GENETIC POLYMORPHISMS AND LABORATORY VARIABLES AS PREDICTORS OF BLOOD PRESSURE RESPONSE TO ANTIHYPERTENSIVE DRUGS

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Academic dissertation

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LIST OF ORIGINAL PUBLICATIONS


* Equal contribution.

Study III also appears in the thesis of Tuula Hannila-Handelberg (2009).

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ABBREVIATIONS

ABP  ambulatory blood pressure
ACE  angiotensin converting enzyme
ACE I/D angiotensin converting enzyme insertion / deletion
ADD1 alpha-adducin
ADRB1 beta1-adrenergic receptor
ADRB2 beta2-adrenergic receptor
AGT  angiotensinogen
Ang I angiotensin I
Ang II angiotensin II
AT1R angiotensin II type 1 receptor
AT2R angiotensin II type 2 receptor
BMI  body mass index
BP   blood pressure
CYP  cytochrome P450
ESC  The European Society of Cardiology
ESH  The European Society of Hypertension
ISH  The International Society of Hypertension
NEDD4L neural precursor cell expressed, developmentally down-regulated 4-like
OBP  office blood pressure
PCR  polymerase chain reaction
PRA  plasma renin activity
RAS  renin-angiotensin system
SNP  single nucleotide polymorphism
WCE  white coat effect
WHO  The World Health Organization
WHR  waist-hip ratio
WNK1 with no K (lysine) protein kinase 1

In addition, standard one-letter and three-letter abbreviations are used for nucleotides and amino acids.
ABSTRACT

**Background.** Hypertension is a common multifactorial disorder associated with significant risk for cardiovascular and renal comorbidity. The advantages of antihypertensive therapy have been clearly demonstrated, but only about one third of hypertensive patients have their blood pressure (BP) controlled by such treatment. One of the reasons for this poor BP control may lie in the difficulty in predicting BP response to antihypertensive treatment. The average BP reduction achieved is similar for each drug in the main classes of antihypertensive agents, but there is a marked individual variation in BP responses to any given drug.

**Aims.** The purpose of the present study was to examine BP response to four different antihypertensive monotherapies with regard to demographic characteristics, laboratory test results and common genetic polymorphisms.

**Subjects and methods.** The subjects are participants in the pharmacogenetic GENRES Study. Altogether, 313 moderately hypertensive Finnish men were screened for the cohort. A total of 208 subjects completed the whole study protocol lasting eight months and including four drug treatment periods of four weeks, separated by four-week placebo periods. The study drugs were amlodipine, bisoprolol, hydrochlorothiazide and losartan. Both office (OBP) and 24-hour ambulatory blood pressure (ABP) measurements were carried out. The ABP responses (post-treatment minus placebo blood pressure level) were considered as the primary efficacy variables, as ABP recordings showed better repeatability during the placebo periods than OBP measurements. BP response to study drugs were related to basic clinical characteristics, pretreatment laboratory test results (plasma renin activity (PRA), serum levels of glucose, sodium, potassium, chloride, total calcium, creatinine, uric acid, aldosterone, total cholesterol, HDL cholesterol, triglycerides and insulin concentrations along with daily urinary excretion of sodium, potassium, chloride and albumin) and common polymorphisms in genes coding for components of the renin-angiotensin system, alpha-adducin (ADD1), beta1-adrenergic receptor (ADRB1) and beta2-adrenergic receptor (ADRB2). Genotyping of the polymorphisms was performed using polymerase chain
reaction followed by restriction enzyme digestion and electrophoretic separation of the restriction fragments on agarose or polyacrylamide gel.

**Results.** The median ABP response (systolic/diastolic) was 11.1/8.4 mmHg for bisoprolol, 9.1/6.1 mmHg for losartan, 7.4/4.9 mmHg for amlodipine, and 4.9/1.7 mmHg for hydrochlorothiazide. ABP levels during the placebo periods were positively associated with ABP responses to study drugs. For both ABP and OBP age was positively correlated with systolic and diastolic response to amlodipine ($P$ values <0.01) and with systolic and diastolic OBP and systolic ABP response to hydrochlorothiazide ($P$ values <0.01), while body mass index was negatively correlated with systolic and diastolic ABP response to amlodipine ($P$ values <0.05). Of the laboratory test results, PRA correlated positively with systolic and diastolic response to losartan for ABP and OBP ($P$ values <0.01), with systolic and diastolic ABP response to bisoprolol ($P$ values <0.05), and negatively with ABP response to hydrochlorothiazide ($P$ values <0.05). There was also a weaker correlation of PRA with ABP response to amlodipine. Uniquely to this study, it was found that serum total calcium level was negatively correlated with ABP and OBP response to amlodipine ($P$ values <0.05), whilst serum total cholesterol level was negatively correlated with ABP response to amlodipine ($P$ values <0.01).

In this study, there were no significant associations of selected polymorphisms of the renin-angiotensin system (angiotensin II type I receptor 1166A/C, rs5186, angiotensin converting enzyme insertion/deletion, rs4341 and angiotensinogen Met235Thr, rs699), ADD1 (Gly460Trp, rs4961), ADRB1 (Ser49Gly, rs1801252 and Gly389Arg, rs1801253) and ADRB2 (Arg16Gly, rs1042713 and Gln27Glu, rs1042714) with BP responses to the four study drugs. As a consequence, this study, carried out with carefully controlled condition and also including ABP measurements could not lend further support to the earlier positive findings demonstrating stronger BP response for the ADD1 460Trp allele or for the ADRB1 Gly389 allele to hydrochlorothiazide and bisoprolol, respectively.

**Conclusions.** This study confirmed the relationship between pretreatment PRA levels and response to three classes of antihypertensive drugs. This study is the first to note a significant inverse relation between serum calcium level and responsiveness to a
calcium channel blocker. However, this study could not replicate the observation that common polymorphisms in angiotensin II type I receptor, angiotensin converting enzyme, angiotensinogen, ADD1, ADRB1, or ADRB2 genes can predict BP response to antihypertensive drugs.
1. INTRODUCTION

Hypertension is one of the major health problems affecting almost a billion people worldwide. Blood pressure (BP) is continuously related to the risk of stroke, ischemic heart disease, heart failure and renal disease, and it has been estimated that elevated BP is responsible for over 7 million deaths per year (MacMahon et al. 1990, Chobanian et al. 2003, Kearney et al. 2005). The prevalence of hypertension is steadily rising due to an aging population. It has been predicted that almost one third of the adult population will be hypertensive by the year 2025. In Finland, the prevalence of hypertension in the middle-aged population (35 to 64 years) is 49% exceeding the European average of 44.2% (Wolf-Maier et al. 2003).

In most cases of hypertension the etiological cause is multifactorial, combining both environmental and genetic factors. In less than 10% of cases there is an identifiable secondary cause behind high BP (Berglund et al. 1976, Sigurdsson et al. 1983, Omura et al. 2004). Among the environmental factors associated with elevated BP are, physical inactivity, being overweight, excess dietary sodium intake and alcohol (Whelton et al. 2002). Based on family and twin studies, it has been estimated that the genetic affect on variation of blood pressure ranges from 20 to 60% (Kurtz et al. 1993). However, no definitive susceptibility genes for common hypertension have yet been identified, and according to recent genome wide association studies, chromosome regions associated with hypertension have only small effects on BP variation (Newton-Cheh et al. 2009, Levy et al. 2009). The most common causes of secondary hypertension are renal diseases, renal artery stenosis and primary aldosteronism. In addition, there are rare endocrine disorders and monogenic diseases causing elevated BP (Chiong et al. 2008).

The primary aim of hypertension management is to reduce cardiovascular and renal morbidity and mortality. According to systematic reviews of clinical trials, treatment of elevated BP significantly reduces the total cardiovascular risk (Collins and MacMahon 1994, Lawes et al. 2004). However, only about one third of hypertensive patients on antihypertensive medication have their BP controlled (Chobanian et al. 2003, Kastarinen et al. 2009). Reasons for this inadequate BP control among subjects on antihypertensive medication may include excessive salt intake, unfavorable lifestyle
habits, poor treatment compliance and individual variation in BP response to antihypertensive drugs.

Although the average BP response is rather similar when hypertensive subjects are treated with any of the drugs from different classes of antihypertensive agents, the individual variation in BP responses to different drugs is significant (Materson et al. 1993, Attwood et al. 1994, Dickerson et al. 1999). Thus it is probable that the variation in BP response to antihypertensive agents is determined by a variety of pharmacokinetic and pharmacodynamic mechanisms and could be partially genetically determined. There have been attempts to predict BP response to antihypertensive treatment based on laboratory variables and demographic factors, mostly with inconsistent results (Laragh et al. 1979, Chapman et al. 2002). During recent years, increasing attention in this field has focused on the effect of genetic variation on BP response to antihypertensive drugs. As a consequence a vast number of papers have been published on the effect of polymorphisms of putative candidate genes, such as those coding for the genes of alpha-adducin (ADD1) and beta1-adrenergic receptor (ADRB1), on antihypertensive responses (Arnett et al. 2009).

Identification of predictors of individual BP response to antihypertensive drugs may help in optimizing individual treatment of hypertension. The aim of this study was to evaluate the effect of basic clinical characteristics, pretreatment laboratory test results and common genetic variations on BP responses to four different antihypertensive agents.
2. REVIEW OF THE LITERATURE

2.1 Blood pressure and hypertension

2.1.1 Definition of blood pressure and hypertension

Arterial blood pressure (BP) is the product of cardiac output and total peripheral resistance. It is regulated by many mechanisms of the body, causing changes in cardiac output and total peripheral resistance (Guyton 1991). Hypertension is defined as chronically elevated BP caused either by increased cardiac output, elevated peripheral resistance or both. However, most individuals with long-term hypertension have elevated peripheral resistance with normal cardiac output (Cowley 1992).

Table 1. Classification of blood pressure levels (mmHg).

<table>
<thead>
<tr>
<th>Category</th>
<th>Systolic</th>
<th>Diastolic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal</td>
<td>&lt;120</td>
<td>&lt;80</td>
</tr>
<tr>
<td>Normal</td>
<td>120-129</td>
<td>80-84</td>
</tr>
<tr>
<td>High normal</td>
<td>130-139</td>
<td>85-89</td>
</tr>
<tr>
<td>Grade 1 hypertension</td>
<td>140-159</td>
<td>90-99</td>
</tr>
<tr>
<td>Grade 2 hypertension</td>
<td>160-179</td>
<td>100-109</td>
</tr>
<tr>
<td>Grade 3 hypertension</td>
<td>≥180</td>
<td>≥110</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Category</th>
<th>Systolic</th>
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<tr>
<td>Normal</td>
<td>&lt;120</td>
<td>&lt;80</td>
</tr>
<tr>
<td>Stage 1 hypertension</td>
<td>140-159</td>
<td>90-99</td>
</tr>
<tr>
<td>Stage 2 hypertension</td>
<td>≥160</td>
<td>≥100</td>
</tr>
</tbody>
</table>
BP is a continuous variable, with the risk of complications from high BP increasing exponentially with rising BP, therefore any numerical definition and classification of hypertension is arbitrary. Theoretically, hypertension can be defined as the level of BP where the benefits of treatment exceed the risks of inaction (Kaplan 1983). However, for clinical use there is a need for a more precise definition and classification of hypertension based on BP levels. Both The Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7) and the European Society of Hypertension (ESH) and European Society of Cardiology (ESC) define hypertension as systolic office BP values $\geq 140$ mmHg and diastolic $\geq 90$ mmHg even though there are slight differences in the classification of BP levels between the two guidelines (Table 1).

2.1.2 Epidemiology and etiology of hypertension

Hypertension is a globally important health problem with a worldwide prevalence among the adult population of approximately 26%, affecting up to 972 million people (Kearney et al. 2005). The overall prevalences in men and women are similar. However, men develop hypertension at an earlier age than women (Kearney et al. 2005). According to the results from the Framingham Heart Study there is approximately a 90% lifetime risk for middle-aged and elderly individuals to develop hypertension (Vasan et al. 2002). The lifetime risk of hypertension increases with advancing age. It has been estimated that due to the aging of the population, prevalence of hypertension will be approximately 29% by the year 2025.

The prevalence of hypertension is 49% in the middle-aged (35 to 64 years) Finnish population, while the European average is 44.2% and North American average is 27.6% (Wolf-Maier et al. 2003). The overall prevalence of hypertension in Finland has been declining during the last 25 years. However, since the year 2002, this trend has only been observed in women (Kastarinen et al. 2009). More than 500 000 people requiring treatment for hypertension are subsidised by the Social Insurance Institution of Finland in the upper compensation class (www.kela.fi).
In over 90% of the cases the cause of hypertension is unknown and multi-factorial, implying the interplay of both environmental and genetic factors. Reduced physical activity, being overweight, excess dietary sodium and alcohol intake along with insufficient intake of fruits, vegetables, and potassium are associated with the development of hypertension (Whelton et al. 2002). It is likely that there are several causal genes for hypertension and the estimates of the influence of genetic variation on BP levels range from 20 to 60% (Kurtz et al. 1993). In less than 10% of patients with elevated BP there is an underlying disease causing secondary hypertension (Berglund et al. 1976, Sigurdsson et al. 1983, Omura et al. 2004). Common causes of secondary hypertension include renal parenchymal disease, renal vascular disease and primary aldosteronism. The other causes of secondary hypertension are rare diseases including endocrine disorders and monogenic diseases causing elevated BP (Chiong et al. 2008).

### 2.1.3 Complications of hypertension

Both systolic and diastolic BP levels are positively and continuously related to the risk of stroke, coronary heart disease, heart failure and renal disease (Kannel et al. 1972, MacMahon et al. 1990, Klag et al. 1996). In younger patients (under 50 years old) diastolic BP is the strongest predictor of coronary heart disease risk (Franklin et al. 2001b). In patients aged from 50 to 59 years diastolic, systolic and pulse pressure are equal risk factors. With older age (over 60 years) pulse pressure becomes the most important risk factor. According to data from observational studies, the risk of death from both coronary heart disease and stroke increases exponentially and progressively from BP levels of >115 mmHg for systolic and >75 mmHg for diastolic, while the mortality from coronary heart disease and stroke is doubled for every 20 mmHg systolic or 10 mmHg diastolic increase in BP (Lewington et al. 2002). Hypertension has a significant impact on life expectancy. In a follow-up study of the Finnish population, there was a 2.7-year difference in men and a 2.0-year difference in women for life expectancy, between normotensive and severely hypertensive people (Kiiskinen et al. 1998).
2.1.4 Measurement of blood pressure

As the diagnosis and treatment decisions of hypertension are based on BP levels, it is essential that BP measurements are carried out in an accurate and standardized way. The challenge in BP measurement is that BP is a constantly fluctuating haemodynamic parameter influenced by many short-term and long-term factors, including the BP measurement itself.

The short-term variability at rest is under the influence of the autonomic nervous system, is related to changes in respiration and heart rate, and is mediated by baroreflex mechanisms (Conway et al. 1984). The daytime variability is related to behavioral factors, with both dynamic physical exercise and mental stress increasing BP values. In addition, the nicotine in tobacco causes a transient BP elevation lasting for approximately 20-30 min (Swampillai et al. 2006) with this effect also seen in addicted smokers (Verdecchia et al. 1995). Caffeine also has an acute haemodynamic effect, as it raises systolic and diastolic BP while it slightly lowers heart rate (Nurminen et al. 1999). The mechanism seems to be that caffeine antagonizes adenosine A1 and A2A receptors (Fredholm et al. 1999) and increases the circulating concentration of catecholamines (Smits et al. 1985). Besides nicotine and caffeine, temperature, meals, alcohol, bladder distension and pain are all related to daily variability in BP. Diurnal variability is related to an approximately 15% nocturnal fall in BP levels, mostly as a result of sleep and inactivity during the night (Staessen et al. 1997a). There is also long-term seasonal variability in BP, with lower BP levels seen in the summer period and the highest during the winter period (Omboni et al. 1998).

BP can be measured traditionally in the office by a physician or nurse, in the home or workplace as self-measurement, and as ambulatory BP recording using automated devices. Fundamental to all these techniques is that the device used is accurate (Beavers et al. 2001) and the effect of BP variability is minimized.

Office BP (OBP) measurement is most commonly used to evaluate BP levels and to diagnose hypertension. It is, however, very sensitive to both biological and measurement related variation. According to the guidelines of ESH and ESC, OBP should be measured either by a mercury sphygmomanometer or by auscultatory or
oscillometric semiautomatic devices validated according to standardized protocols. At least two measurements should be performed, with a 1-2-minute interval, after several minutes rest in the sitting position. Additional measurements are recommended if the first two readings emphatically vary. A standard bladder is normally used, but for fat and thin arms an appropriate bladder-size should be chosen. At the first visit BP should be measured in both arms, with the higher value taken as the reference (Mancia et al. 2007).

Home BP measurement provides important information on BP levels in a daily life setting. Validated semiautomatic devices are recommended for home BP measurement and the patients are instructed to make BP measurements after several minutes rest in sitting position preferably in the morning and in the night (Mancia et al. 2007). The advantage in home BP measurement compared with office measurement is that it offers a series of measurement in the absence of significant white-coat effect (WCE). Home values predict the risk of organ damage and cardiovascular events better than office values (Ohkubo et al. 1998, Sega et al. 2005, Niiranen et al. 2010).

Ambulatory BP (ABP) measurement is required in some clinical situations, as it provides information on 24-hour average BP and the circadian variation of BP levels. During 24-hour ABP recording, the measurements are usually taken every 15 minutes during the daytime and every 30 minutes during the night-time using an automatic device. ABP levels are usually lower than OBP levels, with office values of 140/90 mmHg corresponding to average 24-hour values of 125-130 mmHg systolic and 80 mmHg diastolic (Mancia et al. 1995). The ESC and ESH guidelines particularly recommend ABP measurement when there is considerable variability in OBP values, when office values are high in a subject with otherwise low cardiovascular risk, when there is significant discrepancy between office and home BP values or when resistance to drug treatment or hypotensive episodes are suspected (Mancia et al. 2007). ABP levels correlate better with end-organ damage than OBP levels (Verdecchia et al. 1990, Fagard et al. 1997). Additionally, by using the ABP measurement it is possible to identify subjects with blunted nocturnal decrease in BP (non-dippers), as these individuals seem to be at an increased risk for cardiovascular events (Mancia et al. 2007).
2.2 Regulation of blood pressure

2.2.1 Renin-angiotensin system

The long-term regulation of BP is mainly based on the kidneys’ ability to regulate excretion of fluid and sodium in the urine. According to the pressure-natriuresis model, the elevation of BP above normal causes the kidneys to excrete more water and sodium in the urine. As a consequence of negative body fluid balance, cardiac output is decreased and BP will return to normal levels within couple of hours or days (Guyton 1991). The pressure-natriuresis is modulated by many neurohormonal mechanisms, of which the renin-angiotensin system (RAS) plays the most crucial role.

Renin is an enzyme synthesized and stored in the juxtaglomerular cells of the kidneys. Its secretion is regulated by renal perfusion pressure, tubular sodium load at macula densa cells and sympathetic nerve activity mediated by beta1-adrenergic receptors in the juxtaglomerular cells (Hackenthal et al. 1990). Renin is the rate-limiting enzyme in the RAS, and its secretion is increased by hypotension and hyponatremia. In the circulation it cleaves angiotensinogen, synthesized by the liver, into angiotensin I (Ang I), a decapeptide having mild vasoconstrictor properties. Ang I is converted into the octapeptide angiotensin II (Ang II) in a reaction catalyzed by the angiotensin converting enzyme (ACE) which is present in the endothelium of lung vessels. ACE is also called kininase II, as it catalyses the degradation of the vasodilator bradykinin. The effects of Ang II are mediated by angiotensin II type 1 (AT1R) and type 2 (AT2R) receptors. Ang II elevates BP by two mechanisms mediated via the AT1R. Firstly, it is an extremely potent vasoconstrictor rapidly increasing the total peripheral resistance, and secondly, it decreases the excretion of water and salt in the kidneys, both by acting directly on the kidneys and by inducing the synthesis and release of aldosterone. AT1R also mediates the effects of Ang II on cellular growth, cardiovascular remodelling and inflammatory processes, including atherosclerosis (Fyhrquist et al. 2008). The AT2R-mediated effects of Ang II are counter-regulatory and oppose the AT1R-mediated effects on haemodynamics and inflammatory processes (Hannan et al. 2004). A simplified view of the RAS is shown in Figure 1.
In addition to the major components of the classical circulating RAS, other biologically active angiotensins have been discovered. Angiotensin 2-8 (Ang III), angiotensin 3-9 (Ang IV) and angiotensin 1-7 (Ang 1-7) all modulate the functions of RAS making the system much more complicated than previously understood (Kramkowski et al. 2006). It has also been found that there is local RAS in most tissues which operate both independently and in interaction with the circulating RAS. Tissue RAS is involved in many local functions such as cellular proliferation, protein synthesis and organ functions (Paul et al. 2006).

### 2.2.2 Autonomic nervous system

The autonomic nervous system regulates BP mostly via activation of the sympathetic and parasympathetic nervous system. Nervous control affects the circulation by regulating cardiac output and total peripheral resistance and by redistributing blood flow to different areas of the body. It provides very rapid control of arterial BP through
vasoconstriction and enhancing cardiac output as a consequence of strong sympathetic stimulation (Dampney et al. 2002).

Peripheral autonomic nervous system is controlled by the vasomotor center located bilaterally in the reticular substance of the medulla and the lower third of the pons. The vasomotor center transmits sympathetic impulses to all blood vessels of the body and parasympathetic impulses through the vagus nerves to the heart. The continuous activity of the vasoconstrictor area of the vasomotor center normally maintains a partial state of contraction in the blood vessels called vasomotor tone. The vasomotor center controls activity of the heart either through impulses of the sympathetic nerve fibers causing increased heart rate and contractility or through impulses of the parasympathetic nerves causing decreased heart rate (Dampney et al. 2002).

In addition to increase BP in response to exercise and other types of stress, autonomic nervous system operates constantly to maintain normal arterial BP. This function is mainly based on negative feedback mechanisms of which baroreceptor reflex is best known. These receptors, located in the wall of large arteries of thoracic and neck region, are stimulated in response to stretching and respond rapidly to changes in BP. The stimulation of baroreceptors inhibits vasoconstrictor area of medulla and activates the vagal parasympathetic center leading to vasodilation of the arterioles and veins and decreased heart rate and contraction (Guyenet 2006).

Most sympathetic nerve fibers regulating heart and vascular tone secrete norepinephrine as synaptic transmitter and are said to be adrenergic. In addition, adrenergic nerve fibers from spinal cord to two adrenal glands end directly to modified neuronal cells that secrete epinephrine and norepinephrine into the blood stream (Guyenet 2006).

Adrenergic receptors are targets to both epinephrine and norepinephrine and are members of a large superfamily of receptors (G protein-coupled receptors) linked to guanine-nucleotide-binding proteins (G proteins). They were originally divided to two principal types, alpha- and beta-adrenergic receptors, based on differences in physiologic responses to adrenergic agonists. Based on finding that agonist and antagonist can be used to differentiate adrenergic responses among different tissues, it was subsequently discovered that there are at least three subtypes of beta-adrenergic
receptors, all encoded by separate genes. Beta1-adrenergic receptors (ADRB1), the main subtype in heart, mediate positive chronotropic and inotropic effects leading to increased cardiac output. The activation of beta2-adrenergic receptors (ADRB2) in turn results in vasodilatation, bronchial dilatation and lipolysis. Beta3-adrenergic receptors are expressed mostly in adipose tissue where they enhance lipolysis (Insel 1996). Later on, two subtypes of alpha adrenergic receptors, alpha1 and alpha2 receptors, with six subclasses (alpha1A, alpha1B, alpha1D, alpha2A/D, alpha2B, alpha2C) have been discovered (Guimaraes et al. 2001).

2.3 Treatment of hypertension

2.3.1 Goals of blood pressure treatment

The primary goal of hypertension treatment is to reduce morbidity and mortality due to cardiovascular and renal complications. Whenever possible, antihypertensive treatment should be initiated before any significant end-organ damage has developed. Current treatment guidelines suggest that for all hypertensive patients systolic BP should be reduced at least below 140 mmHg and diastolic at least below 90 mmHg (Mancia et al. 2007). In diabetics and other high-risk patients systolic BP should be below 130 mmHg and diastolic BP below 80 mmHg (Chobanian et al. 2003, Mancia et al. 2007). According to systematic reviews of clinical trials, antihypertensive treatment significantly reduces the risk for nonfatal and fatal cardiovascular events (Collins and MacMahon 1994, Lawes et al. 2004).

Despite effective antihypertensive drugs only 34% of hypertensive patients on medication have their BP controlled to below 140/90 mmHg in USA (Chobanian et al. 2003). In Finland the situation seems to be even more unsatisfactory (Antikainen et al. 2006, Kastarinen et al. 2009). Reasons for poor BP control include unfavorable lifestyle habits, poor compliance to drug therapy, excessive salt intake, poor motivation of physicians to act in order to reach BP goals and individual variation in BP response to antihypertensive drugs (Elliot 2008).
2.3.2 Nonpharmacological treatment of hypertension

The adoption of healthy lifestyles is recommended for all individuals either to prevent or manage hypertension (Chobanian et al. 2003). The lifestyle modifications that are proven to lower BP and/or reduce cardiovascular risk factors are cessation of smoking, weight management, dietary modifications, moderate alcohol consumption and physical activity. However, as the compliance with healthy lifestyles is very weak, implementation of lifestyle modifications should not inappropriately delay the onset of drug treatment, at least with high-risk patients.

Even though cessation of smoking does not reduce BP or may even increase BP (Lee et al. 2001), it is recommended to all hypertensive patients, as smoking is one of the most significant cardiovascular risk factors (Doll et al. 1994). Nicotine replacement and other pharmacological therapy may be effective to facilitate smoking cessation (Tonstad et al. 2003, Stead et al. 2008).

Weight reduction lowers BP in overweight subjects and has favorable effects on other cardiovascular risk factors. Even a modest 4.5 kg reduction of body weight lowers BP significantly (Neter et al. 2003). In addition to weight loss, dietary modifications have other beneficial effects on BP. Reduced sodium intake with diet rich in fruits, vegetables, and low-fat dairy products with reduced content of cholesterol and saturated and total fat (the DASH diet) seem to have BP lowering effects (Sacks et al. 2001). Alcohol consumption is recommended to be limited to 20–30 g of ethanol per day for hypertensive men and to 10-20 g of ethanol per day for women (Mancia et al. 2007).

Regular dynamic endurance training lowers BP, and the BP response to training is more pronounced in hypertensive patients compared with normotensives (Cornelissen et al. 2005). Regular aerobic exercise at least 30 minutes daily is recommended for all hypertensive patients (Mancia et al. 2007).

2.3.3 Pharmacological treatment of hypertension

According to ESC and ESH guidelines, antihypertensive treatment should be initiated in all patients with grade 2 and 3 hypertension (Table 1), i.e. when BP level is ≥160/100.
Antihypertensive treatment is also recommended to grade 1 hypertensive patients after several months’ follow-up even though the evidence of benefits of treatment is more equivocal with these patients. For diabetic patients antihypertensive therapy is recommended when systolic BP is \( \geq 130 \) mmHg or diastolic BP is \( \geq 85 \) mmHg (Mancia et al. 2007), although supporting evidence is still limited (Mancia et al. 2009). Antihypertensive treatment can be initiated with any of the drugs from five main classes of antihypertensive agents, including diuretics, beta-blockers, calcium antagonists, ACE inhibitors and angiotensin receptor antagonists, as the main benefits of antihypertensive therapy are based on lowering of BP (Turnbull 2003). Sites of action of different classes of antihypertensive drugs are shown in Figure 2.

![Sites of action of different classes of antihypertensive drugs. ACE, angiotensin converting enzyme; ATII, angiotensin II type 1.](image)

Only about one third of all hypertensive patients have their BP controlled on one drug and most of the patients will need two or more drugs (Cushman et al. 2002). Antihypertensive agents from different classes can be combined to reach BP goal and it
is shown that low doses of drugs in combination increases efficacy and reduces adverse effects (Law et al. 2003). According to large meta-analysis by Law et al. (2003), the average BP reduction was rather similar for drugs from five main classes of antihypertensive agents used at standard dose. For single-drug treatment, the average BP reduction was 9.1 mmHg for systolic BP and 5.5 mmHg for diastolic BP.

**Diuretics.** Thiazide-type diuretics have long been recommended as first-line antihypertensive agents, but are now seen more as one possibility among others to initiate BP treatment (Mancia et al. 2007). Thiazides lower BP by inhibiting sodium and chloride co-transport across the membrane of the distal convoluted tubule within nephron. Diuretic treatment causes an initial plasma and extracellular fluid volume contraction leading to a decrease in cardiac output. However, with prolonged treatment plasma volume and cardiac output return towards normal as a consequence counter-regulatory mechanisms activating RAS and sympathetic nervous system. The exact BP lowering mechanism of diuretics is not fully known, but during chronic treatment of diuretics peripheral resistance decreases (Hughes 2004). As side effects, thiazides cause electrolyte disturbances including hypokalemia, hypomagnesemia and hyponatremia, and metabolic disorders including hyperuricemia, hyperlipidemia, glucose intolerance and insulin resistance (Dupont 1993). In addition to thiazides, loop diuretics have antihypertensive effects but are not superior to thiazides on efficacy or side effects. Spironolactone may be effective in patients with treatment-resistant hypertension and may also be used in patients with hypertension and hypokalemia (Jansen et al. 2009). Potassium-sparing diuretics triamterene and amiloride are mostly used in combination with a thiazide. Hydrochlorothiazide is a commonly used thiazide-type diuretic in the treatment of hypertension. The plasma half-life of hydrochlorothiazide is 8–15 hours enabling long-term dosing, and it is excreted unchanged by the kidneys (Carter et al. 2004). The recommended daily dose of hydrochlorothiazide is 12.5-50 mg.

**Beta-blockers.** Beta-blockers have served as basis for antihypertensive therapy along with thiazide-type diuretics for many years. However, during the recent years the rationale of using beta-blockers as first-line therapy for hypertension has been questioned after results from two large trials showed a reduced ability of beta-blockers to protect against stroke (Dahlöf et al. 2002, Dahlöf et al. 2005). In addition to hypertension, beta-blockers are used in a wide range of indications including chronic
heart failure, coronary heart disease, atrial fibrillation and other arrhythmias. Beta-blockers bind to beta-adrenergic receptors and antagonize the effects of the endogenous agonists norepinephrine and epinephrine. The competitive inhibition of beta-receptors leads to reduction in cardiac output, attenuation in renin release, adrenergic neuron-inhibiting effects and decrease in central sympathetic nervous activity, but the exact BP lowering mechanism of beta-blockers is not fully known (Prichard et al. 1980). There are various beta-blockers that can be classified by their relative selectivity for ADRB1. In addition, some beta-blockers have also alpha-receptor antagonist activity. The BP lowering effect of beta-blockers does not seem to be related to ADRB1 selectivity but ADRB1-selective agents are less likely to cause bronchial and metabolic side effects. Bisoprolol is a widely used, highly ADRB1-selective beta-blocking agent with a plasma half-life of 10-12 hours. About half of bisoprolol is excreted unchanged by the kidneys and the other half is metabolized by the liver to three inactive metabolites. Usual daily dose of bisoprolol is 2.5-20 mg.

**Calcium antagonists.** Calcium antagonists are among the most widely used antihypertensive drugs. They block L-type voltage-gated calcium channels in the heart and vasculature. Calcium antagonists reduce intracellular calcium levels leading to decreased cardiac contractility and cardiac output in the heart and decreased peripheral resistance in the vasculature. There are three major classes of calcium antagonists based on their relative effects on cardiac versus vascular calcium channels. Dihydropyridines block calcium channels preferentially in vascular smooth muscle, which causes vasodilatation and lowering of BP. Verapamil, a phenylalkylamine, has more effects on the myocardium, and diltiazem, a benzothiazepine, has intermediate effects between the other two groups. Dihydropyridines are most suitable for antihypertensive therapy, and diltiazem and verapamil are recommended for use in hypertensive patients with angina pectoris, carotid atherosclerosis and supraventricular tachycardia (Mancia et al. 2007). The adverse effects of calcium antagonists include gastrointestinal symptoms, mostly seen with verapamil and diltiazem, and vasodilative side effects and gingival hyperplasia with dihydropyridines. Amlodipine, a dihydropyridine, is a long acting vasoselective calcium antagonist with a plasma half-life of 35-50 hours. It is extensively metabolized in the liver and mostly excreted by the kidneys (Reid et al. 1988). The normal daily dose of amlodipine is 2.5-10 mg.
ACE inhibitors. ACE inhibitors, initially developed for treatment of hypertension, are now in widespread use in the treatment of cardiovascular and renal diseases. They inhibit both the conversion of Ang I to Ang II and the degradation of bradykinin. Accordingly, they reduce the vasoconstricting and fluid-retentive effects of Ang II and promote the vasodilatative effects of bradykinin (Brown et al. 1998). As a consequence, ACE inhibitors reduce peripheral vascular resistance without causing significant change in heart rate (Lund-Johansen et al. 1993). Besides antihypertensive effect, ACE inhibitors have cardiac and renal protective effects independent of BP lowering. They increase cardiac output in patients with congestive heart failure (Levine et al. 1980) and improve renal blood flow and sodium excretion (Hollenberg et al. 1981). There are three classes of ACE inhibitors with different chemical structures and pharmacokinetic properties (Brown et al. 1998). The adverse effects of ACE inhibitors include hyperkalemia, decreased renal function, cough and, rarely, angioedema.

Angiotensin receptor antagonists. Angiotensin receptor antagonists act by selectively blocking the binding of Ang II to AT1R, resulting in a decrease in peripheral resistance. The BP lowering and other beneficial effects of angiotensin receptor antagonists are very much the same as with ACE inhibitors (Schmieder 2005). However, as angiotensin receptor antagonists do not affect the degradation of bradykinin, cough is not among side effects. In generally, angiotensin receptor antagonists are well-tolerated even though hyperkalemia and renal dysfunction may be occasionally noted (Burnier et al. 2000). Losartan was the first selective AT1R-blocking agent available on the market. Losartan itself has a short half-life of about 2 hours, but it is converted via cytochrome P450 2C9 (CYP) and CYP3A4 to a longer acting active metabolite, which is responsible for the most of the pharmacological activity of losartan (Sica et al. 2005). Conventional daily dose of losartan is 50-100 mg.

Other antihypertensive drugs. In addition to agents from five main classes of antihypertensive drugs, other pharmacological alternatives are available although, most of them have only minor importance in the treatment of essential hypertension. Centrally acting antihypertensive agents, such as alpha2-adrenergic agonists (e.g., clonidine) and imidazoline receptor agonist (moxonidine), may be useful in patients with treatment-resistant hypertension (Sica 2007). Alpha1-selective adrenergic receptor blockers (e.g., prazosin) have beneficial effects on lipid levels and insulin sensitivity
and relieve the symptoms of benign prostatic hypertrophy which might support their use in treatment of hypertension (Frishman et al. 1999). However, there was an increased incidence of heart failure in the alpha1-blocker doxazosin arm of the ALLHAT study compared with the chlorthalidone (a thiazide diuretic) arm, which argues against the use of alpha-blockers in treatment of hypertension (ALLHAT Officers and Coordinators 2000). Aliskiren, a direct renin inhibitor, is a novel antihypertensive agent. It provides effective BP reduction but the role of aliskiren in the management of cardiovascular diseases is not defined yet as the clinical outcome trials are still going on (Alfie et al. 2011).

2.3.4 Variation in blood pressure response to treatment

There is a marked individual variation in BP responses to antihypertensive agents. In a study where hypertensive men were randomly treated with either hydrochlorothiazide, atenolol, captopril, clonidine, diltiazem or prazosine as a monotherapy, the ranges of BP response were at least four times greater than average BP response (Materson et al. 1993, Materson et al. 1995). Similar variation in BP response to treatment has been seen in other studies (Attwood et al. 1994, Dickerson et al. 1999). In studies with crossover comparisons of BP response in individual patients, response to one drug does not seem to reliably predict response to another drug. However, weak correlations between BP response to ACE inhibitors and beta-blockers and BP response to calcium antagonists and diuretics have been reported (Bidiville et al. 1988, Attwood et al. 1994, Dickerson et al. 1999, Deary et al. 2002).

The variation of individual BP response to an antihypertensive agent is determined by a variety of pharmacokinetic and pharmacodynamic mechanisms modified by environmental and demographic factors (Materson 2007). Attempts have been made to predict BP response to antihypertensive treatment based on laboratory parameters, body size, age, gender and ethnicity, mostly with inconsistent results (Laragh et al. 1979, Chapman et al. 2002). There is also growing evidence that individual variation in BP response is partially genetically determined. To date, more than 60 publications have reported findings from pharmacogenetic studies of antihypertensive treatment, even though the results have been inconsistent (Arnett et al. 2009). Genetic factors might
influence BP response by altering pharmacokinetics of the antihypertensive agent or by changing the activity or quantity of any of the factors involved in the pharmacodynamic effects of the drug.

2.4 Nongenetic predictors of antihypertensive drug response

2.4.1 Demographic factors

The majority of elderly people are hypertensive, and in most of these cases the hypertension is predominantly systolic (Franklin et al. 2001). For older people with essential hypertension, diuretics and calcium antagonists are suggested as the initial antihypertensive agents. This is based on clinical trials showing that treatment of isolated systolic hypertension with a diuretic drug, or a calcium antagonist, has reduced the number of cardiovascular events in elderly people (Dahlöf et al. 1991, Meade 1992, Staessen et al. 1997a). These findings are supported by observations from studies evaluating the efficacy of the antihypertensive drugs for BP response. Materson et al. showed in a randomized double-blind study of 1292 hypertensive men, receiving either placebo, hydrochlorothiazide, atenolol, captopril, clonidine, diltiazem or prazosin for at least one year, that older men had the best BP response to hydrochlorothiazide and diltiazem (Materson et al. 1993, Materson et al. 1995). Correspondingly, Morgan et al. reported a crossover study where each of the 74 study subjects, aged 65-68 years, were receiving ACE inhibitors, beta-blockers, dihydropyridines, thiazide diuretics and placebo as monotherapy. In this randomized open trial the decrease in systolic BP was significantly greater with diuretics and calcium antagonists compared to beta-blockers and ACE inhibitors (Morgan et al. 2001). However, benefits have also been shown for drugs from the three other main classes of antihypertensive agents for the treatment of older hypertensive patients, and therefore any age-dependent strategy for antihypertensive treatment is not recommended (Mancia et al. 2007).

There is some evidence of gender-specific differences in BP responses to antihypertensive agents. In a clinical study with 240 hypertensive men and 265 hypertensive women, that BP response to hydrochlorothiazide was greater among women compared to men (Chapman et al. 2002). The data from the Women’s Health
Initiative Study supports these results, as postmenopausal women on diuretic monotherapy had their BP controlled better compared to those who were receiving monotherapy using either a beta-blocker, an ACE inhibitor or a calcium antagonist, even though there may have been several confounding factors behind these results (Wassertheil-Smoller et al. 2000). It has been speculated that these findings may be related to lower plasma renin activity in hypertensive women compared to men (Alderman et al. 2004), therefore implying, that calcium antagonists and diuretics might be superior to beta-blockers and ACE inhibitors in hypertensive women. However, there are studies showing equal BP responses in elderly women to atenol, enalapril and isradipine (Perry et al. 1994), and to atenol, enalapril and diltiazem (Applegate et al. 1991).

Racial differences in BP response to antihypertensive drugs have also been observed. African Americans are shown to respond less favorably to beta-blockers and ACE inhibitors as monotherapy when compared to European and Hispanic Americans (Materson et al. 1995, Mokwe et al. 2004). Conversely, black hypertensive subjects respond well to diuretics and calcium antagonist (Saunders et al. 1990, Chapman et al. 2002). The difference in BP responses for African Americans was also observed in the ALLHAT study, with over 15 000 black subjects, where ACE inhibitors were demonstrated to be less effective than diuretics or calcium antagonists in lowering BP (ALLHAT Officers and Coordinators 2002). It is thought that these differences are related to an expanded plasma volume and suppressed plasma renin activity in African American hypertensives (Gillum 1979). Other American ethnic groups, Hispanics and Asians, do not seem to differ from Caucasians in response to antihypertensive agents (Jamerson et al. 1996).

Obesity is a major risk factor for hypertension, and it seems that obesity may alter response to antihypertensive agents. In a small study of 18 lean and 18 obese men, with mild to moderate hypertension, there was a better diastolic BP response to isradapine in the lean patients and to metoprolol in the obese patients (Schmieder et al. 1993). Correspondingly, a study with 1292 hypertensive men demonstrated that after one year of treatment obese patients (BMI >30) were 2.5 times more likely to have their BP controlled by atenolol than hypertensive patients with normal weight (BMI <27) (Materson et al. 2003). In this study, there were no other BMI-associated differences in
BP response to the study drugs. It is possible that obese patients might therefore benefit more from beta-blockers, as they have an enhanced sympathetic activity which leads to an increased cardiac output (Rocchini 1992, Grassi et al. 1996). However, there are no specific recommendations for the management of obese patients in current guidelines.

2.4.2 Laboratory tests

From the 1970s until recently, pretreatment PRA in choosing initial antihypertensive drugs has been advocated. This is based on the assumption that patients with high renin values are candidates for monotherapy with ACE inhibitors or beta-blockers, while patients with low renin values are candidates for monotherapy with diuretics or calcium antagonists (Laragh et al. 1979).

The positive association of high pretreatment renin values with BP response to ACE inhibitors and angiotensin receptor antagonists has been demonstrated in several studies (Ikeda et al. 1997, Flack et al. 2003, Canzanello et al. 2008, Minami et al. 2008). In the study of Canzanello et al. (2008), 203 African American and 236 non-Hispanic white subjects with essential hypertension were treated with candesartan for 6 weeks, with pretreatment PRA and other measurements incorporated into linear regression models. Even though pretreatment PRA did predict BP response to candesartan, the predictive ability of PRA in the model was rather low. Furthermore, in another study, inclusion of pretreatment PRA into the logistic regression model made only a borderline contribution to the prediction of BP responses after controlling for baseline diastolic BP, ethnicity and age (Preston et al. 1998).

There is also evidence that low pretreatment PRA is associated with better BP response to thiazide diuretics (Cody et al. 1983, Freis et al. 1983, Blaufox et al. 1992), although, studies with controversial results have also been published (Holland et al. 1979). In a study by Chapman et al (2002), a total of 505 African American and Caucasian hypertensive patients were treated for four weeks with hydrochlorothiazide. In this non-controlled trial, lower pretreatment PRA predicted better response to hydrochlorothiazide. These findings are also supported by the study of Preston et al. (1998), where patients with mild hypertension and a low-renin profile had better BP
response rates with hydrochlorothiazide, diltiazem and prazosin. However, in the study
of Chapman et al. (2002) age and gender were the most important explanatory variables
in a stepwise multiple regression analysis, yet the model accounted for only 33% of the
variation of systolic and 13% of the diastolic BP responses after additive contributions
of age, race, gender, baseline BP, PRA and urinary aldosterone excretion.

The association of high PRA levels with better BP response to beta-blockers is
supported by many reports, even though most of them are open single-drug studies from
the 1970s (Cody et al. 1983, Freis et al. 1983, Blaufox et al. 1992). The association of
BP response to calcium antagonists in patients with low PRA seems to be more
controversial. Some of the studies have been able to observe this association (Erne et al.
1983, Kiowski et al. 1985, Resnick et al. 1987, Kusaka et al. 1991), but there is at least
an equal amount of studies unable to confirm it (Bidiville et al. 1988, Evans et al. 1990,
Cappuccio et al. 1993), some with a large number of study subjects (Preston et al.
1998).

2.4.3 Blood pressure levels

Higher pretreatment BP level is correlated with greater BP response to antihypertensive
drugs. Some of the earlier studies have suggested that this effect is particularly
pronounced with calcium antagonists, and that this drug class might be especially
suitable in patients with very high BP values (MacGregor et al. 1982, Erne et al. 1983,
Muller et al. 1984). However, in a study by Sumner et al., with a total of 255
normotensive and hypertensive subjects, correlations of pretreatment BP level with BP
response to ACE inhibitors, calcium antagonists, direct vasodilators, prazosin and the
ADRB1-selective beta-blocker flusoxolol were all very similar, demonstrating that
correlation of pretreatment BP with BP response is not specific to a particular
antihypertensive drug class or agent (Sumner et al. 1988).
2.5 Genetic predictors of antihypertensive response

2.5.1 Renin-angiotensin system genes

Cloning of the genes coding for the components of RAS (Figure 1) has led to discovery of several polymorphisms in the genes for ACE, angiotensinogen (AGT) and AT1R. Among these polymorphisms, the insertion/deletion (I/D), (rs4341) of the ACE gene, Met235Thr of the AGT gene (rs699) and 1166A/C of the AT1R gene (rs5186) have raised the greatest attention in relation to cardiovascular diseases, including pharmacogenetic studies of hypertension (Koopmans et al. 2003). A summary of pharmacogenetic studies of the genes of the RAS is shown in Table 2.

The ACE I/D polymorphism consists of either the presence or absence of a 287-base-pair fragment in intron 16 of the ACE gene on chromosome region 17q. It has been related to serum ACE activity, with the D allele linked to increased activity (Rigat et al. 1990, Tiret et al. 1992). However, as the ACE I/D polymorphism does not seem to have an effect on ACE expression or function, the true functional genetic variation behind the association probably lies elsewhere in the ACE gene (Zhu et al. 2000). The ACE I/D polymorphism has been associated with hypertension in several studies (Kiema et al. 1996, O'Donnell et al. 1998, Higaki et al. 2000). Although in a meta-analysis of 23 case-control studies, the association of hypertension with the D allele of the ACE I/D does not seem to be significant (Staessen et al. 1997b).

The ACE I/D polymorphism has been shown to be associated with BP response to angiotensin receptor antagonists, ACE inhibitors and diuretics with inconsistent results. Some studies have demonstrated an association of better BP response to a thiazide diuretic, an angiotensin receptor antagonist and different ACE inhibitors to the ACE II genotype (Ohmichi et al. 1997, Haas et al. 1998, O'Toole et al. 1998, Kurland et al. 2001, Sciarrone et al. 2003). While others associate with the DD genotype (Stavroulakis et al. 2000, Li et al. 2003). One study has even suggested a gender-specific association of BP response to hydrochlorothiazide with the ACE I/D genotype (Schwartz et al. 2002). Additionally, there are many earlier studies showing no significant difference in BP response with different angiotensin receptor antagonists, ACE inhibitors or other
antihypertensive drugs between the ACE I/D genotype groups (Hingorani et al. 1995, Dudley et al. 1996, Harrap et al. 2003, Yu et al. 2003, Redon et al. 2005, Schelleman et al. 2006a, Schelleman et al. 2006c, Filigheddu et al. 2008). In the GenHAT study almost 40 000 hypertensive patients were randomized to chlorthalidone, amlodipine, lisinopril or to doxazosin treatment and followed up for 4 to 8 years (Arnett et al. 2005). In that large study, there was no association between ACE I/D genotype group and BP response to study drugs, or with the primary outcomes of the study.

The AGT gene, located on chromosome region 1q42, has proven to be highly polymorphic. The AGT Met235Thr variation is in tight linkage disequilibrium with the -G/6A nucleotide substitution in the promoter region, and has been studied most extensively in relation to hypertension (Jeunemaitre et al. 1999). There is an association between the 235Thr allele and increased plasma AGT levels. However, the G/6A substitution seems to be the functional polymorphism, even if its relationship to the true in vivo biological effect is not fully known (Jeunemaitre 2008).

Some of the earlier studies have found association between the Met235Thr polymorphism and hypertension (Jeunemaitre et al. 1992, Caulfield et al. 1994, Nishiuma et al. 1995) while others have not (Barley et al. 1994, Fornage et al. 1995). Results from a few meta-analyses seem to demonstrate a mild but statistically significant association for the AGT Met235Thr to hypertension, even though there are marked racial and ethnic differences (Staessen et al. 1999, Sethi et al. 2003).

Most of the pharmacogenetic studies have failed to show any association for the AGT Met235Thr polymorphism to BP response to different antihypertensive drugs (Dudley et al. 1996, Katsuya et al. 2001, Kurland et al. 2001, Schelleman et al. 2006a). There are, however, at least two studies suggesting that Met235Thr may be related to antihypertensive responses. Hingorani et al. found that AGT Met235Thr was an independent predictor of BP response to ACE inhibitors, in a non-controlled open study with 125 untreated hypertensives (Hingorani et al. 1995). Kurland et al performed a randomized double-blind study where 97 subjects with mild to moderate hypertension were treated for 12 weeks with either atenolol or irbesartan as monotherapy (Kurland et al. 2004). In that study with a relatively small number of study subjects, BP response to atenolol was enhanced in subjects with the Met235Met genotype or the AGT -6A allele.
In addition to AGT Met235Thr and AGT -G/6A, there are also other polymorphisms in the AGT gene of which AGT -217G/A and -20A/C in the promoter area have been related to BP response to ACE inhibitors (Woodiwiss et al. 2006).

In the AT1R gene on chromosome regions 3q21-3q25, there are more than 20 single-nucleotide polymorphisms (SNPs), all identified in the non-coding regions of the gene (Jeunemaitre 2008). A nucleotide change from adenine to cytosine at position 1166 in the 3’-untranslated region of the AT1R gene (1166A/C) has been linked to cardiovascular diseases (Bonnardeaux et al. 1994). The C allele of 1166 A/C has been associated with hypertension (Hingorani et al. 1995, Kainulainen et al. 1999). However, according to a meta-analysis of 38 studies, the literature is heterogeneous and the evidence for the association is insufficient (Mottl et al. 2008). Most of the pharmacogenetic studies have shown no difference in BP response to ACE inhibitors, angiotensin receptor antagonists or other drugs between the AT1R 1166 A/C genotypes (Hingorani et al. 1995, Katsuya et al. 2001, Kurland et al. 2001, Kurland et al. 2004, Redon et al. 2005, Filigheddu et al. 2008, Gluszek and Jankowska 2008). However, there is one study showing better BP response to hydrochlorothiazide in African-American women with the A/A genotype (Frazier et al. 2004), with two other studies suggesting that the C allele favors increased BP response to an ACE inhibitor and an angiotensin receptor antagonist (Miller et al. 1999, Benetos et al. 1996).
Table 2. RAS gene polymorphisms and blood pressure response to antihypertensive drugs. Summary of the earlier studies.

<table>
<thead>
<tr>
<th>Author / Drug</th>
<th>Polymorphism</th>
<th>No of subjects</th>
<th>Design / Method of BP measurement</th>
<th>Results (BP response)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Studies with positive association:</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Hingorani et al. 1995 Captopril, enalapril, lisinopril, perindopril</td>
<td>AGT Met235Thr, AT1R 1166A/C</td>
<td>125</td>
<td>Hypertensive subjects in a 4-week open study (OBP).</td>
<td>Thr235 &gt; Met235 (SDB, DBP)</td>
</tr>
<tr>
<td>Benetos et al. 1996 Perindopril, nitrrendipine</td>
<td>AT1R 1166A/C</td>
<td>311</td>
<td>Hypertensive subjects in a 2-month open study (OBP).</td>
<td>C carriers &gt;AA with response to perindopril (SBP, DBP)</td>
</tr>
<tr>
<td>Ohmichi et al. 1997 Imidapril</td>
<td>ACE I/D</td>
<td>57</td>
<td>Hypertensive subjects in a 6-week study (OBP).</td>
<td>II &gt; DD and ID (DBP)</td>
</tr>
<tr>
<td>Haas et al. 1998 Enalapril</td>
<td>ACE I/D</td>
<td>36</td>
<td>Hypertensive subjects with renal disease in a 6-month study (OBP).</td>
<td>ACE I carriers &gt; DD (SBP, DBP)</td>
</tr>
<tr>
<td>O’Toole et al. 1998 Captopril, lisinopril</td>
<td>ACE I/D</td>
<td>34</td>
<td>Subjects with heart failure in a 6-week randomized study (ABP).</td>
<td>I &gt; D with captopril (mean ABP)</td>
</tr>
<tr>
<td>Ueda et al. 1998 Enalaprilat 30 min intravenous infusion</td>
<td>ACE I/D</td>
<td>23</td>
<td>Normotensive men in a 10-hour single-dose placebo-controlled study.</td>
<td>II &gt; DD (MBP)</td>
</tr>
<tr>
<td>Miller et al. 1999 Losartan</td>
<td>AT1R 1166A/C</td>
<td>66</td>
<td>A 3-hour single-dose study.</td>
<td>AC/CC &gt; AA (MBP)</td>
</tr>
<tr>
<td>Stavroulakis et al. 2000 Fosinopril</td>
<td>ACE I/D</td>
<td>104</td>
<td>Hypertensive subjects in a 6-month study (OBP).</td>
<td>DD &gt; ID and II (SBP and DBP)</td>
</tr>
<tr>
<td>Kurland et al. 2001 Irbesartan, atenolol</td>
<td>ACE I/D, AT1R 1166A/C, AGT Met235Thr, AGT Thr174Met</td>
<td>86</td>
<td>Hypertensive subjects with LVH in a 3-month open study (OBP).</td>
<td>ACE II &gt; ID and DD with response to irbesartan (DBP)</td>
</tr>
<tr>
<td>Schwartz et al. 2002 Hydrochlorothiazide</td>
<td>ACE I/D</td>
<td>376</td>
<td>Hypertensive subjects in a 2-month double-blind study</td>
<td>II &gt; DI and DD a 4-week study (OBP) (women) DD &gt; DI and II (men) (SBP and DBP)</td>
</tr>
<tr>
<td>Li et al. 2003 Benazepril</td>
<td>ACE I/D</td>
<td>89</td>
<td>Hypertensive subjects in a 2-month study (OBP).</td>
<td>DD &gt;DI and II (SBP and DBP)</td>
</tr>
<tr>
<td>Sciarrone et al. 2003 Hydrochlorothiazide</td>
<td>ACE I/D</td>
<td>87</td>
<td>Hypertensive subjects in a 2-month study (OBP).</td>
<td>II &gt; DD (MBP)</td>
</tr>
<tr>
<td>Kurland et al. 2004 Irbesartan, atenolol</td>
<td>A total of 30 SNPs in candidate genes in the RAS.</td>
<td>97</td>
<td>Hypertensive subjects in a 3-month double-blind study (OBP).</td>
<td>AGT 235Thr &gt; Met with response to atenolol (SBP)</td>
</tr>
<tr>
<td>Spiering et al. 2005 Active metabolite of losartan</td>
<td>AT1R 1166A/C</td>
<td>29</td>
<td>A 90-min single-dose study.</td>
<td>CC &lt; AA during high salt diet (SBP and DBP)</td>
</tr>
<tr>
<td>Woodiwiss et al. 2006 Enalapril, lisinopril, nifedipine</td>
<td>AGT -217G/A, AGT -20A/C</td>
<td>194</td>
<td>Hypertensive black subjects in a 2-month open study (ABP).</td>
<td>217 G carriers &gt;AA, 20 C carriers &gt; AA, with response to ACEi (SBP and DBP)</td>
</tr>
<tr>
<td>Author / Drug</td>
<td>Polymorphism</td>
<td>No of subjects</td>
<td>Design / Method of BP measurement</td>
<td>Results (BP response)</td>
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<tr>
<td>Todd et al. 1995</td>
<td>ACE I/D</td>
<td>27</td>
<td>Healthy men in a 6-hour single-dose study.</td>
<td>I = D (MBP)</td>
</tr>
<tr>
<td>Sasaki et al. 1996</td>
<td>ACE I/D</td>
<td>60</td>
<td>Hypertensive subjects in a 12-month study (OBP).</td>
<td>I = D (SBP and DBP)</td>
</tr>
<tr>
<td>Dudley et al. 1996</td>
<td>ACE I/D, Met235Thr</td>
<td>66</td>
<td>Hypertensive subjects in a randomized placebo-controlled crossover study (ABP).</td>
<td>I = D Met = Thr (SBP and DBP)</td>
</tr>
<tr>
<td>Nakano et al. 1997</td>
<td>ACE I/D</td>
<td>82</td>
<td>Hypertensive subjects in an 1 hour single-dose study.</td>
<td>I = D (SBP and DBP)</td>
</tr>
<tr>
<td>Mondorf et al. 1998</td>
<td>ACE I/D AGT Met235Thr</td>
<td>121</td>
<td>Hypertensive subjects in an 1 hour single-dose study.</td>
<td>I= D, Met= Thr (SBP and DBP)</td>
</tr>
<tr>
<td>Harrap et al. 2003</td>
<td>ACE I/D</td>
<td>5688</td>
<td>Subjects with previous stroke participating the PROGRESS trial (OBP).</td>
<td>I = D (SBP and DBP)</td>
</tr>
<tr>
<td>Yu et al. 2003</td>
<td>ACE I/D</td>
<td>517</td>
<td>Hypertensive subjects in a 6-week randomized study (OBP).</td>
<td>I=D (SBP and DBP)</td>
</tr>
<tr>
<td>Arnett et al. 2005</td>
<td>ACE I/D</td>
<td>37 939</td>
<td>A double-blind outcome trial. 6-months treatment with study drugs (OBP).</td>
<td>I = D (SBP and DBP)</td>
</tr>
<tr>
<td>Redon et al. 2005</td>
<td>ACE I/D, AGT -6A/G AT1R 1166A/C AT1R Cys573Thr</td>
<td>206</td>
<td>Hypertensive subjects in an 12-month study (OBP).</td>
<td>No differences among genotype groups</td>
</tr>
<tr>
<td>Schelleman et al. 2006a</td>
<td>Diuretics, beta-blockers, ACE inhibitors</td>
<td>625</td>
<td>A prospective cohort study. BP data collected from general practitioners and drug response data from pharmacy records (OBP).</td>
<td>I = D (SBP and DBP)</td>
</tr>
<tr>
<td>Schelleman et al. 2006b</td>
<td>Diuretics, beta-blockers, CCBs, ACE inhibitors</td>
<td>3025</td>
<td>A prospective cohort study (OBP).</td>
<td>Met = Thr (SBP and DBP)</td>
</tr>
<tr>
<td>Scelleman et al. 2006c</td>
<td>Diuretics, beta-blockers, CCBs, ACE inhibitors</td>
<td>3025</td>
<td>A prospective cohort study (OBP).</td>
<td>I = D (SBP and DBP)</td>
</tr>
<tr>
<td>Gluszek et al. 1998</td>
<td>AT1R 1166A/C</td>
<td>64</td>
<td>Hypertensive subjects in an 8-week study (OBP, ABP).</td>
<td>A = C (SBP and DBP)</td>
</tr>
<tr>
<td>Filigheddu et al. 2008</td>
<td>ACE I/D, AT1R 1166A/C, aldosterone synthase 344C/T, AGT -6A/G</td>
<td>191</td>
<td>Hypertensive subjects in a 4-week study (OBP).</td>
<td>No differences among genotypes</td>
</tr>
</tbody>
</table>

BP, blood pressure; ABP, ambulatory blood pressure; OBP, office blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; SBP, systolic blood pressure; LVH, left ventricular hypertrophy.
2.5.2 Alpha-adducin gene

Adducin is a ubiquitously expressed cytoskeletal protein that is composed of one α-subunit combined with either a β- or a γ-subunit, all encoded by different genes (Matsuoka et al. 2000). Adducin promotes the organization of a spectrin-actin lattice and controls the rate of actin polymerization (Hughes et al. 1995). It may also be involved in cellular signal transduction, and interacts with Na-K-ATPase (Manunta et al. 2007).

A point mutation, Phe316Tyr, in the α-adducin gene was first discovered in the Milan hypertensive rat strain, and was demonstrated to be involved in BP variation in the Milan rats by affecting kidney tubular ion transport (Bianchi et al. 1994, Tripodi et al. 1996). The human α-adducin (ADD1) gene, on chromosome region 4p16.3, is highly homologous to the rat ADD1 gene. In a subsequent study it was found that the α-adducin locus was associated with hypertension (Casari et al. 1995). Cusi et al. (1997) reported that the Gly460Trp polymorphism (rs4961) in the human ADD1 gene was associated with hypertension, noting that hypertensive patients heterozygous for the 460Trp allele had better BP response to two month treatment with hydrochlorothiazide compared to wild-type homozygotes (14.7 vs. 6.8 mmHg). The mechanism for these findings has been suggested to be that the ADD1 460Trp allele is linked to higher activity of the sodium pump, thereby leading to increased tubular reabsorption of sodium in the kidneys, and ultimately to salt-sensitivity and hypertension (Manunta et al. 1998, Ferrandi et al. 1999, Manunta et al. 1999). Consistent with these findings, it has also been reported that hypertensive patients carrying the Trp allele have a larger BP increase in response to saline infusion and lower PRA compared to the GlyGly homozygotes (Cusi et al. 1997, Glorioso et al. 1999, Barlassina et al. 2000b).

The association of ADD1 Gly460Trp with hypertension, reported by Cusi et al., has been confirmed by some studies (Castellano et al. 1997, Iwai et al. 1997, Barlassina et al. 2000, Province et al. 2000), but not by others (Ishikawa et al. 1998, Kamitani et al. 1998, Kato et al. 1998, Busch et al. 1999, Wang et al. 1999). In addition to hypertension, ADD1 has been related to cardiovascular outcome. In two prospective population studies, the 460Trp allele was associated with increased risk of total and
cardiovascular mortality, as well as to cardiovascular, cardiac and coronary events (Li et al. 2005, Gerhard et al. 2008). In a third prospective study, the 460Trp allele was associated with increased risk to ischemic and hemorrhagic stroke in blacks (van Rijn et al. 2006).

In addition to the study of Cusi et al. (1997), the 460Trp allele has been associated with better BP response to hydrochlorothiazide in three other studies with newly diagnosed hypertensive Italian subjects. Glorioso et al. (1999) performed a prospective study with 143 hypertensive patients from Milan and Sassari and confirmed that BP response to two months of hydrochlorothiazide treatment was better in patients carrying the 460Trp allele. Correspondingly, Sciarrone et al. (2003) demonstrated that hypertensive patients carrying at least one ACE I allele and one ADD1 Trp allele had the best mean BP response to hydrochlorothiazide (12.7 mmHg vs. 3.4 mmHg in DD-GlyGly group). In the most recent study using 193 hypertensive Italian subjects, both systolic and diastolic BP response to one-month treatment of hydrochlorothiazide was significantly better in subjects with the 460Trp allele (Manunta et al. 2008). None of these four studies with positive results have been placebo-controlled. There is a population-based case-control study showing that in subjects carrying the ADD1 460Trp allele, diuretic therapy was associated with a lower risk of combined myocardial infarction and stroke compared to other antihypertensive therapies (Psaty et al. 2002).

Collectively, the potential association of the ADD1 Gly460Trp polymorphism with hypertension and BP response to thiazide-type diuretics has reached extensive proportions in the scientific litterature. In one of the recent review articles on adducin polymorphisms and hypertension, detection of the ADD1 Gly460Trp polymorphism has been stated as “an example of a prospective efficacy of pharmacogenetics and pharmacogenomics” that “may support new strategies aimed at optimizing the use of new antihypertensive agents for the prevention of hypertension-associated organ damage” (Manunta et al. 2007). As a consequence of this enthusiasm, the published literature on hypertension and ADD1 consists of over 190 original articles and over 70 review articles indexed in PubMed (august 2011).
Table 3. Alpha-adducin gene Gly460Trp polymorphism and blood pressure response to antihypertensive drugs. Summary of the earlier studies.

<table>
<thead>
<tr>
<th>Author / Drug</th>
<th>No of subjects</th>
<th>Design / Method of BP measurement</th>
<th>Results (BP response)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Studies with positive association:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cusi et al. 1997 Hydrochlorothiazide</td>
<td>58</td>
<td>Hypertensive subjects in a 2-month open study (OBP).</td>
<td>Gly460Trp &gt; Gly460Gly (MBP)</td>
</tr>
<tr>
<td>Glorioso et al. 1999 Hydrochlorothiazide</td>
<td>143</td>
<td>Hypertensive subjects in a 2-month open study (OBP).</td>
<td>460Trp carriers &gt; Gly460Gly (MBP)</td>
</tr>
<tr>
<td>Sciarrone et al. 2003 Hydrochlorothiazide</td>
<td>87</td>
<td>Hypertensive subjects in a 2-month open study (OBP).</td>
<td>460Trp carriers &gt; Gly460Gly (MBP)</td>
</tr>
<tr>
<td>Manunta et al. 2008 Hydrochlorothiazide</td>
<td>193</td>
<td>Hypertensive subjects in a one-month open study (OBP).</td>
<td>460Trp carriers &gt; Gly460Gly (SBP and DBP)</td>
</tr>
<tr>
<td>Studies with no association:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turner et al. 2003 Hydrochlorothiazide</td>
<td>585</td>
<td>Hypertensive subjects in a 4-week open study (OBP).</td>
<td>Gly460 = Trp460 (SBP and DBP)</td>
</tr>
<tr>
<td>Schelleman et al. 2006a Diuretics, beta-blockers, ACE inhibitors</td>
<td>625</td>
<td>A prospective cohort study. BP data collected from general practitioners and drug data from pharmacy records (OBP).</td>
<td>Gly460 = Trp460 (SBP and DHP)</td>
</tr>
<tr>
<td>Schelleman et al. 2006b Diuretics, beta-blockers, ACE inhibitors, CCBs</td>
<td>3025</td>
<td>A prospective cohort study (OBP).</td>
<td>Gly460 = Trp460 (SBP and DHP)</td>
</tr>
<tr>
<td>Davis et al. 2007 Chlorthalidone, amlodipine lisinopril, doxazosin</td>
<td>36913</td>
<td>A double-blind outcome trial. 6-month treatment with study drugs (OBP).</td>
<td>Gly460 = Trp460 (SBP and DBP)</td>
</tr>
</tbody>
</table>

None of the studies was placebo-controlled. ACE, angiotensin converting enzyme; BP, blood pressure; CCB, calcium channel blocker; DBP, diastolic blood pressure; OBP, office blood pressure; MBP, mean blood pressure; SBP, systolic blood pressure.

Despite the promising results on the association of the ADD1 460Trp allele with enhanced BP response to hydrochlorothiazide in four Italian studies, the same association has not been reproduced in studies using other populations. Turner et al. could not demonstrate any Gly460Trp polymorphism-related effect on BP response to four week treatment with hydrochlorothiazide, in a study of 291 African Americans and 294 non-Hispanic whites (Turner et al. 2003). Negative results were also obtained in two cohort studies from the Netherlands (Schelleman et al. 2006a, Schelleman et al. 2006b). Supporting these results, in the GenHAT study, with a total of 37 000 individuals, there was neither association of the Gly460Trp genotype with BP response to chlorthalidone, amlodipine, lisinopril or doxazosin, nor better primary clinical outcome in subjects carrying the Trp allele whilst on chlorthalidone treatment (Davis et al. 2007). Table 3 provides a summary of pharmacogenetic studies on the ADD1 Gly460Trp polymorphism.
2.5.3 Beta-adrenergic receptor genes

ADRB1 is the main subtype of beta-adrenergic receptors expressed in the heart and its activation leads to positive inotropic and chronotropic effects. The ADRB1 protein is encoded by an intronless gene located on chromosome region 10q24-26 (Frielle et al. 1987). Several SNPs have been identified in the human ADRB1 coding region. However, most of them occur with an allele frequency <1-2% (Brodde 2008). The two main SNPs in the ADRB1 gene are found in codons 49 (rs 1801252) and 389 (rs1801253) (Maqbool et al. 1999), and correspond to amino acid variations Ser49Gly in the amino terminus of the receptor and Arg389Gly in the carboxy terminus of the receptor. As a consequence of the linkage disequilibrium between the codon 49 and 389 polymorphisms, genotype combinations Gly49Gly/Gly389Gly are very rarely seen.

The Ser49Gly and Arg389Gly polymorphisms of ADRB1 are functionally active (Brodde 2008). The Ser49Gly variation does not influence agonist binding or adenylyl cyclase activity of the receptor, while the Gly49 receptor showed enhanced agonist-induced down-regulation compared to the Ser49 receptor, in studies with hamster fibroblasts (Levin et al. 2002, Rathz et al. 2002). Whereas, the Arg389 receptor exhibits higher adenylyl cyclase activity, increased isoprenaline-induced adenylyl cyclase activation and increased agonist-promoted desensitization, when compared to the Gly389 receptor (Rathz et al. 2003, Joseph et al. 2004). The in vitro findings are supported by results from clinical studies on resting haemodynamics. In a study by Ranade et al. (2002), the Ser49 allele was associated with significantly higher resting heart rate. In a sibling-pair study within Finnish population, siblings homozygous for the Arg389 allele had significantly higher diastolic BP and resting heart rate than carriers of the Gly389 allele (Bengtsson et al. 2001a) and another study with Finnish population Arg389 allele was associated with higher systolic BP during exercise (Nieminin et al. 2006). Consistently, in another study with 142 subjects undergoing dobutamine stress echocardiography, subjects homozygous for the Arg389 allele had significantly higher resting heart rate and diastolic BP (Humma et al. 2001). However, there is a study presenting slightly elevated BP in carriers of the Gly389 allele (McCaffery et al. 2002), while studies that show no influence of the Arg389Gly polymorphism on resting haemodynamics have also been published (Buscher et al. 2001, Liu et al. 2003, Sofowora et al. 2003).
Studies examining the possible association of Ser49Gly and Arg389Gly with hypertension have proven to be inconsistent. The association of Arg389Gly with hypertension has been proposed in two case-control studies, reporting increased prevalence of the Arg389 allele in hypertensive patients (Bengtsson et al. 2001a, Shioji et al. 2004), while two other studies have demonstrated no association (Ranade et al. 2002, Filigheddu et al. 2004).

In a prospective study of 40 hypertensive patients, Johnson et al. found that 24 hour and day-time diastolic ABP responses to metoprolol were greater in patients homozygous for the Agr389 allele compared to carriers of the Gly389 allele (-12 % for Arg389Arg vs. -5.1 % for Gly389 carriers) (Johnson et al. 2003). They also discovered that patients with the haplotype Ser49Ser/Arg389Arg had significantly better diastolic ABP response than patients with the haplotype Ser49Gly/Arg389Gly. In that small study, there was a marked racial imbalance between the genotype groups, and the metoprolol doses were varying since they were titrated according to BP responses. However, these findings are supported by a study with 61 Chinese hypertensive patients. This study showed that the best response to four week treatment with metoprolol was in patients homozygous for the Arg389 allele and in patients with the haplotype pair Ser49Arg389/Ser49Arg389, respectively (Liu et al. 2006). They also found that systolic BP response to metoprolol was better in patients homozygous for the Ser49 allele, compared with Ser49Gly heterozygotes. However, subjects with the haplotype pairs Ser49Arg389/Ser49Gly389 and Gly49Arg389/Gly49Arg389 of ADRB1 were excluded from the analyses, with the study population being selected from a total of 223 previously genotyped patients. In addition, two other small studies with healthy volunteers have noted similar results (Liu et al. 2003, Sofowora et al. 2003).

The positive findings of association between the Arg389 allele and increased BP response to beta-blockers (Johnson et al. 2003., Liu et al. 2006) has brought high expectations upon Arg389 as a clinically relevant tool for treatment of hypertension by some of the authors (Shin and Johnson 2007). In one of the review articles of the field, the reports of Johnson et al. and Liu et al. have been seen as key articles to cardiovascular pharmacogenomics (Aquilante et al. 2009). However, three other studies with 52-270 hypertensive patients, and one study with healthy volunteers, failed to

Table 4. Beta1-adrenergic receptor gene polymorphisms and blood pressure response to beta-blockers. Summary of the earlier studies.

<table>
<thead>
<tr>
<th>Author / Drug</th>
<th>Polymorphism</th>
<th>No of subjects</th>
<th>Design / Method of BP measurement</th>
<th>Results (BP response)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Studies with positive association:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Johnson et al. 2003</td>
<td>Ser49Gly Arg389Gly</td>
<td>40</td>
<td>Hypertensive subjects in ≥ 4-week study (ABP).</td>
<td>Arg389 &gt; Gly389 (DBP)</td>
</tr>
<tr>
<td>Liu et al. 2003</td>
<td>Arg389Gly</td>
<td>16</td>
<td>Healthy volunteers in a 3-day exercise and resting trial.</td>
<td>Arg389 &gt; Gly389 (SBP)</td>
</tr>
<tr>
<td>Sofowora et al. 2003</td>
<td>Arg389Gly</td>
<td>34</td>
<td>Healthy volunteers in a single-dose exercise and resting trial.</td>
<td>Arg389 &gt; Gly389 (resting SBP and MBP)</td>
</tr>
<tr>
<td>Liu et al. 2006</td>
<td>Ser49Gly Arg389Gly</td>
<td>61</td>
<td>Selected hypertensive subjects in a 4-week study (OBP).</td>
<td>Arg389 &gt; Gly389 (SBP and DBP) Ser49Ser &gt; Ser49Gly (SBP)</td>
</tr>
<tr>
<td><strong>Studies with no association:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O'Shaughnessy et al. 2000</td>
<td>Arg389Gly</td>
<td>147</td>
<td>Hypertensive subjects in a 4-week open study (OBP).</td>
<td>Arg389 = Gly389</td>
</tr>
<tr>
<td>Kumar et al. 2008</td>
<td>Ser49Gly Arg389Gly</td>
<td>41</td>
<td>Healthy volunteers in a single-dose exercise and resting trial.</td>
<td>Ser49 = Gly49 Arg389 = Gly389</td>
</tr>
</tbody>
</table>

None of the studies was placebo-controlled. BP, blood pressure; ABP, ambulatory blood pressure; OBP, office blood pressure; DBP, diastolic blood pressure; LVH, left ventricular hypertrophy; MBP, mean blood pressure; SBP, systolic blood pressure.

ADRB2 is encoded by an intronless gene on chromosome region 5q31-32 (Kobilka et al. 1987). In the ADRB2 gene, 12 SNPs have been identified (Brodde 2008). Two of them, Arg16Gly (rs1042713) and Gln27Glu (rs1042714), may have functional effects, since there is some evidence that they alter the degree of agonist-promoted down-regulation of receptor expression (Green et al. 1994). There is strong linkage disequilibrium between these codon 16 and 27 SNPs, so that subjects homozygous for Glu27 are almost always homozygous for Gly16.
ADRB2 is a potential candidate gene for hypertension since ADRB2-mediated vasodilatation does contribute to BP regulation. However, the association data for the ADRB2 Arg16Gly and Gln27Glu polymorphisms with hypertension seem very contradictory. There are some studies showing association of the Gly16 allele with hypertension (Kotanko et al. 1997, Bray et al. 2000), while others show association with the Arg16 allele to hypertension (Timmermann et al. 1998, Bengtsson et al. 2001b) with still others showing no association (Jia et al. 2000, Herrmann et al. 2002).

There is only one earlier report on positive association of ADRB2 polymorphisms with antihypertensive drug responses, suggesting that the Gly16 allele predicts better response to an ACE inhibitor (Huang et al. 2004).

### 2.5.4 Other genes

In addition to polymorphisms in the RAS system, alpha-adducin and beta-adrenergic genes, genetic variations of some other genes have been reported to be associated with BP response to different antihypertensive agents, at least in single studies.

There is some evidence suggesting that BP response to diuretics may be associated with the Glu298Asp polymorphism of the nitric oxide synthase gene, NEDD4L rs4149601 polymorphism, and five SNPs in WNK1 gene (Turner et al. 2003, Manunta et al. 2008). In a genome-wide association study of Turner et al., there was an association between BP response to hydrochlorothiazide and SNPs in the lysozyme gene (LYZ) and Yeats domain-containing protein 4 gene (YEATS4), with no evidence of association for variation in the RAS genes (Turner et al. 2008).

Single studies have shown that BP response to atenolol or other beta-blockers associate with polymorphisms in genes for KCNMB1 (Glu65Lys), GNB3 (Cys825Thr) and low-density lipoprotein receptor (C16730T) (Filigheddu et al. 2004, Liljedahl et al. 2004, Kelley-Hedgepeth et al. 2008). Moreover, there is one study demonstrating an association between the Arg347Cys polymorphism of the alpha1A-adrenoceptor gene and BP response to nifedipine (Zhang et al. 2009), while another study suggest an association between polymorphisms of the L-type calcium channel alpha1c and alpha1d
subunit genes with BP response to dihydropyridine calcium channel blockers (Kamide et al. 2009).

Perhaps the most convincing evidence of genetic effects on the responses to antihypertensive therapies comes from studies on polymorphisms of drug metabolizing enzymes. Many of the beta-blockers are substrates of the CYP2D6 enzyme, and 70-80% of metoprolol is metabolized through this highly polymorphic pathway. It has been demonstrated that CYP2D6 genotype has a profound effect on the pharmacokinetics of metoprolol, and there is some evidence that CYP2D6 genotype may be associated with BP response to metoprolol (Bijl et al. 2009). Angiotensin receptor antagonists are metabolized by the CYP2C9 enzyme, with reports showing that BP response to irbesartan and losartan may be modified by genetic variation in CYP2C9 (Hallberg et al. 2002, Sekino et al. 2003). However, the relationship between CYP2C9 variation and losartan responsiveness was not seen in the study of Donner et al. (2009). It has also been shown that polymorphisms in genes for N-acetyltransferase (NAT2) and catechol-o-methyltransferase (COMT) exert a significant effect on the metabolism of hydralazine and alpha-methyldopa (Shepherd et al. 1981, Lachman et al. 1996).

2.5.5 Rationale for the selected candidate gene polymorphisms

In the present study, the tested polymorphisms were chosen on the basis of functional relevance, either to BP regulation, or through reported association with hypertension or antihypertensive drug response.

Of the polymorphisms in the genes encoding the components of the RAS, the ACE I/D (rs4341) and AGT Met235Thr (rs699) polymorphisms were chosen, as they have been reported to have functional significance (Rigat et al. 1990, Jeunemaitre 2008) and to be associated with hypertension (O’Donnel et al. 1998, Higaki et al. 2000, Jeunemaitre et al. 1992, Caulfield et al. 1994) and BP response to different antihypertensive drugs (Ohmichi et al. 1997, Haas et al. 1998, Sciarrone et al. 2003, Hingorani et al. 1995, Kurland et al. 2004). Additionally, the AGTR1 A/C polymorphism (rs5186) was selected, as it has been associated with hypertension and BP response to different

The ADD1 Gly460Trp polymorphism (rs4961) was selected due to it being associated with BP response to diuretics in earlier studies (Cusi et al. 1997, Glorioso et al. 1999, Sciarrone et al. 2003, Manunta et al. 2008). Furthermore, it has been reported that the ADD1 Gly460Trp polymorphism may be associated with hypertension (Cusi et al. 1997, Castellano et al. 1997, Barlassina et al. 2000) with some evidence that it may also be associated with enhanced renal tubular reabsorption of sodium through the activation of Na,K-ATPase (Manunta 1998).

Of the polymorphisms in the gene coding for ADRB1, Arg389Gly (rs1801253) and Ser49Gly (rs1801253) were chosen. These have both been shown to be functionally active (Levin et al. 2002, Rathz et al. 2002, Rathz et al. 2003, Joseph et al. 2004) and associated with hypertension and BP response to beta-blockers (Bengtsson et al. 2001a, Shioji et al. 2004, Johnson et al. 2003, Liu et al. 2006). The Gly16Arg (rs1042713) and Glu27Gln (rs1042714) polymorphisms of the ADRB2 gene were studied as there is some evidence that they may have an effect on receptor function (Green et al. 1994).
3. AIMS OF THE STUDY

The principal aim of the present study was to explore the associations of clinical characteristics, a number of clinically relevant laboratory variables and common genetic polymorphisms, with blood pressure response to an angiotensin II receptor antagonist (losartan), a beta-blocker (bisoprolol), a calcium channel blocker (amlodipine) and a thiazide diuretic (hydrochlorothiazide) in subjects with moderate hypertension. In order to avoid several weaknesses of many previous studies, the present study used a placebo-controlled, double-blind and cross-over design. Moreover, both office and 24-h blood pressure recordings were employed.

The specific aims were as follows:

1. To examine the relationship between blood pressure levels and basic clinical characteristics with blood pressure response to each of the study drugs, and to evaluate a possible correlation of blood pressure responses between the different study drugs in a placebo-controlled, cross-over setting.

2. To evaluate the association of baseline laboratory test results with blood pressure response to four antihypertensive drugs in a placebo-controlled, cross-over setting.

3. To assess the association of common variations in the angiotensin II type I receptor, angiotensin converting enzyme, alpha-adducin, angiotensinogen, beta1-adrenergic receptor and beta2-adrenergic receptor genes with blood pressure response to four antihypertensive drugs in a placebo controlled, cross-over design.
4. SUBJECTS AND METHODS

4.1 Subjects

In all studies (I-IV), the subjects were moderately hypertensive Finnish men aged from 35 to 60 years that were recruited to the GENRES Study (A Randomized Double-Blind Cross-Over Single-Centre Placebo-Controlled Study on Molecular Genetics of Drug Responsiveness in Essential Hypertension) through newspaper advertisements. Hypertension was defined as three previously measured diastolic BP readings ≥95 mmHg or current use of antihypertensive medication. The first study subject was recruited in October, 1999 and the last subject completed the study in February, 2004.

Exclusion criteria included treatment with three or more antihypertensive drugs, secondary hypertension, drug-treated diabetes mellitus, congestive heart failure, coronary heart disease, cerebrovascular disease, kidney disease (serum creatinine >115 µmol/L), obstructive pulmonary disease, a disease treated with corticosteroids, clinically significant liver disease, abuse of drugs or alcohol and obesity (BMI ≥32 kg/m²). The subject was withdrawn during the study if an exclusion criterion was met, the BP level rose to ≥200/120 mmHg or non-compliance was recorded. To compensate for the withdrawals, new subjects were recruited.

Based on a priori power calculations the number of subjects to complete the study was intended to be 192 and for this a total of 313 subjects were screened for the study.

4.2 Study design

The GENRES Study was designed as a randomized double-blind placebo-controlled cross-over study for the pharmacogenomic analysis of hypertension (Figure 3). The study started with a four-week run-in placebo period followed by four treatment periods, during which the subjects used one of four study drugs, in a randomized order, as monotherapy for four weeks. The treatment periods were separated by placebo periods, each also lasting for four weeks. Randomization was done after the first
placebo period. The study drugs were a thiazide diuretic (hydrochlorothiazide 25 mg; Hydrex semi, Orion Pharma), a beta-blocker (bisoprolol 5 mg; Emconcor, Merck KGaA), an angiotensin receptor blocker (losartan 50 mg; Cozaar, Merck & Co.) and a calcium antagonist (amlodipine 5 mg; Norvasc, Pfizer). All preparations, including placebo, were packed in identical capsules with one taken daily between 6 and 9 a.m. Any previous antihypertensive medication, used by 81% of the study subjects, was discontinued before the first placebo period.

Fasting blood samples were taken at the end of the first placebo period for determination of PRA and serum glucose, sodium, potassium, chloride, total calcium, creatinine, uric acid, aldosterone, total cholesterol, HDL cholesterol, triglycerides and insulin concentrations. In addition, urine samples were collected for the determination of daily urinary excretion of sodium, potassium, chloride and albumin.

Of the 313 subjects screened for the study, at least one placebo period was completed by 244 subjects and at least one active drug period was completed by 233 subjects. All four placebo periods were completed by 211 subjects, and all four active drug periods by 208 subjects. Reasons for withdrawal during the study were: BP over 200/120 mmHg (12 subjects), aortic dilatation (7 subjects), significant left ventricular hypertrophy (1 subject), previous myocardial infarction detected in echocardiography (2 subjects), angina pectoris (3 subjects), atrial fibrillation (4 subjects), asthma (2 subjects), normotension (7 subjects), non-compliance (8 subjects), kidney disease (4 subjects), other medical causes (6 subjects) and personal reasons (49 subjects).
4.3 Ethical aspects

The GENRES study was approved by the Ethical Committee of Helsinki University Central Hospital and it was conducted in accordance with the Declaration of Helsinki and Guidelines for Good Clinical Practice. In addition, the study design was approved by the National Agency for Medicines of Finland. All subjects gave signed informed consent before any study-related activities.

Even though placebo was administered, the study did not violate ethical considerations, since placebo periods were short and individuals with severe hypertension or significant comorbidities were excluded or withdrawn. In addition, the study provided useful patient-specific data for individual drug treatment.

4.4 Measurement of blood pressure

4.4.1 Office blood pressure measurement

Office (OBP) and ambulatory (ABP) blood pressure measurements were carried out before the first placebo period and after each placebo and treatment period. OBP measurements were taken between 7.30 and 11 a.m. on the same day of the week within
a two-hour time interval for each subject. The non-dominant arm was used for the measurements. The results of BP measurements were revealed to the subject only if the readings were to cause withdrawal from the study.

The OBP measurements were carried out after a 30-minute rest in the sitting position using a semi-automated oscillometric device (Omron® M4; Omron Healthcare). Three measurements were taken with one-minute intervals and the mean of the last two was used in the analyses. The same device was used for each subject on every visit.

### 4.4.2 Ambulatory blood pressure measurement

ABP measurements were carried out with a device equipped with a QRS complex detector and a position sensor (Diasys Integra; Novacor). Readings were taken every 15 minutes when the subject was in an upright position and every 30 minutes when the subject was in a supine position. Strong physical activity was not permitted, however the subjects were recommended to continue normal life during the recordings.

The 24-hour ABP was calculated as the mean of daytime and nighttime values adjusted according to the number of daytime and nighttime hours. Daytime hours were defined as from 7 a.m. to 10 p.m. and nighttime hours defined as from 10 p.m. to 7 a.m. A minimum number of measurements were required for a recording to be accepted, these were ≥15 for daytime and ≥7 for nighttime.

### 4.4.3 White-coat effect and nocturnal dipping

The systolic and diastolic white-coat effect was determined by subtracting daytime systolic and diastolic ABP levels from sitting systolic and diastolic OBP levels, respectively. The nocturnal dipping was determined as difference between day- and nighttime ABP values.
4.5 Biochemical determinations

4.5.1 Serum and plasma analyses

PRA and serum aldosterone levels were determined by radioimmunoassay (DiaSorin RIA). Serum glucose was measured with an enzymatic hexokinase method (Glucoquant Glucose/HK, Roche Diagnostics GmbH). Serum total cholesterol (Cholesterol CHOD-PAP, Roche Diagnostics GmbH), HDL cholesterol (HDL-C plus 3rd generation, Roche Diagnostics GmbH), triglycerides (Triglycerides, Roche Diagnostics GmbH), creatinine (Crea Plus, Roche Diagnostics GmbH) and uric acid (Uric Acid plus, Roche Diagnostics GmbH) were measured with enzymatic colorimetric tests. Serum sodium, potassium, and chloride were measured by ion-selective electrodes (Roche Hitachi MODULAR, Hitachi Ltd). Serum total calcium was measured using o-cresolphthalein complexone method (Calcium, Roche Diagnostics GmbH) and serum insulin was measured by a time-resolved immunofluorometric assay (Perkin Elmer, Wallac). The laboratory analyses were performed by Helsinki University Central Hospital Laboratory.

4.5.2 Urine analyses

Urinary sodium and potassium were analysed by automated flame photometry (IL 943, Instrument Laboratory (UK) Ltd), urinary chloride by coulometric titration (Corning model 925 chloride analyser, Ciba Corning Diagnostics Corp) and urinary albumin using an immunoturbidimetric assay (Tina-quant Albumin; Roche Diagnostics GmbH).

4.6 Genetic analyses and DNA isolation

The molecular methods are described in detail in the original studies III and IV. DNA was extracted from peripheral leukocytes by the salting-out method (Miller et al. 1988). Genotyping of the polymorphisms was performed using polymerase chain reaction (PCR) followed by restriction enzyme digestion. Electrophoretic separation of the restriction fragments was carried out on agarose or polyacrylamide gel. Positive and
negative controls were included in each PCR reaction. The DD genotypes of ACE I/D polymorphism were doublechecked with insertion-specific primers (Tiret et al. 1992).

4.7 Statistics

The statistical software SPSS (versions 11.0-13.0, SPSS Inc., Chicago, IL, USA) was used for the analysis. Normality of variable distributions was assessed by the Shapiro-Wilk and Kolmogorov-Smirnov tests, analysis of skewness, and visual examination. The data are presented as mean (±SD) or median and interquartile range. Since most of the variables were non-normally distributed, pairwise correlations were analyzed using Spearman’s rho. Mann-Whitney U test was used in comparisons between two groups, and Kruskall-Wallis and Jonckheere-Terpstra trend tests in comparisons between three different groups. The average of placebo BP levels of a single subject from different placebo periods was used in the analysis. The ABP responses (post-treatment minus placebo blood pressure levels) were considered as the primary efficacy variables, since ABP recordings showed better repeatability during the placebo periods than OBP measurements, and as ABP levels are better correlated with end-organ damage than OBP levels (Verdecchia et al. 1990, Fagard et al. 1997).

In Study I, the antihypertensive responses between the study drugs were initially compared using the Friedman test and then pairwise with the Wilcoxon signed ranks test. Within-subject correlations between BP response (post-treatment minus placebo blood pressure levels) and different study drugs were analysed with Pearson’s correlation, using normalized z scores of the response values. In Study II, the association of laboratory variables (PRA, serum aldosterone, glucose, uric acid, creatinine, total cholesterol, HDL cholesterol triglycerides, sodium, potassium, chloride, insulin and total calcium and urinary sodium, potassium, chloride and albumin) with BP response was analyzed using partial correlation, controlling for the corresponding placebo BP level. Laboratory variables with significant correlations were additionally divided into quartiles, mostly for illustrative purposes, and BP response in the lowest quartile was compared against BP response in the highest quartile.
An additive model for genotype effect was used in Study III for all genotypes as a primary analysis. Furthermore, the rare allele homozygotes of the ADD1 and AT1R polymorphisms were pooled with heterozygotes for secondary analysis. In Study IV, a hypothesis-free approach was used to study the effect of the tested polymorphisms on dependent variables. Since the model of the effect (recessive / dominant / additive) of the analysed polymorphisms is not known, the minor allele homozygotes were not combined to heterozygotes in primary analyses. For the analysis of placebo BP levels and BP response to study drugs, comparisons were made between three genotype groups, and if the data suggested genotype-related differences, further analyses were performed using Mann-Whitney U test with major allele homozygotes as the reference group.

All significant findings in the initial analyses were subjected to multivariate analysis. Non-normally distributed response variables were transformed by the Blom method to approximate normal distribution in multivariate analysis. The Levene test was used to confirm the homogeneity of variances.

In Studies I and II the stepwise linear regression procedure was used with a significance level of \( P<0.05 \) as an entry criterion into the multivariate model. In Study I, explanatory variables in the analysis of BP levels and BP response included age, duration of hypertension, number of previous antihypertensive drugs, number of affected parents, BMI, waist-hip ratio (WHR), weight and triceps skinfold thickness all in the same model. In the analysis of laboratory test results in Study II, age, BMI, number of earlier antihypertensive medications and the corresponding BP level on placebo periods were included as explanatory variables (covariates) in multivariate analysis.

In Studies III and IV, multivariate analysis was carried out with the General Linear Model Univariate procedure of SPSS, using BP level or BP response (post-treatment minus placebo blood pressure levels) as the dependent variable. Earlier use of antihypertensive medication and earlier use of a thiazide diuretic (in Study III) were treated as fixed factors, while genotype, age, duration of hypertension, BMI, daily urinary excretion of sodium and potassium, and the corresponding BP level on placebo periods were covariates.
5. RESULTS

5.1 Clinical characteristics of the study subjects (Study I)

Principal clinical characteristics of the hypertensive male subjects are presented in Study I. Their mean age was 50.5 years and mean BMI was 26.8. According to placebo BP levels, the study subjects were moderately hypertensive. Only 19% of the subjects did not previously use antihypertensive medication (Table 5).

5.2 Blood pressure levels during placebo periods (Studies I-IV)

BP levels during the placebo periods are reported in Study I. The mean OBP was 153/100 mmHg and the mean 24-hour ABP 135/93 mmHg (Table 5). Twenty four hour ABP recording showed the best repeatability, with coefficients of variation of 3.6% for systolic and of 3.5% for diastolic BP during the placebo periods, compared to OBP measurements with coefficients of variation of 5.4% for systolic and of 5.2% for diastolic BP. The within-subject correlation between the two types of BP measurements was substantial. The Spearman’s correlation coefficients between OBP and 24-hour ABP values were 0.70/0.67 (systolic/diastolic), between OBP and daytime ABP values they were 0.68/0.64, and between OBP and nighttime ABP values they were 0.63/0.56.

Age was positively correlated with systolic and diastolic OBP (but not ABP) values, and BMI with diastolic OBP values (Study I, Table 2). Pulse pressure in office measurements was also greatly influenced by age. The median value was 46.5 mmHg in the lowest (<45.7 years) and 55.5 mmHg in the highest (>55.7 years) age quartile ($P<0.001$). The average WCE for systolic and diastolic pressures were 7.4 and 0.2 mmHg, respectively. WCE was more pronounced in older subjects. The median systolic and diastolic effects were 11.3 and 1.6 mmHg in the highest age quartile, and 1.0 and -1.7 mmHg in the lowest quartile ($P$ values $<0.001$). Systolic and diastolic WCE was strongly correlated with pulse pressure, even when age was controlled for in multivariate analysis.
The mean nocturnal dipping during the placebo periods was 26.2 for systolic and 18.3 mmHg for diastolic pressure (Study I, Table 1). The diastolic dipping value was negatively correlated with age and positively with BMI.

Table 5. Characteristics of the GENRES study population.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50.5 ± 6.4</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.8 ± 2.7</td>
</tr>
<tr>
<td>Triceps skinfold thickness (mm)</td>
<td>11.2 ± 5.2</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>0.99 ± 0.05</td>
</tr>
<tr>
<td>Duration of hypertension (years)</td>
<td>11.2 ± 8.5</td>
</tr>
<tr>
<td>Number of previous antihypertensive drugs (n / %)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>47 / 19 %</td>
</tr>
<tr>
<td>1</td>
<td>123 / 50 %</td>
</tr>
<tr>
<td>2</td>
<td>74 / 30 %</td>
</tr>
<tr>
<td>Number of parents with hypertension (n / %)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>90 / 37 %</td>
</tr>
<tr>
<td>1</td>
<td>110 / 45 %</td>
</tr>
<tr>
<td>2</td>
<td>44 / 18 %</td>
</tr>
<tr>
<td>BP levels and HR during placebo periods (mmHg)</td>
<td></td>
</tr>
<tr>
<td>Office measurements (sitting values)</td>
<td></td>
</tr>
<tr>
<td>systolic</td>
<td>153 ± 14</td>
</tr>
<tr>
<td>diastolic</td>
<td>100 ± 8</td>
</tr>
<tr>
<td>heart rate</td>
<td>70 ± 8</td>
</tr>
<tr>
<td>pulse pressure</td>
<td>52 ± 9</td>
</tr>
<tr>
<td>heart rate</td>
<td>70 ± 8.5</td>
</tr>
<tr>
<td>Ambulatory recordins</td>
<td></td>
</tr>
<tr>
<td>24 hours:</td>
<td></td>
</tr>
<tr>
<td>systolic</td>
<td>135 ± 11</td>
</tr>
<tr>
<td>diastolic</td>
<td>93 ± 6</td>
</tr>
<tr>
<td>heart rate</td>
<td>73 ± 8</td>
</tr>
<tr>
<td>Daytime:</td>
<td></td>
</tr>
<tr>
<td>systolic</td>
<td>145 ± 11</td>
</tr>
<tr>
<td>diastolic</td>
<td>100 ± 6</td>
</tr>
<tr>
<td>heart rate</td>
<td>79 ± 9</td>
</tr>
<tr>
<td>Nighttime:</td>
<td></td>
</tr>
<tr>
<td>systolic</td>
<td>119 ± 11</td>
</tr>
<tr>
<td>diastolic</td>
<td>82 ± 7</td>
</tr>
<tr>
<td>heart rate</td>
<td>64 ± 8</td>
</tr>
<tr>
<td>White coat effect (mmHg)</td>
<td></td>
</tr>
<tr>
<td>systolic</td>
<td>7.4 ± 10.3</td>
</tr>
<tr>
<td>diastolic</td>
<td>0.2 ± 5.9</td>
</tr>
<tr>
<td>Nocturnal dipping of BP (mmHg)</td>
<td></td>
</tr>
<tr>
<td>systolic</td>
<td>26 ± 8</td>
</tr>
<tr>
<td>diastolic</td>
<td>18 ± 6</td>
</tr>
</tbody>
</table>

The 244 subjects with at least one placebo period completed are included. BP, blood pressure; HR, heart rate.
Pretreatment laboratory tests were correlated with placebo BP levels in Study II. Serum aldosterone concentration was significantly correlated with systolic and diastolic ABP as well as diastolic OBP levels. Less consistent correlations were found between BP levels and other laboratory variables (Study II, Table 2).

The effect of genetic variation in the ADD1, RAS, ADRB1 and ADRB2 genes on placebo BP levels was tested in Studies III and IV. There were no statistically significant differences in placebo BP levels between genotype groups for the ACE I/D, ADD1 Gly460Trp, AGT Met235Thr, AT1R 1166A/C, ADRB1 Ser49Gly and Arg389Gly and ADRB2 Arg16Gly and Gln27Glu polymorphisms (Study III, Table 2 and Study IV, Table 1). However, certain polymorphisms provided small, although nonsignificant, differences in placebo BP levels. Thus systolic and diastolic ABP and systolic OBP tended to decrease for each ADRB1 389Gly allele, while the ADRB2 16Gly allele tended to be associated with slightly higher BP levels.

The ADD1 460Trp and AGT 235Thr alleles were associated with higher systolic WCE, and these associations were statistically significant in multivariate analysis ($P=0.04$ for ADD1 Gly460Trp and $P=0.03$ for AGT Met235Thr). However, there was no significant association between diastolic WCE and the ADD1 or AGT genotypes (Study III, Table 2).

### 5.3 Antihypertensive responses to study drugs (Study I)

When the mean/median BP response of the study drugs are compared, bisoprolol 5 mg had the strongest antihypertensive effect in both ABP and OBP measurements, followed by losartan 50 mg, amlodipine 5 mg, and then hydrochlorothiazide 25 mg (Figure 4). In 24-hour ABP recordings, only 2.9/0 % (systolic/diastolic) of the subjects were non-responders to bisoprolol, defined as a higher BP level compared to placebo. With the other study drugs non-responders comprised 8.3/8.8 % of the subjects on losartan, 12.6/10.2 % on amlodipine, and 20.8/34.3 % on hydrochlorothiazide. None of the subjects was a non-responder for each of the four study medications in ambulatory 24-hour recordings. The daytime ABP response (in mmHg) was higher than the nighttime response for bisoprolol, losartan, and amlodipine. In contrast, hydrochlorothiazide
response during the nighttime was greater than during the daytime ($P=0.15$ for systolic and $P=0.003$ for diastolic response, Wilcoxon signed ranks test).

Figure 4. Antihypertensive responses. A: Office sitting blood pressure. B: Ambulatory 24-hour blood pressure. C: Ambulatory daytime blood pressure. D: Ambulatory nighttime blood pressure. Medians and interquartile ranges are shown. Statistical significances between adjacent medications were calculated with Wilcoxon signed ranks test. BP, blood pressure; HCT, hydrochlorothiazide.
Overall, there was large between-subject variation in the antihypertensive responses to specific drugs. BP response to bisoprolol and losartan showed highest within-subject correlation in all measurement modes, with $r$ values ranging from 0.32 to 0.39, followed by response to amlodipine and hydrochlorothiazide, with $r$ values ranging from 0.20 to 0.38. The lowest correlations were seen for the bisoprolol-amlodipine, bisoprolol-hydrochlorothiazide and losartan-hydrochlorothiazide pairs (Figure 5). The correlations were in most cases higher for ABP than for OBP response.

Figure 5. Correlations of intrindividual ambulatory 24-h systolic (A) and diastolic (B) ambulatory blood pressure responses between the study drugs. Spearman’s correlation coefficients ($r$) are shown.
The WCEs during the active treatment periods did not differ statistically significantly from each other or from the placebo periods, although bisoprolol seemed to reduce the effect slightly. The median systolic effects ranged from 5.5 mmHg (bisoprolol) to 7.5 mmHg (losartan and amlodipine), \( P=0.34 \) in Friedman test, and diastolic effects from -1.5 mmHg (bisoprolol) to 0.0 mmHg (losartan), \( P=0.78 \) in Friedman test.

Pulse pressure in office measurements was reduced by all study drugs \( P \) values <0.002. The median reductions were 4.0 mmHg for bisoprolol, 2.5 mmHg for losartan and hydrochlorothiazide, and 1.4 mmHg for amlodipine.

### 5.4 Non-genetic predictors of antihypertensive response

#### 5.4.1 Demographic factors (Study I)

The relationships between BP response to study drugs and age, BMI, triceps skin fold thickness, WHR, weight, duration of hypertension, number of previous antihypertensive drugs, and number of affected parents were analyzed in Study I.

For bisoprolol and losartan, no significant associations of these explanatory variables with BP response were found in neither pairwise nor multivariate analysis (Table 6).

Age was positively correlated with OBP and ABP response to amlodipine and hydrochlorothiazide, even though the association with diastolic 24-hour ABP response to hydrochlorothiazide became statistically significant only after multivariate analysis. For example, the median 24-hour systolic response to amlodipine was 4.8 mmHg higher in the highest age quartile than in the lowest quartile. Although the duration of hypertension was positively correlated with age \( (r=0.15, P=0.02) \), the associations with BP response to hydrochlorothiazide was in the opposing direction (Table 6).

BMI was negatively correlated with 24-hour systolic and diastolic ABP response to amlodipine (both \( r \) values -0.14, \( P<0.05 \)), but not with OBP response.
Table 6. Correlation matrix of blood pressure responses to study drugs with several variables.

<table>
<thead>
<tr>
<th></th>
<th>Amlodipine</th>
<th>Bisoprolol</th>
<th>Hydrochlorothiazide</th>
<th>Losartan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OBP</td>
<td>ABP</td>
<td>OBP</td>
<td>ABP</td>
</tr>
<tr>
<td>Age</td>
<td>0.26‡/0.20†</td>
<td>0.24‡/0.26‡</td>
<td>-0.07/-0.07</td>
<td>0.10/0.02</td>
</tr>
<tr>
<td>Duration of hypertension</td>
<td>-0.05/0.01</td>
<td>-0.07/-0.04</td>
<td>0.05/0.09</td>
<td>0.09/0.06</td>
</tr>
<tr>
<td>Number of antihypertensive drugs</td>
<td>0.20‡/0.06</td>
<td>0.14*/0.13</td>
<td>-0.08/-0.06</td>
<td>0.02/0.08</td>
</tr>
<tr>
<td>Number of affected parents</td>
<td>-0.13/-0.13</td>
<td>0.08/0.02</td>
<td>-0.06/-0.03</td>
<td>-0.02/0.00</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.14*/-0.14*</td>
<td>-0.01/-0.02</td>
<td>-0.09/-0.03</td>
<td>-0.03/-0.08</td>
</tr>
<tr>
<td>WHR</td>
<td>0.10/0.09</td>
<td>-0.02/0.02</td>
<td>-0.01/-0.02</td>
<td>-0.03/0.06</td>
</tr>
<tr>
<td>Triceps skinfold thickness</td>
<td>0.01/0.04</td>
<td>0.03/0.02</td>
<td>-0.08/-0.07</td>
<td>0.09/0.01</td>
</tr>
</tbody>
</table>

BMI, body mass index; ABP, ambulatory blood pressure; OBP, office blood pressure; WHR, waist hip ratio. The values shown are Spearman’s correlation coefficients ($r$) for systolic / diastolic responses, and a positive coefficient indicates a better drug response with increasing value of explanatory variable. * P < 0.05, † P < 0.01, ‡ P < 0.001.

5.4.2 Blood pressure levels (Study I)

BP levels during the placebo periods were positively associated with BP response to the study drugs (Table 7). The antihypertensive drug response to all of the study drugs was greater in the highest placebo BP quartile compared to the lowest placebo BP quartile (Study I, Figure 3). In 24-hour ABP recordings, this trend was especially pronounced for amlodipine (both systolic and diastolic pressures) and hydrochlorothiazide (systolic pressure).

There were some correlations of pulse pressure with OBP response to study drugs. Both systolic and diastolic ABP and OBP response to amlodipine, and systolic ABP and OBP response to hydrochlorothiazide were positively and statistically significantly correlated with placebo pulse pressure. In contrast, the response to bisoprolol and losartan were stable at different placebo pulse pressure levels.
Table 7. Correlation matrix of blood pressure responses to study drugs with corresponding placebo blood pressure level.

<table>
<thead>
<tr>
<th>Blood pressure response to</th>
<th>Placebo blood pressure level</th>
<th>Ambulatory</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Office</td>
<td>Ambulatory</td>
</tr>
<tr>
<td>Amlodipine</td>
<td>0.39‡ / 0.35‡</td>
<td>0.48‡ / 0.43‡</td>
</tr>
<tr>
<td>Bisoprolol</td>
<td>0.17* / 0.20†</td>
<td>0.21† / 0.25‡</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td>0.21† / 0.23‡</td>
<td>0.30‡ / 0.25‡</td>
</tr>
<tr>
<td>Losartan</td>
<td>0.14* / 0.16*</td>
<td>0.17* / 0.19†</td>
</tr>
</tbody>
</table>

The values shown are Spearman’s correlation coefficients (r) for systolic / diastolic responses.
* P<0.05, † P<0.01, ‡ P<0.001.

ABP response to amlodipine and the OBP response to hydrochlorothiazide were negatively correlated with nighttime dipping during placebo periods. The correlations were statistically significant in multivariate analysis with age and corresponding BP level on placebo included (data not shown). However, the BP response to bisoprolol and losartan were similar at different levels of nighttime BP dipping whilst on placebo.

There was a negative correlation between heart rate in OBP measurements during placebo periods to 24-hour ABP response to bisoprolol (P=0.02 for systolic and P=0.06 for diastolic response). The systolic OBP response to amlodipine was also negatively correlated with HR (P=0.03).

5.4.3 Laboratory tests (Study II)

The correlation between pretreatment laboratory tests to BP response to study drugs was analyzed in Study II. Of the laboratory tests, PRA was the most distinct predictor of the antihypertensive effect of losartan, and correlated positively with all BP responses to losartan in both pairwise and multivariate analysis (Table 8, Figure 6). In accordance with this, losartan exerted a significantly stronger antihypertensive response in the highest PRA quartile compared to the lowest quartile. Additionally, there was a positive correlation between PRA and BP response to bisoprolol (Table 8, Figure 6), with diastolic ABP being more effectively reduced in the high-renin quartile than in the low-renin quartile (Study II, Figure 1 C).
Baseline PRA was negatively correlated with BP response to hydrochlorothiazide, with these correlations being statistically significant in multivariate analysis with diastolic ABP and systolic OBP response (Table 8, Figure 6). The ABP response to hydrochlorothiazide was higher in the lowest, compared to the highest, PRA quartile. There was also a weaker correlation between PRA and BP response to amlodipine (Table 8, Figure 6). The BP lowering effect of amlodipine tended to be more noticeable in the low vs. high PRA quartile, however, the correlation was only statistically significant in the pairwise analysis with systolic ABP response (Study II, Figure 1 C). There was no significant correlation between baseline serum aldosterone levels and BP response to any of the study drugs.

Daily urinary excretion of sodium, chloride and potassium were all negatively correlated with BP response to amlodipine (Table 8). In multivariate analysis, inclusion
of sodium excretion removed the statistical significance of chloride and potassium excretions to ABP response. The ABP response to amlodipine treatment were significantly different \((P<0.05)\) between the lowest and highest quartiles of daily urinary excretion of sodium. No significant association was found for the response of the other three drugs with daily urinary excretion of sodium (Table 8).

Serum total calcium level was negatively correlated with BP response to amlodipine, but not to other drugs, in all measurement modes (Figure 7, Table 8). When systolic and diastolic ABP response to the study drugs was analyzed in the four quartiles of pretreatment serum calcium levels, ABP response to amlodipine was found to be significantly stronger in the lowest calcium quartile than in the highest calcium quartile (Study II, Figure 1C). In multivariate analysis, the association of serum calcium level to amlodipine response was statistically significant for all BP responses except for systolic ABP response \((P=0.08)\).

![Figure 7](image-url)

**Figure 7.** Correlation of serum total calcium level with blood pressure response to amlodipine. Statistical significance was analyzed as partial correlation controlling for the corresponding placebo blood pressure level. ABP, 24-h ambulatory blood pressure; OBP, office blood pressure
Serum total cholesterol level was negatively correlated with ABP response to amlodipine and to a lesser extent with BP response to bisoprolol, both in subjects with or without earlier statin therapy (Table 8). In addition, fasting serum glucose levels were correlated in an inverse manner with losartan response in all BP measurements.

Furthermore, there were less consistent correlations between serum triglyceride, insulin, creatinine, sodium and potassium levels to BP response to the four study drugs (Study II, Table 3).

Table 8. Correlation matrix of blood pressure responses with pretreatment laboratory test results.

<table>
<thead>
<tr>
<th></th>
<th>Amlodipin ABP Syst/diast</th>
<th>Amlodipin OBP Syst/diast</th>
<th>Bisoprolol ABP Syst/diast</th>
<th>Bisoprolol OBP Syst/diast</th>
<th>Hydrochlorothiazide ABP Syst/diast</th>
<th>Hydrochlorothiazide OBP Syst/diast</th>
<th>Losartan ABP Syst/diast</th>
<th>Losartan OBP Syst/diast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renin</td>
<td>-0.17*/-0.11 0.08/-0.02</td>
<td>0.15*/0.15*</td>
<td>0.14*/0.09</td>
<td>-0.16*/-0.18* -0.12/-0.16</td>
<td>0.20‡/0.25† 0.22‡/0.27‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aldosterone</td>
<td>0.01/-0.03 0.02/-0.04</td>
<td>0.05/0.08</td>
<td>0.03/0.03</td>
<td>-0.10/-0.04 -0.02/-0.07</td>
<td>-0.01/-0.04 0.03/0.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dU-Sodium</td>
<td>-0.27‡/-0.17* -0.05/-0.05</td>
<td>-0.05/0.04</td>
<td>0.05/0.09</td>
<td>-0.12/-0.11 -0.04/-0.06</td>
<td>-0.09/-0.03 0.07/0.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dU-Chloride</td>
<td>-0.25‡/-0.16* -0.05/-0.07</td>
<td>-0.03/0.05</td>
<td>0.09/0.14*</td>
<td>-0.09/-0.10 -0.09/-0.07</td>
<td>-0.04/-0.01 0.08/0.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dU-Potassium</td>
<td>-0.21†/-0.14* -0.18†/-0.12</td>
<td>-0.07/-0.04</td>
<td>0.03/0.08</td>
<td>-0.09/-0.10 -0.09/-0.07</td>
<td>-0.05/-0.06 -0.01/0.05</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Glucose</td>
<td>-0.05/-0.06 0.05/0.03</td>
<td>0.02/0.01</td>
<td>-0.01/-0.07</td>
<td>-0.04/-0.01 -0.11/-0.10</td>
<td>-0.14*/-0.16* -0.17*/-0.17*</td>
<td></td>
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</tr>
<tr>
<td>Cholesterol</td>
<td>-0.29‡/-0.23† -0.13/-0.12</td>
<td>-0.13/-0.08</td>
<td>-0.09/-0.17*</td>
<td>-0.02/-0.02 -0.06/-0.09</td>
<td>-0.04/-0.04 0.05/0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>-0.23†/-0.27‡ -0.18†/-0.16*</td>
<td>-0.02/0.00</td>
<td>-0.03/0.00</td>
<td>-0.01/-0.07 0.03/0.03</td>
<td>-0.03/0.02 -0.03/0.01</td>
<td></td>
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</table>

ABP, ambulatory blood pressure; OBP, office blood pressure; syst, systolic; diast, diastolic; dU, daily urinary. The values shown are partial correlation coefficients (r), controlling for the corresponding placebo BP level, for serum values unless otherwise indicated. A positive r value indicates a better antihypertensive response with increasing explanatory variable value. * P<.05, † P<.01, ‡ P<.001 for pairwise analysis.

5.5 Genetic variation as predictor of antihypertensive response

5.5.1 Renin-angiotensin system and alpha-adducin genes (Study III)

The effect of genetic variation in the RAS and ADD1 genes on BP response to the study drugs was evaluated in Study III.
The presence of the ADD1 460Trp allele was associated with lower systolic ABP response to hydrochlorothiazide, both in univariate ($P=0.01$) and in multivariate analysis ($P=0.03$) (Figure 8). Combining ADD1 460Trp allele carriers into a single group, or restriction of the analysis to the 184 subjects without earlier diuretic treatment did not change these results. However, the occurrence of the ADD1 460Trp allele was not significantly associated with diastolic ABP response or OBP response to hydrochlorothiazide treatment (Figure 8).

![Figure 8. Blood pressure responses to hydrochlorothiazide stratified by alpha adducin Gly460Trp genotype. Statistical significance between the genotype groups was calculated with Joncheere-Terpstra test. BP, blood pressure; IQR, interquartile range.](image)

There were no consistent or significant associations between the ADD1 460 polymorphism and BP response to amlodipine, bisoprolol or losartan (Study III, Table 4, Supplementary Information Tables 5 and 6).

There were no significant associations of the ACE I/D, AGT Met235Thr and AT1R 1166 A/C polymorphisms to BP response to any of the study drugs (Study III, Tables 3 and 4, and Supplementary Information Tables 5 and 6), although the AGT 235Thr allele was associated with slightly lower ABP response to losartan ($P=0.04$ for systolic and $P=0.14$ for diastolic ABP response in multivariate analysis). However, the association
was not seen in OBP response to losartan, where the observed trend was even in the opposing direction.

No significant gene-gene interactions associated with BP response to any of the study drugs. In addition, the effect of the combined ACE and ADD1 genotype on BP response was evaluated according to the study by Sciarrone et al. (2003). In this sub-analysis, there were no statistically significant differences between placebo BP levels or BP response to any of the drugs studied, when subjects with the combination of the ACE DD and ADD1 GlyGly genotype (n=37-40) were compared with subjects with the combination of the ACE II and ADD1 GlyTrp+TrpTrp genotype (n=14-18) (data not shown).

5.5.2 Beta-adrenergic receptor genes (Study IV)

The effect of genetic variation in the ADRB1 and ADRB2 genes on BP response to the four antihypertensive drugs was evaluated in Study IV. The ADRB1 Ser49Ser homozygotes tended to have a better BP response to bisoprolol than the Ser49Gly heterozygotes, with 1.6 and 1.4 mmHg differences in systolic and diastolic ABP response ($P=0.04$ and 0.06, in Mann-Whitney test and $P=0.02$ and 0.09 in multivariate analysis, respectively) (Figure 9). The difference in systolic OBP response between these two genotype groups was reminiscent of the ABP response, but was not significant ($P=0.21$ in Mann-Whitney test). However, the Gly49Gly genotype group with seven subjects did not follow this trend and showed no statistically significant difference from the major homozygotes.

ADRB1 Ser49Gly-related ABP response to amlodipine and losartan followed a pattern similar to bisoprolol. The Ser49Ser homozygotes responded better to both of these drugs compared to the Ser49Gly heterozygotes (Figure 9), and the results were comparable in multivariate analysis (data not shown). Moreover, the differences were similar in systolic and diastolic OBP response (data not shown; $P$ values from 0.07 to 0.19). Again, the Gly49Gly homozygotes did not follow this trend and demonstrated no difference from the major homozygotes. There was no statistically significant difference
in ABP response to hydrochlorothiazide between the Ser49Gly genotype groups (Figure 9).

![Graphs showing systolic and diastolic blood pressure responses to study drugs stratified by beta1-adrenergic receptor Ser49Gly genotype.](image)

Figure 9. Ambulatory 24-hour systolic and diastolic blood pressure responses to study drugs stratified by beta1-adrenergic receptor Ser49Gly genotype. Statistical significance was calculated between Ser49Ser and Ser49GlyGly genotypes with Mann-Whitney U test; only p values <0.05 are indicated. ADRB1, beta1-adrenergic receptor; HCT, hydrochlorothiazide; *, P = 0.01-0.05; **, P = 0.003-0.01.

Some of the earlier studies reported augmented BP response to beta-blockers in subjects homozygous for the Arg389 allele of the ARB1 gene (Johnson et al. 2003, Liu et al. 2006). The present study however, shows the opposing trend, since the 13 subjects homozygous for the Gly389 allele had better systolic and diastolic ABP response to bisoprolol, compared to the Arg389Arg homozygotes (Figure 10). When carriers of the Gly389 allele were added to the analysis and compared with the Arg389Arg homozygotes, there were no significant differences in BP response to bisoprolol (data not shown).

The Gly389Gly genotype was also associated with stronger systolic and diastolic ABP response to losartan (P=0.07 and 0.01, respectively) (Figure 10). However, BP response to amlodipine and hydrochlorothiazide was not different between the Arg389Gly genotype groups (Figure 10). There was no statistically significant association between
the Arg389Gly polymorphism and OBP response to any of the study drugs (Study IV, Table 2).

Figure 10. Ambulatory 24-hour systolic and diastolic blood pressure responses to study drugs stratified by beta1-adrenergic receptor Arg389Gly genotype. Statistical significance was calculated between Arg389Arg and Gly389Gly genotypes with Mann-Whitney U test; only p values <0.05 are indicated. ADRB1, beta1-adrenergic receptor; HCT, hydrochlorothiazide; *, P = 0.01-0.05; **, P = 0.003-0.01.

No ADRB2 Gly16Arg and Gln27Glu genotype-associated differences in BP response to any of the study drugs were observed in the present study (Study IV, Table 2).
6. DISCUSSION

6.1 Variation in antihypertensive responses

6.1.1 Variation in individual blood pressure responses

Variation in individual BP response to antihypertensive agents may constitute one reason for unsatisfactory BP control among hypertensive patients, which is a disturbingly common condition (Materson et al. 1993, Attwood et al. 1994, Dickerson et al. 1999). The variation in BP response is supposed to reflect individual-specific mechanisms behind BP elevation, and individual patterns of BP response to different antihypertensive agents may help in characterizing different phenotypes of essential hypertension (Laragh et al. 1988). This is supported by the findings from earlier cross-over studies with two or more antihypertensive drugs, showing that given individuals are not non-responders to all study drugs (Materson et al. 1993). Furthermore, results from the present study demonstrate that none of the GENRES Study subjects was a non-responder for all four antihypertensive drugs tested.

Attempts to predict BP response to different classes of antihypertensive agents based on laboratory tests, such as PRA, and on demographic factors, such as age, gender and race, started in the early 1970’s (Laragh et al. 1979). However, no major progress in understanding the mechanisms behind variation in antihypertensive drug responses has been achieved since then. As a consequence, no specific algorithms for targeting antihypertensive treatment to any sub-population of hypertensive patients have been developed. Exceptions include the ESH guidelines which recommend for example that a renin-angiotensin receptor blocker should always be included in combination treatment for hypertensive patients with diabetes, due to its protective effect against nephropathy (Chobanian et al. 2003, Mancia et al. 2007, Mancia et al. 2009).
6.1.2 Reproducibility of blood pressure responses

Data on the reproducibility of BP response to antihypertensive therapies is distinctly limited. In a non-pharmacological intervention study, the reproducibility of BP response to a short-term low-sodium diet, performed with an average interval of 3.4 months, was poor (Zoccali et al. 1996). In the first of the two published pharmacological studies, the reproducibility of BP response to 5 day treatment of enalapril was moderate at best (Chatellier et al. 1995). Subsequently, Finkielman et al. evaluated the reproducibility of BP response to four-week treatment of hydrochlorothiazide in a study with 22 subjects. Although, the mean levels of systolic and diastolic BP response to hydrochlorothiazide did not differ significantly between the two participations, with an average interval of 26.6 months, the response of individual subjects varied widely between the first and second participation (Finkielman et al. 2002). However, in this study the correlations of BP response was equal to the correlations between BP levels at the first and second participations. Overall, it seems additional studies are needed to establish the reproducibility of BP response to antihypertensive agents, with the existing limited data suggesting that attempts to find predictors of BP response should be as possible as attempts to identify causative factors for hypertension.

6.1.3 Heritability of blood pressure responses

It has been estimated that 30-60% of BP variation is explained by genetic factors. These findings are based mainly on twin and familial aggregation studies (Williams et al. 1990, Kurtz and Spence 1993, Lifton 1995). Since such study strategies can not be easily used to evaluate the heritability of BP responses, there is much less evidence on the effect of genetic factors on BP response to antihypertensive agents. However, the BP response to most of the commonly used antihypertensive agents is a continuously distributed trait suggesting involvement of multiple genetic and environmental factors.

6.2 Methodological aspects of the GENRES Study

The GENRES Study was a randomized, prospective, double-blind, cross-over study that was placebo-controlled and performed in a single center. Moderately hypertensive men without significant co-morbidity were recruited for the study. Participants of the study
were limited to males in order to reduce gender and hormonal effects on BP variation and drug response.

BP response was examined with regard to four different antihypertensive drugs representing the four main classes of antihypertensive agents. Drug dosages were chosen to be sufficient but tolerable. The study design did not aim to compare the pharmacological potency of the selected study drugs, and the drug dosages were not designed to be equipotent. BP response to the study drugs agreed well with previous studies.

Each study subject completed four placebo and four active drug periods, taking at least eight months for each participant to complete the study. Placebo BP levels were especially accurate as they represented average values from four separate periods in most of the subjects. Additionally, the study included ABP measurements, which show better repeatability during placebo periods than OBP measurements. The study protocol was labor-intensive, with about 4700 study visits, over 2000 ABP recordings and over 800 drug periods. As a consequence, it took over four years to finish the clinical aspect of the study. The study was able to reproduce earlier findings on PRA, the most established predictor of BP response to antihypertensive drugs, thus validating the study design and lending reliability to the other results of the study.

There are a few important limitations in the present study. As the study population was limited to white, male, and relatively young individuals, the data may not be valid for women, elderly subjects, or other ethnic groups. Each of the the study subjects went through a long study protocol. Since no dietary or lifestyle advice was given to the study subjects, it is possible that lifestyle habits and living conditions changed during the study, therefore affecting BP levels. Furthermore, clinical and laboratory variables were measured only once, in the beginning of the study. However, this aspect of the study protocol closely resembles normal clinical practice, where most of the laboratory tests are performed only once at the beginning of an antihypertensive therapy, and are not reanalyzed after every change of medication. Due to the number of subjects in the smallest genotype groups (n=6-13), the power of the study may have been too low to detect small differences between some sub-groups.
6.3 Nongenetic predictors of antihypertensive response

6.3.1 Demographic factors

In this study there were no strong correlations between BP response to amlodipine, bisoprolol, hydrochlorothiazide or losartan to demographic factors of the study population. Nonetheless, OBP and ABP response to amlodipine and systolic OBP and ABP response to hydrochlorothiazide showed some correlation with age of the individual. These findings are in line with earlier studies, showing that BP response to calcium antagonists and diuretics is better in the elderly (Materson et al. 1993, Materson et al. 1995, Morgan et al. 2001). However, as a consequence of the design of the GENRES study, these studies are not fully comparable as elderly people were not included in this study.

BMI was negatively associated with ABP response to amlodipine, but did not correlate with any of the other drugs. This study therefore does not support the observation that increased BMI is associated with better BP response to beta-blockers, as suggested by earlier trials that had included obese subjects (BMI >27 and >30, respectively), (Schmieder et al. 1993, Materson et al. 2003). However, markedly obese subjects with BMI ≥32 were excluded from the present study.

6.3.2 Blood pressure levels

There is evidence that higher pretreatment BP is correlated with stronger BP response to different classes of antihypertensive drugs (Sumner et al. 1988). In accordance with this previous finding, the present study noted that BP levels during placebo periods were positively correlated with BP response to all of the study drugs.

Pulse pressure was positively associated with BP response to amlodipine and hydrochlorothiazide. Moreover, there was a negative association with BP response to amlodipine and hydrochlorothiazide to nighttime dipping on placebo. There seems to be no earlier literature on the association of BP response patterns to diurnal variation in BP. The exact mechanisms underlying these associations remain to be investigated.
6.3.3 Laboratory tests

In the present study, there were positive correlations between PRA and BP response to losartan and bisoprolol. Additionally, BP response to hydrochlorothiazide was negatively correlated with PRA, with a similar but weaker negative correlation also observed for amlodipine. These response patterns of the study drugs with regard to PRA are highly congruent with earlier findings (Cody et al. 1983, Freis et al. 1983, Kiowski et al. 1985, Ikeda et al. 1997). The data fits well with the models presented by Laragh et al., suggesting that subjects with high renin levels respond better to ACE inhibitors and beta-blockers, while subjects with low renin levels respond better to diuretics and calcium antagonists (Laragh et al. 1979). It is likely that PRA-associated differences in the BP-lowering effects of different antihypertensive drug classes are related to the diverse pathophysiological mechanisms that underlie elevated BP. Increased PRA may reflect a pronounced contribution of vasoconstriction to elevated BP, and as a consequence predicts a better response to ACE inhibitors, angiotensin receptor antagonists and beta-blockers, all of which inhibit RAS (Prichard et al. 1980, Brown et al. 1998, Schmieder 2005). Conversely, low PRA may associate with a volume dependent type of hypertension, which would predict better BP response to diuretics, and to a lesser extent calcium antagonists that possess some diuretic effect (Zanchetti and Leonetti 1985).

It is conceivable that pretreatment PRA bears only minor importance as a predictor of BP response in routine clinical practice, since it is responsible for only a relatively small proportion of the variability in response to antihypertensive treatment, and does not seem to be a better predictor of BP response than simple demographic characteristics (Freis et al. 1983, Preston et al. 1998). Consistant with this assumption, in the present study, the correlations of PRA with BP response to the study drugs were modest, with wide distributions of BP response within the different quartiles of PRA, for each of the four study drugs.

The negative correlation between serum total cholesterol level and ABP response to amlodipine is supported by data from a previous study where 29 patients with mild to moderate hypertension (aged 35-67 years) were treated with nitrendipine for 6 months (Mazeaud et al. 1991). In this earlier study, hypertensive patients with serum total
cholesterol <6.4 mmol/L had significantly better BP response to nitrendipine, compared to patients with serum total cholesterol ≥6.4 mmol/L. This finding could be due to altered release of vasoactive substances from endothelial cells in patients with hypercholesterolemia, as it has been demonstrated that hypercholesterolemic subjects have blunted vasodilator response to methacholine and nitroprusside (Creager et al. 1990).

Of the other laboratory variables, plasma catecholamines, plasma fasting glucose, serum creatinine and serum uric acid have been to varying degrees associated with BP response to different antihypertensive agents, but do not seem to have any importance in predicting individual BP response (Myers and de Champlain 1983, Mazeaud et al. 1991, Campo et al. 2002, Kjeldsen et al. 2008). Generally, there has been very limited previous data on the effect of metabolic laboratory variables on BP response to antihypertensive drugs.

In the present study, the negative correlation of serum total calcium level to OBP and ABP response to amlodipine is a novel finding that needs to be replicated in other studies. It is, however, supported by two earlier single-drug studies that demonstrated corresponding associations with serum ionized calcium to BP response to calcium antagonists, although our data on serum total calcium is not fully comparable with results from correlations between BP response and serum ionized calcium. The first of these studies was performed in 25 patients receiving a single dose of nifedipine (Midtbo and Hals 1987), and the second in 20 patients treated for four weeks with verapamil (Resnick et al. 1987). Both studies showed a negative correlation between OBP response to the study drug and serum ionized calcium. There appears to be no other reports of association between serum calcium levels and BP response to antihypertensive agents, and therefore the possible mechanism behind this association is poorly understood. Yet, it is possible that lower serum calcium levels are associated with enhanced sensitivity to calcium channel blockers as a consequence of higher cellular uptake of calcium, and increased intracellular calcium concentrations (Erne et al. 1984). Another possibility is that the higher calcium concentration itself may reduce the effect of a calcium channel blockers, in fact, it has been shown that calcium infusion reduces the responsiveness of the resistance vessels to verapamil (Robinson et al. 1984).
6.4 Genetic variation as predictor of antihypertensive response

6.4.1 Alpha-adducin gene

The ADD1 460Trp allele was initially associated with enhanced BP response to hydrochlorothiazide in three studies using Italian subjects (Cusi et al. 1997, Glorioso et al. 1999, Sciarrone et al. 2003). These three single-drug studies, without placebo-control, comprised moderately hypertensive patients without earlier antihypertensive treatment. However, subsequent studies in different populations have failed to show any effect of ADD1 Gly460Trp on BP response to antihypertensive drugs (Turner et al. 2003, Matayoshi et al. 2004, Schelleman et al. 2006b). This was also true for one of the largest pharmacogenetic studies, GenHAT, where the 460Trp allele did not predict better response to chlorthalidone (Davis et al. 2007).

Results from this study do not support the assumption that the 460Trp allele is associated with stronger BP response to diuretics or other antihypertensive drugs. In fact, there was even an opposing trend, as the 460Trp allele was associated with a decreased BP response to hydrochlorothiazide. This controversial result may represent a chance finding, as it was seen only with systolic ABP response. Collectively, the present study along with previously published studies suggests that the ADD1 460Trp allele is not a useful clinical marker of enhanced BP response to thiazide diuretics. There remains the theoretical possibility that the Gly460Trp polymorphism is to some degree in linkage disequilibrium with the true functional variant, and therefore, that the linkage to different Gly460Trp alleles may vary from population to population.

6.4.2 Renin-angiotensin system genes

The results of this study showed no effect for the ACE I/D polymorphism on BP response to losartan, amlodipine, bisoprolol or hydrochlorothiazide. It is possible that higher serum ACE level in subjects with the D allele (Rigat et al. 1990, Zhu et al. 2000) is not reflected to the systemic activity of the RAS, and thus does not predict response to ACE inhibitors or angiotensin receptor antagonists. In fact it has been reported that renin, and not ACE, is the rate-limiting factor in the production of angiotensin II.
(Roulston et al. 1978). This is supported by the results of the GenHAT study and eight other studies which presented no association between the ACE II genotype and BP response to thiazides and other antihypertensive drugs (Hingorani et al. 1995, Dudley et al. 1996, Harrap et al. 2003, Yu et al. 2003, Redon et al. 2005, Schelleman et al. 2006a, Schelleman et al. 2006c, Filigheddu et al. 2008, Arnett et al. 2005). The many positive results in studies that demonstrated better antihypertensive response to different drugs with the ACE II genotype (Ohmichi et al. 1997, Haas et al. 1998, O'Toole et al. 1998, Kurland et al. 2001, Sciarrone et al. 2003), the DD genotype (Stavroulakis et al. 2000, Li et al. 2003) or with both the II and DD genotypes (Schwartz et al. 2002) may in fact represent false positive findings, as these results have been inconsistent with not one of these studies being randomized and placebo-controlled.

The results of the present study, showing no significant association of Met235Thr with BP response to study drugs, are in line with the majority of earlier reports, which do not support any effect of AGT Met235Thr on BP response to different antihypertensive drugs (Dudley et al. 1996, Katsuya et al. 2001, Kurland et al. 2001, Schelleman et al. 2006a). With regard to the two studies with positive results, the first one was a non-controlled open study with different ACE inhibitors (Hingorani et al. 1995), while the results from the other study with positive findings are probably unreliable (Kurland et al. 2004) as in an earlier study, with subjects from the same SILVHIA trial, there was no effect of AGT Met235Thr on BP response to atenolol (Kurland et al. 2001). Collectively, although the AGT Met235Thr polymorphism is truly associated with increased plasma AGT levels, and may show some association with BP levels (Staessen et al. 1999, Sethi et al. 2003, Jeunemaitre 2008), it does not exert significant effects on BP response to antihypertensive drugs.

In the present study there was no association with the AT1R 1166 A/C polymorphism to BP response to the four antihypertensive drugs tested. A finding which is in accordance with most of the earlier studies (Hingorani et al. 1995, Katsuya et al. 2001, Kurland et al. 2001, Kurland et al. 2004, Redon et al. 2005, Filigheddu et al. 2008, Gluszek and Jankowska 2008). It is plausible that the few positive findings, of a relationship between AT1R 1166 A/C and antihypertensive response, represent chance findings (Frazier et al. 2004, Miller et al. 1999, Benetos et al. 1996).
6.4.3 Beta-adrenergic receptor genes

The Arg allele of ADRB1 Arg389Gly polymorphism has been associated with increased BP response to beta-blockers in two earlier studies of hypertensive patients (Johnson et al. 2003, Liu et al. 2006). However, in the study of Johnson et al., there was a marked racial imbalance between the genotype groups, whilst in the study of Liu et al., the subjects were selected according to haplotypes in a way that may appear somewhat arbitrary. On the other hand, four other studies with hypertensive patients have showed no evidence of stronger BP response to beta-blockers in Arg389Arg homozygotes (O'Shaughnessy et al. 2000, Filigheddu et al. 2004, Karlsson et al. 2004).

Results from the GENRES Study give no support to the hypothesis that the ADRB1 Arg389 allele predicts a better BP response to beta-blockers. In fact, an enhanced ABP response to bisoprolol with Gly389Gly homozygotes was noted. However, this finding may represent a chance association, since there were only 13 subjects in this group. Considering the available data as a whole, one may conclude that the Arg389Gly polymorphism is not associated with variation of BP response to beta-blockers.

In the present study ABP response to bisoprolol was slightly better in ADRB1 Ser49Ser homozygotes compared to Ser49Gly heterozygotes. However, the significance of this association remains obscure. Since the difference was only of borderline statistical significance, the Gly49Gly genotype group did not follow the trend compatible with a gene dosage effect, and OBP findings were not fully concordant. Furthermore, there is only one study with parallel results (Liu et al. 2006), as most of the earlier studies have reported no difference in BP response to beta-blockers between the Ser49Gly genotypes (Johnson et al. 2003, Filigheddu et al. 2004, Karlsson et al. 2004, Mahesh Kumar et al. 2008). In summary, even though there is some evidence that the Ser49Gly polymorphism might be functionally active (Brodde 2008), the association of Ser49Gly with BP response to beta-blockers seems to be inconclusive.

In this study there was no association between the Gly16Arg and Glu27Gln polymorphisms with BP response to bisoprolol or the other study drugs. There are only two other published studies on the association of Gly16Arg and Glu27Gln with BP response. One of these studies reported a better BP response to an ACE inhibitor in
Gly16 allele carriers (Huang et al. 2004) while the other revealed no differences in BP response to atenolol between the Gly16Arg genotype groups (Filigheddu et al. 2004). It can therefore be concluded that the available data does not support an association between these two ADRB2 polymorphisms and antihypertensive drug response.

6.5 Challenges in pharmacogenetic studies on blood pressure response

Since Cusi et al. published one of the first studies showing a pharmacodynamic effect of genetic variation on BP response to an antihypertensive drug (Cusi et al. 1997), more than 60 articles have reported results from pharmacogenetic studies of antihypertensive responses by the year 2009 (Arnett et al. 2009). However, results have been mostly inconsistent, and to date no common genetic alteration accounting for a significant proportion of variation in BP response to a given drug has been identified.

According to Kurland et al. (2005), an ideal pharmacogenetic study should be prospective and placebo-controlled and have adequate statistical power. Study subjects should comprise previously untreated individuals and the study should have a cross-over design so that each subject takes each drug, from the main classes of antihypertensive drugs, as monotherapy on a rotational basis and in a random order. Moreover, each study should be replicated independently (Kurland et al. 2005).

In most of the pharmacogenetic studies to date there have been problems in study design. Farahani et al. (2007) explored design-related bias in pharmacogenetic studies involving ACE inhibitors and angiotensin receptor antagonists. Of the total of 16 studies, examining the influence of genetic polymorphisms on BP response or clinical outcome, only 9 were originally designed as a genetic study and only 6 studies were focusing on more than one gene. In most of these studies the sample size was less than 100, with only two of the studies including proper power calculations. Additionally, in many studies different treatments were combined in one group, and study groups comprised subjects from previous studies with different selection criteria. Furthermore, in some of the studies different genotypes of a single polymorphism were combined. It can be presumed that a particular genetic variation will only contribute a small effect on
BP response to an antihypertensive agent. As a consequence of small sample size, heterogeneity in the study population, lack of placebo-controlling and inaccuracy in BP measurement, most of the previous studies have suffered from an insufficient power to detect such genetic effects (Farahani et al. 2007).

Problems have also been encountered in comparisons between different pharmacogenetic studies of hypertension. Differences in age, gender, ethnicity and diagnosis of hypertension between populations may complicate comparisons between studies (Filigheddu et al. 2006). It appears that more carefully conducted studies with larger sample size are urgently needed, in order to identify any true association between genetic variations and BP response to antihypertensive drugs. Furthermore, newer methods such as genome-wide association analysis may provide a novel means to identify genetic variants influencing BP response to different drugs (Wellcome Trust Case-Control Consortium 2007, Cho et al. 2009, Org et al. 2009, Wang et al. 2009).
7. SUMMARY AND CONCLUSIONS

Essential hypertension is associated with significant comorbidity and mortality, and the prevalence of hypertension is rising worldwide, further increasing the burden of the disease. Although there are effective antihypertensive drugs, only about one third of hypertensive subjects achieve acceptable goals of BP treatment. Lack of clinically useful predictors of individual variation in antihypertensive responses to different BP lowering drugs may constitute one reason for the insufficient drug control of hypertension.

The aim of the present study was to evaluate the relationship between placebo BP levels, selected demographic characteristics, baseline laboratory tests and common genetic variations with BP response to four different antihypertensive monotherapies including, an angiotensin receptor blocker, a beta-blocker, a calcium channel blocker and a thiazide diuretic. The study was conducted in a double-blind fashion and included both OBP and ABP measurements. The main conclusions of the present study are as follows:

1. The intra-individual variation in BP response to different study drugs was significant. Concordant with previous studies, pretreatment BP levels were positively correlated with BP response to all of the study drugs. Some modest correlations of BP response to study drugs with certain demographic factors, such as age and BMI, were found. Age was significantly positively correlated with OBP and ABP response to amlodipine and hydrochlorothiazide. BMI was significantly negatively correlated with ABP response to amlodipine.

2. Baseline plasma renin activity correlated positively with BP response to losartan and bisoprolol, and negatively with BP response to hydrochlorothiazide confirming earlier findings. However, pretreatment PRA did not seem to have an important clinical role in predicting individual BP response to antihypertensive drugs, as it explains only a minor proportion of the variability in response to antihypertensive treatment. Serum total calcium correlated negatively with BP response to amlodipine. The possible mechanisms behind this finding are unknown and the results need to be reproduced before further speculation.
3. There were no significant associations between selected polymorphisms of the ACE, AGT, AT1R, ADD1, ADRB1 and ADRB2 genes with BP response to any of the four antihypertensive drugs. Collectively, results from this study and earlier studies suggest that these widely studied polymorphisms cannot be used to select effective antihypertensive drugs in clinical practice. It appears that more systematic genetic approaches, such as genome-wide association studies are needed to identify genetic variants influencing responsiveness to commonly used antihypertensive drugs.
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