Pharmacogenetic Studies of Antihypertensive Treatment

With Special Reference to the Renin-Angiotensin-Aldosterone System

BY

LISA KURLAND
Hypertension is common and constitutes an increased risk of morbidity and mortality of cardiovascular disease. Antihypertensive treatment will reduce this risk; the individual patient's response to treatment, however, is difficult to predict.

Patients with hypertension and left ventricular hypertrophy were randomized to monotherapy with either the angiotensin II type 1 receptor antagonist irbesartan or the beta-adrenoreceptor blocker atenolol, and followed for three months. The aim was to determine whether gene polymorphisms in the renin-angiotensin-aldosterone system were related to the response to treatment.

The ACE II genotype was associated with the most pronounced diastolic blood pressure response, while the aldosterone synthase (CYP11B2) -344 TT genotype showed the greatest systolic blood pressure response. The angiotensinogen 174 TM genotype showed the most pronounced regression in left ventricular mass, independent of the change in blood pressure. These associations were exhibited only in response to treatment with the angiotensin II type 1 receptor antagonist irbesartan.

In a sample of apparently healthy subjects, those with both the D allele and the angiotensinogen 174 TM variant in combination showed a decreased endothelium-dependent vasodilation.

These results suggest that the response to antihypertensive treatment is associated with polymorphisms in the genes reflective of the pathophysiological pathway the drug targets. The present study is an encouragement for future investigation, such as large scale studies of multiple polymorphisms and combinations thereof in an attempt to identify a panel of genotypes that can be used as a predictor of an individual patient's response to antihypertensive treatment.

Key words: Pharmacogenetics, hypertension, renin angiotensin aldosterone system, gene polymorphisms.

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“If it were not for the great variability among individuals
medicine might as well be a science not an art.”
Sir William Osler
1892
Pharmacogenetic Studies of Antihypertensive Treatment

This thesis is based on the following studies which will be referred to by their Roman numerals:

I. “Angiotensin converting enzyme gene polymorphism predicts blood pressure response to angiotensin II receptor type 1 antagonist treatment in hypertensive patients.”
Lisa Kurland, Håkan Melhus, Julia Karlsson, Thomas Kahan, Karin Malmqvist, Peter Öhman, Fredrik Nyström, Anders Hägg and Lars Lind

II. “Aldosterone synthase (CYP11B2) -344 C/T polymorphism is related to antihypertensive treatment.”
Lisa Kurland, Håkan Melhus, Julia Karlsson, Thomas Kahan, Karin Malmkvist, Peter Öhman, Fredrik Nyström, Anders Hägg and Lars Lind
In review

III. “Polymorphisms in the angiotensigen and AT1-receptor gene are related to regression of left ventricular mass during antihypertensive treatment.”
Lisa Kurland, Håkan Melhus, Julia Karlsson, Thomas Kahan, Karin Malmqvist, Peter Öhman, Fredrik Nyström, Anders Hägg and Lars Lind
Journal of Hypertension, in press

IV. “Polymorphisms in the renin-angiotensin system and endothelium-dependent vasodilation in normotensive subjects.”
Lisa Kurland, Håkan Melhus, Mahziar Sarabi, Jonas Millgård, Sverker Ljunghall, Lars Lind
Clinical Physiology 2001;21;3:343-349

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## Abbreviations

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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ACE</td>
<td>angiotensin converting enzyme</td>
</tr>
<tr>
<td>AGT</td>
<td>angiotensinogen</td>
</tr>
<tr>
<td>AS</td>
<td>aldosterone synthase</td>
</tr>
<tr>
<td>AT1R</td>
<td>angiotensin II type 1 receptor</td>
</tr>
<tr>
<td>DBP</td>
<td>diastolic blood pressure</td>
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<tr>
<td>EDV</td>
<td>endothelium-dependent vasodilation</td>
</tr>
<tr>
<td>EIDV</td>
<td>endothelium-independent vasodilation</td>
</tr>
<tr>
<td>LVH</td>
<td>left ventricular hypertrophy</td>
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<tr>
<td>LVM</td>
<td>left ventricular mass</td>
</tr>
<tr>
<td>LVMI</td>
<td>left ventricular mass index</td>
</tr>
<tr>
<td>RAAS</td>
<td>renin-angiotensin-aldosterone system</td>
</tr>
<tr>
<td>SBP</td>
<td>systolic blood pressure</td>
</tr>
<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
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ACE I/D
insertion (I) or deletion (D) in intron 16 of the ACE gene.

aldosterone synthase C -344T
cytosine (C) to thymine (T) transition at base pair -344 in the aldosterone synthase gene.

allele
one of several alternative forms of a gene or DNA sequence at a specific chromosomal location (locus).

AT1R A1166C
adenine (A) to cytosine (C) transversion at base pair 1166 in the angiotensin II type 1 receptor gene.

dizygotic twins
fraternal twins

endothelial vasodilatory function
a measurement of the capacity of the endothelium to cause nitric oxide-dependent vasodilation in response to chemical and/or physical stimuli.

Frank-Starling mechanism
the relationship between the filling of the left ventricle and the stroke volume.

genotype
the genetic constitution of an individual either overall or at a specific locus.

genome
the total genetic complement of an organism or virus.

heritability
the proportion of the causation of a character that is due to genetic causes.

incidence
the number of new cases of a disease that develop in a population in a given time period.

M235T
a point mutation in the angiotensinogen gene resulting in methionine to threonine substitution at residue 235.

monozygotic twins
identical twins

pharmacogenetics
the study of heritability on drug response.

phenotype
the observable characteristics of a cell or an organism.

polymorphism
the existence of two or more variants (alleles, phenotypes, sequence variants, chromosomal structural variants) at significant frequencies in the population.

prevalence
the number of subjects with a specific disease at a given point in time.

pulse pressure
the difference between systolic and diastolic pressure.

T174M
a point mutation in the angiotensinogen gene resulting in threonine to methionine substitution at residue 174.
Introduction

Hypertension is prevalent, affecting approximately 20-25% of the adult population in the Western world. Coronary heart disease is the leading cause of death, and approximately one half of all people who have a first heart attack and two-thirds of those with a first stroke have high blood pressure levels. By treating hypertension and factors that contribute to cardiovascular disease both mortality and morbidity can be reduced. Many drugs have proven to be effective in treating hypertension, though an individual patient’s response to these drugs is unpredictable. Today there are no effective methods with which to individualize antihypertensive treatment. This is the essence of the question addressed in this thesis; How do we know which drug to choose for the individual hypertensive patient?

There is no one specific cause of primary hypertension, which defines the term. Most likely, multiple pathophysiological mechanisms are involved, including gene-gene and gene-environment interaction. Between 30-60% of blood pressure variation is determined by genetic factors. The renin-angiotensin-aldosterone system is a powerful hormonal system that strives to increase blood volume and constrict blood vessels, and thereby raise blood pressure. This led us to the assumption that common genetic variants (polymorphisms) in the renin-angiotensin-aldosterone system were likely to be involved in the regulation of blood pressure and therefore also associated with the response to specific antihypertensive therapy.

Based on a clinical trial designed to compare the efficacy of two antihypertensive drugs (atenolol and irbesartan) the question we asked was; Can we relate the individual patient's antihypertensive response to his/her genotype? Our results imply that this is possible and lead us to conclude that the present study should be viewed as an encouragement for future investigation, such as large-scale studies in which multiple polymorphisms and combinations thereof may be investigated in an attempt to identify a panel of genotypes that can be used as predictors of an individual patient's response to commonly used antihypertensive drugs.
Background

Hypertension

“[Hypertension] afflicts us from middle age onwards [and] might simply represent “unfavorable” genes that have accumulated to express themselves in the second half of our lives. This could never be corrected by any evolutionary pressure since such pressures act only on the first half of our lives: once we have reproduced, it does not matter that we grow “sans teeth, sans eyes, sans taste, sans everything.”

Anonymous, Editorial, Lancet 1993

The above passage would suggest that modern medicine has taken on a challenge of colossal proportions—the task of dissecting and mitigating the hypertensive phenotype, a genetic composition that has taken more than 100,000 years to evolve.

Blood pressure

Blood pressure may be defined as a product of the pumping action of the heart (cardiac output, a product of heart frequency and stroke volume) and the tone of the peripheral arteries (vascular resistance). Any factor that affects these components will also affect the blood pressure. Blood pressure is measured as systolic blood pressure (SBP), corresponding to the contraction of the heart, and diastolic blood pressure (DBP) to its relaxation. The difference between SBP and DBP is referred to as pulse pressure and reflects the pulsatile character of blood pressure.

Hypertension

As summarized by Pickering (Pickering 1972), “arterial pressure [is] a quantity and the consequence numerically related to the size of the quantity”, making the definition of hypertension somewhat arbitrary. However, the World Health Organization-International Society of Hypertension (WHO-ISH) Guidelines Committee (1999) has published guidelines for management of chronically elevated blood pressure based on evidence from epidemiological studies and clinical trials. According to these guidelines, hypertension is defined as an SBP of 140 mmHg or higher and/or a DBP of 90 mmHg or greater. (See Table 1 for definitions and classification.)

Primary hypertension (earlier referred to as “essential” or “benign”), hypertension of unknown cause, as distinguished from secondary hypertension whose cause is known, represents as much as 90-95% of all hypertension. In the following text, unless otherwise stated, hypertension refers to primary hypertension.

Epidemiology of hypertension

In some countries, the United States, for example, as many as 1 in every 4 adults has hypertension. In the US, this represents 43 million people (Burt et al. 1995). Roughly the same prevalence is seen in Sweden. In the developing countries hypertension rates are on the rise, prevalences close to, or in some cases even exceeding those mentioned here.

Most hypertensives are 50 years of age or older (74%), with a predominance of isolated systolic hypertension (80%), as defined in Table 1. In this age category, the majority are women (58%), whereas hypertensives below the age of 50 are predominantly men (62%) (Franklin et al. 2001).
70% of hypertension in men and 61% in women is directly attributable to obesity, with a 4.5 mmHg increase in systolic BP for every 10-pound increase in weight (Kannel et al. 1993).

Table 1.

<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>&lt; 130</td>
<td>&lt; 85</td>
</tr>
<tr>
<td>High - normal</td>
<td>130 - 139</td>
<td>85 - 89</td>
</tr>
<tr>
<td>Grade 1 hypertension (mild)</td>
<td>140 - 159</td>
<td>90 - 99</td>
</tr>
<tr>
<td>Subgroup: borderline</td>
<td>140 - 149</td>
<td>90 - 94</td>
</tr>
<tr>
<td>Grade 2 hypertension (moderate)</td>
<td>160 - 179</td>
<td>100 - 109</td>
</tr>
<tr>
<td>Grade 3 hypertension (severe)</td>
<td>≥ 180</td>
<td>≥ 110</td>
</tr>
<tr>
<td>Isolated systolic hypertension</td>
<td>≥ 140</td>
<td>&lt; 90</td>
</tr>
<tr>
<td>Subgroup: borderline</td>
<td>140 - 149</td>
<td>&lt; 90</td>
</tr>
</tbody>
</table>

When a patient’s systolic and diastolic blood pressures fall into different categories, the higher categories should apply.

What causes hypertension?

In young borderline hypertensives cardiac output is raised without change in vascular resistance (hyperkinetic circulation) (Julius and Conway 1968). In established hypertension, however, cardiac output is normal or even reduced and blood pressure is elevated by increased vascular resistance (Cowley 1992), the benchmark of established hypertension. This conversion is demonstrated in a 20 year follow-up of mild hypertensives (Lund-Johansen 1989), where it was observed that as the overall blood pressure rose, cardiac output fell and peripheral resistance increased.

Increased sympathetic nerve activity raises cardiac output by increasing both heart rate and myocardial contractility. This is believed to be the mechanism behind hypertension in young adults (Julius 1988). In addition to the direct pressor effect, catecholamines are trophic to vascular tissue (Yu et al. 1996).

The blood volume filling the heart is a major determinant of stroke volume (through the Frank-Starling mechanism), and one of the key determinants of circulating blood volume is sodium balance and its regulation effectuated by the kidney. A high sodium intake may increase blood volume, as shown from both epidemiological and experimental data. Recent trials show that lowering dietary salt (sodium chloride) intake also lowers blood pressure (Greenland 2001; Sacks et al. 2001). As a result, maintaining a moderate sodium intake has become part of the dietary recommendations given by the American Heart Association (Krauss et al. 2000). The observation of varying responses of blood pressure to short periods of low and high-sodium intake
lead to the definition of salt sensitivity (Weinberger et al. 1986). Salt-sensitive individuals (more common in hypertensives than in normotensives) will increase their blood pressure more in response to sodium than salt-resistant subjects. There are several theories that attempt to explain why the kidneys are unable to excrete excess sodium and restore sodium and fluid volume balance (de Wardener and MacGregor 1980; Guyton 1987; Brenner et al. 1988; Sealey et al. 1988; Guyton 1989). All of these support a defective sodium output in relation to blood pressure level in salt-sensitive subjects, resulting in chronically increased blood volume.

The renin-angiotensin-aldosterone system (RAAS) plays a key role in the regulation of blood pressure by influencing both the salt-water homeostasis and vascular tone. Decreased intravascular volume presents a threat, necessitating a rapid response to support circulation. This is effectuated by the pressor actions of angiotensin II, resulting in vasoconstriction, increased aldosterone secretion leading to salt retention, increased thirst and release of antidiuretic hormone to conserve water, increased cardiac contractility to maintain cardiac output, and the potentiation of the sympathetic nervous system which synergizes with angiotensin II. The RAAS is also responsible for more long-term effects, such as structural remodeling of the cardiovascular system.

Vascular remodeling (rearranging of existing vascular cells around a smaller lumen) of resistance arteries may represent an adaptation in response to chronically elevated blood pressure (Heagerty et al. 1993; Mulvany 1995), but, as pointed out above, may also develop in response to trophic hormones, such as angiotensin II (Griffin et al. 1991) and endothelin (Moreau et al. 1997). Hypertension is thought to be perpetuated by vascular hypertrophy “making permanent” the increased peripheral resistance (Lever and Harrap 1992). Chronically elevated blood pressure, trophic factors and atherosclerosis affect the large central arteries, rendering them less compliant which is manifested as isolated systolic hypertension.

In summary, hypertension involves three main factors: abnormalities in blood volume and salt regulation, increased peripheral vascular tone and vessel wall remodeling. Multiple pathophysiological pathways are involved, interwoven and interacting with each other in a complex balance. There is therefore no simple way to discern one specific pathophysiological mechanism that gives rise to hypertension in an individual hypertensive patient.

**Is there a genetic component?**

It is widespread clinical awareness that hypertension and cardiovascular disease run in families. The inherited nature of hypertension is supported by several reports showing that blood pressure is more similar between relatives than between unrelated individuals (Hamilton et al. 1954; Miall and Oldham 1955; Johnson et al. 1964; Miall et al. 1967; Havlik et al. 1979). The similarities in blood pressure within families are not restricted to hypertensives, but to all levels of blood pressure (Miall and Oldham 1963; Harrap 1994).

Twin studies (Havlik et al. 1979; Slattery et al. 1988; Hunt et al. 1989; Ditto 1993; Fagard et al. 1995; Pausova et al. 2001) show that as much as 40-70% of blood pressure variance can be explained by genetic factors (heretability). The genetic makeup of monozygotic twins is virtually identical. When raised together, environmental factors are also shared. Study of monozygotic twins brought up in
separate environments shows 34-44% of the variance in blood pressure can be explained by genetic factors (Hong et al. 1994).

Based on the blood pressure distribution of hypertensive patients and their siblings Platt launched the “single gene theory” (Platt 1947). This would mean that the presence of this gene in homozygous form would cause severe hypertension, and in heterozygous form moderate hypertension. This theory was opposed by Pickering (Pickering 1959) arguing polygenic inheritance, which also reflects the current consensus.

Blood pressure is a quantitative trait. Any characteristic dependent on the additive action of a large number of small, individually independent causes will show a normal distribution in the population. This is the case for the blood pressure distribution of which hypertension represents the upper tail. However, there are many factors that complicate the dissection of blood pressure as a genetic trait, and the individual genetic contributions are most likely not independent, due to gene-gene and gene-environment interaction. In addition, the same genetic background can be responsible for more than one phenotype (the observable characteristics). This is exemplified by the genetic clustering of hypertension, diabetes and obesity (Rice et al. 1994; Pausova et al. 2001). The “reverse” may also occur, that different genetic make-ups may give rise to the same phenotype (phenocopies).

In summary, primary hypertension is most likely not a singular disease but a clinical syndrome attributable to a variety of underlying pathophysiological mechanisms, genes and genetic variants interacting with environmental factors.
The renin-angiotensin-aldosterone system

Schematic overview of the RAAS and RAAS polymorphisms.

Circulating angiotensinogen is derived mainly from hepatocytes and secreted in a constitutive manner. Several lines of evidence support angiotensinogen contributing to the hypertensive phenotype. Gene titration studies in mice show that angiotensinogen and blood pressure levels increase in proportion to the copy number of the angiotensinogen gene (Kim et al. 1995; Smithies 1997). Conversely, angiotensinogen deficient mice are hypotensive (Tanimoto et al. 1994). In humans, polymorphisms (alternative genetic variants) within the angiotensinogen gene have been linked to hypertension, in particular the M235T polymorphism (Jeunemaitre et al. 1992; Kunz et al. 1997; Staessen et al. 1999).

Since renin catalyzes angiotensin I formation, which is conventionally viewed as the rate-limiting step leading to the formation of angiotensin II, renin activity is used as an index for endogenous angiotensin II formation. Plasma renin is secreted from the juxtaglomerular cells in the kidney, in response to a decrease in blood pressure. Sodium depletion is another important stimulus. Juxtaglomerular cells are innervated by sympathetic nerves, and β-adrenergic stimulation also increases renin release. No conclusive associations between the renin gene and hypertension have been presented (Jeunemaitre et al. 1992; Frossard et al. 2001).

Angiotensin I (inactive) is converted to the active octapeptide angiotensin II by angiotensin converting enzyme (ACE). Most ACE is membrane bound and in the vascular bed located to the surface of the endothelial cells (constitutive expression) and to the vascular smooth muscle cells of the vascular wall (inducible expression). Plasma ACE levels are stable throughout life, but show a marked interindividual variability. Approximately half of this variability is accounted for by a major gene effect (Cambien et al. 1988). The insertion/ deletion polymorphism of a 287 base pair alu repeat sequence in intron 16 of the ACE gene has been associated with both plasma (Rigat et al. 1990; Tiret et al. 1992) and tissue levels of ACE (Danser et al. 1995). This is seen in both normotensives and hypertensives, see Figure 1.
Figure 1. Serum angiotensin converting enzyme (ACE) levels (µkat/l) and the ACE insertion (I)/deletion (D) polymorphism, with the genotypes: II, ID and DD. The serum ACE level showed a highly significant difference depending on ACE genotype (p< 0.0001 for both hypertensives and normotensives, adjusted for gender, age and weight). There was no significant difference in serum ACE level between the hypertensive and normotensive groups.

The rationale behind the ACE I/D polymorphism's functionality is the assumption that increased levels of ACE render increased angiotensin II levels. Angiotensin II levels are, however, not shown to be increased in subjects with the ACE DD genotype (Lachurie et al. 1995; Ueda et al. 1995; Chadwick et al. 1997; Danser et al. 1999; van Dijk et al. 2000). Neither is there support for increased conversion of angiotensin I to angiotensin II (Lachurie et al. 1995; Chadwick et al. 1997; Danser et al. 1999; van Dijk et al. 2000). Nevertheless, these results do not rule out potential differences in angiotensin I conversion rates at specific tissue sites, where the influence of the ACE I/D polymorphism on the amount of tissue ACE may be the limiting factor. This line of reasoning is supported by an increased vascular responsiveness to angiotensin I infusion in ACE DD subjects despite a lack of association with plasma angiotensin II levels (Ueda et al. 1995; van Dijk et al. 2000).

Interestingly, gene titration experiments in mice show increased ACE levels, but not blood pressure, with increased ACE gene copy number (Krege et al. 1997; Smithies 1997). Mice lacking the carboxy-terminal of the ACE molecule (which anchors it to the plasma membrane) have significant plasma levels, but no tissue bound enzyme (Esther et al. 1997). These mice present a phenotype with low blood pressure, signifying the importance of tissue bound ACE for blood pressure control.

The ACE I/D polymorphism has been extensively investigated, sparked by the accessibility of the method and the clinical implications. As is discussed in the reviews (Butler et al. 1997; Schunkert 1997; Wang and Staessen 2000; Danser and Schunkert 2000) and meta-analyses (Samani et al. 1996; Agerholm-Larsen et al. 1997; Staessen et al. 1997), there is no conclusive evidence of this polymorphism's relation to hypertension. However, a number of more recent studies have associated the ACE I/D polymorphism with hypertension in men (Fornage et al. 1998; O'Donnell et al. 1998; Higaki et al. 2000; Uemura et al. 2000). Wang et al. suggests that the ACE D allele be viewed as a risk factor that requires long-term exposure to other additional risk factors.
to alter phenotype presentation (Wang and Staessen 2000). This may explain why ACE DD carries a higher risk of hypertension in men, an increased risk of LVH in untreated hypertension (Kuznetsova et al. 2000) and an increased risk of nephropathy in the presence of diabetes (Fujisawa et al. 1998).

The angiotensin II type 1 receptor (AT_1-receptor) mediates most of the effects of angiotensin II - vasoconstriction, the stimulation of sodium resorption, the stimulation of aldosterone biosynthesis, cellular growth and hypertrophy, and facilitation of sympathetic nerve transmission - and is expressed in the tissues required for these functions. Mice with a disrupted AT_1-receptor gene present hypotension (Sugaya et al. 1995). The blood pressure and vascular response to infused angiotensin II is in proportion to copy number (Ito et al. 1995). The AT_1-receptor A1166C polymorphism is located in the 3’ untranslated region of the AT_1-receptor gene and has been associated with hypertension (Bonnardeaux et al. 1994; Wang et al. 1997; Kainulainen et al. 1999) and aortic stiffness (Benetos et al. 1996). There is evidence of the 1166 C allele being related to increased angiotensin II response (Amant et al. 1997; Spiering et al. 2000). There are however no mechanistic explanations for the functionality of this polymorphism.

Aldosterone is a mineral corticoid synthesized in the adrenal gland, and acts on renal, and other, epithelial cells to enhance sodium absorption and potassium and hydrogen ion excretion. Along with sodium retention, follows water retention, leading to volume expansion. Aldosterone synthase (CYP11B2) is the enzyme responsible for the final steps in the biosynthesis of aldosterone. Its expression regulates the level of aldosterone secretion, which is in turn regulated by angiotenin II and potassium levels. Gene polymorphisms, in particular C-344T, have been shown to influence aldosterone levels and been associated with hypertension (Brand et al. 1998; Davies et al. 1999).

The circulating (endocrine) RAAS is thought to be responsible for short-term regulation of the cardiovascular system, and tissue (local) RAAS the long-term regulation and the adjustment of regional blood flow (Dzau 1993). Many tissues can synthesize angiotensin II independent of circulating renin, and all RAAS components are present in the blood vessels and the heart. There is evidence, as reviewed by De Mello et al. (De Mello and Danser 2000), that angiotensin II synthesis occurs in cardiac tissue under normal conditions, dependent of renin and angiotensinogen from the circulation. However, in response to pathological conditions, cardiac renin and angiotensinogen genes may be “switched on”. There is also evidence to indicate that angiotensin II has physiologically important “intracrine” actions, such as the regulation of heart contractility and impulse propagation (Re 2000).

Despite adequate long-term ACE inhibition, angiotensin II levels are not decreased (Mento and Wilkes 1987), suggesting enzymes other than ACE to play an important role in the conversion of angiotensin I to angiotensin II. There is evidence that chymase (Urata et al. 1990) (Richard et al. 2001) may be the main enzyme involved in angiotensin II formation in cardiovascular tissue, while ACE is of most importance for angiotensin II formation in circulation.
Left ventricular hypertrophy

Left ventricular mass is normally distributed in the general population (Levy et al. 1987). The cut-off for left ventricular hypertrophy (LVH) was originally determined arbitrarily as the left ventricular mass index (LVMI) exceeding 2 standard deviations in healthy middle aged men (Levy et al. 1987), i.e. corresponding to the upper end of the LVMI distribution curve (Casale et al. 1986; Koren et al. 1991; Verdecchia et al. 1994). The prevalence of echocardiographic LVH in the general population is approximately 16-19% in young-middle aged subjects (Levy et al. 1987) and 25% in elderly subjects (Levy et al. 1988; Andren et al. 1996). In middle-aged hypertensives, the prevalence of echocardiographic LVH is approximately 35% (Ganau et al. 1992). The range of LVH, however, is large and varies according to the selected LVH criteria (Cuspidi et al. 2001).

Mechanical forces are thought to be the principle determinants of cardiac hypertrophy. Due to an increased afterload, chronically elevated blood pressure is assumed to lead to cardiac myocyte hypertrophy and an increase of extracellular protein. Interestingly, LVH has also been shown to precede and been proposed to contribute to, the development of hypertension (de Simone et al. 1991; Devereux et al. 1991; van Hooft et al. 1993; Post et al. 1994; Post et al. 1994). This is most likely the result of the multifactorial nature of LVH in which hemodynamic, neurohumoral and genetic factors are causative. Analyses of juvenile twins have estimated the inter-individual variability of left ventricular mass to be strongly determined by inherited factors (Bielen et al. 1991; Verhaaren et al. 1991), a finding also supported by data from the Framingham Heart Study (Post et al. 1997). In a study by Schunkert et al. (Schunkert et al. 1999), the risk of LVH is shown to be substantially increased in siblings of affected individuals. Furthermore, RAAS gene polymorphisms have also been associated with LVH (Schunkert et al. 1994; Jeng 1999).

LVH represents a powerful predictor for cardiovascular morbidity and mortality independent of other cardiovascular risk factors, such as blood pressure, in both the general population (Levy et al. 1989; Levy et al. 1990) and in hypertensive patients (Koren et al. 1991; Bikkina et al. 1994; Schillaci et al. 2000). The relation between LVMI and cardiovascular risk has been shown to be linear (Levy et al. 1989; Vasan et al. 1997; Schillaci et al. 2000), analogous with the above discussion on blood pressure. However, these findings are opposed by data showing that it is only in the highest quartile of LVMI that there is a substantial risk increase (Levy et al. 1990). Though results from large intervention studies are still needed, regression of LVH may be associated with an improved prognosis (Levy et al. 1994; Muiesan et al. 1995; Verdecchia et al. 1998). ACE inhibitors (Dahlof et al. 1992; Schmieder et al. 1998) and more recently AT1-receptor antagonists (Thurmann et al. 1998; Malmqvist et al. 2001) appear to be superior to other antihypertensive agents in inducing regression of LVH, independent of the effect on blood pressure reduction, implicating the essential role played by the RAAS in the development of LVH.

Only part of the change in LVMI in response to antihypertensive treatment can be explained by the change in blood pressure. In addition, the individual patient's response, measured as change in LVMI in response to antihypertensive treatment, is not possible to predict. This large variation in response to 3 months of antihypertensive treatment is illustrated in Figure 2. It was therefore postulated that polymorphisms in the RAAS are associated with the change in LVMI in response to antihypertensive treatment.
Figure 2. Distribution of the change in left ventricular mass index (ΔLVMI, %) after 3 months antihypertensive treatment with either the AT1-receptor antagonist irbesartan or the β-blocker atenolol. Count (n). This figure illustrates the wide range of response measured as ΔLVMI (%) to antihypertensive treatment.
Endothelial function

The endothelium is a monolayer of cells coating the innermost surface of all blood vessels. Previously believed to be an inert and passive layer, the current understanding is that the endothelium plays a key role in the control of vascular patency and tone. Nitric oxide is viewed as the most important vasodilator produced by the endothelial cells, counterbalanced mainly by endothelin-1 in concert with angiotensin II. The vasodilatory capacity (endothelium-dependent vasodilation, EDV) of the endothelium is one measure of its function. The technique used in this thesis serves as an indirect measure of the endothelial cells capacity to produce nitric oxide.

EDV is reduced in normotensive subjects with familial history of hypertension (Taddei et al. 1992), and in subjects presenting the classic risk factors for cardiovascular disease; e.g. aging (Gerhard et al. 1996), male gender (Taddei et al. 1996), smoking (Celermajer et al. 1993), hyperlipidemia (Creager et al. 1990), diabetes (Johnstone et al. 1993) and hypertension (Panza et al. 1990). There is evidence that basal nitric oxide formation is decreased in essential hypertension (Forte et al. 1997), which may account for the abnormalities in vascular function. Moreover, hypertensives with LVH have a more pronounced blunting of endothelial function than that of other hypertensives (Perticone et al. 1999; Millgard et al. 2000). LVH and endothelial dysfunction in hypertensive patients may be considered indicative of preclinical cardiovascular disease that may be reversed by therapeutic intervention.

Decreased EDV has shown to be reversible with lipid lowering drugs, L-arginine (a nitric oxide precursor), free radical scavengers, antioxidants (vitamin C and E, folic acid), physical exercise and estrogen replacement therapy in postmenopausal women (see reviews (Celermajer 1997; Drexler and Hornig 1999)). In young healthy subjects, inhibition of the RAAS has been shown to improve EDV (Millgard and Lind 1998). ACE inhibitors (Hirooka et al. 1992; Mancini et al. 1996; Millgard and Lind 1998) and more recently, AT1-receptor antagonists (Ghiadoni et al. 2000; von Zur Muhlen et al. 2001) have also shown to improve endothelial function in hypertensives. There are, however, conflicting results (Kiowski et al. 1993; Creager and Roddy 1994) and there is yet no conclusive evidence determining whether RAAS blocking agents are superior to other antihypertensive drugs in this context.

Endothelial dysfunction may be regarded as a forerunner of hypertension and atherosclerotic presentations of cardiovascular disease (Celermajer et al. 1992; Sorensen et al. 1994; Luscher and Noll 1996), and has been shown to be an independent predictor of disease progression and cardiovascular event rate (Schachinger et al. 2000; Suwaidi et al. 2000; Perticone et al. 2001). Thus, evaluating endothelial function may serve as an integrated index of overall cardiovascular risk factors and an early intermediate phenotype of hypertension. It is therefore of interest to study gene polymorphisms in the RAAS in relation to endothelial function. This has been done only in a few studies (Celermajer et al. 1994; Buikema et al. 1996; Perticone et al. 1998; Butler et al. 1999; Arcaro et al. 2001; Rossi et al. 2001), with inconclusive results. In this thesis endothelial function was assessed by analyzing EDV in relation to polymorphisms in the RAAS in a population sample of apparently healthy, normotensive individuals.
Antihypertensive treatment

Why do we treat hypertension?

A lower blood pressure confers a lower risk of stroke and coronary heart disease and there is no evidence of a blood pressure threshold below which this is not the case. This was clearly shown for DBP levels in the classic study by MacMahon (MacMahon et al. 1990). Prior to the Fifth Joint National Committee report on the detection, evaluation and treatment of high blood pressure (JNC V) 1993, hypertension classification was based solely on DBP criteria. At present, the debate concerning the importance of SBP is ongoing (Neaton and Wentworth 1992). Several groups suggest that SBP should become the major criterion for diagnosis, staging and therapeutical management, particularly in middle-aged and older hypertensives (Black 1999; Lloyd-Jones et al. 1999; Izzo et al. 2000; Staessen et al. 2000; Franklin et al. 2001). Others advocate the superiority of pulse pressure to both SBP and DBP in predicting cardiovascular events (Franklin et al. 1999; O'Rourke and Frohlich 1999; Wilkinson et al. 2000).

When considering pharmacological treatment, assessment of the individual's total cardiovascular risk in addition to blood pressure level is essential. This management strategy is emphasized in the guidelines of the World Health Organization-International Society of Hypertension, 1999. (See Table 2 for a summary). Lifestyle measures should be initiated for all patients, and treatment of other major cardiovascular risk factors implemented when present. If an individual exhibits several risk factors and/or target organ damage, thus presenting a high absolute risk, antihypertensive treatment can be beneficial even at blood pressure levels which may otherwise not have been treated in a low-risk patient.

Table 2. Adapted from 1999 World Health Organization - International Society of Hypertension Guidelines for the Management of Hypertension. TOD = target organ damage, V = very.

Stratification of Risk to Quantify Prognosis

<table>
<thead>
<tr>
<th>Blood pressure (mm Hg)</th>
<th>Grade 1 (mild hypertension)</th>
<th>Grade 2 (moderate hypertension)</th>
<th>Grade 3 (severe hypertension)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other Risk Factors &amp; Disease History</td>
<td>SBP 140-159 or DBP 90-99</td>
<td>SBP 160-179 or DBP 100-109</td>
<td>SBP ≥ 180 or DBP ≥ 100</td>
</tr>
<tr>
<td>I no other risk factors</td>
<td>LOW RISK</td>
<td>MED RISK</td>
<td>HIGH RISK</td>
</tr>
<tr>
<td>II 1 - 2 risk factors</td>
<td>MED RISK</td>
<td>MED RISK</td>
<td>V HIGH RISK</td>
</tr>
<tr>
<td>III 3 or more risk factors or TOD or diabetes</td>
<td>HIGH RISK</td>
<td>HIGH RISK</td>
<td>V HIGH RISK</td>
</tr>
<tr>
<td>IV Associated clinical conditions</td>
<td>V HIGH RISK</td>
<td>V HIGH RISK</td>
<td>V HIGH RISK</td>
</tr>
</tbody>
</table>

From a public health-perspective, reducing blood pressure, by even a few mmHg, might save large numbers from premature deaths and disabling strokes. Yet, despite increased awareness of the importance of lowering blood pressure levels, in the case of the US, less than half of the patients with hypertension are treated, of whom an approximate 1/4 are adequately controlled (1997; Burt et al. 1995). Similar numbers are also found in Sweden (Björklund et al. 2000). This poor blood pressure control is
largely attributed to inadequate control of SBP (Lloyd-Jones et al. 2000; Franklin et al. 2001).

**What drugs are used to treat hypertension?**
In early studies of malignant hypertension (Mohler and Freis 1960; Björk et al. 1961) the benefit of antihypertensive drugs was so great that randomized trials were not undertaken and the value of pharmacological treatment of this condition gained rapid, widespread acceptance. Reports suggesting therapy of nonmalignant hypertension to be beneficial began to appear during the 1960s. The first definitive proof that this is the case came from the Veterans Administration Cooperative Study (Veterans Administration Cooperative Study Group on Antihypertensive Agents 1967), and was followed by other large studies of milder forms of hypertension. The benefit of treatment in the elderly with systolic hypertension (Dahlof et al, 1991; Mulrow et al, 1994; Curb et al; 1996; Staessen et al, 1997) was later proven.

Drugs used in the early studies were mainly diuretics and β-blockers (Collins et al. 1990). Today our first-line antihypertensive drugs are diuretics, β-blockers, calcium channel blockers and ACE inhibitors, with AT₁-receptor antagonists on the rise and α-blockers used as additional treatment when necessary. With the certainty that lowering blood pressure is beneficial, the question is whether newer drugs, such as ACE inhibitors and calcium antagonists, are better than diuretics and β-blockers at reducing morbidity and mortality due to cardiovascular disease. This question was addressed in the STOP Hypertension-2 study (Hansson et al. 1999), which concluded that there was no added benefit from these “newer” drugs. These findings are also supported by recent results from the WHO-ISH Blood Pressure Lowering Treatment Trialists' Collaboration (1998) (Neal and MacMahon 1999; Neal et al. 2000).

There is still no conclusive evidence that the major benefits of treating hypertension are due to any particular drug property, but rather to the lowering of blood pressure per se. At the same time, the individual blood pressure variation in response to specific antihypertensive drugs is great (Laragh et al. 1988; Lind et al. 1995). This is confirmed in our studies (I-III), as shown in Figure 3, illustrating the wide range of response in SBP after 3 months of antihypertensive treatment. Attempts to predict blood pressure response to antihypertensive treatment effect based on plasma renin levels and salt sensitivity (Laragh et al. 1979; Buhler 1988; Oshima et al. 1988), insulin levels, obesity, and insulin resistance (Lind et al. 1995) have not been conclusive. This stated, we come to the essence of this thesis. How do we know which drug to choose for the individual hypertensive patient?
Figure 3. Distribution of the change in systolic blood pressure (ΔSBP, mmHg) after 3 months antihypertensive treatment with either the AT$_1$-receptor antagonist irbesartan or the β-blocker atenolol. This figure illustrates the wide range of SBP response to antihypertensive treatment.
Pharmacogenetics

The concept of a familial component modulating drug response was described in the 1950s, often in connection with case-reports of unexpected drug response (Hughes et al. 1954; Carson et al. 1956; Kalow 1956; Evans et al. 1960). The variation in drug metabolism (pharmacokinetics) was ascribed to different metabolic rates in the enzymes either activating or inactivating the drug. The term pharmacogenetics (the study of heritability on drug response) was coined prior to current knowledge in molecular biology. The current explosion of interest for this field stems from technological advances, such as the mapping of the human genome and SNP (single nucleotide polymorphism) maps constituting the basis for our understanding of individual genetic diversity, and the fact that the results of these efforts are publicly accessible.

Drug selection today is based on statistical information gained from clinical trials undertaken in the general population with inference to the individual patient, i.e. population averages rather than individual profiles. Pharmacogenetics holds the promise of individualizing drug treatment (Evans and Relling 1999; McLeod and Evans 2001), which will lead to more effective drug treatment based on the individual patient’s unique genetic profile and in turn decrease the risk of unwanted side-effects. Pharmacogenetics may in addition serve as a tool for drug development (Lindpaintner 1999; Roses 2000; Shi et al. 2001).

Pharmacogenetics can be separated into two basic components, pharmacokinetics (drug metabolism) and pharmacodynamics (how a drug acts). Polymorphisms in the CYP2D6 gene (a cytochrome P450 enzyme) account for polymorphic responses to 25% of all currently available prescription drugs (Marshall 1997; Kleyn and Vesell 1998). The variation in therapeutic response to warfarin treatment due to CYP2C9 polymorphisms (Furuya et al. 1995; Steward et al. 1997; Aithal et al. 1999) is an example of pharmacokinetics. Another essential aspect of pharmacokinetics is drug absorption. Hoffmeyer et al. (Hoffmeyer et al. 2000) has demonstrated that polymorphisms in the multidrug-resistance gene-1 influence digoxin absorption.

Pharmacodynamics reflects the activity and thus the efficacy of a drug on its target. The drug-target interaction is not to be looked at in isolation but in the context of a complex physiological system, as illustrated by the heterogeneity of therapeutic response to asthma medication (Drazen et al. 1999). More closely related to the work in this thesis is the antihypertensive response to ACE inhibitor treatment in relation to the ACE I/D genotype. Several investigators showed no genotype related effect on antihypertensive response (Hingorani et al. 1995; Dudley et al. 1996; Kohn et al. 1999). Ohmichi et al. (Ohmichi et al. 1997) however, demonstrated that hypertensive patients with the ACE II genotype showed the most pronounced antihypertensive response.

There are numerous other genetic polymorphisms in drug-metabolizing enzymes, transporters and targets, a compilation of which can be found at http://www.sciencemag.org/feature/data/1044449.shl (Evans and Relling 1999).

This thesis addresses the pharmacodynamics of antihypertensive treatment, more specifically of the β-blocker atenolol and the AT1-receptor antagonist irbesartan, the hypothesis being that polymorphisms in the RAAS are in part responsible for the individual variation in drug response. Ultimately, the most complete pattern of individual drug responses is rendered by combining pharmacodynamics and pharmacokinetics.
Aims of the study

The hypothesis of studies I-III was that the response to antihypertensive treatment is in part genetically determined. The aim was therefore to determine whether specific gene polymorphisms in the renin-angiotensin-aldosterone system were related to:

- the antihypertensive response to the AT₁-receptor antagonist irbesartan and the β-blocker atenolol (studies I and II).

- the change in left ventricular mass in response to antihypertensive treatment with the AT₁-receptor irbesartan and the β-blocker atenolol (study III).

- endothelial function, measured as the endothelium-dependent vasodilation in healthy adults (study IV).
**Study Population**

**Study I-III**
The study material comes from the SILVHIA (Swedish Irbesartan Left Ventricular Hypertrophy Investigation vs Atenolol) trial (Malmqvist et al. 2001). This is a multicenter trial designed to evaluate the efficacy of the AT1-receptor antagonist irbesartan, in comparison with the beta-blocker atenolol, on the regression of LVH in hypertensive patients during optimal blood pressure control. A total of 115 patients were randomized to receive irbesartan 150 mg or atenolol 50 mg once daily as monotherapy, in a double-blinded parallel group trial. The dose was doubled after 6 weeks if the diastolic blood pressure was equal to or exceeded 90 mmHg. Following randomization, 14 patients discontinued the trial. We analyzed a random sample of 86 (study I and II) and 84 (study III) subjects with DNA sampling. Data presented here relates to blood pressure response and reduction of LVMI after three months treatment.

Men above the age of 18 and postmenopausal women with primary mild to moderate hypertension and LVH, verified by echocardiography were enrolled. The inclusion criteria for hypertension constituted a diastolic blood pressure of 90-115 mmHg at two examinations within one week, with values differing no more than 8 mmHg. Secondary hypertension was excluded by physical examination and appropriate laboratory analyses. In addition, we measured plasma renin activity and serum aldosterone in plasma. All patients were Caucasians. All antihypertensive agents were withdrawn before the start of a 4-6 week, single-blind, placebo lead-in period.

**Study IV**
The study population (32 males and 27 females) was randomly selected from the population registry (Sarabi et al. 1999). These individuals were apparently healthy and were not using any regular medication, including estrogen replacement therapy or contraceptive pills. All subjects were Caucasian and ranged from 24 to 69 years of age. The blood pressure mean was 122±14/79±8 mmHg.
Methods

Blood pressure measurements (study I-III)
Blood pressure was measured by trained nurses, using a mercury sphygmomanometer, after the patients had rested for at least 10 minutes in the seated position, and was determined as the average of three measurements taken one minute apart. During treatment blood pressure was measured at trough (24 ± 3 hours after the last dose).

Echocardiography (study I-III)
Echocardiography was performed with the patient in the left semilateral position. The ultrasound devices used were the Acuson 128 X P/10 (Mountain View, CA, USA), Vingmed CFM 750 (Vingmed Sound, Horten, Norway) and HP SONOS 2500 (Andover, MA, USA). Measurements were performed on three to five consecutive beats, from which mean values were calculated. Basic measurements of LV dimensions in diastole (LVEDD) and intraventricular septum (IVS) thickness and posterior wall thickness (PWT) were made by M-mode technique. The Penn convention was used for calculation of left ventricular mass, which was corrected for body mass index (LVMI) (Devereux et al. 1986). The body weight was the actual weight measured in conjunction to each echocardiographic examination. Left ventricular hypertrophy was considered present if LVMI was >131 g/m² for men and >100 g/m² for women (Devereux et al. 1986). For details see Malmqvist et al. (Malmqvist et al. 2001).

Genotyping
Genomic DNA was prepared using spin columns (QIAamp DNA Blood Mini Kit, QIAGEN, Germany). The ACE I/D, M235T and the T174M restriction fragment length polymorphisms were analyzed as previously described (Shanmugam et al. 1993; Caulfield et al. 1994). Both the AT₁-receptor A1166C polymorphism (Kainulainen et al. 1999) and the aldosterone synthase (CYP11B2) T-344C polymorphism (Kurland et al. 2001) were analyzed by solid-phase minisequencing (Syvänen et al. 1993).

Hormone analysis (study I-III)
Blood samples for hormone analyses were obtained in the morning after overnight fasting. Following blood pressure and heart rate measurements, an indwelling venous catheter was placed in an antecubital vein and blood samples were drawn after an additional 30 minutes (at minimum) rest in the supine position. Following centrifugation (1,500 x g for 15 minutes), all samples were stored at -70°C until analyzed at the core laboratory. Serum ACE was determined using the artificial substrate 3-(2-furyl-akryloyl)-L-phenylalanyl-glycyl-glycin (FAPGG) from which 3-(2-furyl-akryloyl)-L-phenylalanine (FAP) was formed and its concentration determined by spectrophotometry (Buttery and Stuart 1993). Serum aldosterone was analyzed by radioimmunoassay (McKenzie and Clements 1974).

Forearm blood flow and vascular function (study IV)
All studies were performed with the subjects in supine position, in a quiet air-conditioned room at a constant temperature (20° C), after overnight fasting. The subjects were instructed to refrain from smoking the day of the study.

Forearm blood flow (FBF) was measured by venous occlusion plethysmography. A 20-gauge polyethylene catheter was inserted into the brachial artery for drug infusion. This arm was slightly elevated above the level of the right atrium. A mercury-filled sialic strain gauge was placed on the widest part of the forearm and connected to a calibrated
plethysmograph. A cuff placed on the upper arm was inflated to 40 mmHg with a rapid cuff inflator in order to exclude venous outflow from the forearm. Vasodilations were performed on one arm, while the other arm was used as a control.

All participating subjects rested for 30 minutes after arterial cannulation before data collection. EDV was assessed by intra-arterial metacholine infusion (2 and 4 µg/min, each for 5 minutes). We have recently shown in a pilot study in healthy volunteers that local infusion of metacholine (4 mg/min) increased the forearm release of nitrate and nitrite, the stable breakdown products of NO, ten-fold, thus validating that methacoline induces EDV by increased NO production (Lind et al. 2000). EIDV was assessed by an intra-arterial sodium nitroprusside infusion (5 and 10 µg/min, each for 5 minutes). The drugs were administered in random order at a rate of 1 ml/min, with a 30 minutes washout period. The mean of five measurements was obtained at each time point. EDV and EIDV are given as the percental change from baseline FBF at the highest doses used for the two vasodilators.

The reproducibility of the test of EDV and EIDV has previously been evaluated in 10 healthy young volunteers in whom the investigation was performed before and after 2 hours of i.v. saline infusion and was repeated after 2-3 weeks. Baseline resting FBF showed a variation of less than 10%, while FBF during vasodilation induced by either metacholine or sodium nitroprusside showed a variation of less than 5%, both in the short-term (2 hours) as well as in the long-term (2-3 weeks) perspective (Lind et al. 1998).

The appropriate university ethics committees approved these studies (I-IV). The patients gave informed consent and the studies were performed in accordance with institutional guidelines.

Data are presented as mean values ±SD. Differences between groups were calculated with ANOVA. In study II and III, the response is calculated as the change in blood pressure or left ventricular mass after treatment divided by the baseline level. Adjustments for changes in diastolic and systolic blood pressure responses were done with multiple regression analysis (study III). Chi-square analysis was performed to examine if the gene polymorphisms were related (study III). Calculations were done using the StatView version 4.5 software program. p<0.05 was regarded as significant.
Results and Discussion

Baseline characteristics (study I-III)

No significant differences were seen between the two treatment groups regarding age (mean 54±8 SD years), gender (34% women), blood pressure (mean SBP 162±20 SD mmHg and DBP 103±8 mmHg) or LVMI (mean 144±31 SD g/m^2) before treatment. Neither were there significant differences in blood pressure or LVMI at baseline depending on genotype for the two treatment groups.

Blood pressure response (study I-II) and regression of LVMI (study III) in relation to treatment

After three months treatment with either irbesartan or atenolol as monotherapy, significant reductions in blood pressure were achieved in both groups. The mean doses of irbesartan and atenolol were 247 mg and 72 mg o.d., respectively. 67% of the patients treated with irbesartan and 45% of the patients treated with atenolol received the higher doses (300 mg vs 100 mg). The blood pressure reduction was -14/-11±19/7 mmHg with irbesartan and -11/-9±15/11 mmHg with atenolol, with no significant difference between drug groups. There was no association between dose and blood pressure response after 3 months treatment with either drug.

ACE gene polymorphism is associated with the diastolic blood pressure response to AT₁ receptor antagonist treatment (study I)

Patients with the ACE II genotype showed a significantly more pronounced reduction in diastolic blood pressure after three months treatment with the AT₁-receptor antagonist than those with the DD or ID genotype (p=0.01), see Figure 4. Approximately 89% of the patients homozygous for the I allele showed a reduction in diastolic blood pressure equal to or exceeding 10 mmHg, compared to 42% of those with the D allele. This was not the case for patients being treated with the β-blocker. The interaction term between type of treatment and ACE II genotype was significant (p=0.02). A similar trend was observed for the systolic blood pressure response during AT₁-receptor antagonist treatment (-24±15 mmHg for those without the D allele vs -12±19 mmHg for those with the D allele, p=0.09).

Figure 4. Change in diastolic blood pressure (ΔDBP, mmHg) after 3 months treatment with either the AT₁-receptor antagonist irbesartan or the β-blocker atenolol and ACE genotypes. In the irbesartan group the difference in systolic blood pressure reduction between the ACE genotypes was significant (p=0.01), ANOVA. Count in each genotype group (n). Mean values ± SEM.
Why is the ACE II not the DD genotype associated with diastolic and not systolic blood pressure response?
The ACE genotype is associated with serum ACE levels, Figure 1. However, serum ACE levels do not predict antihypertensive response to ACE inhibitors, as shown by Todd et al. (Todd et al. 1995) and Reneland et al. (Reneland et al. 1999). Also, serum ACE is not associated with skeletal muscle ACE, showing that the circulating ACE level does not reflect the tissue level in a simple manner (Reneland et al. 1999). In addition, it has been shown that plasma levels of angiotensin II show no variation with ACE genotypes (Harrap et al. 1993). Thus, from a mechanistic point of view, it is presently unclear why the ACE II genotype predicts the blood pressure response to AT1-receptor antagonist treatment. However, we can speculate that since diastolic blood pressure is more closely related to peripheral resistance than the systolic blood pressure, that the vascular control at the tissue level may be more closely related to the ACE genotype without necessarily being related to the circulating levels of ACE. Our findings are supported by Ueda et al. (Ueda et al. 1998) showing the effect of enalaprilat to be significantly greater and longer lasting in subjects with the ACE II genotype.

Aldosterone synthase gene polymorphism is associated with the systolic blood pressure response (study II)
The systolic blood pressure lowering response differed between the aldosterone synthase -344 C/T genotypes when all the patients were analyzed together (p=0.02). However, when the treatment groups were studied separately, the systolic blood pressure reduction was significant only in the irbesartan group (p=0.04 for irbesartan and p=0.43 for atenolol). The patients treated with irbesartan with the TT variant had the most pronounced response, Figure 5. There was no association between the CYP11B2 -344 C/T polymorphism and the diastolic blood pressure response for the entire sample (p=0.21) or for the two treatment groups when considered separately (irbesartan treatment: TT (n=17) -12±11 mmHg, TC (n=18) 6±11 mmHg, CC (n=8) -6±10 mmHg, p=0.17 and atenolol: TT (n=17) -12±7 mmHg, TC (n=19) -11±8 mmHg, CC (n=7) -11±7 mmHg, p=0.93). There was no association between the dose and the genotype.

There was no association between the CYP11B2 polymorphism and serum aldosterone levels. Neither baseline serum aldosterone levels nor the change in aldosterone levels after treatment were associated with the change in systolic or diastolic blood pressure after three months treatment.

Why is the aldosterone synthase polymorphism related to systolic and not diastolic blood pressure response?
Expression of aldosterone synthase has been found in human vascular endothelium (Takeda et al. 1996), suggesting that aldosterone is synthesized in these tissues. Increased expression of this gene could lead to increased local aldosterone levels, potentially leading to vascular hypertrophy and fibrosis without necessarily raising the circulating levels of aldosterone (Brilla et al. 1993; Young et al. 1994). Fibrosis of conduit arteries could lead to reduced arterial compliance and hence increased systolic blood pressure. Indeed, the aldosterone synthase -344 C/T polymorphism has been associated with increased pulse wave velocity, a marker of reduced arterial compliance (Pojoga et al. 1998). Therefore we speculate that the aldosterone synthase -344 C/T
polymorphism could be more closely associated with the regulation of systolic than that of diastolic blood pressure.

Serum aldosterone is by definition within the normal range in primary hypertension, but is on average abnormally high for the concurrent renin level. The -344 C/T polymorphism may be directly implicated in transcriptional control or it could be in linkage disequilibrium with a yet unidentified genetic variant. Whether this polymorphism represents a functional variant that can lead to increased expression of the CYP11B2 gene is still unclear. Inconsistent findings have been reported regarding the associations between this polymorphism and plasma and urinary aldosterone levels (Pojoga et al. 1998; Davies et al. 1999; Schunkert et al. 1999). In the present study we did not find an association between the -344 C/T polymorphism and baseline aldosterone levels. Neither serum aldosterone levels nor the reductions in serum aldosterone (Kahan et al. 1998) were correlated to the blood pressure response to treatment. Thus, although aldosterone levels may be related to blood pressure levels in untreated hypertensives, they do not appear to be related to the antihypertensive response to treatment; if the -344 C/T polymorphism is a functional variant or not remains to be proven.

Figure 5. Change in systolic blood pressure (ΔSBP, mmHg) after 3 months treatment with the AT1-receptor antagonist irbesartan or the β-blocker atenolol for the CYP11B2 genotypes. In the irbesartan group the difference in systolic blood pressure reduction between the CYP11B2 genotypes was significant (p=0.04), ANOVA. Mean values ± SEM. Count in each genotype group (n).

AGT and AT1-receptor gene polymorphisms are related to regression of LVMI (study III)

The angiotensinogen polymorphisms
Hypertensive patients with the angiotensinogen 174 TM genotype were associated with a greater regression in LVMI than those with the TT genotype in the group treated with irbesartan (p=0.005, see Figure 6). This association was still significant (p=0.005) when adjusted for the change in diastolic and systolic blood pressure induced by treatment. No subjects homozygous for the M allele were found in this sample.

The angiotensinogen 235 polymorphism was associated with the change in LVMI induced by irbesartan treatment (p=0.04, Figure 6), also after adjustment for the percentile diastolic and systolic blood pressure change (p=0.02). For this genotype,
those carrying the T allele showed the largest reduction in LVMI. None of the two angiotensinogen polymorphisms were associated with the change in LVMI during treatment with atenolol.

Chi-square analysis showed the two angiotensinogen polymorphisms to be related to each other (p=0.025), while no such associations were seen between the other polymorphisms.

Figure 6. Change in left ventricular mass index (ΔLVMI g/m²)±SEM and the angiotensinogen T174M and the angiotensinogen M235T after 3 months treatment with either the AT₁ receptor antagonist irbesartan or the β-blocker atenolol. In the irbesartan group for the T174M polymorphism, p=0.005 and for M235T p=0.02 when adjusted for change in blood pressure. Count in each genotype group (n).

The T allele of the M235T polymorphism has previously been associated with increased angiotensinogen levels and hypertension (Jeunemaitre et al. 1992; Staessen et al. 1999). In the case of cardiac hypertrophy some studies support an association (Jeng
and others not (Ishanov et al. 1997). The M235T and the T174M are at some distance from both the angiotensinogen cleavage site and the promoter region and are most likely in linkage disequilibrium with another variant, such as the A-6G variant (Inoue et al. 1997), which may cause a change in the angiotensinogen gene expression. Increased cardiac angiotensinogen levels could lead to an increased local RAAS activity inducing cardiac hypertrophy. Thus, the present results provide additional support for angiotensigen gene polymorphisms to be involved in the development and maintenance of LVH.

**The AT1 receptor 1166 polymorphism**

Also the AT1-receptor 1166 polymorphism was associated with the regression of LVMI in those treated with irbesartan (p=0.02) and remained significant when adjusted for the diastolic and systolic blood pressure response (p=0.02). Patients with the AT1-receptor 1166 AC genotype exhibited the most pronounced reduction in LVMI. Nor was this polymorphism associated with the response to treatment with atenolol.

Using a stepwise multiple regression analysis (with age, gender, and change in blood pressure as possible confounders together with the angiotensinogen 174 and 235 and the AT1-receptor 1166 genotypes as independent variables) the significant independent determinant of change in LVMI, after three months treatment with irbesartan was the angiotensinogen 174 polymorphism (p=0.005).

![Graph showing change in left ventricular mass index (ΔLVMI, g/m²)±SEM and the AT1-receptor A1166C polymorphism after 3 months treatment with either the AT1 receptor antagonist irbesartan or the β-blocker atenolol. Counts for each genotype group (n).](image)

**Figure 7.** Change in left ventricular mass index (ΔLVMI, g/m²)±SEM and the AT1-receptor A1166C polymorphism after 3 months treatment with either the AT1 receptor antagonist irbesartan or the β-blocker atenolol. In the irbesartan group, p=0.02 when adjusted for change in blood pressure. Count for each genotype group (n).

In a transgenic mouse model, Paradis et al (Paradis et al. 2000) showed that angiotensin II can induce cardiac hypertrophy and heart failure via human myocardial AT1-receptor stimulation in the absence of blood pressure changes. The AT1-receptor A1166C polymorphism has been implicated in essential hypertension (Bonnardeaux et al. 1994). However, there is no evidence for a C allele related change in AT1-receptor density and/or affinity (Danser and Schunkert 2000). This renders the mechanism of the
association between this polymorphism and regression of LVH in the irbesartan treated patients in this study unclear at present.

**The ACE and aldosterone synthase polymorphisms were not related to regression of LVMI (study III)**

Neither the ACE I/D nor the aldosterone synthase (CYP11B2) -344 C/T polymorphisms were associated with the reduction in LVMI after treatment with either drug.

**The ACE I/D polymorphism**

The association between the ACE I/D polymorphism and LVH has been studied extensively. Kuznetsova et al. (Kuznetsova et al. 2000) demonstrated in a meta-analysis the overall excess risk of developing LVH in relation to the D allele not to be significant, but when considering untreated hypertensives the D allele may behave as a marker for LVH. There are a few studies examining the effect of the ACE I/D polymorphism in relation to response to treatment with ACE inhibitors, however, the results are inconclusive. Sasaki et al. (Sasaki et al. 1996) showed the ACE DD to respond with a greater regression of LVH in hypertensive patients and Kohno et al. (Kohno et al. 1999) the opposite. In the present study, the ACE polymorphism was not related to LVMI at baseline or to the regression of LVMI during treatment.

**The aldosterone synthase polymorphism**

We expected the aldosterone synthase (CYP11B2) T-344C polymorphism to be related to the reduction in LVMI in patients treated with irbesartan since studies have shown the aldosterone level to be associated with LVH (Schunkert et al. 1997) and this polymorphism has been associated with circulating aldosterone levels (Brand 1998; Davies 1999). The mechanism behind aldosterone-induced hypertrophy is thought to be both an indirect effect, by increasing cardiac preload by salt and volume retention, and a direct effect via cardiac mineralcorticoid receptors leading to fibrosis (Lijnen and Petrov 2000). Indeed, the -344 polymorphism has been associated with left ventricular mass (Kupari et al. 1998) in a Finnish sample of young normotensive subjects, but this was not confirmed in another population study (Schunkert et al. 1999). However, in the present study no association between the -344 polymorphism and LVMI at baseline or to the change in LVMI after 3 months antihypertensive treatment could be found.

**Why are are the RAAS polymorphisms associated with response to AT1-receptor antagonist treatment and not β-blockade (study I-III)?**

The investigated polymorphisms were associated with the antihypertensive response to the AT1-receptor antagonist irbesartan, but not to the beta-blocker atenolol. This agrees with the assumption that these drugs target different antihypertensive mechanisms. One could infer that also beta-blockers affect the renin-angiotensin-aldosterone system by decreasing the renin release from juxtaglomerular cells. Although proposed as one of several modes of action for beta-blockers the present and previous reports favor mechanisms other than blockade of the renin-angiotensin-aldosterone system to be important for the antihypertensive effects of beta-blockers, as reviewed elsewhere (Prichard and Cruickshank 1995).

**Study IV**

No significant differences in age, gender, BMI, fasting blood glucose, serum cholesterol level, blood pressure or smoking habits were observed between the different ACE, angiotensinogen and AT1-receptor genotypes.
Genotype distribution in the healthy, normotensive subjects
The distribution of the ACE genotypes DD, ID and II was 22% (n = 13), 51% (n = 30), and 27% (n = 16), respectively. For the angiotensinogen gene at locus 174 with the genotypes TT, TM and MM, the distribution was 79% (n = 46), 19% (n = 11) and (n = 1), respectively. At locus 235 with the genotypes TT, MT and MM it was 22% (n = 13), 47% (n = 28) and 31% (n = 18), respectively. While no subjects showed the CC AT1-receptor genotype, 44% (n =26) were AC and 56% (n =33) AA. For the CYP11B2 gene; 22% (n=13) were homozygous for T, 51% (n=30) heterozygous TC and 27% (n=16) homozygous for C.

Subjects with ACE D allele in combination with the angiotensinogen 174 TM genotype have a blunted EDV
There were no significant differences in EDV or EIDV in relation to the ACE, angiotensinogen and CYP11B2 genotypes when analyzed separately. However, there was a tendency that EDV was reduced in individuals with the angiotensinogen genotype T174M when compared with 174 TT (p=0.06).

Subjects with the ACE D allele in combination with the 174 TM genotype (n=9) showed a reduction in EDV (but not EIDV) when compared to the rest of the sample (p< 0.05, see figure 8).

Figure 8 Endothelial-dependant vasodilation (EDV, %) in subjects for the T174M polymorphism with respect to the ACE I/D genotype. Means ± SEM are shown. * = p < 0.05, vs other genotypes.

The AT1-receptor polymorphism is associated with both EDV and EIDV
The subjects with the AC AT1-receptor genotype showed a reduction in both EDV (p=0.05) and EIDV (p=0.04) when compared to those with the AA genotype (Figure 9).

When studying the subjects with the presence of the ACE D allele, angiotensinogen T174M and the AT1-receptor AC variant (n=4), there was a substantial decrease in both EDV (267±55% compared to 443±198% for the rest of the study population, p=0.08) and EIDV (196±90% compared to 357±179%, p=0.08).
Figure 9. Endothelium dependent vasodilation (EDV, %) and endothelium independent vasodilation (EIDV, %) in subjects with the AA and AC angiotensin II type 1 receptor genotypes. Means ± SEM are shown. * = p < 0.05. AT, R 1166 AA (n=33), AC (n=26).

ACE D allele and endothelial function
The current study in apparently healthy subjects showed no association between the ACE I/D polymorphism, when studied separately, in relation to EDV. These findings are supported by previous studies (Celermajer et al. 1994; Perticone et al. 1998; Rossi et al. 2001). However, Butler et al. (Butler et al. 1999) showed a blunting of both EDV and EIDV in subjects with ACE DD. Some of the discrepancies may be a result of differing methodology. Celermajer et al. used an ultrasound based technique, while the others, as well as in the current study, used the perfused forearm technique. Recently Arcano et al. (Arcaro et al. 2001) showed that when using the forearm method in young healthy subjects, no association was found between the ACE I/D polymorphism and EDV. Interestingly, when using ultrasound methodology, those with the ACE D allele show a blunted EDV in the common femoralis artery, but not the brachial artery. Arcano et al. suggests that these site-related differences can be explained by the local (tissue) differences in vascular beds and types of arteries.

Subjects with hypertension (Perticone et al. 1998) and patients undergoing coronary by-pass surgery (Buikema et al. 1996) show an association between EDV and the ACE DD genotype or the ACE D allele. This may explain the results of the present study where it is only in subjects with the ACE D allele and the angiotensinogen 174 TM variant in combination that there is an association with a blunted EDV. We suggest that the ACE D allele requires exposure to additional risk factors, in the case of the present study, an additional genetic risk factor, the 174 TM variant in order to be associated with a decreased endothelial function.

Functional aspects of ACE I/D and AT1-receptor polymorphisms
There are several potential mechanisms connecting increased RAAS activity and endothelial dysfunction, such as angiotensin II stimulated endothelial NADH/NADPH oxidase and superoxide anion production (Griendling et al. 1994; Harrison 1997) leading to increased nitric oxide degradation. Angiotensin II also increases preproendothelin gene transcription (Moreau et al. 1997).

Moreover, bradykinin induces vasodilation by the formation of nitric oxide, which has been suggested to be one of the mechanisms behind the vasodilation induced by ACE inhibitors (Vanhoutte et al. 1989). An increased bradykinin degradation in relation to ACE levels and ACE I/D genotype has been demonstrated (Brown et al. 1998; Murphey et al. 2000). However, in the study by Murphey et al. (Murphey et al. 2000),
despite an increased bradykinin degradation in proportion to the number of ACE D alleles present, the vasodilatory response was increased. The authors explained this as the effect of the B$_2$ receptor upregulation predominating over bradykinin degradation.

In addition, stimulation of the AT$_1$-receptor, both on the endothelium and vascular smooth muscle cells, leads to vascular contraction which may explain why subjects with the AT$_1$-receptor 1166 AC polymorphism show both a blunted EDV and EIDV. The AT$_1$-receptor A1166C polymorphism has previously been associated with hypertension (Bonnardeaux et al. 1994; Kainulainen et al. 1999), aortic stiffness (Benetos et al. 1995) and increased coronary artery vasoconstriction, in vitro (Henrion et al. 1998; van Geel et al. 2000) and in vivo (Amant et al. 1997). However, the AT$_1$-receptor A1166C polymorphism is located in the 3’untranslated region of the AT$_1$-receptor gene, and no explanation to its functionality has been given, as reviewed (Danser and Schunkert 2000; Wang and Staessen 2000; Duncan et al. 2001).

In summary, subjects with the ACE D allele in combination with the angiotensinogen T174M genotype showed a reduced endothelium-dependent vasodilation. The AT$_1$-receptor 1166 polymorphism was associated with an impaired vasodilatory function in general.
Limitations

The greatest limitation in these studies is the relatively small sample size. Therefore this study should be regarded as hypothesis generating and needs to be reproduced in prospective studies with larger samples.

Roughly 80% of the patients in study I-III were previously treated hypertensives. The wash-out period of 4-6 weeks has been assessed to be sufficient for hormone levels and blood pressure to return to habitual levels. However, when it comes to LVM, there will most certainly be a residual effect of the previous antihypertensive treatment. This could contribute to the lack of association between gene polymorphisms and LVMI at baseline. Also, three months can be considered a short period of time in order to properly evaluate the long-term effect of antihypertensive therapy on the change in LVH. Thus, the conclusions from this study may only apply to the early phase of LVH regression. Despite these limitations, three polymorphisms were significantly associated with the change in left ventricular mass index induced by AT₁-receptor antagonist treatment.

Another limitation is that these studies are based on a clinical study (Malmqvist et al. 2001) and not primarily designed as a pharmacogenetic study. However, study I-III follow the same strategy as clinical antihypertensive treatment does, with titration of the drug to a blood pressure goal.

Furthermore, the patients in study I-III are hypertensive patients with LVH, which will restrict the ability to generalize the results to all hypertensives.
General Discussion and Future Perspectives

In Study III, the angiotensinogen polymorphism (174 TM and 235 T allele) and AT₁-receptor (1166 AC) were associated with the most pronounced regression of left ventricular mass index in patients treated with the AT₁-receptor antagonist. These results indicate that it is the polymorphisms related to higher cardiovascular risk that benefit most from treatment. Concerning the apparent lack of association with the ACE I/D polymorphism and response to treatment, this may be explained by the fact that the majority of these patients were receiving antihypertensive therapy prior to the study; possibly overshadowing the potential risk the ACE D allele represents.

Interestingly, the patients with the ACE II variant show the most pronounced blood pressure reduction in response to treatment. Irbesartan is a competitive AT₁-receptor antagonist. In the case of ACE II, there is, theoretically, less angiotensin II to compete with, thus a greater reduction in blood pressure.

Hypertension is thought to lead to LVH and, in some cases, LVH also precedes hypertension. Whether these “forms” of LVH represent different risks and mechanisms remains to be seen. Young men with the ACE DD genotype exhibit a greater hypertrophic response to exercise-induced LVH (Montgomery et al. 1997). In this context, LVH appears an advantage. As a response to hypertension, however, LVH constitutes a disadvantage, suggesting analogy with the “thrifty gene theory” and diabetes. From an evolutionary standpoint, these genetic compositions constituted an advantage, but in our current environment they pose a disadvantage.

Both LVH and decreased EDV can precede hypertension, suggesting a clustering of genes in which individual polymorphic loci may represent small individual risks, and the overall genome the sum of these risks. Environmental factors may be the triggers that modify this disease risk.

As we learn more about the pathophysiological mechanisms and the genes that are connected with hypertension, it is likely we will find well-defined subcategories of hypertension. In some cases, we may learn of monogenetic causes, although the majority will likely remain multifactorial and complex. In clinical studies, it is imperative to stringently define the individual hypertensive phenotype.

How do we explain all the conflicting results of genetic association studies in hypertension? The contribution of individual genotypes is most likely small, rendering the outcome of the studies especially sensitive to ethnic heterogeneity (a surrogate of genetic composition), and varying and sometimes imprecise definition of clinical phenotype. Also, assuming that each individual contribution from a specific polymorphism is small, an association with a disease may not be unmasked until provoked. Pharmacological treatment may be one way of standardizing the environmental impact and disclosing the effect of gene polymorphisms that would otherwise not be seen.

Pharmacogenetics holds the promise of individualized (antihypertensive) drug treatment and accurate dosage. With this, follows increased drug safety and avoidance of side-effects. Another result of improved pharmacotherapy is better patient compliance to treatment and likely also increased cost-effectiveness. Molecular genetics will also make it possible to analyze an individual person’s disease risk and
identify high-risk individuals. Thus, in future, we can see a shift in medical intervention from diagnosis to prediction and from therapy to prevention.

The difference between pharmacogenetics of the 1950s and that of the 21st century is the genomic revolution, brought forth by great technological advances in molecular biology and computational sciences, crowned by the publication of the human genome sequence earlier this year. The International SNP Map Working Group recently presented an initial map of the genome sequence variation containing over 1 million SNPs. The SNP map offers the potential of characterizing and correlating genes with complex traits and drug response. High throughput genomic methods will lead to faster and less expensive pharmacogenetic analyses. Differential gene expression profiling is another useful approach with which to dissect the variability of drug response. Large-scale genotyping is crucial to the identification of the genetic make-ups that underlie the onset of disease and individual variations in drug response.

The pharmaceutical industry is showing great interest in the field and large investments are being made. Pharmacokinetic studies are now being applied in early phase I trials, and samples being collected from larger ongoing phase III and IV trials for genetic analysis. At present, much investment in drug development goes to waste due to adverse drug reactions that cannot rationally be avoided, or due to non-responders being part of the trial sample. Moreover, if a specific responder subgroup were to be identifiable, fewer subjects could be included in future clinical trials. Naturally, it is in the interest of the industry to avoid side-effect prone individuals, i.e. to increase drug safety and to adjust dose accurately, as well as to identify novel drug targets.

Comparing the gene expression of “good responders” and “bad responders” would be an interesting extension of the results in this study. Another would be a prospective pharmacogenetic trial in hypertensives using a cross-over-design in which individual patient are treated with antihypertensive drugs representing each of the main drug classes.

**Closing words**

I would like to close with a quote from the World Health Organisation (http://www.who.int/inf-fs/en/fasct209.html) on human genetics and noncommunicable disease “Current and future trends in genetic approaches to disease prevention and control are strongly linked with the progress of international human genome research. Based on these trends, it is estimated that:

**Now:** It is possible to reduce the effect on mortality, disability and reproductive fitness in just under a third of single gene disorders. About 50% congenital abnormalities, 10% of inherited diseases and 2% of chromosomal disorders can be treated or corrected.

**Within five years:** Genetic approaches will become integral in many aspects of medical practice and it will be important for most health workers to have a basic understanding of medical genetics.

There will be further developments in genetic counseling, based on family-oriented approach, neonatal screening and individual testing. The number of common diseases, including cancer, diabetes, heart disease and autoimmune disorders being treated in clinical trials of gene therapy will increase.

**Within 20 years:** All human genes will have been mapped and identified. The genetic mechanisms of each disorder will have been described. Medicine will become more predictive and preventive and diagnosis and therapy will become more specific and effective. Genetic diagnosis and counseling will be integrated into an increasing range
of medical services. Genetic methodology will become a basic approach for health improvement and disease control. Gene therapy will be a universal method for disease prevention and treatment.”
Brief summary

* Hypertensive patients with the ACE II genotype presented the most pronounced diastolic blood pressure response to the AT1-receptor antagonist irbesartan (Study I).

* Hypertensive patients with the aldosterone synthase (CYP11B2) -344 TT genotype showed the most pronounced systolic blood pressure response to the AT1-receptor irbesartan (Study II).

* Polymorphisms in the angiotensinogen gene, foremost at the 174 locus, and in the AT1-receptor gene, were associated with the change in left ventricular mass during treatment with the AT1-receptor antagonist irbesartan, independent of the change in blood pressure (Study III).

* Healthy normotensive subjects with the ACE D allele in combination with the angiotensinogen 174 TM genotype show decreased endothelial function measured as endothelium-dependent vasodilation (Study IV).

* These results suggest that the response to antihypertensive treatment is associated with polymorphisms in the genes in the pathophysiological pathway the drug targets. The present study is an encouragement for future investigation, such as large scale studies of multiple polymorphisms and combinations thereof in an attempt to identify a panel of genotypes that can be used as a predictor of an individual patient's response to antihypertensive treatment.
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