zoals beschreven in **hoofdstuk 4.3**. **Hoofdstuk 4.4** bevat gegevens uit de Doetinchem Cohort Studie. Dit cohort is een gedeelte van het bevolkingsonderzoek naar risicofactoren van hart- en vaatziekten in Nederland (MORGEN project). In totaal hadden 625 personen met hypertensie complete informatie over bloeddruk, geneesmiddelgebruik en genotypes. Er werd geen geneesmiddel-gen interactie gevonden met het ACE I/D, AGT M235T, AGTR1 1166A/C, of ADD1 G460W polymorfisme. Alleen het GNB3 825C/T polymorfisme veranderde de systolische bloeddruk niveau's in diuretica gebruikers (C-allele versus TT; 4,33 mmHg; 95%BI: 0,14-8,54). Mensen met één of twee kopieën van het 825C allel zouden misschien minder baat hebben bij het gebruik van diuretica. Bovendien werden er vier significante geneesmiddel-gen-gen interacties gevonden met consequenties voor de systolische of diastolische bloeddruk.

   Hoofdstuk 5 bevat gegevens van twee studies naar de associatie tussen geneesmiddel-gen interacties en uitkomsten op de lange termijn. Deze studies zijn uitgevoerd met data uit het ERGO onderzoek. In **hoofdstuk 5.1**, werd onderzocht of het ACE I/D, AGT M235T of AGTR1 573C/T polymorfisme geassocieerd was met de mate van atherosclerose in patiënten, die behandeld werden met ACE-remmers of β-blokkers. We gebruikten drie subklinische parameters voor atherosclerose, namelijk perifeer vaatlijden, atherosclerose van de arteria carotis en atherosclerose van de aorta. Er werd geen consistente geneesmiddel-gen interactie gevonden met deze drie uitkomstmaten. **Hoofdstuk 5.2** geeft het risico op een hart- en herseninfarct en de interactie tussen het AGT M235T polymorfisme en het gebruik van ACE-remmers of β-blokkers. De interactie tussen het AGT M235T polymorfisme en het gebruik van ACE-remmers was multiplicatief op het risico van een hartinfarct (HR=4,00; 95%BI: 1,32-12,11) in personen met het MT of TT genotype ten opzichte van mensen met het MM genotype. Ook was er een (niet significant) hoger risico op een herseninfarct (HR=1,83; 95%BI: 0,95-3,54). Er werd geen interactie gevonden tussen gebruik van β-blokkers en het AGT M235T polymorfisme op het risico van een hart- of herseninfarct. Mensen met tenminste één kopie van het 235T allel op het AGT gen

lijken minder baat te hebben bij behandeling met ACE-remmers.

Hoofdstuk 6 bevat de belangrijkste resultaten van dit proefschrift en bespreekt de sterke punten, de klinische relevantie en beperkingen van studies uit dit proefschrift. Daarnaast worden aanbevelingen gegeven voor toekomstig onderzoek.

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List of publications

List of Publications

Schelleman H, Klungel OH, Kromhout D, de Boer A, Stricker BHCh, Verschuren WMM. Prevalence and determinants of undertreatment of hypertension in the Netherlands. J Hum Hypertens 2004; 18:317-24.

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Schelleman H, Stricker BHCh, Verschuren WMM, de Boer A, Kroon AA, de Leeuw PW, Kromhout D, Klungel OH. Interactions between five candidate genes and antihypertensive therapy on blood pressure. Pharmacogenomics J; *in press*

Curriculum Vitae

Curriculum Vitae

Hedi Schelleman was born on February 16, 1979 in Tiel, the Netherlands. In 1997, she finished secondary school at the 'Cals College' in Nieuwegein. In the same year she started her study Biomedical Sciences at Utrecht University and obtained her Master of Science degree in 2002. In March 2002 she started work on the studies described in this thesis at the Department of Epidemiology & Biostatistics of the Erasmus University MC, Rotterdam, and the Department of Pharmaco-epidemiology and Pharmacotherapy of Utrecht Institute for Pharmaceutical Sciences, Faculty of Pharmaceutical Sciences of the Utrecht University. In addition, she received a Master of Science degree in Genetic Epidemiology at the Netherlands Institute for Health Sciences in Rotterdam in 2003. In May 2006 she will start in a postdoctoral position at the Center for Clinical Epidemiology & Biostatistics at the University of Pennsylvania School of Medicine, United States of America.

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