# Tailored-therapy of ACE-inhibitors in Coronary Artery Disease:

## Pharmacogenetic Profiling of Treatment Benefit

J. J. Brugts

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# Tailored-therapy of ACE-inhibitors in Coronary Artery Disease: Pharmacogenetic Profiling of Treatment Benefit

Gericht voorschrijven van ACE-remmers
in patiënten met coronair lijden.

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**Jan Jasper Brugts**

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*Voor mijn ouders en zus*

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**Chapter 6**

J.J.Brugts, E.Boersma, M.Chonchol, J.W.Deckers, M.Bertrand, W.Remme, R.Ferrari, K.Fox, M.L.Simoons. The cardioprotective effects of the ACE-inhibitor perindopril in patients with stable coronary artery disease are not modified by mild to moderate renal insufficiency: results from the EUROPA trial. *J Am Coll Cardiol.* 2007;50(22):2148-55.

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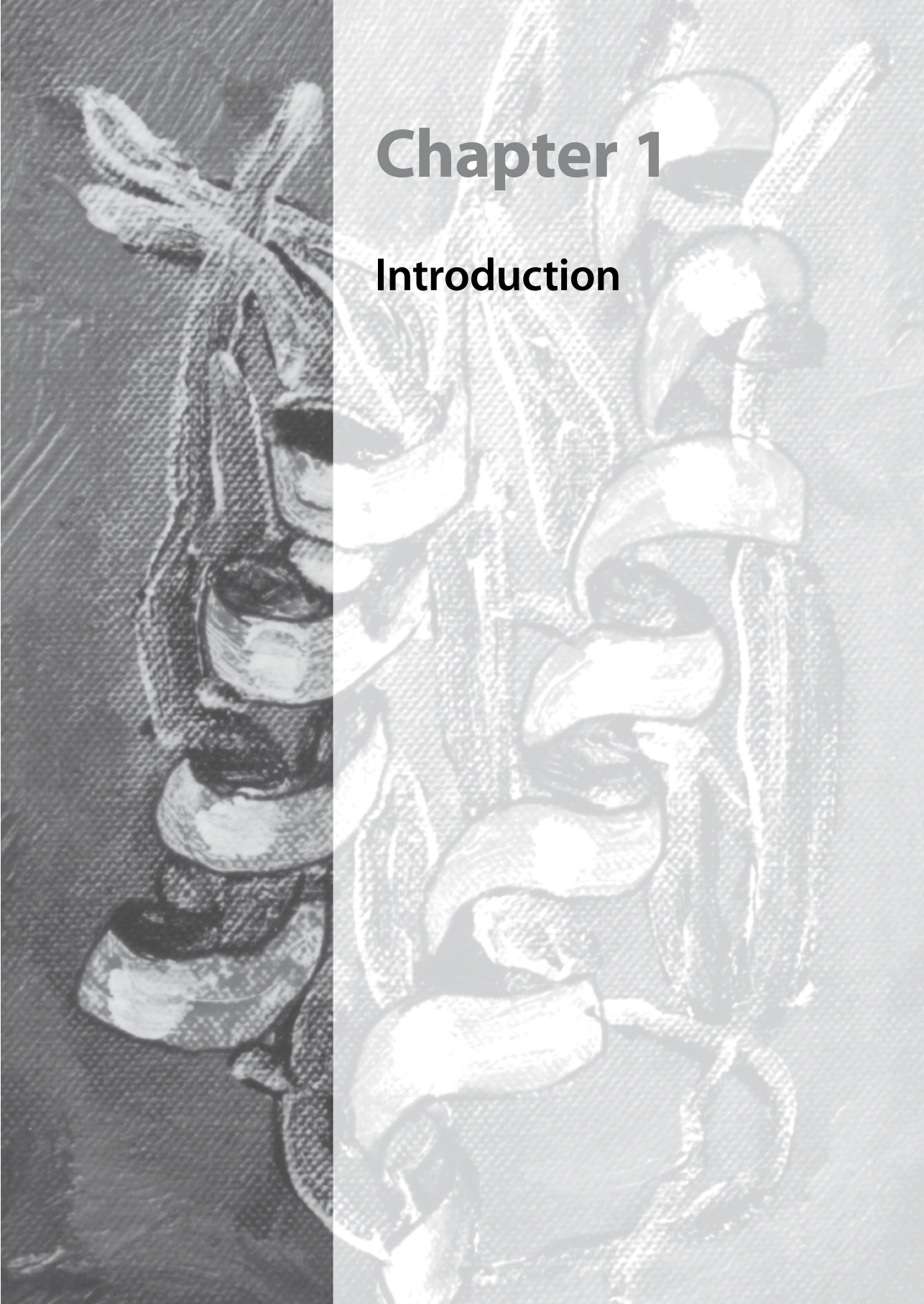


# Chapter 1

## Introduction

The renin-angiotensin-aldosteron system (RAAS) is an important regulator of the hemodynamic stability in the human body by controlling circulating volume and electrolyte balances. The RAAS accomplishes this function by regulating extracellular fluid volume, sodium balance and cardiovascular function through direct and indirect effects on several organ systems and it interacts with the autonomic nervous system and several vasoactive hormones [1]. The RAAS is activated in response to threats which compromise blood pressure stability and extracellular fluid volume homeostasis. A decrease in the perfusion of the juxtaglomerular apparatus raises the production of renin from the kidney. A cascade of hormones is initially triggered by the release of renin [2, 3]. Renin is a proteolytic enzyme that has a local action on angiotensinogen in the kidney as well as in the circulation. Angiotensinogen is a protein precursor produced in the liver and is cleaved by renin to form an inactive peptide angiotensin I (AT-I), which is finally converted to the active octapeptide angiotensin II (AT-II) by the angiotensin-converting enzyme (ACE). ACE is a largely tissue-based zinc metalloprotease, mainly generated by the lungs, the cell membranes of the kidneys and the endothelial cells of the vasculature [1-3]. Therefore, the serum concentrations of ACE determine the levels of AT-II, which is the active metabolite of the system through which the RAAS mediates its main unfavorable effects (pro-atherosclerotic) in the human body. Angiotensin II mediates its effects through the AT-1 receptor which results in arteriolar vasoconstriction and water and salt retention. Excessive or maladaptive stimulation of this hormonal cascade causes pathologic changes in a wide variety of organ systems. For example, an overactive RAAS is associated with hypertension, renal injury, atherosclerosis and left ventricular dysfunction [4]. These conditions have been associated with high levels of tissue ACE [4]. Likewise, the blockade of an activated RAAS has become a key therapeutic target in a wide variety of patients, such as patients with hypertension, heart failure, renal disease, and atherosclerotic (cardio-)vascular disease. The clinically most important examples of pharmacologic agents that block the RAAS currently are the ACE-inhibitors, and AT1 receptor blockers (ARBs).

## TREATMENT EFFECT OF ACE-INHIBITORS

The clinical efficacy of ACE-inhibitors has been clearly demonstrated in a wide variety of patient groups [5-11], such as the EUROPA trial which studied 12.218 patients with stable coronary artery disease (CAD) randomized to the ACE-inhibitor perindopril versus placebo [11]. Perindopril was associated with a 20% reduction in the event rate of cardiovascular mortality, myocardial infarction and resuscitated cardiac arrest during 4 years of follow-up (HR 0.80; 95% CI 0.71-0.91) P-value 0.0003). As a results, the European Society of Cardiology (ESC) clinical treatment guidelines recommend the use of ACE inhibitors as routine secondary prevention for the broad group of patients with known CAD with, respectively, a class I (level of evidence A) recommendation for ACE-inhibitor therapy in CAD patients with hypertension, heart failure, left ventricular dysfunction, previous myocardial infarction with left ventricular dysfunction or diabetes and a

class IIa recommendation in all patients with angina pectoris and confirmed coronary disease (level of evidence B) [12]. Currently, the ACE-inhibitors, as antihypertensive and cardioprotective drug class, compromise one of the most frequently prescribed drugs in cardiovascular patients.

Still, in a patient population of stable CAD, several considerations need to be made, as these patients compromise a relatively low-risk group. In the EUROPA trial, the absolute risk reduction of the primary endpoint (cardiovascular mortality, myocardial infarction and resuscitated cardiac arrest) by perindopril was 2 % during 4 years of treatment, which means that 50 patients needed to be treated for 4 years to prevent one cardiovascular event in EUROPA [12]. Therefore, it is important to study whether the treatment benefit of ACE-inhibitors in stable CAD is equal to all patients studied, in other words a high consistency in treatment benefit across clinical subgroups of patients, or whether specific subgroups of patients can be identified that do not experience the full treatment benefit. By elucidating heterogeneity in the treatment effect of ACE-inhibitors, doctors could target prescription of ACE-inhibitors only to those patients most likely to benefit and by doing so reduce the number of patients treated with such prolonged prophylactic treatment [13,14]. In recent years, the consistency of the treatment effect of ACE-inhibitors has been tested according to clinical characteristics, risk factors and concomitant medication use [14-18]. A risk model based on clinical characteristics related to the incidence of the primary endpoint in EUROPA further showed no modification of treatment effect of perindopril (figure 1) [16]. Prior attempts to identify responding or non-responding patients to ACE-inhibitor therapy appeared not feasible based on clinical characteristics [14-18]. Therefore,



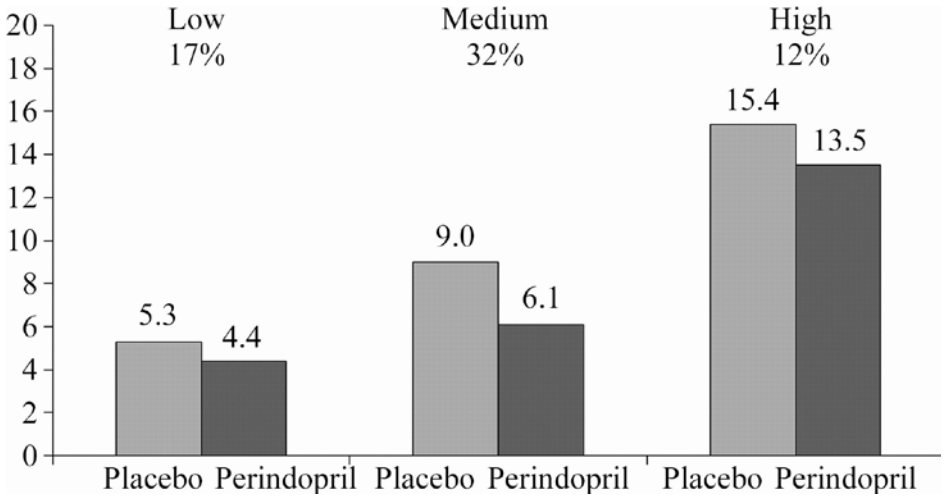



**Figure 1** The consistency of treatment benefit of perindopril according to a risk model based on clinical characteristics. Tertiles of baseline risk based upon clinical characteristics related to the incidence of the primary endpoint in the EUROPA-trial. Adapted from Deckers J, et al. Eur Heart J. 2006;27:796-801 with permission.

at current, all patients fitting the indication of stable CAD (as complete group) are treated with ACE-inhibitors for secondary prevention of recurrent cardiovascular events according to international treatment guidelines [12].

## TAILORED-THERAPY OF ACE-INHIBITORS

To optimally treat patients, and to develop ways to guide ACE-inhibitor treatment, it remains essential to identify those patients most likely to benefit from therapy. New research to elucidate such heterogeneity is necessary. If feasible, guided-therapy of ACE-inhibitors will have a large impact on clinical practice by increasing patient's benefit of drug prescriptions and reducing healthcare costs: to get the right drug to the right patient.

*"The quest for the Holy Grail: tailoring drug therapies to individual patients"*

The purpose of this thesis was to investigate a new approach to guide ACE-inhibitor therapy using patient specific genetic characteristics [19]. We studied the feasibility of pharmacogenetic profiling of the treatment benefit of ACE-inhibitor therapy. We focused on the common genetic variation in the candidate genes of the direct pharmacodynamic pathway of ACE-inhibitors: the renin-angiotensin-system and kallikrein-bradykinin system. These studies were conducted within the randomized placebo-controlled EUROPA-trial studying the ACE-inhibitor perindopril versus placebo in ten thousand patients with stable coronary artery disease.

The main research questions we examined were as follows:

- Is the treatment benefit (reduction of cardiovascular events) of ACE-inhibitor therapy modified by genetic variation between patients?
- Is the level of blood pressure and blood pressure reduction by ACE-inhibitor therapy modified by genetic variation between patients?
- Can we develop a pharmacogenetic profile to individualize ACE-inhibitor therapy and optimize patients' benefit in stable coronary artery disease?

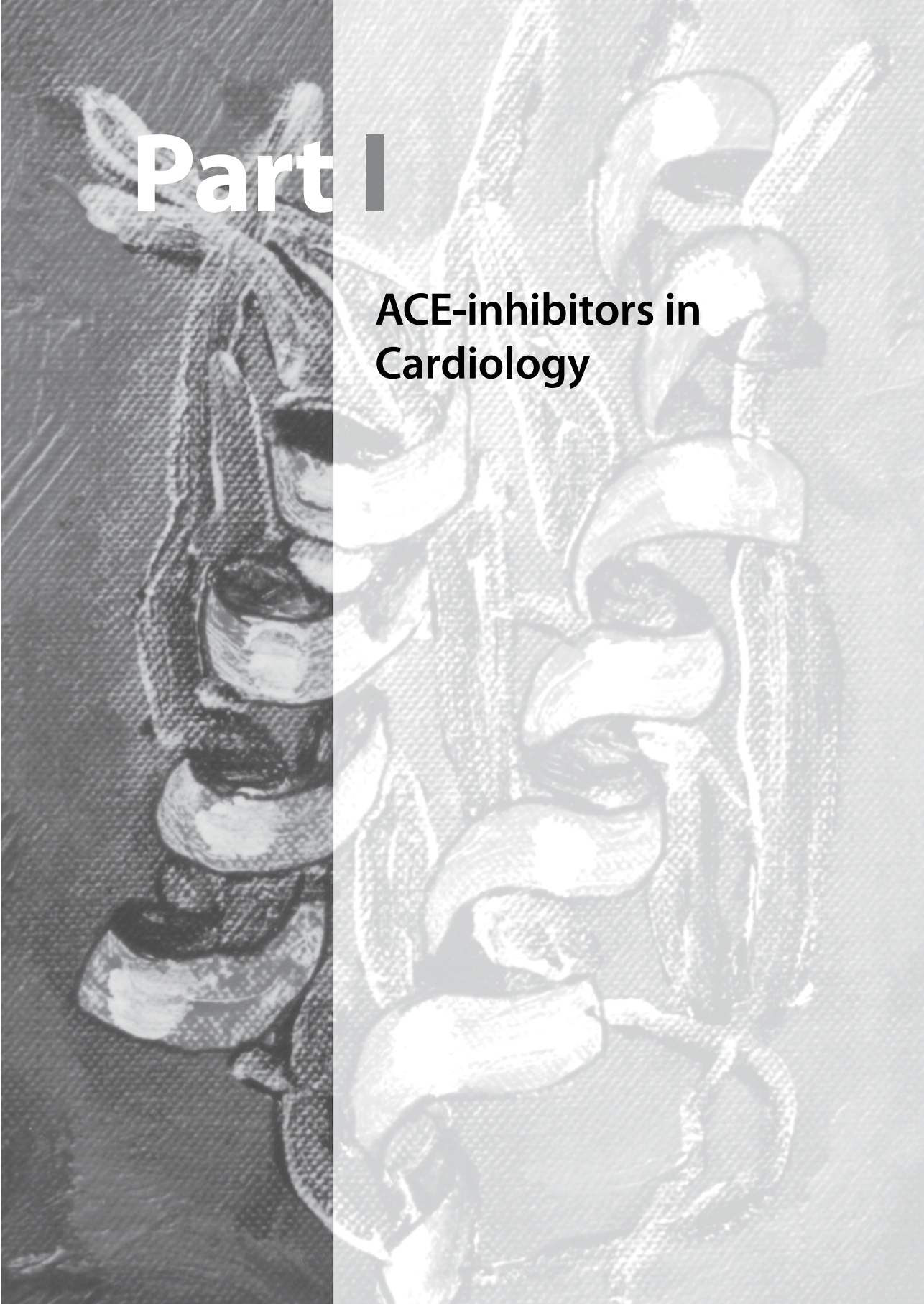
## OUTLINE OF THE THESIS

In the following chapters, several issues are discussed. Part I focuses on the clinical efficacy of ACE-inhibitors, especially perindopril (chapter 2), as demonstrated in various clinical trials in different patient groups. Also, the lessons learned from previous clinical trials with ACE-inhibitors are discussed (chapter 3). Part II focuses on the search for the heterogeneity in treatment benefit of ACE-inhibitors by clinical subgroup analyses. In particular, we focus on the risk

marker – renal insufficiency – which we investigated with regard to the incidence of cardiovascular events in relatively healthy subjects (chapter 4), as well as in patients with coronary artery disease (chapter 5). As renal insufficiency proved to be an important marker of cardiovascular risk and considering the effect of ACE-inhibitors in renal insufficiency, we further investigated whether renal insufficiency was a modifier of treatment benefit of ACE-inhibitors which could be used to guide ACE-inhibitor therapy (chapter 6). Part III focuses on the consistency of the treatment effect of ACE-inhibitor therapy in a combined analysis of several ACE-inhibitor trials with in-depth subgroup analyses using clinical characteristics as well as blood pressure and blood pressure response to ACE-inhibitor therapy as potential mediators of treatment effect (chapter 7). Part IV summarizes all prior attempts to target ACE-inhibitor therapy by our group and other research groups (chapter 8) and advocates a new approach of targeting therapy to those patients most likely to benefit of treatment using (pharmaco-) genetic factors (chapter 9). In part V, the study design and rationale of the PERindopril GENEtic association (PERGENE) study is presented (chapter 10) and the results of this large-scale pharmacogenetic analysis are revealed (chapter 11 and 12). In part VI, the feasibility of pharmacogenetic profiling is discussed with respect to the findings of the PERGENE study (chapter 13) and other successes in pharmacogenetic research of cardiovascular drugs. As the current pharmacogenetic approach can be applied for other cardiovascular drugs as well, and should be integrated in future randomized clinical trials, we advocate the use of pharmacogenetics in statins, one of the worlds most frequently prescribed drugs, to optimize drug response. In Part VII, we study the treatment benefit of statins and the consistency according to clinical characteristics. Due to the strong consistency as observed, the same pharmacogenetic concept of optimizing treatment benefit of statins should be proposed in future pharmacogenetic analyses in similarity with the ACE-inhibitors (chapter 14). Finally, in the general discussion (chapter 15), methodological considerations are addressed and the main findings of this thesis are placed in a broader context, and the potential clinical implications (number needed to treat) and directions for future pharmacogenetic research of ACE-inhibitors are discussed.

## REFERENCES


1 Skeggs LT, Dorer RE, Kahn JR, Lentz KE,Levine M: The biochemistry of the renin-angiotensin system and its role in hypertension. *Am J Med* 1976; 60: 737–748.
2 Erdos EG: Angiotensin I converting enzyme and the changes in our concepts through theyears. *Hypertension* 1990; 16: 363–370.
3 Perazella MA, Setaro JF: Renin-angiotensin aldosterone system: fundamental aspects and clinical implications in renal and cardiovascular disorders. *J Nucl Cardiol* 2003;10: 184–194.
4. Ferrario WS. Role of the Renin-Angiotensin-Aldosterone System and Proinflammatory Mediators in Cardiovascular Disease. *Am J Cardiology*; 98:121-128.
5. Pfeffer MA, Braunwald E, Moye LA, Basta L, Brown EJ Jr, Cuddy TE, Davis BR, Geltman EM, Goldman S, Flaker G, for the SAVE Investigators. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction and myocardial infarction: results of the survival and ventricular enlargement trial. *N Engl J Med* 1992; 327: 669–77.
6. The SOLVD investigators. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. *N Engl J Med* 1991; 325: 293–302.
7. The Acute Infarction Ramipril Efficacy (AIRE) study investigators. Effect of ramipril on mortality and morbidity of survivors of acute myocardial infarction with clinical evidence of heart failure. *Lancet* 1993; 342: 821–28.
8. Torp-Pedersen C, Kober L for the TRACE Study Group. Effect of ACE inhibitor trandolapril on life expectancy of patients with reduced left-ventricular function after acute myocardial infarction. *Lancet* 1999; 354: 9–12.
9. The Heart Outcomes Prevention Evaluation study investigators. Effects of an angiotensin-converting enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. *N Engl J Med* 2000;342:145–53.
10. The PEACE Trial Investigators. Angiotensin-converting-enzyme inhibition in stable coronary artery disease. *N Engl J Med* 2004; 351: 2058–68.
11. The European trial on reduction of cardiac events with perindopril in patients with stable coronary artery disease investigators. Efficacy of perindopril in reduction of cardiovascular events in stable coronary artery disease: randomized, double-blind, placebo-controlled, multicentre trial (the EUROPA study). *Lancet* 2003; 362: 782–88.
12. The Task Force on the Management of Stable Angina Pectoris of the European Society of Cardiology. Guidelines on the management of stable angina pectoris. *Eur Heart J* 2006 27, 1341–81.
13. Danser AHJ, Schunkert H. Renin–angiotensin system gene polymorphisms: potential mechanisms for their association with cardiovascular diseases. *Eur J Pharmacology* 2000.303–316.
14. Brugts JJ, den Uil CA, de Maat M, Danser AHJ. The renin-angiotensin-aldosterone system: Approaches to Guide Angiotensin-Converting Enzyme Inhibition in Patients with Coronary Artery Disease. *Cardiology* 2008 Oct 2;112(4):303-312.
15. Dagenais GR, Pogue J, Fox K, Simoons ML, Yusuf S. Angiotensin-converting-enzyme inhibitors in stable vascular disease without left ventricular systolic dysfunction or heart failure: a combined analysis of three trials. *Lancet* 2006; 368: 581–88
16. Deckers JW, Goedhart DM, Boersma E, et al. Treatment benefit by perindopril in patients with stable coronary artery disease at different levels of risk. *Eur Heart J* 2006; 27: 796–801.
17. Brugts JJ, Boersma E, Chonchol M, Deckers JW, Bertrand M, Remme WJ, Ferrari R, Fox K, Simoons ML. The cardioprotective effects of the angiotensin-converting enzyme inhibitor perindopril in patients with stable coronary artery disease are not modified by mild to moderate renal insufficiency: insights from the EUROPA trial. *J Am Coll Cardiol*. 2007;50:2148-55.
18. Brugts JJ, Ninomiya T, Boersma E, Remme WJ, Bertrand M, Ferrari R, Fox K, MacMahon S, Chalmers J, Simoons ML. The consistency of the treatment effect of an ACE-inhibitor based treatment regimen in patients with vascular disease or high risk of vascular disease: a combined analysis of individual data of ADVANCE, EUROPA, and PROGRESS trials. *Eur Heart J*. 2009;30(11):1385-94.
19. Brugts JJ, de Maat MPM, Boersma E, Witteman JCM, van Duijn C, Uitterlinden AG, Bertrand M, Remme W, Fox K, Ferrrari R, Danser AHJ, Simoons ML. The Rationale and Design of the Perindopril Genetic Association Study: A Pharmacogenetic analysis of ACE-inhibitor therapy in patients with stable coronary artery disease. *Cardiovasc Drugs Ther* 2009;23(2):171-81.



# Part I

## ACE-inhibitors in Cardiology

# Chapter 2

## Drug profile of the ACE-inhibitor perindopril in the treatment of patients with cardiovascular disease.

J. J. Brugts, R. Ferrari, M. L. Simoons

*Expert Rev Cardiovasc Ther. 2009;7(4):345-360*

## ABSTRACT

The angiotensin-converting enzyme (ACE) inhibitor perindopril (Coversyl®) is a long-acting lipophilic drug with a high-tissue affinity for the angiotensin-converting enzyme. ACE-inhibition by perindopril has two main effects: it inhibits the angiotensin II formation and potentiates bradykinin. Perindopril is one of the ACE-inhibitors, which has been extensively studied in randomized clinical trials within various patient populations. The clinical efficacy has been demonstrated in patients with hypertension, diabetes mellitus, cerebrovascular disease, stable coronary artery disease (CAD), and heart failure. Also, perindopril has a positive safety and tolerability profile. Therefore, perindopril, as ACE-inhibitor, has an established place in the clinical treatment guidelines of the European Society of Cardiology, European Society of Hypertension, European Association for the Study of Diabetes and the American Heart Association and American College of Cardiology. The Action in Diabetes and Vascular disease: preterAx and diamicroN Controlled Evaluation (ADVANCE) trial, the Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm (ASCOT-BPLA), the European trial on Reduction Of cardiac events with Perindopril among patients with stable coronary Artery disease study (EUROPA), and the Perindopril pROtection aGainst REcurrent Stroke Study (PROGRESS), have shown that an antihypertensive treatment with perindopril reduces and prevents cardiovascular events in a large range of patients with established vascular disease or high-risk of vascular disease. The observed cardioprotective benefits of perindopril were independent of blood pressure. The outcome of these and other trials support the concept of specific cardioprotective properties of ACE-inhibition by perindopril in addition to the blood-pressure lowering effects, such as anti-atherosclerotic, anti-inflammatory and anti-thrombotic properties. In addition, the observed consistency of the treatment benefit across subgroups indicates that the absolute benefits conferred by treatment are established mainly by each patient's future risk of vascular complications, rather than their initial blood pressure level or other risk factors. This review describes these issues according to the main studies with perindopril or perindopril based-regimens, concluding that the blood pressure dependent and independent cardioprotective effects extend to all patients with vascular disease. This concept supports the provision of ACE-inhibitor based treatment, not on the basis of arbitrary cut-off points for blood pressure but rather on assessment of vascular risk, which is raised in patients with stable CAD, diabetes and stroke. Therefore, perindopril should be considered as first line agent in patients with heightened (cardio-)vascular risk considering its properties and the clinical evidence on efficacy, safety profile and tolerability.

## INTRODUCTION

The renin-angiotensin aldosteron system (RAAS) is an important regulator of the hemodynamic stability in the human body by controlling circulating volume and electrolyte balances (Figure 1). The RAAS accomplishes this function by regulating extracellular fluid volume, sodium balance and cardiovascular function through direct and indirect effects on several organ systems and interaction with the autonomic nervous system and several vasoactive hormones[1]. The RAAS is activated in response to signals of compromises in the blood pressure stability or extracellular fluid volume homeostasis. A decrease in pressure of the juxtaglomerular apparatus, raises the production of renin from the kidney[2,3]. Renin is a proteolytic enzyme that has a local action on angiotensinogen in the kidney as well as in the circulation. Angiotensinogen is a protein precursor produced in the liver and is cleaved by renin to form an inactive peptide angiotensin I (AT-I), which is subsequently converted to the active octapeptide angiotensin II (AT-II) by the angiotensin-converting enzyme (ACE). ACE is a largely tissue-based zinc metalloprotease, mainly generated by the lungs, the cell membranes of the kidneys and endothelial cells of the vasculature[1-3]. Therefore, the concentrations of ACE influence the levels of AT-II, which is the active metabolite of the system through which the RAAS mediates the majority of unfavorable effects (pro-atherosclerotic) in the human body (Table 1). Renin production is the rate limiting step in the system[4]. Excessive or maladaptive stimulation of this hormonal cascade causes pathologic changes in a wide variety of organ systems. For example, an overactive RAAS results in excessive vasoconstriction, which is associated with hypertension, renal injury, atherosclerosis and left ventricular dysfunction[4]. These conditions have been associated with high levels of tissue ACE[4]. Likewise, the blockade of an activated RAAS has become a key

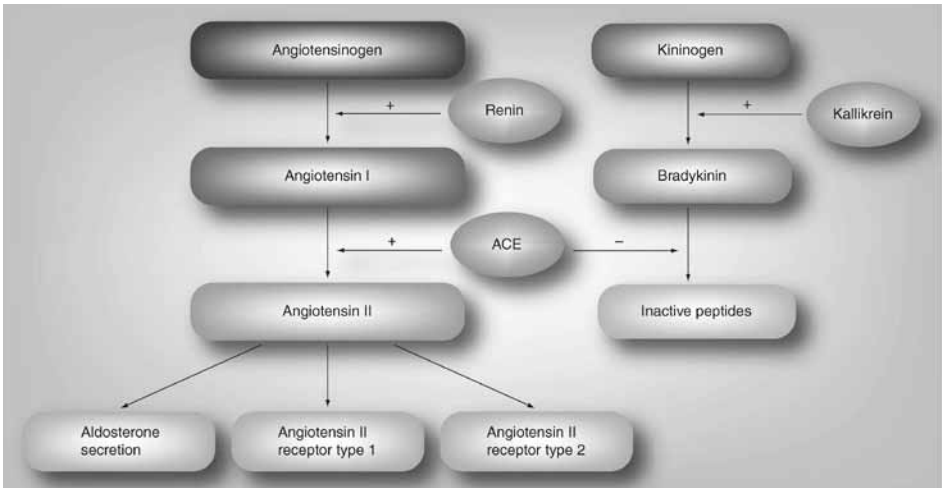



**Figure 1** Renin-angiotensin-aldosteron system
ACE = angiotensin-converting enzyme

**Table 1.** Main effects of angiotensin-II in the human body

| **Effect of AT1 receptor stimulation** |
|---|
| Arteriolar vasoconstriction |
| Increased Na+ and Cl- reabsorption and K+ excretion |
| Aldosteron synthesis |
| Increased plasminogen activator inhibitor 1 activity (thrombogenic) |
| Increased sympathetic activity |
| ADH secretion: $H_2O$ absorption by collecting duct |
| Vascular smooth muscle growth |
| Endothelial dysfunction by: |
| - Increased connective tissue and low-density lipoprotein accumulation in vascular media |
| - Increased uptake and oxidation of LDL by macrophages and endothelial cells |
| - Oxidative stress |
| - Activation of adhesion molecules; monocyte chemo-attractants |
| - Matrix degradation (increased matrix-metalloproteinases) |
| - Activation of inflammatory cytokines (Il-6, TNFa) |
| - Enhanced expression of MMP and oxyradical production (NADH/NADH oxidase activity, cNOS) |

Reference: Ferrari et al. Exp Rev Cardiovasc Ther. 2005 (ref 38).

therapeutic target in a wide variety of patients, such as patients with hypertension, diabetes mellitus, renal disease, atherosclerotic cardiovascular disease and heart failure. The clinically most important examples of pharmacologic agents that block the RAAS are currently the ACE-inhibitors, and AT1 receptor blockers (ARBs).



The ACE-inhibitors were introduced almost three decades ago for the treatment of hypertension. From that moment onwards, ACE-inhibitors have been associated with significant advances in the secondary prevention of a wide range of cardiovascular diseases. This important role of ACE inhibitors has been established by several large clinical trials that demonstrated the efficacy of this drug class in patients at high risk of cardiovascular disease, including those with post-myocardial infarction left ventricular ejection fraction of less than 40%, heart failure or a history of cerebrovascular disease [the Survival and Ventricular Enlargement (SAVE) study, Studies of Left Ventricular Dysfunction (SOLVD), the Acute Infarction Ramipril Efficacy (AIRE) study and the Trandolapril Cardiac Evaluation (TRACE) study], and those with a lower risk of cardiovascular events, in particular patients with stable coronary artery disease (CAD) without overt heart failure [the Heart Outcomes Prevention Evaluation (HOPE) study, and the EUROPA-trial (while the PEACE-trial was neutral) (Figure 2) [5-11].

The use of ACE-inhibitors is now recognized and recommended in the European Society of Cardiology/American Heart Association/American College of Cardiology/European Society of Hypertension/European Association for the Study of Diabetes guidelines on the management of hypertension, stable CAD, myocardial infarction, heart failure, and in the prevention of the progression of renal insufficiency in diabetes mellitus-related kidney disease (specific indication see Table 2) [12-17]. For example, the European Society of Cardiology 2006 clinical treatment guidelines recommend the use of ACE-inhibitors in secondary prevention for the broad group of patients with known CAD with, respectively, a class I (level of evidence A) recommendation

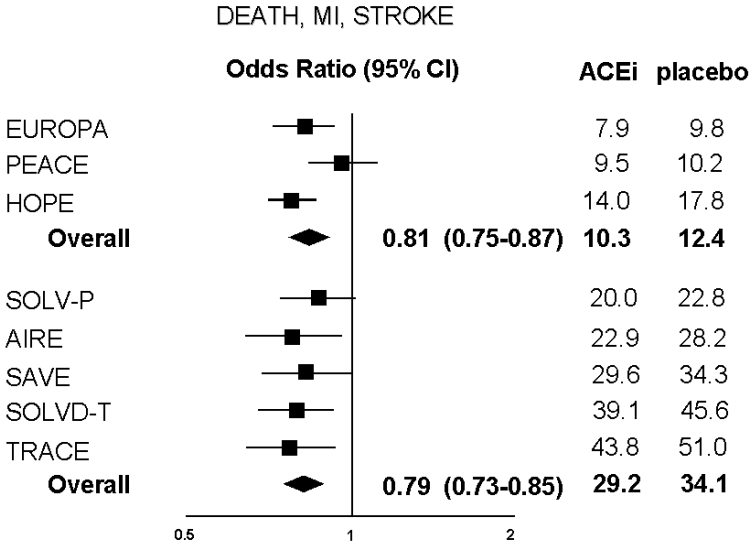



**Figure 2** Efficacy of ACE-inhibitors in patients at high risk of cardiovascular disease (post-MI, LVEF,40%, history of CVA) or relatively lower risk of cardiovascular disease (stable CAD without overt heart failure). Adapted from Dagenais et al. Lancet 2006; 368:581–8 with permission (reference 49)

**Table 2.** ESC guidelines on administration of ACE-inhibitors in specific patient categories.

| Indication | Specifics | Recommendation level |
|---|---|---|
| Hypertension | - Patients with heart failure, systolic LVD, diabetes mellitus, previous MI or CVA, high coronary artery disease risk | I-A |
| | - Control based on blood pressure levels in hypertensives | I-A |
| Heart failure | - Symptomatic heart failure and reduced LVEF | I-A |
| | - LVSD after AMI | I-A |
| | - Asymptomatic reduced LVEF (40-45%), no previous MI | I-A |
| | - Diastolic heart failure | IIa-C |
| Acute MI, after stabilization | - High-risk patients (heart failure, LVD, no perfusion, large infarct) | I-A |
| | - All patients | IIa-A |
| Evolving AMI (>24h), post-MI | - Clinical heart failure or asymptomatic LVD (LVEF <45%) | I-A |
| | - Diabetes Mellitus or other high risk patients | I-A |
| Secondary Prevention | - Patients with evidence of cardiovascular disease (independent of LVEF) or diabetes and 1 other risk factor. | I-A |
| | - All patients with LVEF<40% and in patients with diabetes mellitus, hypertension or chronic kidney disease. | I-A |

Class I: evidence or general agreement that a given treatment is beneficial, useful and effective. Class II: conflicting evidence and/or divergence of opinion about the efficacy of the treatment. Class IIa: weight of evidence is in favor of efficacy. Level of evidence A: data derived from multiple randomized clinical trials or meta-analyses. Level of evidence B: data derived from a single randomized clinical trial or non randomized studies. Level of evidence C: consensus of opinion of the experts and/or small studies, or standard of care. Modified from ESC treatment guideline statements (ref 12-15). LVEF= left ventricular ejection fraction; LVSD left ventricular systolic dysfunction; AMI= acute myocardial infarction; LVD= left ventricular dysfunction; MI= myocardial infarction.

for ACE-inhibitor therapy in CAD patients with hypertension, heart failure, left ventricular dysfunction, previous myocardial infarction with left ventricular dysfunction or diabetes and a class IIa recommendation in all patients with angina pectoris and confirmed coronary disease (level of evidence B). In angina pectoris without co-existing indications for ACE-inhibitor treatment, the anticipated benefit should be weighted against the costs and risks of side effects, and the dose and agent used should be of confirmed efficacy for this indication[12].

Although there are other agents modulating the renin–angiotensin system which have some overlapping pharmacological and clinical effects, ACE-inhibitors remain a unique drug class because of the broad range of their confirmed benefits and tolerability, forming a solid basis for the treatment of cardiovascular disease, including potential combinations with other established drugs, such as statins and antiplatelet agents. At current, ACE-inhibitors are the gold standard in secondary prevention guidelines for blockade of the RAAS, other agents such as ARBs can be considered in cases of intolerance to ACE-inhibitors. Results of ARB trials have been inconsistent until now. The ACE-inhibitors are considered as a homogenous drug class. Among the available ACE-inhibitors important differences exist in their chemical structure and pharmacokinetic aspects, of which the most important is the affinity for tissue-bound and circulating ACE[18].



## Short overview of perindopril

The ACE-inhibitor perindopril has been extensively studied in several large randomized clinical trials such as the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT), the Perindopril Protection Against Recurrent Stroke Study (PROGRESS), the Action in Diabetes and Vascular Disease: Preterax and Diamicron-MR Controlled Evaluation (ADVANCE) study, and the EUROPA-trial of which each has made its own contribution to the expansion of treatment strategies in cardiovascular disease[11,19-21]. A lot of data has been gathered on the safety profile and tolerability of perindopril[22,23]. In a 12 month post marketing study of perindopril, no unexpected hazards were observed in a large cohort of patients treated with perindopril (n=47351 patients) [22]. Research with perindopril has extended our knowledge of the maladaptive responses and pathophysiological processes in the renin–angiotensin–aldosteron system. Several sub studies of EUROPA, have established that perindopril has additional effects beyond blood pressure reduction alone, such as the improvement of endothelial function, improvement of the neurohumoral balance and reduction of unfavorable remodeling of the coronary arteries[24-26]. Perindopril has several unique properties above other ACE-inhibitors in its class, such as a higher affinity for tissue and circulating ACE, which is thought to be related to the penetration capacity in atherosclerotic plaques[18] and 24 h duration of action with once daily administration.

The above mentioned studies and aspects of perindopril will be discussed in the current review focusing at the pharmacokinetics, pharmacodynamics, safety profile and clinical efficacy of the ACE-inhibitor perindopril in the management of cardiovascular disease.

## Chemical structure of perindopril

All ACE inhibitors are 2-methylpropionyl- L - proline analogues, but they differ from each other by their individual chemical structure[28] by which they are currently classified. Some contain a sulfydryl group (captopril), a phosphinyl group (fosinopril) but most have a carboxyl moiety (ie. ramipril and perindopril). ACE-inhibitors exert their effect on ACE by chelating $Zn^{2+}$ in the active centre. The functional ($Zn^{2+}$ -chelating) group binding to ACE is the primary structural difference among these agents. Some of the specific characteristics of these agents may be linked to these different binding groups[29].

Perindopril was discovered in 1982 by Laboratoires Servier [30]. Perindopril ((2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[[(2*S*)-1-ethoxy-1-oxopentan-2-yl]amino]propanoyl]-2,3,3a,4,5,6,7,7a-octahydroindole-2-carboxylic acid) is a potent long-acting lipophilic ACE-inhibitor[28-30] (Figure 3). It is a prodrug ester that, after oral administration, is converted to the active diacid perindoprilat by hydrolysis in the liver and plasma. Perindopril is orally administered in the form of tablets containing its salts (1:1) with erbumine (*tert*-butylamine) and l-arginine (perindopril erbumine and perindopril l-arginine). The new formulation of the ACE inhibitor perindopril as an arginine salt improves the stability of the product and increases its shelf life. Pharmacokinetic studies indicate that perindopril-arginine can be expected to have equivalent antihypertensive efficacy to the previous formulation, with a revised dosage due to the difference in molecular weight of the two salts: perindopril-arginine 5–10 mg replaces perindopril *tert*-butylamine 4–8 mg [31]. In general, it is expected that with the pharmacokinetic results of perindopril-arginine the pharmacodynamic effect are at least equivalent with the prior formulation. Therefore, the benefits demonstrated in large-scale trials performed with the perindopril-*tert*-butylamine formulation are likely to apply to perindopril-arginine formulation.

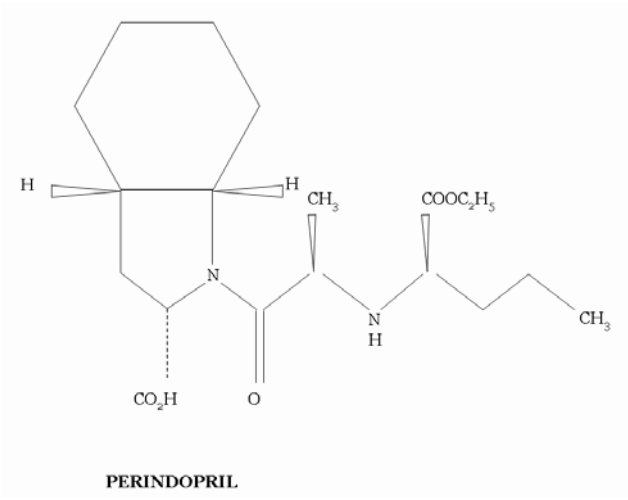



**Figure 3** Chemical structure of perindopril (C19H32N2O5).

## PHARMACODYNAMICS

The biological effects of angiotensin II are 1) vasoconstriction, which increases blood pressure, 2) constriction of the efferent renal arterioles, which increases glomerular perfusion pressure of the glomeruli, 3) stimulation of the adrenal cortex to release aldosteron, which acts on renal tubules to retain sodium and chloride ions and excrete potassium (this leads to water retention which increases blood volume and hence blood pressure), also angiotensin acts on the proximal tubule causing sodium retention, 4) stimulation of the posterior pituitary gland into releasing vasopressin (also known as anti-diuretic hormone (ADH)) which also acts on the kidneys to increase water retention, 5) ventricular remodelling processes in the heart leading to ventricular hypertrophy and heart failure as presented in Table 1 [1-4, 32-34]. The ACE-inhibitors competitively block the conversion of A-I into A-II. This blockade results in a decrease in circulating and local levels of A-II, thus inhibiting the effects of A-II (Table 3).

It is important to realize that ACE-inhibitors do not antagonize the AT1 receptor and thus do not inhibit the unfavorable effects of A-II completely. In addition, it needs to be appreciated

**Table 3.** Pharmacodynamic effects of ACE-inhibitors

| **ACE-inhibitors** |
|---|
| Inhibit ACE in plasma and tissue and blood vessels |
| - Reduce ATII (inhibits conversion of AT-I in AT-II) |
| - Increase Bradykinin |
| Reduce plasma aldosteron levels |
| - SBP and DBP ↓ |
| - Systemic vascular disease ↓ |
| Arterial diameter ↑ (reduces vasoconstrictive effects of AT-II) |
| Arterial compliance ↑ |
| Arterial blood flow ↑ |
| Pulse wave velocity ↓ |
| - Aortic, carotid, femoral |
| Lower albuminuria in HT or diabetic nephropathy |
| Lower plasma uric acid levels in hypertensives |
| Reduce thrombogenesis and improves fibrolysis |
| Reverse endothelial damage |
| Prevent endothelial cell apoptosis |
| Reduced monocyte adhesion |
| Reduced ECM degradation |
| Increase NO availability |
| Reduce remodeling |
| - Less SMC growth, proliferation and migration |
| Anti-oxidative function |
| - Reduced free radical production |
| Anti-atherosclerotic effects |
| No adverse effect on plasma glucose or plasma lipid profiles. |

References 1-4, 32-34

that when ACE is inhibited, the formation of A-II is restored, at least in part, due to the reactive renin rise that occurs when blocking the A-II-induced negative feedback on renin release. Still, an additional beneficial effect of ACE-inhibitors, in contrast to ARBs, is the increase in bradykinin levels by a decrease in degradation of bradykinin in inactive peptides (Table 4) [34]. The increase in bradykinin levels induced by ACE-inhibitors leads to the release of nitric oxide and prostaglandins, resulting in additional vasodilatation [35]. The bradykinin-mediated effect is linked to at least some extent of the beneficial effect of ACE-inhibitors [35] such as the anti-atherosclerotic, anti-thrombotic and anti-inflammatory effects which have not been demonstrated by ARBs. Yet, in the comparison of ACE-inhibitors to ARBs a unsolved dilemma remains since in clinical trials ARBs do have a similar effect as compared to ACE-inhibitors, but when compared to placebo there is a greater inconsistency (as compared to ACE-inhibitors) in convincingly reducing cardiovascular events. These observations have not been explained momentarily.

**Table 4.** Bradykinin effect of ACE-inhibitors.

| **Increased bradykinin levels:** |
|---|
| - counteracts the negative effects of AT-II |
| - increased nitric oxide levels (NO) |
| - preserves endothelial function |
| - cardiovascular anti-remodelling activities |
| - exerts an indirect anti-oxidant effect |
| - increases tPA and fibrinolysis |
| - results in monocyte anti-adhesion |
| - increases eNOS expression |

References 34,35.

### *ACE-inhibitors versus ARBs*

As ACE inhibitors have been shown to reduce mortality and cardiovascular morbidity among a wide variety of patient groups at different level of risk, these agents are being compared to ARBs for their clinical effect. The VALIANT trial compared the effect of the ACE inhibitor captopril versus the ARB valsartan in this population of patients and showed that valsartan is as effective as captopril in patients who are at high risk for cardiovascular events after myocardial infarction [36]. In patients with vascular disease or diabetes without heart failure, ACE inhibitors reduce mortality and morbidity from cardiovascular causes, but the role of ARBs in such patients is unknown. The ONTARGET investigators studied the ACE inhibitor ramipril, the ARB telmisartan and the combination of the two drug combinations [37]. The investigators concluded that telmisartan was equivalent to ramipril in patients with vascular disease or diabetes. Telmisartan was as effective as captopril in reducing the rates of death and other cardiovascular outcomes (including the prevention of myocardial infarction). The combination of the two drugs was associated with more adverse events without an increase in benefit. There is currently no

clinical data on either agent being superior to the other. At the moment, the ACE-inhibitor perindopril has not been compared to ARBs in a comparative trial.

The mechanism through which perindoprilat lowers blood pressure is believed to be primarily inhibition of ACE-activity[18]. ACE is a peptidyl dipeptidase that catalyzes conversion of the inactive decapeptide, angiotensin I, to the vasoconstrictor, angiotensin II. The concentration of an ACE-inhibitor in a particular tissue depends on the physicochemical characteristics of its molecule, e.g. molecular size, dissociation constant, lipophilicity, as well as the presence of blood–tissue barriers and the ability of the tissue to transform inactive prodrugs into active form[18]. Perindopril also affects endothelial function and has been shown to reverse endothelial damage, increase NO availability, and improve fibrinolytic balance which may all underlie the anti-atherosclerotic actions of perindopril[18,24-27,38,39], which will be discussed in more detail elsewhere below.

## PHARMACOKINETICS AND METABOLISM



Following oral administration, perindopril is rapidly absorbed with peak plasma concentrations being reached within 1 hour with a mean bio-availability of 95% [40,41]. Approximately 20-50% of the perindopril absorbed is rapidly converted during its first pass of the liver into the biologically active metabolite perindoprilat (elimination half-life mean 1-2 hours). Peak plasma concentrations of perindoprilat occur after 3-7 hours but perindoprilat is already detectable within 30 minutes of administration. Approximately 60% of circulating perindopril is bound to plasma proteins, and only 10 to 20% of perindoprilat is bound, which reduces the amount of potential drug interactions through effects on protein binding [40,41]. During repeated once-daily oral dosing with perindopril, perindoprilat accumulates about 1.5 to 2 fold and attains steady state plasma levels in 3 to 6 days. Perindopril does not accumulate with a once-a-day multiple dosing regimen. Mean total body clearance of perindopril is 219 to 362 mL/min and its mean renal clearance is 23.3 to 28.6 mL/min [40,41]. Perindopril has a long half-life and 24 hour persistence of action allowing 24 hour BP-control, especially during the sleeping and awakening hours [40,41].

Perindoprilat has a strong affinity for ACE. In patients with CAD, perindopril has been shown to reduce both plasma and vascular levels of ACE, such as endothelial and adventitial ACE and to increase the expression of eNOS in the endothelium and in vascular smooth muscle cells [18,42]. The relative tissue affinity of perindoprilat compares favourably with other ACE-inhibitors [18,38,43] (Figure 4). The exact meaning of these difference is still unclear.

The bioavailability of perindopril is influenced when taking the drug during meals. Co-administration with food did not affect the pharmacokinetics of perindopril, but did reduce the conversion of perindopril to perindoprilat and hence its bioavailability[44,45]. Therefore, perindopril should be taken before food (see section 6) [45]. In patients with severe liver failure or

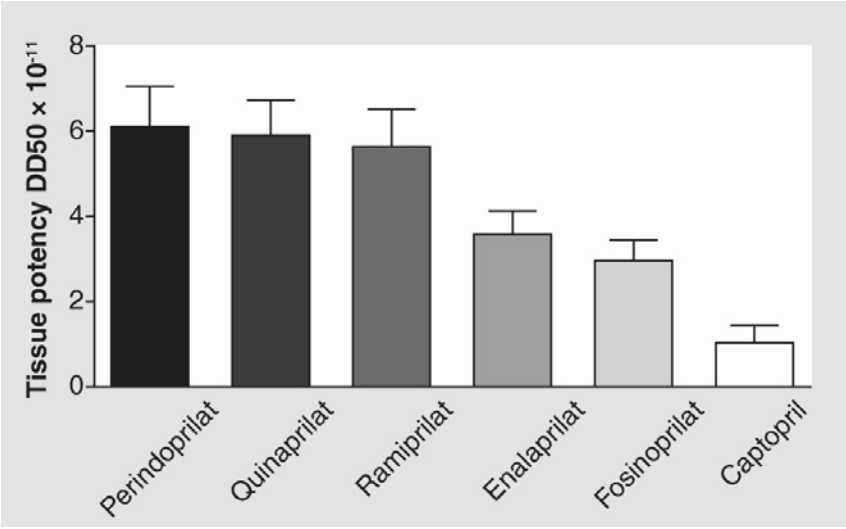



**Figure 4** Tissue potency of different ACE-inhibitors**.** Modified from (38) Ferrari R. Angiotensin-converting enzyme inhibition in cardiovascular disease: evidence with perindopril. *Expert Rev Cardiovasc Ther.* 2005;3(1):15–29 with permission.

compensated liver cirrhosis, the metabolic clearance of perindopril is reduced [46]. The AUC of a single-dose perindopril 8 mg in patients with mild to severe hepatic cirrhosis was 2-fold higher than that in healthy volunteers, but the AUC of perindoprilat was similar to that in healthy volunteers. Therefore, no dosage adjustment is required in patients with hepatic impairment [45,46]. ACE inhibitors, including perindopril, may increase serum lithium concentrations and increase the risk of lithium toxicity when administered concomitantly with lithium [45,47]. Since the clearance of perindoprilat and its metabolites is almost exclusively renal, also elderly in whom renal function is impaired and renal impaired hypertensive patients should have an adjusted dosage of the drug in these circumstances [45,48].

## CLINICAL EFFICACY

The introduction of ACE-inhibitors has had a wide impact on cardiovascular medicine since it has been associated with a reduction of cardiovascular risk a wide variety of patients with hypertension, heart failure, left ventricular dysfunction, post myocardial infarction, diabetic nephropathy, peripheral vascular disease, diabetes mellitus and stroke. A brief overview of the effect of the ACE-inhibitor perindopril within the some of these specific patient groups (hypertension, diabetes mellitus, cerebrovascular disease and stable coronary artery disease) is summarized below:

## Hypertension

The ASCOT-BPLA trial investigated the long term effects on cardiac outcomes of a conventional antihypertensive regimen (beta-blocker -diuretic) with a newer regimen (calcium channel antagonist -ACE-inhibitor) in patients with hypertension at moderate risk of developing cardiovascular events [19]. In ASCOT, 19.257 patients with hypertension and at least three other cardiovascular risk factors were randomized to stepwise regimens of amlodipine 5–10 mg/day adding perindopril 4 or 8 mg/day as required, or atenolol 50–100 mg/day adding bendroflumethiazide 1.25–2.5 mg/day plus potassium as required. Patients were aged 47–79 (mean 63) years and mean BP at baseline was 164/95 mm Hg. The primary endpoint was the combined endpoint of non fatal MI and fatal coronary heart disease. The trial was stopped prematurely (median follow-up of 5.5 years) because the amlodipine-perindopril regimen, compared with atenolol-bendroflumethiazide recipients, reduced all-cause mortality by 11% (738 vs 820 events; $p = 0.025$). The risk of the primary endpoint (nonfatal MI and fatal CHD) was not significantly lowered by 10% with the amlodipine-perindopril compared with the atenolol-based regimen, but the amlodipine-perindopril regimen did show a significant reduction in total coronary events (by 13%; 753 vs 852 events), total cardiovascular events and procedures (by 16%; 1362 vs. 1602 events), cardiovascular mortality (by 24%; 263 vs 342 events), fatal and non fatal stroke (by 23%; 327 vs 422 events) and new-onset diabetes (by 30%; 567 vs 799 events; $p < 0.0001$). During the trial, the mean difference in systolic and diastolic blood pressure measurements in the amlodipine-perindopril compared with the atenolol-thiazide regimen was 2.7/1.9 mm Hg. The findings demonstrate that an antihypertensive drug regimen starting with amlodipine adding perindopril is better than one starting with atenolol adding thiazide in terms of reducing death and cardiovascular risk.



## Diabetes Mellitus

In type 2 diabetes mellitus, controlling blood pressure levels is important to further prevent the risk of macrovascular and microvascular complications of type 2 diabetes. This concept was studied in the ADVANCE study which is a randomized controlled trial performed in 11.140 patients from 215 collaborating centres in 20 countries from Asia, Europe, and North America [20]. Patients were eligible if they had been diagnosed with type 2 diabetes mellitus at the age of 30 years or older and were aged 55 years or older at study entry. Eligible patients also needed to have at least one of the following: a history of major cardiovascular disease (stroke, myocardial infarction, hospital admission for transient ischaemic attack, hospital admission for unstable angina, coronary revascularization, peripheral revascularization, or amputation secondary to vascular disease), or at least one other risk factor for cardiovascular disease. Detailed descriptions of in- and exclusion criteria can be found elsewhere [20]. There were no blood pressure criteria for inclusion. The primary study outcomes were composites of major macrovascular (cardiovascular mortality, MI, and non-fatal stroke) and microvascular events (new or worsening nephropathy or retinopathy). In ADVANCE, 11.140 patients with type 2 diabetes were randomized to treatment with a fixed combination of perindopril and indapamide or matching

placebo, in addition to current therapy [20]. Compared with patients assigned placebo, those assigned active therapy had a mean reduction in systolic and diastolic blood pressure of 5.6/2.2 mm Hg. After a mean of 4.3 years of follow-up, the perindopril-based regimen reduced the risk of major macrovascular or microvascular events by 9% (HR 0.91, 95% CI 0.83–1.00, 15.5% vs 16.8%; p=0·04) (Figure 5). The separate reductions in macrovascular and microvascular events were similar, but were not independently significant (macrovascular HR 0.92; 95% CI 0.81–1.04, microvascular HR 0.91; 95% CI 0.80–1.04). The relative risk of cardiovascular mortality was reduced by 18% (HR 0.82, 95% CI 0.68–0.98; 3.8% vs 4.6%) and all-cause mortality was reduced by 14% (HR 0.86, 95% CI 0.75–0.98; 7.3% vs. 8.5%) [20]. There was no evidence that the effects of the study treatment differed by initial blood pressure level or concomitant medication use. The results suggest that over 5 years, 79 patients needed to be treated to prevent one death (all-cause) [20]. The administration of a fixed combination of perindopril and indapamide was

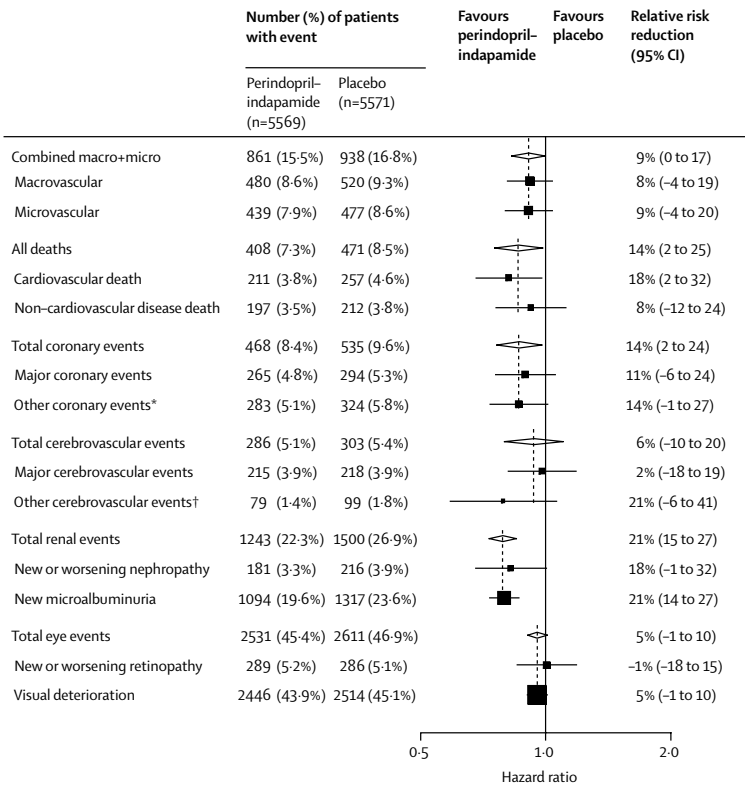



**Figure 5** ADVANCE-trial: result of a perindopril-based regimen in patients with type II diabetes mellitus. Adapted from (20) Patel A; ADVANCE Collaborative Group, et al. Effects of a fixed combination of perindopril and indapamide on macrovascular and microvascular outcomes in patients with type 2 diabetes mellitus (the ADVANCE trial): a randomised controlled trial. *Lancet*. 2007 Sep 8;370(9590):829-40 with permission.

safe and well-tolerated. Treatment guidelines now recommend intensive lowering of blood pressure for diabetic patients with hypertension.

## Cerebrovascular disease

The PROGRESS study was designed to determine the effects of a perindopril-based antihypertensive regimen in patients with a history of stroke or transient ischaemic attack [21]. In PROGRESS, 6105 individuals from 172 centres in Asia, Australasia, and Europe were randomly assigned active treatment (n=3051) or placebo (n=3054). Active treatment comprised a flexible regimen based on perindopril (4 mg daily), with the addition of the diuretic indapamide at the discretion of treating physicians. The primary outcome was total stroke (fatal or non-fatal). Over 4 years of follow up, active treatment reduced blood pressure by 9/4 mm Hg and reduced the primary

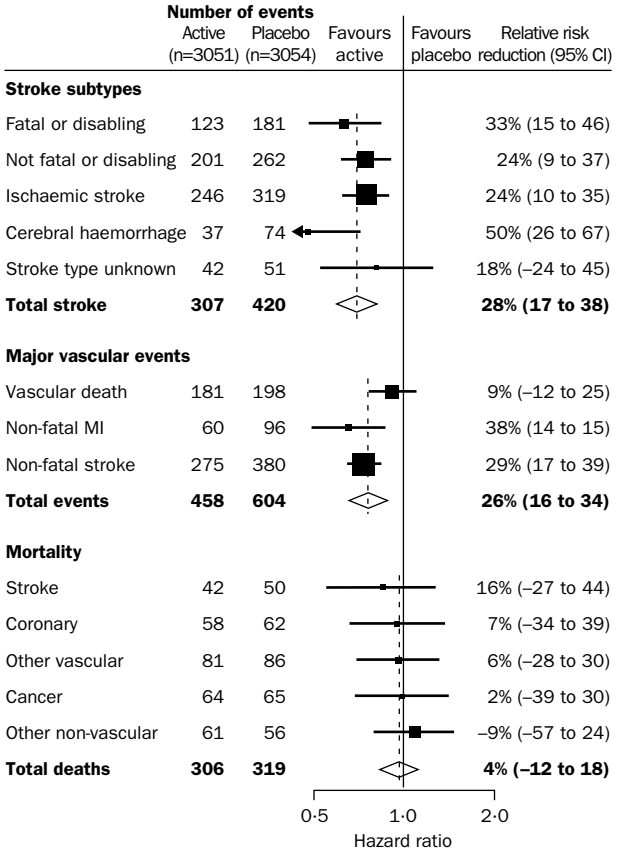



**Figure 6** PROGRESS trial: treatment benefit of perindopril-based regimen in patients with cerebrovascular disease. Adapted with permission from: (21) PROGRESS Collaborative Group. Randomised trial of a perindopril-based blood-pressure-lowering regimen among 6,105 individuals with previous stroke or transient ischaemic attack. *Lancet*. 2001;29;358(9287):1033-41.

endpoint of fatal and non-fatal stroke by 28% (HR 0.72, 95% CI 0.62-0.83; 307 vs 420 events) and total major vascular events by 26% (HR 0.74;95% CI 0.66-0.84; 458 vs 604 events) (Figure 6). There were similar reductions in the risk of stroke in hypertensive and non-hypertensive subgroups (all p<0·01). Combination therapy with perindopril plus indapamide reduced blood pressure even more by 12/5 mm Hg and increased the treatment benefit on stroke risk to 43%. This blood-pressure-lowering regimen reduced the risk of stroke among both hypertensive and non hypertensive individuals with a history of stroke or transient ischaemic attack [21]. Only the combination of the two drugs, perindopril and indapamide was associated with the beneficial effects as single therapy was not associated with significant treatment benefits.

## Stable coronary artery disease

The EUROPA trial studied the ACE-inhibitor perindopril in a relatively low-risk population with stable coronary artery disease and no apparent heart failure [11]. In EUROPA, 12.218 patients were randomly assigned perindopril 8 mg once daily (n=6110), or matching placebo (n=6108). The primary endpoint was cardiovascular death, myocardial infarction, or cardiac arrest. Mean age of patients was 60 years and 85% were male, 92% were taking platelet inhibitors, 62% beta-blockers, and 58% statins. During a mean follow-up of 4.2 years, perindopril was associated with a 20 % relative reduction in the primary endpoint (HR 0.80; 95% CI 0.71-0.91; 8% vs 10%) (Figure 7). These benefits were consistent in clinical subgroups and secondary endpoints. Results were independent of baseline blood pressure and on top op concomitant medication use. Perindopril was safe and well tolerated. Among patients with stable coronary heart disease without apparent heart failure, perindopril significantly improves outcome. To prevent one major cardiovascular event, about 50 patients with stable CAD need to be treated for a period of 4 years [11].

An analysis of three trials of ACE inhibitors in stable vascular disease without left ventricular systolic dysfunction (LVSD) or heart failure, which involved 29 805 patients, demonstrated that, when the findings of HOPE, EUROPA and PEACE were combined [49], ACE-inhibitors significantly reduced all-cause mortality (7.8% versus 8.9%, $P = 0.0004$), cardiovascular mortality (4.3% versus 5.2%, $P = 0.0002$), non-fatal myocardial infarction (5.3% versus 6.4%, $P = 0.0001$), stroke (2.2% versus 2.8%, $P = 0.0004$), heart failure (2.1% versus 2.7%, $P = 0.0007$), coronary artery bypass graft surgery (6.0% versus 6.9%, $P = 0.0036$) but not percutaneous coronary intervention (PCI) (7.4% versus 7.6%, $P = 0.48$). The ACE-inhibitors used in these three trials (HOPE ramipril, EUROPA perindopril, PEACE trandolapril) share several pharmacological characteristics and have been shown to reduce cardiovascular events in patients with heart failure and myocardial infarction or stroke [18,49]. However, in stable CAD trandolapril showed no significant benefit in the PEACE trial, which is possibly related to the reduced power caused by greater crossover and inefficacy of the dosage used. However, the combined analysis confirms that ACE-inhibitors consistently reduce major vascular events in patients with atherosclerosis with preserved LV function. The benefits of ACE inhibitors were independent of baseline blood pressure and apparent in patients taking ß-blockers, lipid-lowering agents and antiplatelet therapy individually or together [49].

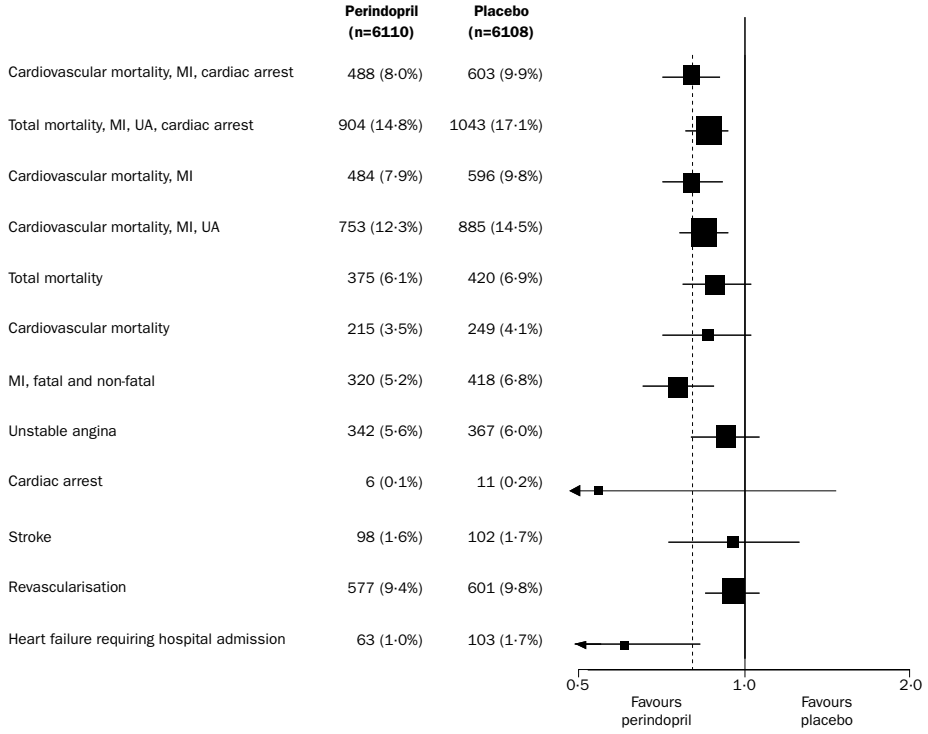



**Figure 7** Treatment benefit of perindopril on primary endpoint and selected secondary endpoints in the EUROPA-trial. MI=myocardial infarction. UA=unstable angina. Size of squares proportional to number of patients in that group. Dashed line indicates overall relative risk. Adapted with permission (ref 11) (*Lancet* 2003; 362: 782–88).



## Elderly

In patients 80 years of age or older, the benefit of a perindopril-indapamide regimen has been demonstrated in the HYVET trial [50]. The HYVET study randomly assigned 3845 elderly patients with a sustained systolic blood pressure of 160 mmHg or more to receive the diuretic indapamide or matching placebo, the ACE-inhibitor perindopril was added if necessary to achieve the target blood pressure of 150/80 mmHg. In the active treatment group, at 2 years, 25.8% were receiving indapamide only, 23.9% indapamide and perindopril 2 mg, and 49,5% indapamide and perindopril 4 mg. The active treatment group was associated with a 30% reduction in the rate of fatal or nonfatal stroke (95% CI -1 to 51, P 0.06) and 21 reduction in the rate of death (95% CI 4-35, P 0.02) and 64% in the rate of heart failure (95% CI 42-78; P <0.001). The HYVET study provides evidence that antihypertensive treatment with indapamide or a combination of indapamide with perindopril is beneficial in persons aged 80 years or older [50]. Additionally, ACE-inhibitors have demonstrated beneficial effects in improving outcome in patients with (diabetic) nephropathy, however, perindopril has not been studies in this patient category and not incorporated in this review.

## CONSISTENCY OF CLINICAL EFFECTS

To ascertain whether or not there is a threshold above which ACE inhibitors have no effect, data from the control group on this composite outcome from HOPE and EUROPA were divided in tertiles of low, medium and high risk according to a risk model for baseline characteristics [51]: for HOPE, the annual rates in the placebo were 2.2% for low risk, 3.6% for medium risk and 6.0% for high risk, and for EUROPA the annual placebo rates according to the tertiles were 1.4% for low risk, 2.4% for medium risk and 4.0% for high risk. Even for low annual rates, below or equal to the 2.1% rate in PEACE, the percentage reductions in odds were between 18% and 28%. Results showing these benefits in lower and intermediate-risk patients complement existing evidence of similar benefit in higher-risk patients with LVSD or heart failure. There was no clear indication for a threshold of ACE inhibitor therapy according to baseline risk. A second analysis on the consistency of the treatment benefit of perindopril was performed in relation to renal insufficiency with similar results [52].

### Meta-analysis of EUROPA, ADVANCE, and PROGRESS

A recent meta-analysis using non-patient level data of the three largest perindopril trials, EUROPA, ADVANCE and PROGRESS, which combines patients with a variety of cardiovascular disease risk states, coronary artery disease, diabetes mellitus, and stroke but all have in common that the vascular bed is diseased [53]. So, the combined population is one of vascular disease or heightened risk of vascular disease. Figure 8 shows a combined analysis of three perindopril trials (EUROPA, PROGRESS and ADVANCE). When these findings were combined, perindopril significantly reduced all-cause mortality (OR 0.89; 95% CI 0.82–0.97), and cardiovascular mortality, myocardial infarction (OR 0.82; 95% CI 0.74–0.90) [53]. This meta-analysis shows that perindopril reduced cardiovascular events irrespective of the type of patients and level of risk, which is in line with previous meta-analyses and risk models [49,51]. This consistency of the treatment benefit by perindopril in patients with stable CAD, type 2 diabetes mellitus, or a history of stroke indicate that the absolute benefits conferred by treatment will be established mainly by each patient's future risk of vascular complications. It becomes more important to base decisions on the treatment of individual patients, on assessment of total cardiovascular risk, rather than on arbitrary cut-off points for single risk factors [53].

## ADDITIONAL EFFECTS BEYOND BLOOD PRESSURE LOWERING

Among ACE inhibitors, perindopril has been extensively studied with regard to the blood pressure-independent effects. This was first suggested by the ASCOT–BPLA investigators, because the observed risk reduction by amlodipine/perindopril was larger than might be expected by the reduction in blood pressure which was 3/2 mmHg greater as compared to a

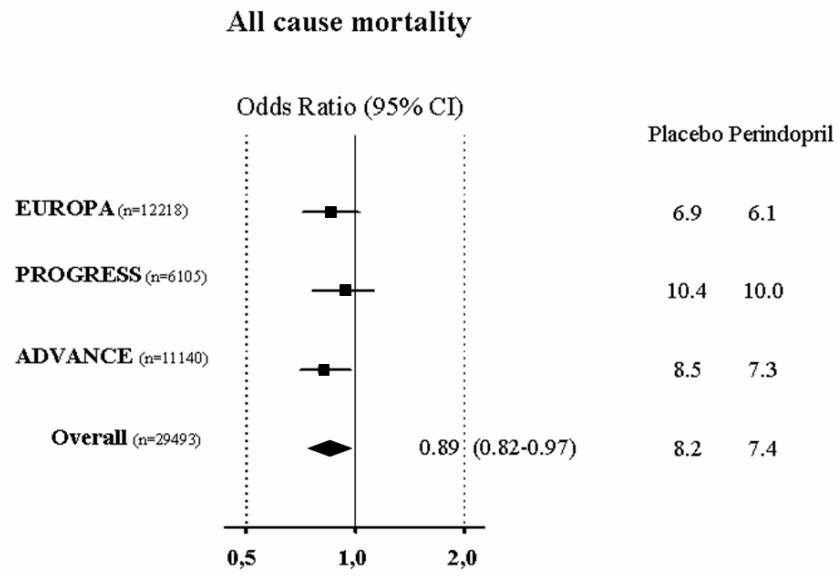



**Figure 8** Meta-analysis of EUROPA, ADVANCE* and PROGRESS trials using perindopril-based regimens versus placebo in patients with vascular disease or high-risk of vascular disease.
Adapted from Brugts JJ, Simoons ML with permission. Meta-analysis of ACE-inhibitors in CAD: lessons from trials in stable coronary artery disease. In: Perindopril. A major contribution to the prevention and treatment of cardiovascular disease. Ferrari R, Fox KM (editors). Paris: Wolters Kluwer Health - Servier; 2008. pp 107-115. Reference 53



ß-blocker/ diuretic regimen in hypertensive patients. This concept that the observed benefits of ACE-inhibitors, such as perindopril, in reducing cardiovascular outcomes is greater than might be expected from the mere blood pressure reductions alone is observed in the other clinical trials as well [11,19-21], including the HOPE trial with ramipril [9]. The observed benefits in ADVANCE, EUROPA and PROGRESS were independent of the level of baseline blood pressure. This suggests that, in addition to blood pressure dependent effects, other factors also contribute to the benefits of ACE inhibition in patients with CAD.

The Blood Pressure Lowering Treatment Trialists' Collaboration (BPLTCC) evaluated the blood pressure-dependent and independent effects of ACE-inhibitors and ARBs on major cardiovascular events using data from 26 large-scale trials comparing ACE-inhibitors or an ARBs with placebo or another drug class [54,55]. From a total of 146.838 individuals with high blood pressure or an elevated risk of cardiovascular disease, 22.666 major cardiovascular events were documented during follow-up. The analyses showed comparable blood pressure-dependent reductions in risk with ACE-inhibitors and ARBs [54,55]. However, the analyses also showed that ACE-inhibitors produced an additional blood pressure-independent reduction in the relative risk of CHD of approximately 9% (95% confidence interval 3–14%) [54,55]. This blood-pressure independent effects have not been observed for ARBs, and there was evidence of a significant heterogeneity between ACE-inhibitors and ARB in this regard ($p = 0.002$). The

authors concluded that there are similar blood pressure-dependent effects of ACE-inhibitors and ARBs, but only for ACE-inhibitors there is evidence of blood pressure-independent effects on the risk of major coronary disease events [54,55]. Also the ON-Target study is relevant in this regard, however the BP reduction of the treatment regimen of either ramipril or telmarsitan in On-Target were comparable, with a slightly higher BP reduction by telmarsitan (0.9/0.6 mmHg), with equivalence in risk reduction. Also, no difference in the treatment effect was observed with combination therapy which was related to an additional 2.4/1.4 mmHg BP lowering as compared to ramipril alone [37].

A recent analysis of EUROPA-investigators focused on this particular issue of effects beyond BP lowering of perindopril [56]. The authors discussed the potential alternative mechanisms to explain the beneficial effects of perindopril in EUROPA. A special feature was that the slight differences between ACE-inhibitors may depend on the degree of tissue affinity of specific ACE-inhibitors (lipophilicity), but also different effects on bradykinin [57,58] Perindopril has high tissue affinity for ACE [59] and, compared with a non-lipid-soluble ACE-inhibitor such as enalapril, markedly increases local tissue bradykinin production [60]. Perindopril has the highest selectivity for the bradykinin binding sites and significantly reduces endothelial cell apoptosis compared to other ACE-inhibitors [61,62]. Bradykinin, by increasing cNOS expression, improves endothelial dysfunction, has anti-oxidant effects, enhances fibrinolysis (tPA release), and reduces cardiovascular remodeling, as such counteracting the effects of angiotensin II [63]. The bradykinin mediated effects could also be an explanation for the observed blood pressure independent effects which absent with ARBs in the BPLTCC analyses. Unfortunately, ACE-inhibitors have not been compared prospectively, and therefore the exact clinical meaning of these specific properties is still unknown.

Several sub studies of EUROPA have been performed to explain the additional beneficial effects observed with perindopril: [the Perindopril – Function of the Endothelium in Coronary Artery Disease Trial (PERFECT), Perindopril's Prospective Effect on Coronary Atherosclerosis by Angiography and Intravascular Ultrasound Evaluation (PERSPECTIVE) and the Perindopril – Thrombosis, Inflammation, Endothelial Dysfunction and Neurohumoral Activation Trial (PERTINENT) [24-27, 39]. The PERSPECTIVE study evaluated the effect of perindopril on coronary plaque progression as assessed by quantitative coronary angiography and intravascular ultrasound [27]. The initial analysis revealed no progression of CAD by quantitative angiography and intravascular ultrasound with long term administration of either perindopril or placebo (median follow-up 3 years), possibly because most patients were on concomitant treatment with a statin. A further analysis showed an association of long-term administration with perindopril and constrictive remodeling patterns without affecting the lumen, suggesting that this treatment is associated with plaque stabilization. The PERTINENT study examined the effects of perindopril on endothelial function and concluded that abnormal endothelial function occurs in patients with CAD (up regulated ACE) and this can be reversed by perindopril [26]. Perindopril significantly increased bradykinin and eNOS levels after one year of treatment and decreased von Willebrand factor [26]. The

PERFECT study also concluded that the beneficial effects of perindopril on cardiovascular morbidity and mortality in the EUROPA study might be at least partly explained by an improvement in endothelial function [24]. In the PREAMI study investigated 1252 elderly post-myocardial infarction patients with preserved left ventricular function in which perindopril reduced the progressive left ventricular deterioration and remodeling [39]. These studies with perindopril demonstrates its additional effects beyond blood pressure lowering by improving endothelial function, thrombolysis and neurohumoral balance and reducing atherosclerosis, and inflammation.

## SAFETY AND TOLERABILITY

At initiation of an ACE-inhibitor, the physician has to check renal function parameters and serum electrolyte. After initiation, up-titration is possible after 2-4 weeks of treatment when necessary. Renal function and serum electrolytes should be monitored after initiation. Usually, up-titration is done slowly but more rapid titration is possible in closely monitored patients. An ARB is recommended as an alternative in patients intolerant of ACE-inhibitor.

### Adverse effects

Common adverse drug reactions (≥1% of patients) include: hypotension, cough, hyperkaliemia, headache, dizziness, fatigue, nausea, renal impairment [12-17,64]. A persistent dry cough is a relatively common adverse effect (10%) and is believed to be associated with the increases in bradykinin levels produced by ACE-inhibitors, although the role of bradykinin in producing these symptoms remains disputed. Recent safety data from EUROPA (n=12218) and PROGRESS (n=6105) reported relatively low withdrawal rates [11,21]. In EUROPA, 13655 patients were registered, and during run-in period 290 patients did not proceed to randomization due to hypotension (2.1%), 149 due to raised potassium or creatinine concentrations (1.1%) and 332 due to other intolerance (2.4%). After randomization, withdrawal associated with dry cough was 2.7 and 2.2% in EUROPA and PROGRESS respectively [11,21]. "However, patients in these studies were selected and the cough rate could be under-estimated". Since this is one of the main arguments for general practitioners to switch to ARBs (ARBs do not influence bradykinin levels), these withdrawal rates seem rather low and the persistent dry cough discomfort, when apparent, must be evaluated for the individual patient. The potential benefit of the bradykinin mediated effects, as described in this review, is also important to when assessing the demonstrated anti-atherosclerotic, anti-inflammatory and anti-thrombotic effects of ACE-inhibitors by bradykinin. The second common side effect of ACE-inhibitors first dose hypotension, but with perindopril because of its slow onset has a very low incidence of this side-effect as compared to other in its class. Again, safety data from EUROPA and PROGRESS show only 1.0 and 2.1% of the patients withdrew from treatment as a result of hypotension [11,21].

Renal impairment is a significant adverse effect of all ACE-inhibitors [12-17,64]. The reason for this is still unknown. Some suggest that it is associated with their effect on angiotensin II-mediated homeostatic functions such as renal blood flow. In hypertensive patients, renal blood flow may be affected by angiotensin II because it vasoconstricts the efferent arterioles of the glomeruli of the kidney, thereby decreasing glomerular filtration rate (GFR) and a rise in serum creatinin. Hence, by reducing angiotensin II levels, ACE-inhibitors may reduce GFR, a marker of renal function. The afferent arteriole is not under the influence of AT-II and when blood pressure is reduced there is a fall in glomerular capillary pressure, which prevents progression of glomerular damage [64]. In some cases, fe. renal artery stenosis, ACE-inhibitors can induce or exacerbate renal impairment. ACE-inhibitors may further cause hyperkalemia, because angiotensin II increases aldosteron release. Since aldosteron is responsible for increasing the excretion of potassium, ACE inhibitors ultimately cause retention of potassium. Some patients develop angioedema due to increased bradykinin levels [64].

### Contraindications and precautions

The ACE-inhibitors are contraindicated in patients with bilateral renal artery stenosis ( or unilateral stenosis with a solitary functioning kidney) and patients with previous angioedema associated with ACE-inhibitor therapy [12-17,64]. Additional caution with the prescription of ACE inhibitors is needed in patient with renal insufficiency, cardiac outflow obstruction (aortic valve stenosis), hypovolemia, dehydration, and patients with haemodialysis. Potassium supplementation should be used with caution and under medical supervision owing to the hyperkalaemic effect of ACE inhibitors.

### ACE-inhibitors in pregnancy (ADEC category D)

ACE-inhibitors should be avoided in women who are likely to become pregnant or are pregnant [12-17,64]. There is a risk of birth defects when taking during the second and third trimester. When pregnancy is detected, ACE inhibitors should be discontinued as soon as possible.

## REGULATORY AFFAIRS

In Europe, perindopril is approved by the EMEA for the treatment of hypertension, heart failure, and more recently, based on the EUROPA trial results, for the reduction of cardiac events in patients with a history of myocardial infarction and / or revascularization. In the USA, perindopril is approved by FDA for the use in patients with hypertension and in patients with stable coronary artery disease to reduce the risk of cardiovascular mortality or non-fatal myocardial infarction. Perindopril is available in a fixed combination with indapamide for the treatment of hypertension, and more recently, in combination with amlodipine for the treatment of hypertension and/or stable coronary artery disease.

## EXPERT OPINION & FIVE-YEAR VIEW

The ACE-inhibitors have influenced cardiovascular medicine in a wide variety of patient groups as no other. Especially, perindopril has been studied extensively from basic research into mechanisms of action and tissue penetrance to clinical efficacy in large randomized trials in various patient groups. The great consistency of the treatment benefit across several clinical subgroups, on top of concomitant medication, have established that ACE-inhibitors, perindopril, are first-line choice in the cardioprotective treatment of heart failure, hypertension, diabetics, stroke and stable CAD. In patients at high-risk of events, ACE-inhibitors therapy is indicated since benefit is expected in all cases. However, in patients at relatively low risk, for example stable CAD, one would like to target ACE-inhibitors to those patients most likely to benefit of such prolonged prophylactic treatment. Research focused at the consistency of the treatment effects is important, since it may elucidate heterogeneity in the treatment effect. It is likely that the treatment benefit differs between patients, some will benefit, other will not. Also, many patients need to be treated against modest benefits (NNT in EUROPA was 50). Several prior approaches to target therapy to those patients most likely to benefit have not been successful. These prior approaches have mainly focused at simple clinical patient characteristics [65]. One option to tailored therapy might be to look into pharmacogenetic aspects of ACE-inhibitors therapy in stable CAD. Until now, cardiovascular pharmacogenetic research is in a formative stage, but it has the potential to guide ACE-inhibitor therapy in patients with stable CAD. Currently, the PERindopril GENEtic Association study is being performed focused at this particular issue of elucidating a heterogeneity in the treatment effect of perindopril which can be used to target therapy to those patients most likely to benefit of treatment [66]. The PERGENE study will investigate the feasibility of pharmacogenetic profiling of ACE-inhibitors. If proven successful, this would revolutionize CVD practice at many other levels but will also lead to an increased clinical efficacy and cost-effectiveness of ACE-inhibitors in patients with stable CAD.



## KEY ISSUES

- Perindopril has a good safety and tolerability profile.
- Several differences between ACE-inhibitors exist and perindopril has been shown to have unique properties such as:
  - a higher tissue ACE-affinity as compared to other ACE-inhibitors.
  - the highest selectivity for the bradykinin binding sites and higher effect on bradykinin levels as compared to other ACE-inhibitors.
  - the clinical meaning of these differences between ACE-inhibitors is yet unknown
- Perindopril has a long half-life and 24 hour persistence of action allowing BP to be controlled during sleep and the awakening hours.

- ACE-inhibition by perindopril has additional effects beyond BP reduction such anti-inflammatory, anti-atherosclerotic and antithrombotic properties.
- Perindopril has been demonstrated to be effective in reducing cardiovascular events in a wide range of patient populations. ACE-inhibitors are first-line choice in treatment guidelines of ESC/AHA/ACC of stable CAD, hypertension, cardiovascular disease and heart failure.
- Perindopril reduced cardiovascular events irrespective of the type of patients and level of baseline risk of events.
- The consistency of the treatment benefit by perindopril in patients with stable CAD, type 2 diabetes mellitus, or a history of stroke indicate that the absolute benefits conferred by treatment will be established mainly by each patient's future risk of vascular complications rather than on arbitrary cut-off points for single risk factors such as blood pressure.
- Treatment decisions on the treatment of individual patients could therefore be based on the assessment of total cardiovascular risk.

## REFERENCES


1 Skeggs LT, Dorer RE, Kahn JR, Lentz KE,Levine M: The biochemistry of the renin-angiotensin system and its role in hypertension. *Am J Med* 1976; 60: 737–748.

2 Erdos EG: Angiotensin I converting enzyme and the changes in our concepts through theyears. *Hypertension* 1990; 16: 363–370.

3 Perazella MA, Setaro JF: Renin-angiotensin aldosterone system: fundamental aspects and clinical implications in renal and cardiovascular disorders. *J Nucl Cardiol* 2003;10: 184–194.

4. Ferrario WS. Role of the Renin-Angiotensin-Aldosterone System and Proinflammatory Mediators in Cardiovascular Disease. *Am J Cardiology*; 98:121-128.

5. Pfeffer MA, Braunwald E, Moye LA, Basta L, Brown EJ Jr, Davis BR, Geltman EM, Goldman S, Flaker G, for the SAVE Investigators. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction and myocardial infarction: results of the survival and ventricular enlargement trial. *N Engl J Med* 1992; 327: 669–77.

6. The SOLVD investigators. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. *N Engl J Med* 1991; 325: 293–302.

7. The Acute Infarction Ramipril Efficacy (AIRE) study investigators. Effect of ramipril on mortality and morbidity of survivors of acute myocardial infarction with clinical evidence of heart failure. *Lancet* 1993; 342: 821–28.

8. Torp-Pedersen C, Kober L for the TRACE Study Group. Effect of ACE inhibitor trandolapril on life expectancy of patients with reduced left-ventricular function after acute myocardial infarction. *Lancet* 1999; 354: 9–12.

9. The Heart Outcomes Prevention Evaluation study investigators. Effects of an angiotensin-converting enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. *N Engl J Med* 2000;342:145–53.

10. The PEACE Trial Investigators. Angiotensin-converting-enzyme inhibition in stable coronary artery disease. *N Engl J Med* 2004; 351: 2058–68.

11. The European trial on reduction of cardiac events with perindopril in patients with stable coronary artery disease investigators. Efficacy of perindopril in reduction of cardiovascular events in stable coronary artery disease: randomized, double-blind, placebo-controlled, multicentre trial (the EUROPA study). *Lancet* 2003; 362: 782–88.

12. The Task Force on the Management of Stable Angina Pectoris of the European Society of Cardiology. Guidelines on the management of stable angina pectoris. *Eur Heart J* 2006 27, 1341–81.

13. Wood D, DeBacker G, Faergam O, Graham I, Mancia G, Pyörälä K and the task force, Prevention of coronary disease in clinical practice: recommendation of the second Joint Task Force of European and other societies on Coronary Prevention. *Atherosclerosis* 1998;140: 99–270.

14. Authors/Task Force Members, Lars Rydén, Eberhard Standl, Malgorzata Bartnik, Greet Van den Berghe, John Betteridge, Bengt Jönsson, Markku Laakso, Klas Malmberg, Silvia Priori, Jan Östergren, Jaakko Tuomilehto, Inga Thrainsdottir, Other Contributors, Ilse Vanhorebeek, Marco Stramba-Badiale, Peter Lindgren, Qing Qiao, ESC Committee for Practice Guidelines (CPG), and David Wood. Guidelines on diabetes, pre-diabetes, and cardiovascular diseases: executive summary: The Task Force on Diabetes and Cardiovascular Diseases of the European Society of Cardiology (ESC) and of the European Association for the Study of Diabetes (EASD). *Eur. Heart J.* 2007; 28: 88 - 136.

15. Task Force Members, José López-Sendón, Karl Swedberg, John McMurray, Juan Tamargo, Aldo P. Maggioni, Henry Dargie, Michal Tendera, Finn Waagstein, Jan Kjekshus, Philippe Lechat, and Christian Torp Pedersen. Expert consensus document on angiotensin converting enzyme inhibitors in cardiovascular disease: The Task Force on ACE-inhibitors of the European Society of Cardiology. *Eur Heart J.* 2004; 25: 1454 - 1470.

16. Josep Redon, Frank Ruschitzka, Juan Tamargo, Pieter van Zwieten, Margus Viigimaa, Bernard Waeber, Bryan Williams, and Jose Luis Zamorano. 2007 Guidelines for the management of arterial hypertension: The Task Force for the Management of Arterial Hypertension of the European Society

of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur. Heart J.* 2007; 28: 1462 - 1536.

17. Gibbons RJ, Abrams J, Chatterjee K, Daley J, Deedwania PC, Douglas JS, Ferguson TB Jr, Fihn SD, Fraker TD Jr, Gardin JM, O'Rourke RA, Pasternak RC, Williams SV; American College of Cardiology; American Heart Association Task Force on practice guidelines (Committee on the Management of Patients With Chronic Stable Angina). ACC/AHA 2002 guideline update for the management of patients with chronic stable angina—summary article: a report of the American College of Cardiology/American Heart Association Task Force on practice guidelines (Committee on the Management of Patients With Chronic Stable Angina). *J Am Coll Cardiol* 2003;41:159-168.
18. Ferrari R, Pasanisi G, Notarstefano P, Campo G, Gardini E, Ceconi C. Specific properties and effect of perindopril in controlling the renin-angiotensin system. *Am J Hypertens*. 2005 Sep;18(9 Pt 2):142S-154S..
19. B Dalhöf, PS Sever, NR Poulter et al. and for the ASCOT investigators, Prevention of cardiovascular events with an antihypertensive regimen of amlodipine adding perindopril as required versus atenolol adding bendroflumethiazide as required, in the Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm (ASCOT-BPLA): a multicentre randomised controlled trial, *Lancet* 2005;366:895–906**.**
20. ADVANCE *Collaborative Group*. Effects of a fixed combination of perindopril and indapamide on macrovascular and microvascular outcomes in patients with type 2 diabetes mellitus (the ADVANCE trial): a randomized controlled trial. *Lancet* 2007; 370: 829–40
21. *PROGRESS Collaborative Group* Randomised trial of a perindopril-based blood-pressure-lowering regimen among 6105 individuals with previous stroke or transient ischaemic attack. *Lancet* 2001; 358: 1033–41.
22. Speirs C, Wagniart F, Poggi L. Perindopril post marketing surveillance: a 12 month study in 47,351 hypertensive patients. *Br J Clin Pharmacol*. 1998 Jul;46(1):63-70.
23. Clark LT. Safety profile of perindopril. *Am J Cardiol.* 2001 Oct 4;88(7A):36i-40i.
24. M. L. Bots, W. J. Remme, T. F. Lüscher, K. M. Fox, M. Bertrand, R. Ferrari, M. L. Simoons, D. E. Grobbee. ACE Inhibition and Endothelial Function: Main Findings of PERFECT, a Sub-Study of the EUROPA Trial. *Cardiovasc Drugs Ther* (2007) 21:269–279
25. Gastón A. Rodriguez-Granillo, Jeroen Vos, Pim J. De Feyter. Long-Term Effect of Perindopril on Coronary Atherosclerosis Progression (from the PERindopril's Prospective Effect on Coronary aTherosclerosis by Angiography and IntraVascular Ultrasound Evaluation [PERSPECTIVE] Study) *Am J Cardiol* 2007;100:159 –163)
26. Ceconi C, Fox KM, Remme WJ, Simoons ML, Bertrand M, Parrinello G, Kluft C, Blann A, Cokkinos D, Ferrari R; EUROPA Investigators; PERTINENT Investigators and the Statistical Committee. ACE inhibition with perindopril and endothelial function. Results of a substudy of the EUROPA study: PERTINENT. *Cardiovascular Research* 73 (2007) 237–246.
27. Gastón A. Rodriguez-Granillo, Sebastiaan de Winter, Nico Bruining, Jurgen M.R. Ligthart, Héctor M. García-García, Marco Valgimigli, and Pim J. de Feyter on behalf of the EUROPA/PERSPECTIVE Investigators. Effect of perindopril on coronary remodelling: insights from a multicentre, randomized study. *Eur. Heart J* 2007; 28: 2326 - 2331.
28. Piepho RW: Overview of the angiotensin converting-enzyme inhibitors. *Am J Health Syst Pharm* 2000; 57:S3–S7.
29. Brown NJ, Vaughan DE: Angiotensin-converting enzyme inhibitors. *Circulation* 1998;97: 1411–1420.
30. Vincent M, Marchand B, Remond G et al. Synthesis and ACE inhibitory activity of stereoisomers of perindopril (S-9490) and perindoprilat (S-9780). *Drug Des. Discov*. 1992;9:11-28
31. Telejko E. Perindopril-arginine: benefits of a new formulation of the ACE-inhibitor perindopril. *Curr Med Res Opin* 2008;23:953-960.
32. Unger T, Culman J, Gohlke P: Angiotensin II receptor blockade and end-organ protection: pharmacological rationale and evidence. *J Hypertens Suppl* 1998; 16:S3–S9.

33. Pellacani A, Brunner HR, Nussberger J: Plasma kinins increase after angiotensin enzyme inhibition in human subjects. *Clin Sci* (Lond)1994; 87: 567–574.
34. Goodfriend TL, Elliott ME, Catt KJ: Angiotensin receptors and their antagonists. *N Engl J Med* 1996; 334: 1649–1654.
35. Gainer JV, Morrow JD, Loveland A, King DJ, Brown NJ. Effect of bradykinin-receptor blockade on the response to angiotensin converting-enzyme inhibitor in normotensive and hypertensive subjects. *N Engl J Med* 1998;339:1285–92.
36 Pfeffer MA, McMurray JJ, Velazquez EJ, Rouleau JL, Køber L, Maggioni AP, Solomon SD, Swedberg K, Van de Werf F, White H, Leimberger JD, Henis M, Edwards S, Zelenkofske S, Sellers MA, Califf RM: Valsartan, captopril, or both in myocardial infarction complicated by heart failure, left ventricular dysfunction, or both. *N Engl J Med* 2003; 349:1893–1906.
37. Yusuf S, Teo KK, Pogue J, Dyal L, Copland I, Schumacher H, Dagenais G, Sleight P, Anderson C: Telmisartan, ramipril, or both in patients at high risk for vascular events. *N Engl J Med* 2008; 358: 1547–1559.
38. Ferrari R. Angiotensin-converting enzyme inhibition in cardiovascular disease: evidence with perindopril. *Expert Rev Cardiovasc Ther*. 2005 Jan;3(1):15-29. Review.
39. The PREAMI Investigators. Effects of Angiotensin-Converting Enzyme Inhibition with Perindopril on Left Ventricular Remodelling and Clinical Outcome. Results of the Randomized Perindopril and Remodelling in Elderly With Acute Myocardial Infarction (PREAMI) Study. *Arch Intern Med* 2006; 166: 659–66.
40. Devissaguet JP, Ammoru N, Devissaguet M, Perret L. Pharmacokinetics of perindopril and its metabolites in healthy volunteers. Fundam *Clin Pharmacol*. 1990;4:175-189.
41. Alfakih K, Hall AS. Drug evaluation of perindopril. *Expert Opin. Pharmacother*. 2006;7:63-71.
42. Zhuo J, Mendelsohn F, Ohishi M. Perindopril alters vascular angiotensin-converting enzyme, AT1 receptor, and nitric oxide synthase expression in patients with coronary heart disease. *Hypertension* 2002;39:634-638.
43. Johnston C, Fabris B, Yamada H, Mendelsohn FA, Cubela R, Sivell D, Jackson B. Comparative studies of tissue inhibition by angiotensin-converting enzyme inhibitors. *J Hypertens* 1989;7(Suppl 5):S11-S16.
44. Busson JP, d'Amore TF, Perret L, Porchet M, Nussberger J, Waeber B, Brunner HR, Single and repeated dosing of the converting enzyme inhibitor perindopril to normal subjects. *Clin Pharmacol Ther* 1986;39:554-558.
45. Servier Laboratories Ltd. Coversyl: summary of product characteristics [online]. Available from URL: http://www.emc.medicines.org.uk [Accessed 2008 Nov 10]
46. Thiollet M, Funck-Bretano C, Grande JD et al. The pharmacokinetics of perindopril in patients with liver cirrhosis. *Br J Clin Pharmacol* 1992; 33:326-328.
47. Solvay Pharmaceuticals Inc. Aceon: US prescribing and safety information [online]. Available from URL: http://www.solvaypharmaceuticals-us.com [Accessed 2008 Nov 10]
48. Bellissant E, Giudicelli J-F. Pharmacokinetic-pharmacodynamic model for perindoprilat regional haemodynamic effects in healthy volunteers and in congestive heart failure patients. *Br J Clin Pharmacol* 2001 Jul; 52 (1): 25-33.
49. Dagenais GR, Pogue J, Fox K, Simoons ML, Yusuf S. Angiotensin converting-enzyme inhibitors in stable vascular disease without left ventricular systolic dysfunction or heart failure: a combined analysis of three trials. *Lancet* 2006; 368:581–8.
50. Beckett NS, Peters R, Bulpitt CJ. Treatment of hypertension in patients 80 years of age or older. *New Engl J Med* 2008;358**.**
51. Deckers JW, Goedhart DM, Boersma E, et al. Treatment benefit by perindopril in patients with stable coronary artery disease at different levels of risk. *Eur Heart J* 2006; 27:796–801.
52. Brugts JJ, Boersma E, Chonchol M, et al. The cardioprotective effects of the angiotensin-converting enzyme inhibitor perindopril in patients with stable coronary artery disease are not modified by mild to moderate renal insufficiency: insights from the EUROPA trial. *J Am Coll Cardiol* 2007; 50:2148–55.

53. Brugts JJ, Simoons ML. Meta-analysis of angiotensin-converting enzyme inhibitors in coronary artery disease: lessons from trials in stable coronary artery disease. In: Perindopril. A major contribution to the prevention and treatment of cardiovascular disease. Ferrari R, Fox KM (editors). Paris: Wolters Kluwer Health - Servier; 2008.pp 107-115.
54. Blood Pressure Lowering Treatment Trialists' Collaboration Blood pressure-dependent and independent effects of agents that inhibit renin-angiotensin system. *J Hypertension* 2007;25:951-8**.**
55. Blood Pressure Lowering Treatment Trialists' Collaboration. Effects of different regimens to lower blood pressure on major cardiovascular events in older and younger adults: meta-analysis of randomised trials. *BMJ* 2008;336:1121-1123.
56. Remme WJ, Deckers JW, Fox KM, Ferrari R, Bertrand M, Simoons ML; EUROPA Investigators. Secondary Prevention of Coronary Disease with ACE Inhibition-does Blood Pressure Reduction with Perindopril Explain the Benefits in EUROPA? *Cardiovasc Drugs Ther.* 2009 Apr;23(2):161-70.
57. Dzau VJ, Bernstein K, Celermajer D, Cohen J, Dahlof B, Deanfield J, et al. Pathophysiologic and therapeutic importance of tissue ACE: a consensus report. *Cardiovasc Drugs Ther* 2002;16:149–60. 498
58. Remme WJ. Effect of ACE inhibition on neurohormones. *Eur Heart J* 1998;19:J16–23.
59. Zhuo JL, Mendelsohn FAO, Ohishi M. Perindopril alters vascular angiotensin-converting enzyme, AT1 receptor and nitric oxide synthase expression in patients with coronary artery disease. *Hypertension* 2002;39:634–8. 539
60. Su JZ, Chen SC, Wu KG, Chen DG, Rui HB, Wang XY, et al. Effects of perindopril, propranolol and dihydrochlorothiazide on cardiovascular remodeling in spontaneously hypertensive rats. *Zhongguo-Yao-Li-Xue-Bao* 1999;20:923–8. 543
61. Ceconi C, Francolini G, Olivares A, Comini L, Bachetti T, Ferrari R. Angiotensin-converting enzyme (ACE) inhibitors have different selectivity for bradykinin binding sites of human somatic ACE. *Eur J Pharmacol* 2007;577:1–6. 535.
62. Ceconi C, Francolini G, Bastianon D, Gitti GL, Comini L, Ferrari R. Differences in the effect of angiotensin-converting enzyme inhibitors on the rate on the rate of endothelial cell apoptosis: in vitro and in vivo studies. *Cardiovasc Drugs Ther* 2007;21:423–9. 547
63. Matsumoto T, Minai K, Horie H, Ohira N, Takashima H, Tarutani Y,et al. Angiotensin-converting enzyme inhibition but not angiotensin II type 1 receptor antagonism augments coronary release of tissue plasminogen activator in hypertensive patients. *J Am Coll Cardiol* 2003;41:1373–9.
64. Clark LT. Safety profile of perindopril. *Am J Cardiol*. 2001 Oct 4;88(7A):36i-40i.
65. Brugts JJ, Danser AH, de Maat MP, den Uil CA, Boersma E, Ferrari R, Simoons ML. Pharmacogenetics of ACE inhibition in stable coronary artery disease: steps towards tailored drug therapy. *Curr Opin Cardiol*. 2008 Jul;23(4):296-301.
66. Brugts JJ, de Maat MP, Boersma E, Witteman JC, van Duijn C, Uitterlinden AG, Bertrand M, Remme W, Fox K, Ferrari R, Danser AH, Simoons ML; On behalf of the EUROPA-PERGENE investigators. The Rationale and Design of the Perindopril Genetic Association Study (PERGENE): A Pharmacogenetic Analysis of Angiotensin-Converting Enzyme Inhibitor Therapy in Patients with Stable Coronary Artery Disease. *Cardiovasc Drugs Ther.* 2009 Apr;23(2):171-81.

# Chapter 3

# Lessons learned from ACE-inhibitor trials in stable CAD

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## ABSTRACT

Angiotensin converting enzyme (ACE) inhibitors have consistently demonstrated a reduction in cardiovascular endpoints, independently of blood pressure reductions, in patients with coronary artery disease (CAD). The persistent effects of perindopril across different populations suggest that it reduces cardiovascular risk via mechanisms in addition to blood pressure reduction.

These effects were demonstrated in the EUROPA study, in which patients with stable CAD receiving perindopril experienced reductions in the risk of cardiovascular mortality, myocardial infarction, cardiac arrest and non-fatal myocardial infarction. The results of these studies support the initiation of ACE inhibitors in patients with stable CAD to reduce the risk of future cardiovascular events based on the individual's cardiovascular risk rather than initial blood pressure.

## INTRODUCTION

The angiotensin-converting enzyme (ACE) inhibitors were introduced almost three decades ago for the treatment of hypertension. Since that time, ACE-inhibitors have been associated with significant advances in the secondary prevention of a wide range of cardiovascular diseases. This important role of ACE-inhibitors has been established by several large clinical trials that demonstrated the efficacy of this drug class (Figure 1) in patients at high risk of cardiovascular disease, including those with post-myocardial infarction left ventricular ejection fraction of less than 40%, heart failure or a history of cerebrovascular accidents [the Survival and Ventricular Enlargement (SAVE) study, Studies of Left Ventricular Dysfunction (SOLVD), the Acute Infarction Ramipril Efficacy (AIRE) study and the Trandolapril Cardiac Evaluation (TRACE) study], and those with a lower risk of cardiovascular events, in particular patients with stable coronary artery disease (CAD) without overt heart failure [the Heart Outcomes Prevention Evaluation (HOPE) study, the Prevention of Events with ACE inhibition (PEACE) study and the European Trial on Reduction of Cardiac Events with Perindopril among Patients with Stable Coronary Artery Disease (EUROPA)] [1-7].

The use of ACE-inhibitors is now recognized and recommended in the European Society of Cardiology / American Heart Association / American College of Cardiology / European Society of Hypertension / European Association for the Study of Diabetes guidelines on the

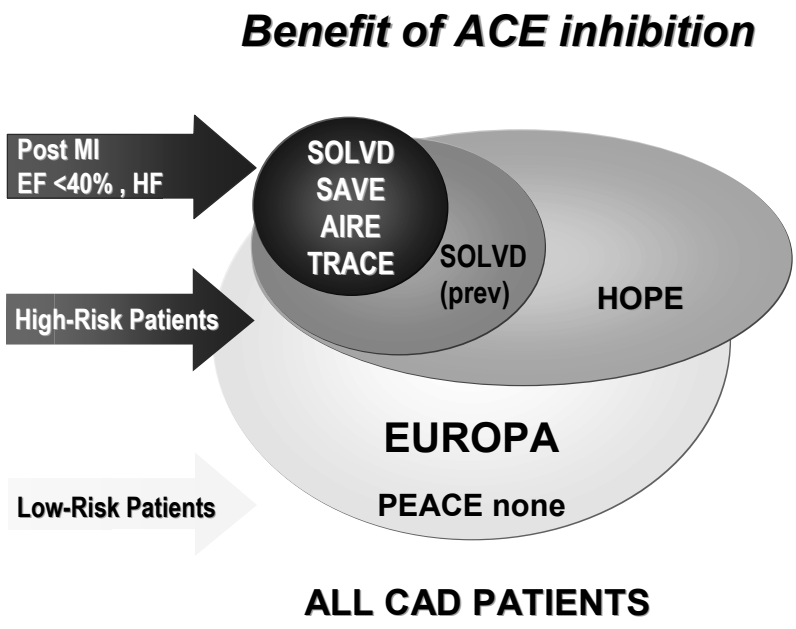



**Figure 1** Benefit of angiotensin-converting enzyme inhibition. ACE = Angiotensin-converting enzyme; CAD = coronary artery disease; EF = ejection fraction; HF = heart failure; MI = myocardial infarction.

management of hypertension, stable CAD, myocardial infarction (MI), heart failure, and in the prevention of the progression of renal insufficiency in diabetes mellitus-related kidney disease [8-10] For example, the European Society of Cardiology 2006 clinical treatment guidelines recommend the use of ACE-inhibitors as routine secondary prevention for the broad group of patients with known CAD with, respectively, a class I (level of evidence A) recommendation for ACE-inhibitor therapy in CAD patients with hypertension, heart failure, left ventricular dysfunction, previous myocardial infarction with left ventricular dysfunction or diabetes and a class IIa recommendation in all patients with angina pectoris and confirmed coronary disease (level of evidence B). In angina pectoris without co-existing indications for ACE-inhibitor treatment, the anticipated benefit should be weighted against the costs and risks of side effects, and the dose and agent used should be of confirmed efficacy for this indication [8] Although other agents modulating the renin–angiotensin system can provide some overlapping pharmacological and clinical effects, ACE-inhibitors remain unique in the range of their confirmed benefits, forming a solid basis for the prevention and treatment of cardiovascular disease, including potential combinations with other established drugs, such as statins and anti-platelet agents.

The ACE-inhibitor perindopril has been extensively studied in several large clinical trials such as EUROPA, the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT), the Perindopril Protection Against Recurrent Stroke Study (PROGRESS), the Perindopril and Remodeling in the Elderly with Acute Myocardial Infarction (PREAMI) study and the Action in Diabetes and Vascular Disease: Preterax and Diamicron-MR Controlled Evaluation (ADVANCE) study, of which each has contributed to the extension of treatment strategies in cardiovascular disease [7,11-14]. Furthermore, ACE-inhibitor research has led to a better understanding of pathophysiological processes and maladaptive responses in the renin–angiotensin–aldosterone system. In particular, several substudies of EUROPA, [the Perindopril – Function of the Endothelium in Coronary Artery Disease Trial (PERFECT), Perindopril's Prospective Effect on Coronary Atherosclerosis by Angiography and Intravascular Ultrasound Evaluation (PERSPECTIVE) and the Perindopril – Thrombosis, Inflammation, Endothelial Dysfunction and Neurohumoral Activation Trial (PERTINENT)], have established that ACE-inhibitors have additional effects beyond blood pressure reduction alone, such as the improvement of endothelial function, improvement of the neurohumoral balance and reduction of unfavourable remodeling of the coronary arteries[15-17].

In this chapter, we will discuss and compare different ACE-inhibitor trials with respect to their confirmed efficacy in stable CAD (HOPE, EUROPA, PEACE). We will analyze the relationship between the effect on blood pressure by perindopril and the reduction in cardiovascular risk in different types of patients in three large trials with this agent (EUROPA, PROGRESS, ADVANCE), and we will summarize the lessons learned from over three decades of research with ACE-inhibitors in cardiovascular disease.

## ANGIOTENSIN-CONVERTING ENZYME INHIBITOR TRIALS IN STABLE CORONARY ARTERY DISEASE

Three large clinical trials have examined the beneficial effect of ACE inhibition on the incidence of cardiovascular events in high-risk patients who had evidence of vascular disease or diabetes plus one other cardiovascular risk factor (HOPE) or in relatively low-risk patients with stable CAD (EUROPA, PEACE). HOPE demonstrated that the ACE-inhibitor ramipril reduced the incidence of cardiovascular events during long-term follow-up in high-risk patients[5]. EUROPA revealed a significant reduction in the incidence of cardiovascular events during long-term follow-up in low-risk patients with stable CAD without overt heart failure receiving the ACE-inhibitor perindopril[7]. In a comparable patient population, however, PEACE did not show a reduction in cardiovascular events and all-cause mortality with the ACE inhibitor trandolapril[6]. The PEACE investigators claimed that the absence of any treatment benefits in the trial might have been related to a lower baseline risk of cardiovascular events compared with the HOPE and EUROPA trials, and referred to the high proportion of patients who had previously undergone revascularization and were using more concomitant medication. A comparison of the baseline characteristics of EUROPA, PEACE and HOPE is shown in Table 1. In contrast with the assumption by the PEACE investigators,

**Table 1.** Baseline characteristics HOPE, EUROPA, PEACE-trials

| | HOPE (n=9297) | EUROPA (n=12218) | PEACE (n=8290) |
|---|---|---|---|
| Characteristics (%) | | | |
| Age (years) | 66 (7) | 60 (9) | 64 (8) |
| Female | 26.8 | 14.6 | 17.5 |
| Previous myocardial infarction | 52.7 | 64.8 | 55.0 |
| PCI | 17.9 | 29.3 | 41.5 |
| CABG | 25.8 | 29.4 | 39.0 |
| Previous stroke or TIA | 10.9 | 3.4 | 6.5 |
| Peripheral artery disease | 43.6 | 7.3 | NA |
| Current smokers | 14.2 | 15.2 | 14.5 |
| Diabetes mellitus | 38.5 | 12.3 | 17.0 |
| Hypertension | 46.8 | 27.1 | 45.5 |
| Serum cholesterol (mg/L)* | 65.9 | 63.3 | 192 (40) |
| Systolic blood pressure (mm Hg) | 139 (20) | 137 (15) | 134 (17) |
| Diastolic blood pressure (mm Hg) | 79 (11) | 82 (8) | 78 (10) |
| Medication use | | | |
| Antiplatelet agents | 76.1 | 92.3 | 90.5 |
| β-blockers | 39.5 | 61.7 | 60.0 |
| Lipid-lowering agents | 28.6 | 57.6 | 70.0 |
| Diuretics | 15.3 | 9.2 | 13.0 |
| Calcium channel blockers | 47.1 | 31.4 | 35.5 |

PCI=percutaneous coronary intervention. CABG=coronary-artery bypass graft. * Percentage of patients with serum cholesterol ≥201 mg/L for HOPE; or >250 mg/L or on a lipid-lowering treatment for EUROPA; for PEACE, serum cholesterol data are mean (± SD). Data are mean (± SD) unless otherwise indicated.

subgroup analyses in EUROPA showed a remarkable consistency in the beneficial treatment effect of ACE inhibition by perindopril, independent of concomitant therapy (ß-blockers, statins and antiplatelet drugs), previous revascularization or the level of baseline risk[7,18]. Notably, the mortality rates in the placebo group were even lower in EUROPA than in the PEACE trial.

Until recently, the PEACE investigators did not present subgroup analyses to support their remarks on the possibilities on the difference in risk or concomitant therapy, which could have explained the neutral results. A recent subgroup analysis by the PEACE investigators did note renal insufficiency defined as an estimated glomerular filtration rate of less than 60 ml/min per 1.73 $m^2$ as a target subgroup of patients with a beneficial effect of trandolapril [19]. The relatively low presence of renal insufficiency in the main study population, in line with the presumed lower risk level, might explain the absence of an overall treatment benefit with trandolapril in PEACE. As the treatment effect of trandolapril in the entire PEACE study was neutral, retrospective analyses to define patient subgroups with positive effects should be regarded cautiously and verified in comparable patients. Therefore, the EUROPA investigators performed a subgroup analysis to examine a possible interaction between renal function and the treatment benefit of perindopril[20]. The authors concluded that the treatment effect of perindopril remained consistent and was not modified by renal insufficiency (with a comparable estimated glomerular filtration rate distribution in both studies). This indicates that the apparent neutral results of PEACE are not explained by the level of risk or the background therapies used, but are possibly related to the reduced power caused by greater crossover and shorter follow-up than in the other studies. PEACE may have been inadequately powered to detect moderate differences in spontaneously occurring clinical outcomes that are unaffected by clinical judgement or variations in practice patterns. In support of this possibility are the results for all-cause mortality, as well as the composite outcome of cardiovascular mortality, non-fatal myocardial infarction and stroke in PEACE, which tended to be favourable [odds ratio (OR) 0.93; 95% confidence interval (CI) 0.81–1.08] with confidence intervals overlapping with those of HOPE and EUROPA. In EUROPA, patients were assigned to receive a relatively high dose of perindopril (8 mg), which was achieved rapidly and in a high proportion of patients, whereas in PEACE, trandolapril was up-titrated to the target dose (4 mg) only 6 months after random selection. At 3 years, the target dose was achieved in 57.8% of patients in PEACE and 93.0% of patients in EUROPA[6,7]. Both agents are in a broadly similar ACE-inhibitor subgroup, share chemical moieties, are lipophilic, and were used in doses that showed important pharmacological effects. Still, without head-to head trials the possibility cannot be excluded that there are pharmacological differences between perindopril and trandolapril that are important for their clinical efficacy to reduce cardiovascular endpoints. The absence of a treatment effect in PEACE may also be a mere chance finding. Among ACE inhibitors, perindopril at the dose of 8 mg and ramipril at the dose of 10 mg have been proved to be effective in the prevention of cardiovascular events in stable CAD patients without overt heart failure, in contrast to other agents studied[5,7].

## COMBINED ANALYSIS OF HOPE, EUROPA, PEACE

An analysis of three trials of ACE-inhibitors in stable vascular disease without left ventricular systolic dysfunction (LVSD) or heart failure, which involved 29.805 patients, demonstrated that, when the findings of HOPE, EUROPA and PEACE were combined, ACE inhibitors significantly reduced all-cause mortality (7.8% versus 8.9%, $P = 0.0004$), cardiovascular mortality (4.3% versus 5.2%, $P = 0.0002$), non-fatal myocardial infarction (5.3% versus 6.4%, $P = 0.0001$), stroke (2.2% versus 2.8%, $P = 0.0004$), heart failure (2.1% versus 2.7%, $P = 0.0007$), coronary artery bypass graft surgery (6.0% versus 6.9%, $P = 0.0036$) but not percutaneous coronary intervention (PCI) (7.4% versus 7.6%, $P = 0.48$). Except for stroke and revascularization, these results were similar to those of the five trials in patients with heart failure or LVSD[21]. For the pooled participants, the composite outcomes of cardiovascular mortality, myocardial infarction and stroke occurred in 10.3% of patients of the ACE inhibitor group and in 12.4% of patients of the placebo group (OR 0.81; 95% CI 0.75–0.87; $P < 0.0001$) as presented in Figure 2 [21]. There was no evidence of heterogeneity across the trials (heterogeneity $P = 0.083$). The 2.1% absolute risk reduction implies that to prevent one cardiovascular death, myocardial infarction or stroke, 48 patients needed to be treated for 4.5 years [21]. The same composite outcome, except that cardiovascular death is replaced by total mortality, in the SAVE, AIRE, TRACE, SOLVD trials, occurred in 29.2% of the patients receiving an ACE inhibitor and in 34.1% of the patients who received placebo (OR 0.79; 95% CI 0.73–0.85; $P < 0.0001$; heterogeneity

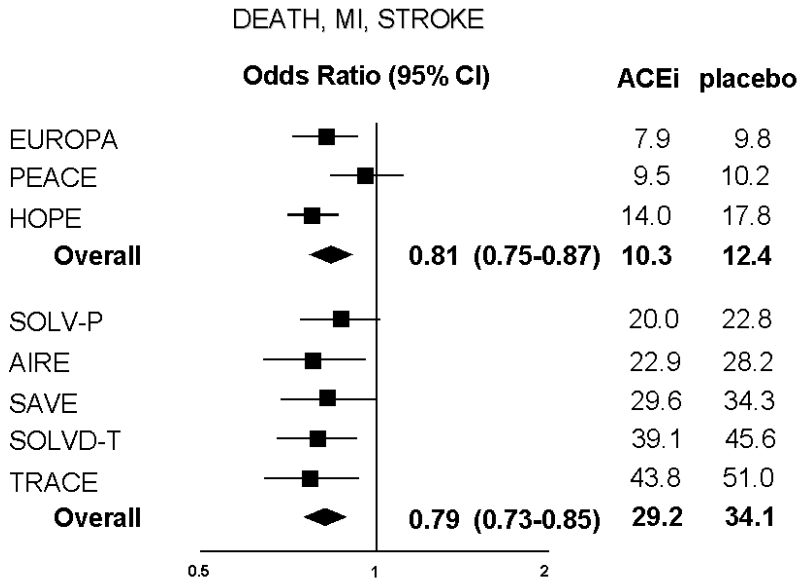



**Figure 2** Combined analysis angiotensin-converting enzyme inhibitor trials. ACE-I = Angiotensin-converting enzyme inhibitor; MI = myocardial infarction. Reproduced from Dagenais et al. Lancet 2006;368:581-8 with permission.

$P = 0.835$). The 4.9% absolute risk reduction implies that to prevent one event, 20 patients needed to be treated for approximately 3 years. There was no apparent heterogeneity in effects between the two categories of trials ($P = 0.455$). Overall, there was a highly significant reduction in the composite of these outcomes: 16.0% versus 18.9% (OR 0.80; 95% CI 0.76–0.84; $P < 0.0001$) [21].

Table 2 shows the percentage reduction in odds for the composite outcome of cardiovascular death, myocardial infarction and stroke in the different trials of ACE inhibitors[21]. The reduction varies between 15% and 30% for the different trials irrespective of their annual rates of events in the placebo groups of each trial except for PEACE, which had a 7% OR reduction. To ascertain whether or not there is a threshold above which ACE inhibitors have no effect, data from the control group on this composite outcome from HOPE and EUROPA were divided in tertiles of low, medium and high-risk according to a risk model for baseline characteristics, and are presented as annual rates: for HOPE, the annual rates in the placebo were 2.2% for low risk, 3.6% for medium risk and 6.0% for high risk, and for EUROPA the annual placebo rates according to the tertiles were 1.4% for low risk, 2.4% for medium risk and 4.0% for high risk. Even for low annual rates, below or similar to the 2.1% rate in PEACE, the percentage reductions in odds were between 18% and 28% [21].

The ACE inhibitors used in these three trials (HOPE ramipril, EUROPA perindopril, PEACE trandolapril) share several pharmacological characteristics and have been shown to reduce cardiovascular events in patients with heart failure and myocardial infarction or stroke. Analysis of the combined results of the HOPE, EUROPA and PEACE studies shows significant reductions in





**Table 2.** Reduction in odds (percentage) of cardiovascular death, non-fatal MI, or stroke for PEACE, HOPE, and EUROPA, and for trials of patients with heart failure or LVSD

| Trials | Number of patients | Annual rates in placebo group | OR (95% CI) | P-value |
|---|---|---|---|---|
| PEACE | 8290 | 2.13 | 7 (–8 - 19) | 0.328 |
| HOPE total | 9297 | 3.95 | 25 (16 - 32) | 0.0001 |
| - HOPE lower-risk | 3083 | 2.17 | 18 (–4 - 35) | |
| - HOPE medium-risk | 3100 | 3.58 | 20 ( 3 - 33) | |
| - HOPE high-risk | 3114 | 5.98 | 24 (12 - 34) | |
| EUROPA total | 12 218 | 2.60 | 19 (8 - 28) | 0.0007 |
| - EUROPA lower-risk | 3976 | 1.40 | 19 (–5 - 38) | |
| - EUROPA medium-risk | 3975 | 2.41 | 28 (11 - 41) | |
| - EUROPA high-risk | 3975 | 4.00 | 10 (–4 - 22) | |
| AIRE | 1986 | 22.6 | 24 (7 - 38) | 0.0068 |
| TRACE | 1749 | 17.0 | 25 (9 - 33) | 0.0028 |
| SOLVD-P | 4228 | 7.4 | 15 (2 - 27) | 0.0252 |
| SOLVD-T | 2569 | 13.1 | 23 (10 - 33) | 0.0009 |
| SAVE | 2231 | 9.8 | 20 (4 - 33) | 0.0168 |

LVSD= left ventricular systolic dysfunction. In trials of patients with heart failure or LVSD, total mortality was used instead of cardiovascular mortality. P for heterogeneity 0.083 for HOPE, EUROPA and PEACE. P-value for heterogeneity 0.835 for the trials of patients with heart failure or LVSD (Dagenais et al. *Lancet* 2006; 368: 581–88)

a broad range of cardiovascular outcomes with no significant differences between the studies. There was, however, little overall effect on reducing PCI, which could be caused by indications for PCI having varying thresholds in different countries. This combined analysis confirms that ACE inhibitors consistently reduce serious vascular events in patients with atherosclerosis either with or without known evidence of heart failure or LVSD. Results showing these benefits in lower and intermediate-risk patients complement existing evidence of similar benefit in higher-risk patients with LVSD or heart failure. At least in these patients with vascular disease, there was no clear indication for a threshold of ACE inhibitor therapy according to baseline risk. Therefore, the use of ACE inhibitors should be considered in all patients with atherosclerosis. The benefits of ACE inhibitors were apparent in patients taking ß-blockers, lipid-lowering agents and antiplatelet therapy individually or together. Furthermore, ACE inhibitors benefited patients who underwent coronary revascularization and were additionally taking all three drugs. This analysis confirms the consistency with which ACE inhibitors reduce the risk of fatal and non-fatal cardiovascular events.

## BLOOD PRESSURE REDUCTION AND IMPROVED OUTCOME WITH ACE-INHIBITORS

The mean blood pressure reductions in the three trials were 3/2 mmHg in HOPE, 5/2 mmHg in EUROPA, and 5/3 mmHg in PEACE. At 3-year follow-up, adherence to the ACE inhibitor was 82.2% in HOPE, 81.0% in EUROPA, and 74.5% in PEACE. The number of patients who were receiving the target dose of ACE inhibitors at 3-years' follow up were 93.0% in EUROPA, 70.9% in HOPE and 68.6% in PEACE [5-7].

With a comparable blood pressure reduction by ramipril and perindopril, the risk reduction in cardiovascular death, myocardial infarction or stroke was similar in HOPE and EUROPA (Table 3). In PEACE, with a similar population of relatively low-risk patients with stable CAD, the same reduction in blood pressure by trandolapril occurred but a weaker risk reduction in cardiovascular death, non-fatal myocardial infarction and stroke. This suggests that, in addition to blood pressure-lowering effects, other factors also contribute to the benefits of ACE inhibition in patients with CAD. This was also suggested by the ASCOT–BPLA investigators, because the event reduction by amlodipine/perindopril was larger than might be expected by the reduction in blood pressure with this treatment regimen in comparison with a ß-blocker/diuretic regimen.

Further support for the benefit of the ACE inhibition by perindopril is provided in studies in patients with cerebrovascular disease and diabetes: The PROGRESS study investigated whether a perindopril 4 mg (with or without indapamide) or placebo-based regimen in 6105 patients with cerebrovascular disease reduced the incidence of recurrent stroke/cerebrovascular accidents[12] After 4 years of treatment, a mean blood pressure reduction of 9/4 mmHg was observed with active treatment compared with placebo. For the total group, a 28% reduction

**Table 3** Summary of effects of ACE-inhibitors on BP and clinical outcomes in clinical trials of CAD patients.

| | Compliance at 3 years | Target dose at 3 years | Mean BP reduction | RRD in CV death, MI, Stroke (95% CI) |
|---|---|---|---|---|
| **HOPE** (ramipril 10 mg) | 82.2 % | 70.9 % | -3/2 mmHg | 0.78 (0.70-0.86) |
| **EUROPA** (perindopril 8 mg) | 81.0 % | 93.0 % | -5/2 mmHg | 0.81 (0.72-0.92) |
| **PEACE** (trandolapril 4 mg) | 74.5 % | 68.6 % | -5/3 mmHg | 0.93 (0.81-1.07) |

Abbreviations: CV = cardiovascular, MI = myocardial infarction, RRD = relative risk reduction, CI = confidence interval. Mean follow-up (yrs) HOPE (4.5), EUROPA (4.2), PEACE (4.8).

in the risk of stroke was observed, which was as expected from the observed blood pressure reduction. The perindopril/indapamide regimen reduced the incidence of major coronary events by 26% and non-fatal myocardial infarction by 38%, which is in line with the results of the ACE inhibitor trials (HOPE, EUROPA) with coronary events as study endpoint. These results were independent of the baseline blood pressure levels at study entry.

The ADVANCE trial studied 11.140 patients with type 2 diabetes who were randomly assigned to a perindopril 4 mg/ indapamide-based regimen or placebo. Blood pressure was reduced by 6/2 mmHg with perindopril/indapamide compared with placebo. Perindopril/indapamide lowered the incidence of major macro- and microvascular events, cardiovascular deaths, total coronary events and renal events [hazard ratio (HR) 0.91; 95% CI 0.83–1.00; $P = 0.04$] during 4.3 years of follow-up. There was no evidence that the effects of the study treatment differed by initial blood pressure or the concomitant use of other treatments at baseline. Interestingly, the reduction in cardiovascular mortality was greater than expected from the blood pressure effect alone (HR 0.82; 95% CI 0.68–0.98), which is comparable with other trials using perindopril.

Figure 3 shows a combined analysis of three perindopril trials (EUROPA, PROGRESS and ADVANCE). When these findings were combined, perindopril significantly reduced all-cause mortality (OR 0.89; 95% CI 0.82–0.97), cardiovascular mortality (OR 0.85; 95% CI 0.75–0.96) and cardiovascular mortality, myocardial infarction (OR 0.82; 95% CI 0.74–0.90). This combined analysis shows that perindopril reduced cardiovascular events by approximately 20% irrespective of the type of patients and level of risk, which is in line with previous meta-analyses and risk models [18,21]. This consistency of the treatment benefit by perindopril in patients with stable CAD, type 2 diabetes mellitus, or a history of stroke is important. Interestingly, in patients with a history of stroke (PROGRESS), the addition of indapamide resulted in a higher blood pressure reduction (12/5 mmHg) and particularly attenuated the reduction in risk of recurrent stroke. With regard to patients with cerebrovascular disease, the effect of blood pressure reduction seems to be more directly related to the reduction in the risk of recurrent stroke than to cardiovascular risk reduction.

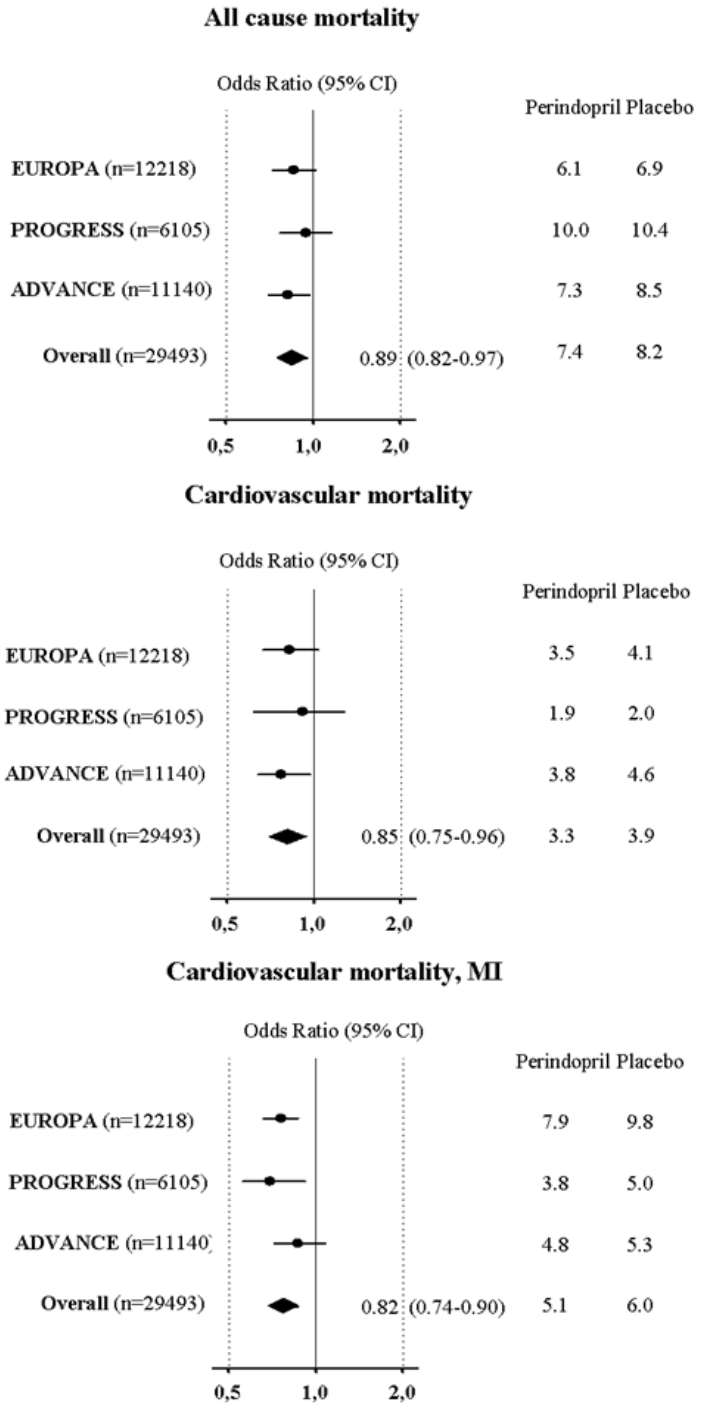



**Figure 3.** Meta-analysis of combined data from EUROPA, ADVANCE and PROGRESS.

## ADDITIONAL EFFECTS BEYOND BLOOD PRESSURE REDUCTION

Among ACE inhibitors, perindopril has been extensively studied with regard to the blood pressure-independent effects: The PERFECT study concluded that the beneficial effects of perindopril on cardiovascular morbidity and mortality in the EUROPA study might be at least partly explained by an improvement in endothelial function [15]. The PERSPECTIVE study evaluated the effect of perindopril on coronary plaque progression as assessed by quantitative coronary angiography and intravascular ultrasound. The initial analysis revealed no progression of CAD by quantitative angiography and intravascular ultrasound with long term administration of either perindopril or placebo (median follow-up 3 years), possibly because most patients were on concomitant treatment with a statin [16]. A further analysis showed an association of long-term administration with perindopril and constrictive remodeling patterns without affecting the lumen, suggesting that this treatment is associated with plaque stabilization[22]. The PERTINENT study examined the effects of perindopril on endothelial function and concluded that abnormal endothelial function occurs in patients with CAD (up-regulated ACE) and this can be reversed by perindopril[17]. The PREAMI study investigated 1252 elderly post-myocardial infarction patients with preserved left ventricular function randomly assigned to receive perindopril 8 mg or placebo. Perindopril significantly reduced the combined primary endpoint (death, hospitalization for heart failure and remodelling) and reduced the progressive left ventricular deterioration and remodelling occurring in the presence of small infarct size[13].



## LESSONS LEARNED FROM ACE-INHIBITORS TRIALSIN STABLE CORONARY ARTERY DISEASE

In the clinical trials in patients with CAD or vascular disease, as discussed in this chapter, the use of an ACE inhibitor showed a consistent reduction in cardiovascular endpoints (except for the PEACE trial). This reduction in the risk of cardiovascular disease seems larger than can be expected from the modest reduction in blood pressure reported in these trials (approximately 6/2 mmHg). The consistency across different risk populations (CAD, diabetes, stroke) by perindopril and the additional effects shown in several substudies make it likely that perindopril also has important blood pressure-independent effects related to the reduction in cardiovascular risk across the continuum of (cardio-)vascular disease (Figure 4).

The benefit of perindopril was most evident in stable CAD patients with a risk reduction of 20% for cardiovascular mortality, myocardial infarction and cardiac arrest to 22% for nonfatal myocardial infarction[7]. This may be related to the dose of the ACE inhibitor because in EUROPA perindopril was used at a dose of 8 mg. The dose of an ACE inhibitor may be important, if the assumed additional effects are involved in their protective effects. The additional effects are probably more likely to occur at higher dosages of ACE inhibition.

The consistency of the relative effects across subgroups indicates that the absolute benefits conferred by treatment will be established mainly by each patient's future risk of vascular complication, rather than their initial level of blood pressure alone. These results support the provision of treatment, not on the basis of arbitrary cut-off points for blood pressure (ADVANCE and EUROPA showed a treatment benefit in normotensive patients), but rather on assessment of vascular risk, which is raised in patients with stable CAD, diabetes and stroke as shown in EUROPA, ADVANCE and PROGRESS.

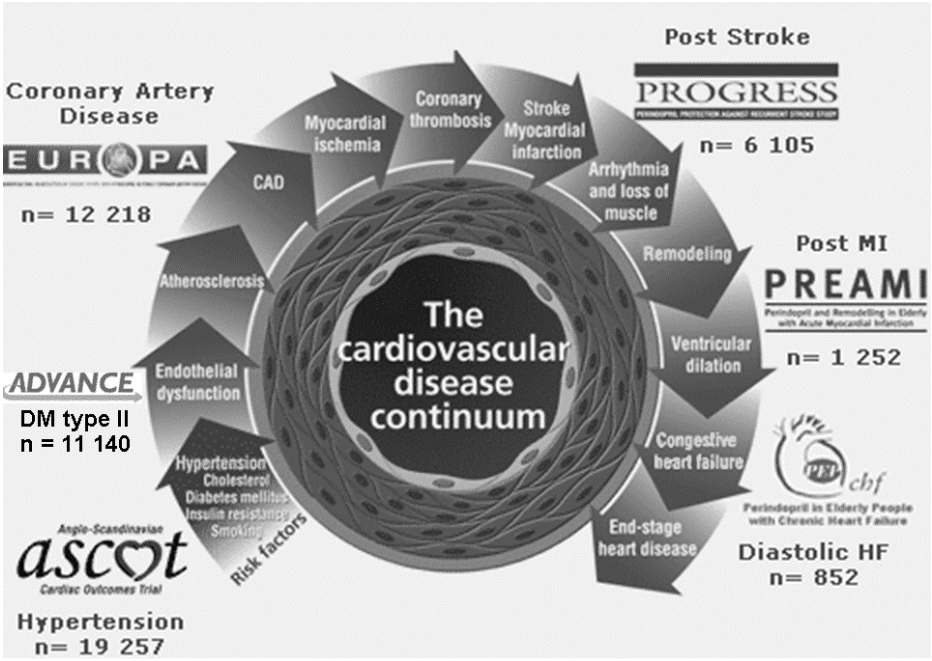
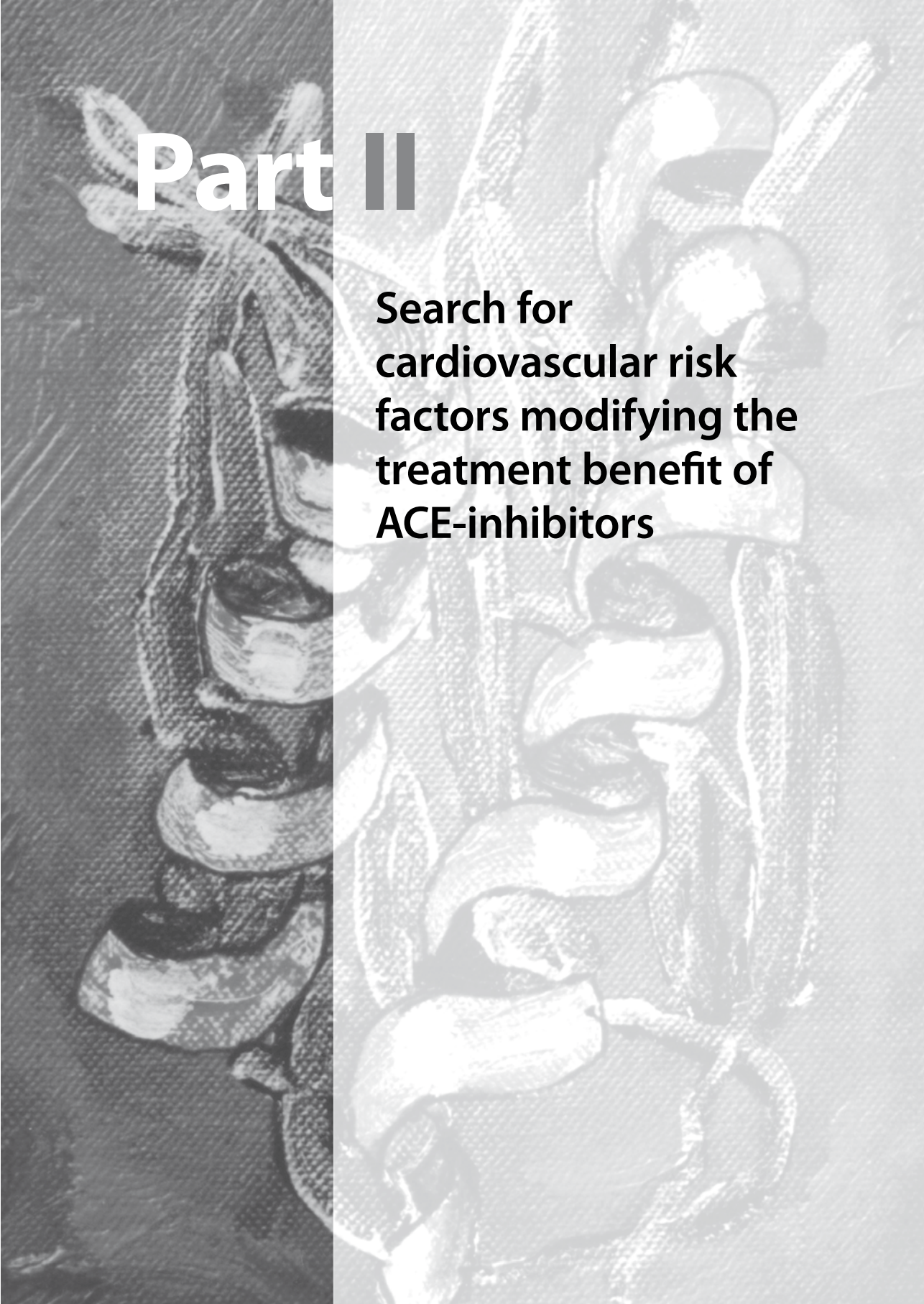



**Figure 4** Treatment effect of perindopril in the continuum of cardiovascular disease.

## REFERENCES


1. Pfeffer MA, Braunwald E, Moye LA, et al., for the SAVE Investigators. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction and myocardial infarction: results of the survival and ventricular enlargement trial. *N Engl J Med* 1992; 327:669–77.
2. The SOLVD investigators. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. *N Engl J Med* 1991; 325:293–302.
3. The Acute Infarction Ramipril Effi cacy (AIRE) study investigators. Effect of ramipril on mortality and morbidity of survivors of acute myocardial infarction with clinical evidence of heart failure. *Lancet* 1993; 342:821–8.
4. Torp-Pedersen C, Kober L, for the TRACE Study Group. Effect of ACE inhibitor trandolapril on life expectancy of patients with reduced left-ventricular function after acute myocardial infarction. *Lancet* 1999; 354:9–12.
5. The Heart Outcomes Prevention Evaluation study investigators. Effects of an angiotensin-converting enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. *N Engl J Med* 2000; 342:145–53.
6. The PEACE Trial Investigators. Angiotensin-converting-enzyme inhibition in stable coronary artery disease. *N Engl J Med* 2004; 351:2058–68.
7. The European Trial on Reduction of Cardiac Events with Perindopril in Patients with Stable Coronary Artery Disease investigators. Efficacy of perindopril in reduction of cardiovascular events in stable coronary artery disease: randomised, double-blind, placebo controlled, multicentre trial (the EUROPA study). *Lancet* 2003; 362:782–8.
8. The Task Force on the Management of Stable Angina Pectoris of the European Society of Cardiology. Guidelines on the management of stable angina pectoris. *Eur Heart J* 2006; 27:1341–81.
9. Wood D, DeBacker G, Faergam O, Graham I, Mancia G, Pyörälä K, and the task force. Prevention of coronary disease in clinical practice: recommendation of the second Joint Task Force of European and other societies on Coronary Prevention. *Atherosclerosis* 1998; 140:99–270.
10. Gibbons RJ, Abrams J, Chatterjee K, et al. ACC/AHA 2002 guideline update for the management of patients with chronic stable angina – summary article: a report of the American College of Cardiology/American Heart Association Task Force on practice guidelines (Committee on the Management of Patients With Chronic Stable Angina). *J Am Coll Cardiol* 2003; 41:159–68.
11. Dahlöf B, Sever PS, Östergren J. Prevention of cardiovascular events with an antihypertensive regimen of amlodipine adding perindopril as required versus atenolol adding bendroflumethiazide as required, in the Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm (ASCOT–BPLA): a multicentre randomized controlled trial. *Lancet* 2005; 366:895–906.
12. PROGRESS Collaborative Group. Randomised trial of a perindopril based blood pressure-lowering regimen among 6105 individuals with previous stroke or transient ischemic attack. *Lancet* 2001; 358:1033–41.
13. The PREAMI Investigators. Effects of Angiotensin-Converting Enzyme Inhibition with Perindopril on Left Ventricular Remodeling and Clinical Outcome. Results of the Randomized Perindopril and Remodeling in Elderly With Acute Myocardial Infarction (PREAMI) Study. *Arch Intern Med* 2006; 166:659–66.
14. ADVANCE Collaborative Group. Effects of a fixed combination of perindopril and indapamide on macrovascular and microvascular outcomes in patients with type 2 diabetes mellitus (the ADVANCE trial): a randomised controlled trial. *Lancet* 2007; 370:829–40.
15. Bots ML, Remme WJ, Lüscher TF, et al. ACE inhibition and endothelialfunction: main findings of PERFECT, a sub-study of the EUROPA trial. *Cardiovasc Drugs Ther* 2007; 21:269–79.
16. Rodriguez-Granillo GA, Vos J, De Feyter PJ. Long-term effect ofperindopril on coronary atherosclerosis progression (from the PERindopril's Prospective Effect on Coronary aTherosclerosis by Angiography and IntraVascular Ultrasound Evaluation [PERSPECTIVE] Study). *Am J Cardiol* 2007; 100:159–63.

17. Ceconi C, Fox KM, Ferrari R, et al. ACE inhibition with perindopril and endothelial function. Results of a substudy of the EUROPA study: PERTINENT. *Cardiovasc Res* 2007; 73:237–46.
18. Deckers JW, Goedhart DM, Boersma E, et al. Treatment benefit by perindopril in patients with stable coronary artery disease at different levels of risk. *Eur Heart J* 2006; 27:796–801.
19. Solomon SD, Rice MM, Braunwald E, et al. Renal function and the effectiveness of angiotensin-converting enzyme inhibitor therapy in patients with chronic stable coronary disease in the Prevention of Events with ACE Inhibition (PEACE) trial. *Circulation* 2006; 114:26–31.
20. Brugts JJ, Boersma E, Chonchol M, et al. The cardioprotective effects of the angiotensin-converting enzyme inhibitor perindopril in patients with stable coronary artery disease are not modified by mild to moderate renal insufficiency: insights from the EUROPA trial. *J Am Coll Cardiol* 2007; 50:2148–55.
21. Dagenais GR, Pogue J, Fox K, Simoons ML, Yusuf S. Angiotensin converting-enzyme inhibitors in stable vascular disease without left ventricular systolic dysfunction or heart failure: a combined analysis of three trials. *Lancet* 2006; 368:581–8.
22. Rodriguez-Granillo GA, de Winter S, Bruining N, et al. Effect of perindopril on coronary remodelling: insights from a multicentre, randomized study. *Eur Heart J* 2007; 28:2326–31.



# Part II

## Search for cardiovascular risk factors modifying the treatment benefit of ACE-inhibitors

# Chapter 4

## Identification of renal insufficiency as important risk factor for cardiovascular disease in relatively healthy subjects

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## ABSTRACT

**Background:** Renal insufficiency is a risk factor for cardiovascular disease in patients with renal disease or coronary heart disease; however, it is unknown whether renal function is an independent predictor of cardiovascular disease in the general population.

**Methods:** We investigated whether the level of renal function, estimated by glomerular filtration rate, was associated with the risk of incident myocardial infarction among 4484 apparently healthy subjects in the Rotterdam Study (mean age, 69.6 years). We estimated the glomerular filtration rate by Cockcroft-Gault and abbreviated modification of diet in renal disease equations and used Cox regression analysis to estimate hazard ratios adjusted for cardiovascular risk factors, atherosclerosis, and medication use.

**Results:** During the follow-up period (mean, 8.6 years), 218 subjects (4.9%) had a myocardial infarction. A 10 mL/min per 1.73m$^2$ decrease in glomerular filtration rate was associated with a 32% increased risk of myocardial infarction ($P$<0.001). Compared with subjects in the fourth quartile, the multivariate-adjusted hazard ratios for the risk of myocardial infarction increased from 1.64 (95% confidence interval [CI], 1.03-2.59) in the third quartile to 1.94 (95% CI, 1.21-3.10) in the second quartile and 3.06 (95% CI, 1.80-5.19) in the quartile with the lowest glomerular filtration rate estimated by the Cockcroft-Gault equation. Using the abbreviated modification of diet in renal disease equation, the risk estimates for the third to first quartiles were 1.34 (95% CI, 0.89-2.01), 1.66 (95% CI, 1.14-2.49), and 1.90 (95% CI, 1.25-2.90), respectively.

**Conclusions:** The present study shows that renal function is a graded and independent predictor of the development of myocardial infarction in an elderly population. Early detection of decreased renal function may identify subjects who are at heightened risk of coronary heart disease.

## INTRODUCTION

Recent studies have shown that renal failure is an independent risk factor for cardiovascular mortality and morbidity in high-risk populations such as patients with chronic kidney or cardiovascular disease and patients with cardiovascular risk factors [1-7]. It remains uncertain whether the level of renal function within asymptomatic ranges predicts the risk of cardiovascular disease in the general population. Until now, population-based studies [8-12] on renal insufficiency and cardiovascular disease gave conflicting results. Most prospective studies have been conducted with middle-aged populations. In some of these studies [8,9], renal insufficiency has been associated with increased risk of cardiovascular disease. In others [10,11], renal insufficiency was not an independent predictor after adjustment for cardiovascular risk factors. The Cardiovascular Health Study (CHS), a prospective population-based study of subjects 65 years or older, is the only study that has investigated the association between renal insufficiency and cardiovascular disease in elderly individuals [12,13]. In this study, an increased risk of cardiovascular disease was found in subjects with elevated serum cystatin C, whereas a much weaker association was found between serum creatinine level and risk of cardiovascular disease. No significant association between glomerular filtration rate (GFR) and risk of myocardial infarction was found [12,13]. The elderly population is growing and, because renal function decreases with age, renal insufficiency will become an increasingly important problem. Therefore, we examined the association between renal function, estimated by glomerular filtration rate (eGFR), and risk of myocardial infarction in the Rotterdam Study, a large population-based study in men and women 55 years and older.

## METHODS

### Study population

The Rotterdam Study is an on-going prospective population based cohort study aimed at assessing the incidence and determinants of chronic diseases in the elderly population[14]. In short, all inhabitants 55 years or older of Ommoord, a suburb of Rotterdam, the Netherlands, were invited to participate in the study. A total of 7983 men and women (response rate, 78%) agreed to participate, and 6950 participants visited the research center for the required physical examination. The main reason for not visiting the research center was that an individual lived in a nursing home. Baseline data were collected from March 1990 to July 1993. The medical ethics committee of the Erasmus Medical Center, Rotterdam, approved the study, and written informed consent was obtained from all participants.

### *Baseline examination*

At baseline, a trained research assistant obtained information on smoking habits and medication use from each subject during a home interview. Individuals were classified as never having smoked, a past smoker, or a current smoker. Clinical measurements were obtained during the visit to the research center. Height and weight were measured, and body mass index was calculated as weight in kilograms divided by the square of height in meters[2] Blood pressure was calculated as the average of 2 consecutive measurements at the right brachial artery with a random zero sphygmomanometer. Serum total cholesterol was measured by an automated enzymatic procedure in a non fasting blood sample. Serum high-density lipoprotein cholesterol was measured similarly after precipitation of the non-high-density lipoprotein fraction. Diabetes mellitus was defined as the use of antidiabetic medication and/or a random or postload serum glucose level above 198.2 mg/dL (11.0 mmol/L). C-reactive protein levels were measured by enzyme-linked high-sensitivity immunoassays. Carotid intima media thickness, as a measurement of generalized atherosclerosis, was assessed by ultrasonography. We computed values of intima media thickness by averaging the anterior and posterior walls of the left and right common carotid arteries (distal)[15]. Prevalent myocardial infarction at baseline was considered present in the case of a self-report, verified by a general practitioner or hospital discharge data, or confirmed by electrocardiogram measurements [16].



### *Assessment of renal function*

Serum creatinine was assessed by a nonkinetic alkaline picrate (Jaffe) method [17]. Creatinine clearance was computed with the Cockcroft-Gault equation and standardized for body surface area using the Dubois formula [18,19]. Creatinine clearance generally exceeds GFR by 10% to 15% due to urinary creatinine derived from tubular secretion [20]. The eGFR was therefore calculated using a correction factor of 0.90. In an additional analysis, we used the abbreviated Modification of Diet in Renal Disease (MDRD) equation [21].

### *Assessment of incident myocardial infarction*

After all baseline examinations were performed, general practitioners in the research area (Ommoord) reported incident cardiovascular events to the study center. An incident myocardial infarction was considered to have occurred when the event led to hospitalization and the hospital discharge records indicated a diagnosis of myocardial infarction based on symptoms, electrocardiographic recordings, and repeated laboratory investigations during the patient's hospital stay. Research assistants collected all information by checking medical records at the general practitioners' offices, including discharge reports from medical specialists. Subsequently, two research physicians independently coded all reported events according to the *International Classification of Diseases, 10th Revision*. If research physicians disagreed on diagnoses, these were discussed to reach consensus. Finally, an expert in the field of cardiology reviewed all events. In case of disagreement between the medical expert and the research

physicians, the expert's judgment was considered final. Information on the vital status of the participants was obtained at regular intervals from the municipal authorities in Rotterdam.

## Population for analysis

Of the 6950 subjects who visited the research center, 808 (12%) were excluded because of prevalent myocardial infarction. Blood samples for measurement of serum creatinine level were available for 4568 (74.4%) of the remaining 6142 subjects. Data were available on eGFRs for 4484 (73.0%) of 6142 subjects. Baseline data on cardiovascular risk factors were missing for 212 subjects (<5%). For missing data on cardiovascular risk factors, the mean of the study population was imputed. Of the study population, 8 individuals (0.1%) were lost to follow-up. For these subjects, the follow-up time was computed until the last date of contact. Follow-up data on incident myocardial infarction were completed until January 1, 2002.

## Statistical analysis

Cox proportional hazard regression analysis was used to estimate hazard ratios for incident myocardial infarction with corresponding 95% confidence intervals (CIs). Analyses were conducted with eGFRs divided into sex-specific quartiles. The quartile with the highest eGFR was used as the reference quartile. Analyses were conducted for crude values (model 1), adjusted for age, sex, body mass index, systolic and diastolic blood pressure, smoking habits, total and high-density lipoprotein cholesterol, and diabetes mellitus (model 2) and further adjusted for use of cardiac medication (angiotensin-converting enzyme inhibitors, diuretics, and beta-blocking agents) and non steroidal anti-inflammatory drugs, C-reactive protein, and carotid intima media thickness (full model). The association between the eGFR and the risk of myocardial infarction was also assessed with the eGFR as a continuous variable. Tests for trend were performed, using the quartiles of the eGFR as a categorical measurement. In an additional analysis, the eGFR was estimated using the abbreviated MDRD equation rather than the Cockcroft-Gault equation. Kaplan-Meier survival plots of quartiles of the eGFR in relation to the risk of myocardial infarction were examined with log-rank test. The attributable risk and population-attributable risk percentage of renal insufficiency associated with myocardial infarction were calculated using an eGFR below 60 mL/min per 1.73 $m^2$ as the cut-off point[22]. For purposes of comparison, we calculated the attributable risk and population-attributable risk of the 4 major, classical cardiovascular risk factors. All measurements of association are presented with 95% CIs. A *P* value of less than 0.05 was considered statistically significant. All analyses were performed using SPSS statistical software (version 12.0; SPSS Inc, Chicago, Ill) for Windows.

## RESULTS

Baseline characteristics of the study population are shown in Table 1. The mean ± SD age was 69.6 ± 8.8 years, and 63.7% of the population were women. The eGFRs (mean ± SD, 61.9 ± 14.7) ranged from 6.1 to 142.6 mL /min per 1.73 $m^2$. A mild degree of renal insufficiency, with an eGFR between 60 and 89, was present in 2317 subjects (51.7%). The mean/SD follow-up time was 8.6 ± 2.8 years. During follow-up, 38479 person-years were collected. Incident myocardial infarction occurred in 218 subjects (4.9%; 125 men and 93 women). The risk of myocardial infarction is significantly increased in the lower 3 quartiles compared with the highest quartile of eGFR (*P* value for trend, 0.001) (Table 2). Risk estimates using quartiles of eGFRs based on the abbreviated MDRD equation were somewhat smaller, but a similar trend was seen (Table 3). In the following analyses, the eGFR is based on the Cockcroft-Gault equation. The survival curves for quartiles of eGFRs are shown in Figure 1. The log-rank test gave *P* values of 0.056, 0.002, and less than 0.001, respectively, corresponding to descending quartiles of eGFRs. The attributable and population-attributable risks of renal insufficiency associated with myocardial infarction were 32.8% and 14.7%, respectively (Table 4). If renal insufficiency is assumed to be causally related to myocardial infarction, our findings suggest that it contributed to 32.8% of the cases affected by renal insufficiency and that it was involved in the pathogenesis of 14.7% of all myocardial infarctions in the study sample.



**Table 1.** Baseline Characteristics of the Study Population

| Characteristic | All subjects (n=4484) | Males (n=1628) | Females (n=2856) |
|---|---|---|---|
| Age, yrs. | 69.6 ± 8.8 | 68.8 ± 8.1 | 70.1 ± 9.1 |
| Body mass index, kg/$m^2$ | 26,2 ± 3.7 | 25.6 ± 3.0 | 26.6 ± 4.1 |
| Systolic blood pressure, mmHg | 139.6 ± 22.3 | 139.4 ± 21.9 | 139.7 ± 22.5 |
| Diastolic blood pressure, mmHg | 73.8 ± 11.7 | 74.9 ± 12.1 | 73.2 ± 11.4 |
| Current smokers, % | 23.3 | 31.0 (505) | 18.9 (539) |
| Former smoker, % | 38.8 | 59.2 (963) | 27.1 (775) |
| Total cholesterol, mmol/l | 6.6 ± 1.2 | 6.3 ± 1.2 | 6.8 ± 1.2 |
| HDL-cholesterol, mmol/l | 1.3 ± 0.4 | 1.2 ± 0.3 | 1.4 ± 0.4 |
| Diabetes mellitus, % | 10.0 | 9.3 (152) | 10.3 (295) |
| C-reactive protein, mg/l | 3.1 ± 5.7 | 3.4 ± 5.5 | 2.9 ± 5.8 |
| Cardiac Medication, % | 29.8 | 23.8 (387) | 33.2 (948) |
| - ACE-inhibitors, % | 4.5 | 5.4 (88) | 4.0 (114) |
| - Diuretics use, % | 14.7 | 8.4 (137) | 18.3 (524) |
| - β-blockers use, % | 13.3 | 12.0 (196) | 14.0 (401) |
| NSAID use, % | 8.2 | 5.6 (91) | 9.6 (275) |
| Body surface area ($m^2$)* | 1.8 ± 0.2 | 1.9 ± 0.1 | 1.7 ± 0.1 |
| Carotid intima media thickness, mm | 0.8 ± 0.1 | 0.8 ± 0.1 | 0.8 ± 0.1 |

Data are percentages for dichotomous variables and mean ± SD for continuous variables.
* Body surface area is computed by (0.007184 * $weight^{0.425}$ * $height^{0.725}$).

**Table 2.** Hazard Ratios for Incident Myocardial Infarction associated with GFR-levels (based on adjusted Cockcroft and Gault equation)

| | | | **Unadjusted** | **Traditional** | |
|---|---|---|---|---|---|
| | | | **Hazard Ratios** | **CVD-risk factors†** | **Full Model‡** |
| | GFR* | Total/events | HR 95% CI | HR 95% CI | HR 95% CI |
| Males | | | | | |
| Reference | 81.4 | 407/19 | 1.00 | 1.00 | 1.00 |
| Third quartile | 68.2 | 407/29 | 1.56 (0.88-2.78) | 1.60 (0.87-2.89) | 1.60 (0.89-2.89) |
| Second quartile | 59.4 | 407/35 | 1.97 (1.13-3.45) | 2.10 (1.14-3.85) | 2.06 (1.12-3.79) |
| First quartile | 45.9 | 407/42 | 3.04 (1.77-5.24) | 3.17 (1.58-6.34) | 2.96 (1.48-5.95) |
| Females | | | | | |
| Reference | 79.8 | 714/13 | 1.00 | 1.00 | 1.00 |
| Third quartile | 65.2 | 714/19 | 1.51 (0.74-3.05) | 1.71 (0.82-3.54) | 1.64 (0.78-3.42) |
| Second quartile | 55.9 | 714/23 | 1.88 (0.95-3.71) | 1.87 (0.88-3.96) | 1.74 (0.81-3.70) |
| First quartile | 42.2 | 714/38 | 4.02 (2.14-7.56) | 3.43 (1.50-7.87) | 3.34 (1.45-7.66) |
| Total | | | | | |
| Reference | 80.4 | 1121/32 | 1.00 | 1.00 | 1.00 |
| Third quartile | 66.4 | 1121/48 | 1.54 (0.98-2.41) | 1.64 (1.04-2.60) | 1.64 (1.03-2.59) |
| Second quartile | 57.2 | 1121/58 | 1.92 (1.25-2.96) | 1.98 (1.24-3.17) | 1.94 (1.21-3.10) |
| First quartile | 43.0 | 1121/80 | 3.42 (2.27-5.16) | 3.22 (1.90-5.47) | 3.06 (1.80-5.19) |

Upper (fourth) quartile was used as reference category. * GFR: mean glomerular filtration rate (ml/min/1.73 $m^2$). CVD: cardiovascular disease. † adjusted for age, gender, body mass index, systolic and diastolic blood pressure, smoking-habits, total and HDL-cholesterol, and diabetes mellitus. ‡ further adjusted for, cardiac medication (ACE-inhibitors, diuretics and beta-blocking agents), NSAID-use, C-reactive protein (hs-CRP) and carotid IMT. GFR as continuous variable (for every 10 ml/min per 1.73 $m^2$: HR 1.32 (1.18-1.47), HR 1.30 (1.11-1.50) in males, HR 1.37 (1.16-1.59) in females

**Table 3.** Hazard Ratios for Incident MI associated with GFR-levels (based on MDRD equation)

| | Mean eGFR | Total/events | Unadjusted Hazard Ratios | Adjusted for CVD risk factors† | Full Model‡ |
|---|---|---|---|---|---|
| | | | HR 95% CI | HR 95% CI | HR 95% CI |
| Reference | 94.2 | 1121/44 | 1.00 | 1.00 | 1.00 |
| Third quartile | 78.4 | 1121/50 | 1.12 (0.74-1.67) | 1.35 (0.90-2.03) | 1.34 (0.89-2.01) |
| Second quartile | 69.5 | 1121/58 | 1.34 (0.90-1.98) | 1.70 (1.14-2.55) | 1.66 (1.14-2.49) |
| Lowest quartile | 55.9 | 1121/66 | 1.68 (1.15-2.46) | 1.99 (1.31-3.03) | 1.90 (1.25-2.90) |

Upper (fourth) quartile was used as reference category .Abbreviations: BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; MDRD, modification of diet in renal disease. † adjusted for age, gender, body mass index, systolic and diastolic blood pressure, smoking-habits, total and HDL-cholesterol, and diabetes mellitus. ‡ further adjusted for cardiac medication (ACE-inhibitors, diuretics and beta-blocking agents), NSAID-use, hs-CRP and carotid IMT.

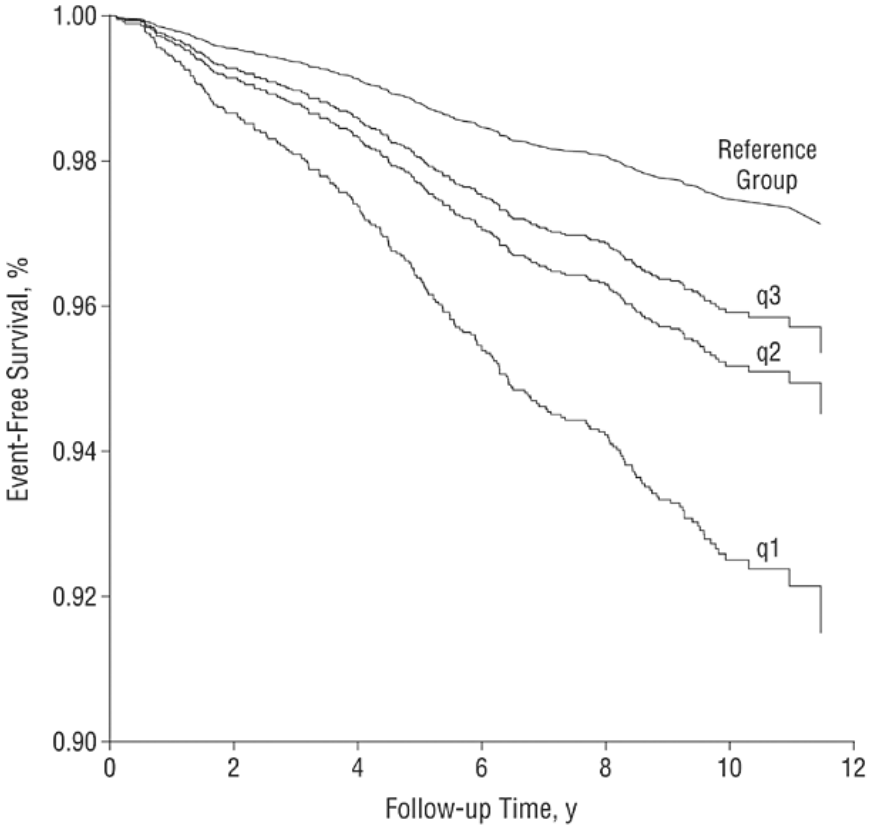


**Figure 1** Event-free survival curves for quartiles of estimated glomerular filtration rate (eGFR) (Cockcroft-Gault equation). Kaplan-Meier survival curves were used to calculate the event-free survival curves in the descending quartiles of eGFR. The lines correspond to the survival curves of the fourth quartile as reference group, third quartile (q3), second quartile (q2), and the lowest quartile of the eGFR (q1), respectively.



**Table 4.** Attributable risk and population attributable risk percentages associated with incident MI.

| Risk Factor | Prevalence | Age-Adjusted Hazard Ratio [1] | Attributable Risk | Population Attributable Risk |
|---|---|---|---|---|
| | % | | | % |
| Diabetes Mellitus | 10.0 | 1.6 | 38.1 | 3.8 |
| Hypertension | 25.6 | 1.5 | 34.5 | 8.8 |
| Smoking | 23.3†/38.8‡ | 1.9/1.6 | 48.2/38.8 | 11.2/15.0 |
| Hypercholesterolemia | 60.9 | 1.4 | 26.8 | 16.3 |
| Renal Insufficiency[2] | 44.9 | 1.5 | 32.8 | 14.7 |

[1] Determined by Cox proportional hazards regression analysis. [2] Renal insufficiency with eGFR below 60 ml/min per 1.73 $m^2$ as cut-off point. † Current compared to never smokers. ‡ Past compared to never smokers.

## DISCUSSION

In this population-based cohort study of adults 55 years or older, we found that impaired renal function was common and associated with an increased risk of myocardial infarction. Even the earlier stages of renal function loss, before there are any symptoms of renal disease, are associated with an increased risk of myocardial infarction. This association is independent of cardiovascular risk factors and atherosclerosis. The population attributable risk is substantial and within range of traditional cardiovascular risk factors.

A previous study [7] demonstrated the association between renal insufficiency and adverse outcomes in patients after a myocardial infarction. Relatively few studies [8-12] have evaluated the relationship between renal function and risk of incident coronary heart disease in the general population. The results of these studies were inconsistent. Most studies [8-11] reported on the association between renal function and cardiovascular disease in middle-aged subjects. The Framingham Heart Study [10] and the National Health and Nutrition Examination Survey [11] (NHANES I) found no association between elevated serum creatinine level and cardiovascular events after adjustment for cardiovascular risk factors. The Atherosclerotic Risk in Communities study [9] in subjects aged 45 to 64 years (mean age, 54.2 years) showed an association between renal insufficiency and atherosclerotic cardiovascular disease as a composite outcome. Although the investigators in that study found an increased risk of 1.38 (95% CI, 1.02-1.87) for subjects with an eGFR below 60 mL/min per 1.73 $m^2$ and a risk of 1.16 (95% CI, 1.00-1.34) for subjects with an eGFR above 60 mL/min per 1.73 $m^2$ compared with subjects with an eGFR above 90 mL/min per 1.73 $m^2$, these risks are relatively small compared with those found in the present study and were not adjusted for C-reactive protein and a measurement of atherosclerosis. No separate analysis of myocardial infarction was performed.

A recent study [8] in a middle-aged population of 1.120.295 insured adults 20 years or older (mean age, 52 years) reported an increased risk of cardiovascular events (heart failure, stroke, coronary heart disease, and peripheral artery disease) in subjects with chronic kidney disease, defined as an eGFR below 60 mL/min per 1.73 $m^2$. This risk8 increased substantially at an eGFR below 45 mL/min per 1.73 $m^2$. Although we support their results, our study differs in several important aspects. First, we found an increased risk at higher levels of renal function, namely, in asymptomatic subjects with an eGFR well above the level of patients with chronic kidney disease. Second, the authors of the California study9 were not able to adjust for smoking, cholesterol and C-reactive protein levels, blood pressure, use of cardiac medication, and measurements of atherosclerosis. In addition, we excluded subjects with a history of myocardial infarction at baseline. Our study is the first to show a graded and independent association between renal function and the risk of myocardial infarction in an elderly population. The only previous study that evaluated the association between renal function and cardiovascular disease in an elderly population was the CHS. The CHS (mean age of subjects, 72.9 years) found an association between elevated serum creatinine level and total cardiovascular disease as a combined

outcome. In that study[12], a 1.5 times increased risk was found for subjects with elevated serum creatinine level, defined as 1.5 mg/dL (132.6 µmol/L) or higher in men or 1.3 mg/dL (114.9 µmol/L) or higher in women, compared with subjects with creatinine levels in the reference range. In contrast with our study, however, no association of elevated serum creatinine level with incident myocardial infarction was found after adjusting for cardiovascular risk factors.[12] A recent report of the CHS concluded that serum cystatin C level is a stronger predictor of the risk of cardiovascular outcomes than serum creatinine level and eGFR[13]. The study showed a relationship between serum cystatin C level and the risk of death from cardiovascular disease; the association of cystatin C level with incident myocardial infarction was less strong than was the case for association with mortality outcomes. In multivariate analysis, only a subgroup of the highest quintile (quintile subgroup 5c) was at significantly increased risk of myocardial infarction. Creatinine level and eGFR estimated by MDRD equation had no significant association with myocardial infarction in either unadjusted or adjusted analyses. Risk estimates were not adjusted for measurements of atherosclerosis, low-density lipoprotein cholesterol, or history of smoking [13]. We have no explanation for the discrepancy with our findings. However, there are important differences between the two studies in population (the CHS population is somewhat older), ascertainment methods, and analysis.

The reason why mild renal insufficiency is associated with an increased risk of cardiovascular disease in our study is not clear. It has been suggested the increased risk can be explained by co-occurrence of a high prevalence of cardiovascular risk factors at baseline.[5,10] In that case, renal insufficiency would be a marker for cardiovascular risk factors and their severity rather than an independent risk factor. However, we still observed an independent association after adjusting for cardiovascular risk factors. Another possible explanation is the presence of atherosclerosis, commonly thought to pre-exist in individuals with mild renal insufficiency. However, after adjustment for carotid intima media thickness, as a measurement of generalized atherosclerosis, an increased risk remained associated with lower levels of renal function. Therefore, pre-existing atherosclerotic vascular disease cannot fully explain the relationship, although the possibility exists that it is mediated by small vessel disease rather than large vessel disease. Finally, renal insufficiency itself might initiate and accelerate cardiovascular disease.

The US National Kidney Foundation has provided guidelines for the assessment of renal function. Definitions of stages 1 to 5 of renal function correspond to eGFR levels of 90 or higher (reference range), 60 to 89, 30 to 59, 15 to 29, and less than 15 mL/min per 1.73 $m^2$. Stage 3 is the first stage at which a patient shows symptoms of renal insufficiency, and it is considered the cut-off point for chronic kidney disease[2]. The results of our study show an increased risk of myocardial infarction starting at earlier stages of renal function loss than commonly thought, namely, well above the levels of chronic kidney disease. Using the Cockcroft-Gault equation, the third quartile with a mean eGFR of 66.3 mL / min per 1.73 $m^2$ was associated with a 64% increased risk of myocardial infarction. An additional analysis with the MDRD equation in the second quartile with a mean eGFR of 69.5 mL/min per 1.73 $m^2$. Both equations show a

significantly increased risk at these mildly decreased eGFR levels, with the difference in size of risk estimates related to the difference in mean eGFR levels of the quartiles. These mildly decreased eGFR levels, corresponding to stage 2, represent subjects at an early stage of renal insufficiency, without symptoms or signs of renal function loss. With descending quartiles of eGFR, the risk of myocardial infarction increased gradually to a 2-fold (MDRD equation; mean eGFR, 54 mL/min per 1.73 $m^2$) to a 3-fold (Cockcroft-Gault equation; mean eGFR 44 mL/min per 1.73 $m^2$) increased risk.

A mild degree of renal insufficiency with an eGFR of 60 to 89 mL/min per 1.73 $m^2$ (based on the Cockcroft-Gault equation) was present in 51% of the study population. In aging populations, such as that of Western Europe, mild degrees of renal insufficiency will be progressively more important because renal function declines rapidly with age but life expectancy is increasing. Early detection of decreased renal function, using eGFR measurements as a relatively simple screening method, may identify subjects at high risk of coronary heart disease. Because renal insufficiency is associated with a high prevalence of traditional risk factors, early risk factor reduction measures may be of benefit in these subjects. Therefore, the early stages of renal insufficiency may be a key target to prevent worsening renal function as well as to reduce the risk of cardiovascular disease. Some of the factors detrimental to kidney function, such as smoking, dyslipidemia, and elevated blood pressure, can be modified. Recent studies [3,23] report that angiotensin converting enzyme inhibitors may have renoprotective effects and delay the progression of renal insufficiency. Because the subjects in our study were predominantly white, the generalizability of the study to other racial groups is limited. Moreover, data on proteinuria were unavailable in this database because urinalyses were not performed in the Rotterdam Study[14]. Microalbuminuria is an early marker of diabetic nephropathy and cardiovascular disease; however, a recent study [24] showed that the GFR is a predictor of cardiovascular events independent of the presence of microalbuminuria in patients with asymptomatic diabetes mellitus. The CHS study found that cystatin C was a stronger predictor of cardiovascular events than serum creatinine level and eGFR. Cystatin C levels were not available in our study, but the use of an eGFR in our study provided risk estimates for myocardial infarction stronger than those based on cystatin C levels in the CHS study[13]. A study on the diagnostic accuracy of eGFR formulas and cystatin C concluded that cystatin C is superior to serum creatinine in estimating renal function but similar to estimates of GFR by Cockcroft-Gault or MDRD equations[25]. Whether serum cystatin C has potential clinical value and could be a useful prognostic tool in the evaluation of elderly patients needs to be confirmed in future studies[13,25]. The use of serum creatinine alone may lead to some underrecognition of renal insufficiency, particularly in elderly subjects, because muscle mass tends to decline with age[26,27]. We used the Cockcroft-Gault equation corrected for body surface area and tubular secretion to estimate eGFR more accurately in elderly individuals[18,20,26,27]. The new MDRD equation has not yet been validated in elderly individuals and in healthy subjects, and the accuracy of this formula in these popu-

lations is still being discussed[21,28,29]. Using the MDRD equation in our study yielded similar results.

In this prospective, population-based study of older individuals, we found that renal insufficiency was highly prevalent and predicted the onset of myocardial infarction even in subjects without symptoms or signs of renal disease. The population-attributable risk was substantial and within the range of that of traditional cardiovascular risk factors. Therefore, the impact of renal insufficiency on the development of coronary heart disease may be larger than commonly thought. This suggests that assessment and treatment of decreased renal function at an early stage may help in the prevention of coronary heart disease.

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## REFERENCES


1. Sarnak MJ, Levey AS, Schoolwerth AC, et al. Kidney disease as a risk factor for development of cardiovascular disease: statement from the American Heart Association councils on kidney in cardiovascular disease, high blood pressure research, clinical cardiology, and epidemiology and prevention. *Circulation*. 2003; 108:2154-2169.
2. Levey AS, Coresh J, Balk E, et al. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Ann Intern Med*. 2003;139:137-143. Mann JF, Gerstein HC, Pogue J, Bosch J, Yusuf S. Renal insufficiency as a predictor of cardiovascular outcomes and the impact of ramipril: the HOPE randomized trial. *Ann Intern Med*. 2001;134:629-636.
4. Beddhu S, Allen-Brady K, Cheung AK, et al. Impact of renal failure on the risk of myocardial infarction and death. *Kidney Int*. 2002;62:1776-1783.
5. Shlipak MG, Fried LF, Crump C, et al. Cardiovascular disease risk status in elderly persons with renal insufficiency. *Kidney Int*. 2002;62:997-1004.
6. Edwards MS, Craven TE, Burke GL, Dean RH, Hansen KJ. Renovascular disease and the risk of adverse coronary events in the elderly: a prospective population based study. *Arch Intern Med*. 2005;165:207-213.
7. Anavekar NS, McMurray JJ, Velazquez EJ, et al. Relation between renal dysfunction and cardiovascular outcomes after myocardial infarction. *N Engl J Med*. 2004; 351:1285-1295.
8. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu C. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med*. 2004;351:1296-1305.
9. Manjunath G, Tighiouart H, Ibrahim H, et al. Level of kidney function as a risk factor for atherosclerotic cardiovascular outcomes in the community. *J Am Coll Cardiol*. 2003;41:47-55.
10. Culleton BF, Larson MG, Wilson PW, Evans JC, Parfrey PS, Levy D. Cardiovascular disease and mortality in a community-based cohort with mild renal insufficiency. *Kidney Int*. 1999; 56:2214-2219.
11. Garg AX, Clark WF, Haynes RB, House AA. Moderate renal insufficiency and the risk of cardiovascular mortality: results from the NHANES I. *Kidney Int*. 2002; 61:1486-1494.
12. Fried LF, Shlipak MG, Crump C, et al. Renal insufficiency as a predictor of cardiovascular outcomes and mortality in elderly individuals. *J Am Coll Cardiol*. 2003; 41:1364-1372.
13. Shlipak MG, Sarnak MJ, Katz R, et al. Cystatin C and the risk of death and cardiovascular events among elderly persons. *N Engl J Med*. 2005;352:2049-2060.
14. Hofman A, Grobbee DE, de Jong PT, van den Ouwenland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. *Eur J Epidemiol*. 1991;7:403-422.
15. Iglesias del Sol A, Bots ML, Grobbee DE, Hofman A, Witteman JC. Carotid intima media thickness at different sites: relation to incident myocardial infarction. *Eur Heart J*. 2002;23:934-940.
16. de Bruyne MC, Mosterd A, Hoes AW, et al. Prevalence, determinants, and misclassification of myocardial infarction in the elderly. *Epidemiology*. 1997;8:495-500.
17. Perrone RD, Madias NE, Levey AS. Serum creatinine as an index of renal function: new insights in old concepts. *Clin Chem*. 1992;38:1933-1953.
18. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron*. 1976;16:31-41.
19. Wang Y, Moss J, Thisted R. Predictors of body surface area. *J Clin Anesth*. 1992; 4:4-10.
20. Vervoort G, Willems HL, Wetzels JF. Assessment of glomerular filtration rate in healthy subjects and normoalbuminuric diabetic patients: validity of a new (MDRD) prediction equation. *Nephrol Dial Transplant*. 2002;17:1909-1913.
21. Levey A, Bosch J, Lewis J, et al. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. *Ann Intern Med*. 1999;130:461-470.
22. Hennekens C, Buring J. *Epidemiology in Medicine*. Boston, Mass: Little, Brown; 1987.
23. Barnett A, Bain S, Bouter P, et al. Angiotensin-receptor blockade versus converting enzyme inhibition in type 2 diabetes and nephropathy. *N Engl J Med*. 2004; 351:1952-1961.

24. Knobler H, Zornitzki T, Vered S, et al. Reduced glomerular filtration rate in asymptomatic diabetic patients: predictor of increased risk for cardiac events independent of albuminuria. *J Am Coll Cardiol*. 2004;44:2142-2148.
25. Harmoinen A, Lehtimaki T, Korpela M, Turjanmaa V, Saha H. Diagnostic accuracies of plasma creatinine, cystatin C,and glomerular filtration rate calculated by the Cockcroft-Gault and Levey (MDRD) formulas. *Clin Chem*. 2003;49:1223-1225.
26. Swedko PJ, Clark HD, Paramsothy K, Akbari A. Serum creatinine is an inadequate screening test for renal failure in elderly patients. *Arch Intern Med*. 2003; 163:356-360.
27. Corsonello A, Pedone C, Corica F, Mussi C, Carbonin P, Incalzi R. Concealed renal insufficiency and adverse drug reactions in elderly hospitalized patients. *Arch Intern Med*. 2005;165:790-795.
28. Nicoll SR, Sainsbury R, Bailey RR, et al. Assessment of creatinine clearance in healthy subjects over 65 years of age. *Nephron*. 1991;59:621-625.
29. Lamb EJ, Webb MC, Simpson DE, et al. Estimation of glomerular filtration rate in older patients with chronic renal insufficiency: is the modification of diet in renal disease formula an improvement? *J Am Geriatr Soc*. 2003;51: 1012-1017.

# Chapter 5

# Identification of renal insufficiency as risk factor for recurrent cardiac events in patients with coronary artery disease

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## ABSTRACT

**Background**: Proteinuria was associated with cardiovascular events and mortality in community-based cohorts. The association of proteinuria with mortality and cardiovascular events in patients undergoing percutaneous coronary intervention (PCI) was unknown. The association of urinary dipstick proteinuria with mortality and cardiovascular events (composite of death, myocardial infarction, or non-hemorrhagic stroke) in 5,835 subjects of the EXCITE trial was evaluated.

**Methods**: Dipstick urinalysis was performed before PCI, and proteinuria was defined as trace or greater. Subjects were followed up for 210 days/7 months after enrolment for the occurrence of events. Multivariate Cox regression analysis evaluated the independent association of proteinuria with each outcome.

**Results**: Mean age was 59 years, 21% were women, 18% had diabetes mellitus, and mean estimated glomerular filtration rate was 90 ml/min/ 1.73 $m^2$. Proteinuria was present in 750 patients (13%). During follow-up, 22 subjects (2.9%) with proteinuria and 54 subjects (1.1%) without proteinuria died (adjusted hazard ratio 2.83, 95% confidence interval [CI] 1.65 to 4.84, $p < 0.001$). The severity of proteinuria attenuated the strength of the association with mortality after PCI (low-grade proteinuria, hazard ratio 2.67, 95% CI 1.50 to 4.75; high-grade proteinuria, hazard ratio 3.76, 95% CI 1.24 to 11.37). No significant association was present for cardiovascular events during the relatively short follow-up, but high-grade proteinuria tended toward increased risk of cardiovascular events (hazard ratio 1.45, 95% CI 0.81 to 2.61).

**Conclusion:** Proteinuria was strongly and independently associated with mortality in patients undergoing PCI. These data suggest that such a relatively simple and clinically easy to use tool as urinary dipstick may be useful to identify and treat patients at high risk of mortality at the time of PCI.

## INTRODUCTION

We evaluated the predictive value of proteinuria, determined using standard urinary dipstick, in patients with coronary artery disease (CAD) at the time of percutaneous coronary intervention (PCI). We conducted a post hoc analysis of the Evaluation of Oral xemilofiban in Controlling Thrombotic Events (EXCITE) trial, a randomized placebo controlled trial designed to evaluate whether long-term administration of an oral glycoprotein IIb/IIIa receptor inhibitor would be associated with lower cardiovascular events and death rates. We hypothesized that the presence of proteinuria would be associated with higher mortality and cardiovascular event rates independent of traditional cardiovascular risk factors, diabetes mellitus, and renal function (estimated glomerular filtration rate [eGFR]) in patients with CAD undergoing PCI. Urinary dipstick, as a relatively simple and clinically easy-to-use tool to detect proteinuria, may identify and treat patients at high risk of mortality at the time of PCI.

## METHODS

The EXCITE trial was a double-blind randomized placebo controlled study conducted at 412 centers in North and South America, Europe, Israel, Australia, New Zealand, and South Africa. The protocol and main results of the trial have been described elsewhere[1]. In brief, 7,232 patients with angiographic evidence of clinically significant CAD were randomly assigned to receive 20 mg of an oral glycoprotein IIb/IIIa inhibitor (xemilofiban) or placebo 30 to 90 minutes before PCI, with maintenance doses of 10 or 20 mg of xemilofiban or placebo administered 3 times/day for up to 182 days. Patients with high-risk features, including unstable angina, acute myocardial infarction (MI), diabetes mellitus, left ventricular ejection fraction <30%, anticipated need for placement of >1 stent, and multivessel CAD, were specifically sought according to the study protocol. Exclusion criteria included serum creatinine >1.5 mg/dl, history of bleeding disorders or active bleeding, thrombocytopenia (platelet count >120,000 cells/$mm^3$), coagulation factor deficiency, uncontrolled hypertension, major trauma or surgery within the previous three months, thrombolytic treatment within 6 hours before PCI, inability to discontinue oral anticoagulant therapy, non hemorrhagic stroke within the previous two months, history of hemorrhagic stroke, or inability to provide informed consent. The primary results of this study showed no significant effect of xemilofiban for prevention of mortality or cardiovascular events[1]. Nineteen percent (n = 1,397) of EXCITE study subjects had missing data regarding proteinuria at baseline and were excluded from analyses. However, there were complete follow-up data for the remaining 5,835 subjects. Subjects with missing proteinuria data were of similar age (59 years) and gender (23% women) and had a similar eGFR (96 ml/min/1.73 $m^2$) and prevalence of diabetes mellitus (20%). Additionally, clinical outcomes of these subjects were compared, and no relevant differences were observed.

A urine sample was collected during a 7-day period before PCI. The presence of proteinuria was determined using a standard urinary dipstick that measured albumin through a colorimetric reaction between albumin and tetrabromophenol blue, producing different shades of green according to the concentration of albumin in the sample[2]. Each color was semiquantified by central laboratory personnel as negative, trace (protein 15 to 30 mg/dl), $1^+$ (30 to 100), $2^+$ (100 to 300), $3^+$ (300 to 1,000), or $4^+$ (>1,000) proteinuria. eGFR was determined using the abbreviated (4-variable) Modification of Diet in Renal Disease Study equation[3]. Patients with eGFR <60 ml/min/1.73 $m^2$ were considered to have moderate chronic kidney disease, consistent with stage 3 or higher chronic kidney disease using the National Kidney Foundation classification[4]. Subjects were evaluated within 24 hours from 10 to 21 days after PCI and again 60 days after PCI. Subsequent monitoring for cardiac events, safety, laboratory values, concurrent medications, and compliance was performed monthly using telephone or site visits. For patients who did not have a diagnosis of acute MI at the time of enrolment, the criterion for a new MI occurring within 24 hours after PCI was creatine kinase-MB ≥3 times the upper limit of normal range. For patients undergoing PCI within 24 hours after the onset of acute MI, criteria for the diagnosis of reinfarction within 24 hours after the procedure was defined as CK≥MB twice as high as the lowest increased value before PCI. For all patients, MI ≥24 hours after PCI was defined as CK ≥2 times the upper limit of the normal range or electrocardiographic appearance of new Q waves of 0.04 seconds in duration with a depth >1/4 of the corresponding R wave amplitude in ≥2 contiguous leads. When CK-MB was not available, total CK was used. Serum samples for cardiac enzymes were collected at baseline and 8, 16, and 24 hours after PCI. An independent Clinical Endpoints Committee reviewed and adjudicated all cardiac clinical events. In addition, a central electrocardiographic laboratory reviewed the screening and final electrocardiograms for each patient to determine whether a Q-wave MI occurred during the study. Non hemorrhagic stroke was defined as the onset of a new neurologic deficit that occurred any time after PCI, persisted ≥24 hours, and was confirmed using computed tomography or magnetic resonance imaging studies. Subjects who died for any reason during follow-up were censored for the all-cause mortality outcome. Subjects were censored for a cardiovascular event if they experienced ≥1 of the outcomes during follow-up of MI, non-hemorrhagic stroke, or all-cause mortality.

Subjects were categorized into 2 groups on the basis of the presence or absence of proteinuria (trace or greater vs none). Baseline characteristics were compared across proteinuria groups using Student's *t* test or Kruskal-Wallis test for continuous variables or chi-square test or Fisher's exact test for dichotomous variables, as appropriate. Univariate and multivariable Cox proportional hazard regression analysis evaluated the association of proteinuria with mortality and cardiovascular events. We developed 3 models. The first was unadjusted. The second model, termed demographic adjusted, was adjusted for age, gender, race, diabetes, and eGFR. The final model, termed fully adjusted, was adjusted for any additionally important potential confounding variables selected on the basis of previous published research (age, gender, race, diabetes, eGFR, left ventricular ejection fraction, number of diseased vessels on coronary angiography,

systolic and diastolic blood pressure, body mass index, and use of ACE inhibitors or angiotensin receptor blocking medications, beta-blockers, statins, or anti-platelet inhibitors and allocated treatment [xemilofiban or placebo]). The primary analysis evaluated proteinuria as a dichotomous predictor variable. In a companion analysis, indicator variables were created for severity of proteinuria (none, low-grade [trace (protein 15 to 30 mg/dl) to $1^+$ (20 to 100)], and high-grade [$2^+$ (100 to 300) or greater (>300)]), and tests for trend were evaluated to determine whether severity of proteinuria was associated with each outcome. In addition, we evaluated for effect modification on the basis of eGFR (<60 vs >60 ml/min/1.73 $m^2$) and presence of diabetes mellitus. For all tests, $p < 0.05$ (two-sided) was considered statistically significant. Statistical analyses were performed using SAS, version 8.0 software package (SAS Institute, Cary, North Carolina).

## RESULTS

Mean age of the study population (n = 5,835) was 59 years, 79% were men, 88% were Caucasian, and 19% had diabetes mellitus. Coronary stents were placed at the time of PCI in 71% subjects. Mean eGFR was 90 ml/min/1.73 $m^2$. Three hundred ninety-two subjects (5.4%) had an eGFR <60 ml/min/1.73 $m^2$. There was no loss to follow-up during the observation period. Seven hundred fifty subjects (13%) had trace or greater proteinuria. Subjects with proteinuria had a higher prevalence of diabetes, hypertension, and eGFR <60 ml/min/1.73 $m^2$; were more likely to be using renin-angiotensin system inhibitors; and had higher blood pressure and body mass index. Randomized allocation to xemilofiban by study protocol did not differ by proteinuria status (P-value 0.10; Table 1).

During the 210-day follow-up, 22 subjects (2.9%) with proteinuria and 54 subjects (1.1%) without proteinuria died (Figure 1). Of 76 all-cause deaths, 69 (91%) were cardiac deaths with similar distribution in the proteinuria and nonproteinuria groups. Subjects with proteinuria had a nearly 3-fold odds of death compared with those without proteinuria, an association that was essentially unaltered after extensive statistical adjustment for potential confounding variables, including allocated treatment (Table 2). Moreover, the association was similar between persons with eGFR <60 or >60 ml/min/1.73 $m^2$ and between subjects with and without diabetes mellitus (p for interaction = 0.72 and 0.88, respectively). Additionally, increasing severity of proteinuria was associated with increased risk of all-cause mortality in both unadjusted and adjusted analyses (Table 2).

During follow-up, there were 558 cardiovascular events (454 MIs, 28 strokes, and 76 deaths). Sixty-six subjects (8.8%) with proteinuria and 456 subjects (9%) without proteinuria reached the composite secondary outcome. In adjusted analysis, there was no association of proteinuria with this outcome, with 95% confidence intervals (CIs) excluding an odds ratio >30% in either direction. We found no evidence of effect modification on the basis of eGFR <60 ml/min/1.73 $m^2$ or diabetes mellitus (p for interaction = 0.23 and 0.78, respectively). Severity of proteinuria

**Table 1.** Baseline characteristics of patients with or without proteinuria.

| | *Proteinuria* | | |
|---|---|---|---|
| Variable | No | Yes | P-value |
| N | 5085 | 750 | |
| Age(years) | 59 (8) | 58 (8) | 0.20 |
| Men | 78.1 % | 80.5 % | 0.13 |
| Race | | | 0.02 |
| Caucasian | 88.4 % | 88.0 % | |
| Black | 1.5 % | 1.9 % | |
| Other | 10.1 % | 10.0 % | |
| Diabetes | 17.1 % | 28.0 % | <0.01 |
| Hypertension | 45.7 % | 50.0 % | 0.026 |
| Prior percutaneous coronary intervention | 13.6 % | 13.9 % | 0.86 |
| Prior coronary artery bypass graft | 17.6 % | 18.4 % | 0.59 |
| Prior myocardial infarction | 25.1 % | 24.1 % | 0.59 |
| Prior Stroke | 2.0 % | 2.0 % | 0.98 |
| ACE-inhibitor use | 32 % | 39 % | <0.01 |
| Beta-blocker use | 68 % | 70 % | 0.40 |
| Statin use | 54 % | 51 % | 0.13 |
| Glomerular filtration rate <60 ml/min/1.73m$^2$ | 5.2 % | 8.3 % | <0.01 |
| Systolic blood pressure, mmHg* | 130 (11) | 130 (10) | 0.021 |
| Diastolic blood pressure, mmHg* | 76 (6) | 78 (8) | 0.006 |
| Body mass index (kg/m$^2$)* | 27.5 (2.4) | 28.3 (2.2) | <0.01 |
| Left ventricular ejection fraction ≤30% | 2.0 % | 2.2 % | 0.71 |
| Diseased Coronary Vessel | | | |
| Right coronary artery | 22.4 % | 24.5 % | 0.18 |
| Left anterior descendens | 42.8 % | 42.8 % | 1.0 |
| Left circumflex artery | 35.0 % | 35.2 % | 0.89 |
| Left main | 1.0 % | 1.6 % | 0.14 |
| Multivessel Disease | 45.5 % | 47.9 % | 0.21 |
| Allocated to Xemilofiban | 66.0 % | 69.1 % | 0.10 |

Values are n (%) unless marked otherwise. PCI= percutaneous coronary-intervention. CABG= coronary bypass surgery, ACE=angiotensin-converting enzyme, GFR= glomerular filtration rate, RCA= right coronary artery, LAD= left anterior descendens, LCX= left circumflex artery, LM= left main. * Median (Inter-Quartile Range).

showed a trend toward increased risk of incident cardiovascular events with more severe proteinuria status (low-grade proteinuria hazard ratio 0.89, 95% CI 0.66 to 1.21; high grade proteinuria hazard ratio 1.45, 95% CI 0.81 to 2.61; Table 2).

## DISCUSSION

This study showed that proteinuria, measured using standard urinary dipstick, was associated with a nearly 3-fold increased odds of all-cause mortality in patients with established CAD at the

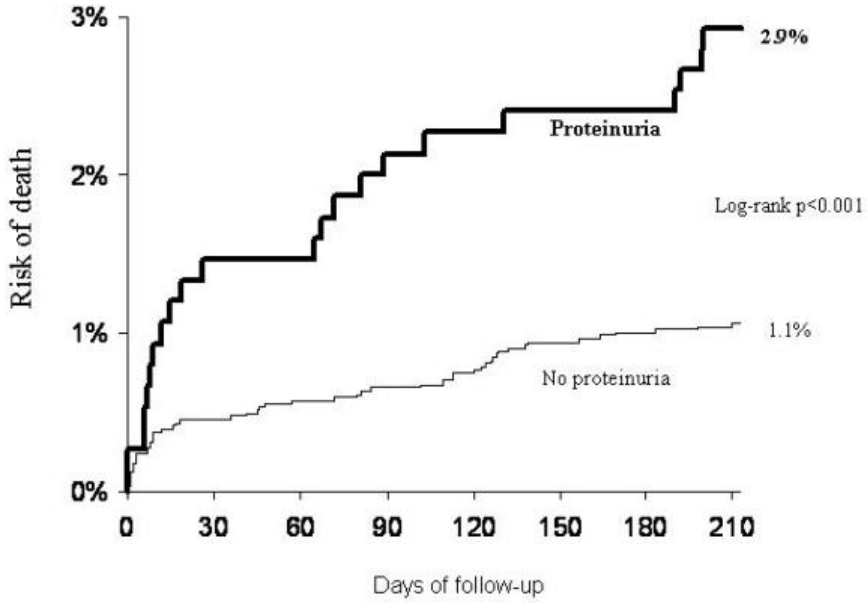


**Figure 1** Kaplan-Meier curve of mortality by the presence or absence of proteinuria. *Bold black line,* patients with proteinuria; *thin black line,* patients without proteinuria.

**Table 2.**

| | All-cause Mortality | | | |
|---|---|---|---|---|
| Variable | Events / N at risk | Unadjusted | Demographic Adjusted† | Fully Adjusted‡ |
| No proteinuria | 54/5085 (1.1%) | 1.00 | 1.00 | 1.00 |
| Proteinuria | 22/750 (2.9%) | 2.81 (1.70 – 4.65) | 2.64 (1.57 – 4.44) | 2.83 (1.65 – 4.84) |
| - low-grade | 18/643 (2.8%) | 2.68 (1.56 – 4.60) | 2.53 (1.44 – 4.43) | 2.67 (1.50 – 4.75) |
| - high-grade | 4/107 (3.7%) | 3.61 (1.28 – 10.17) | 3.03 (1.04 – 8.86) | 3.76 (1.24 – 11.37) |
| | P-value for trend | <0.001 | <0.001 | <0.001 |
| | Cardiovascular events | | | |
| No proteinuria | 456/5085 (9%) | 1.00 | 1.00 | 1.00 |
| Proteinuria | 66/750 (8.8%) | 0.98 (0.74 – 1.28) | 0.95 (0.72 – 1.26) | 0.97 (0.74 – 1.28) |
| - low-grade | 52/643 (8.0%) | 0.89 (0.66 – 1.20) | 0.87 (0.64 – 1.18) | 0.89 (0.66 – 1.21) |
| - high-grade | 14/107 (13%) | 1.52 (0.86 – 2.70) | 1.46 (0.82 – 2.60) | 1.45 (0.81 – 2.61) |
| | P-value for trend | 0.70 | 0.83 | 0.76 |

Association of Proteinuria* and the severity of proteinuria with mortality and cardiovascular events among patients undergoing PCI.

*Dipstick urinalysis with proteinuria defined as trace or greater. Low-grade proteinuria defined as trace to 1+ and high-grade as 2+ or greater. § Composite outcome of mortality, myocardial infarction, and stroke. † Adjusted for age, gender, race, diabetes, and eGFR. ‡ Adjusted for demographic adjusted variables and ejection fraction, number of diseased vessels, systolic and diastolic blood pressure, body mass index, and use of ACE/ARB, beta-blockers, statins, anti-platelet inhibitors and allocated treatement (xemilofiban).

time of PCI. This association was essentially unaltered despite extensive statistical adjustment for traditional cardiac risk factors, chronic kidney disease, or diabetes mellitus. Surprisingly, despite the independent association of proteinuria with mortality, we observed no strong associations of proteinuria with cardiovascular events in the EXCITE cohort, although the risk of cardiovascular events increased with more severe proteinuria.

Proteinuria is the earliest manifestation of kidney dysfunction in patients with several forms of kidney disease [5] because it is a marker of loss of the normal selective barrier at the glomerular filtration slits. It also strongly correlated with markers of endothelial dysfunction [6-11] Previous epidemiologic studies consistently showed that proteinuria predicted mortality in populations with and without diabetes or cardiovascular disease [1,11-14] However, the predictive value of proteinuria at the time of PCI has not been extensively studied. Marso et al [15] showed that proteinuria at the time of PCI was strongly associated with mortality in patients with diabetes mellitus in a large single-center study. However, Reeder et al [16] refuted this finding, showing that serum creatinine was a stronger predictor of death than proteinuria in persons with diabetes undergoing PCI. Therefore, debate existed regarding the prognostic significance of proteinuria in diabetic subjects undergoing PCI, and to our knowledge, no previous study has evaluated the prognostic significance of proteinuria in persons without diabetes in this clinical setting. Thus, the observations presented here contributed to existing reports in several ways. First, the large sample size, multinational nature of the study cohort, and use of coronary stents in most subjects made our results generalizable to diverse populations and to practices that closely resemble those used in current clinical practice. Second, we showed that the association of proteinuria with mortality was similar between diabetic and nondiabetic subjects and persons with or without moderate chronic kidney disease. Because urinary dipstick was routinely available and inexpensive, it may be clinically useful to identify and treat subjects at high risk of mortality after PCI irrespective of diabetes status or kidney function. Ibsen et al [17] showed that treatments that decreased proteinuria translated into decreased cardiovascular events during follow-up in persons with hypertension. Third, the increase in risk of all-cause mortality and cardiovascular events observed with an increase in severity of proteinuria in patients undergoing PCI was a new and clinically relevant finding. Whether medications that decrease proteinuria might also be associated with a decrease in mortality in persons undergoing PCI, or alternatively, whether proteinuria is simply a marker of early kidney disease or endothelial dysfunction is an important question to be addressed in future studies.

Mild to moderate kidney disease was also associated with cardiac structural abnormalities [18] and conduction system disease [19]. Therefore, we hypothesized that proteinuria may be associated with an increased risk of sudden cardiac death, which could explain the stronger relation to mortality. This outcome was not specifically adjudicated in EXCITE and can therefore not be evaluated in the context of the present study. However, regardless of cause, because we observed the association of proteinuria with mortality during a relatively short 7-month follow-up, persons undergoing PCI who have proteinuria may benefit from closer surveillance

during this time frame. Future studies are required to evaluate the cause of death in this setting and determine whether proteinuria is associated with mortality or cardiovascular events during longer periods of observation after PCI.

This study had several limitations. The present analysis mainly addressed patients with mild to moderate renal insufficiency according to the National Kidney Foundation kidney disease outcomes quality initiative (KDOQI) guidelines [4] because patients with a serum creatinine >1.5 mg/dl were excluded in the trial. We used standard urinary dipstick evaluation for assessment of proteinuria, a relatively insensitive marker of urinary protein excretion compared with microalbuminuria. Whether microalbuminuria may represent a more sensitive test to identify persons at increased risk of death after PCI requires evaluation in future studies. Additionally, random urine dipstick assessment of proteinuria may vary by the concentration of the urine sample. However, such variation should have biased the results toward the null hypothesis. We emphasize that urinary dipstick is an inexpensive, easy-to-use, and simple tool to identify the presence of proteinuria. The majority of EXCITE study subjects were men and Caucasian, and results may not generalize to other patient populations. Nineteen percent of EXCITE study subjects did not have urinary dipstick proteinuria measurement and were excluded from the present study. However, clinical characteristics and event rates were similar in persons with and without proteinuria data. Last, the observation period in EXCITE was relatively short at 7 months, which could be inadequate to answer this question adequately for incident cardiovascular events. Still, it is important to emphasize that more sensitive tests of proteinuria are warranted, regardless of whether it is less convenient, because proteinuria was convincingly shown to be an important risk factor. Whether the association of proteinuria with mortality remains or the association with cardiovascular events develops at longer follow-up should be evaluated in future studies.

## REFERENCES


1. O'Neill WW, Serruys P, Knudtson M, van Es GA, Timmis GC, van der Zwaan C, Kleiman J, Gong J, Roecker EB, Dreiling R, Alexander J, Anders R. Long-term treatment with a platelet glycoprotein-receptor antagonist after percutaneous coronary revascularization. EXCITE Trial Investigators. Evaluation of Oral xemilofiban in Controlling Thrombotic Events. *N Engl J Med* 2000;342:1316 –1324.
2. Kim MS, Corwin HL. Urinalysis. In: Schrier RW, ed. Diseases of the Kidney and Urinary Tract. 8th Ed. Philadelphia: Lippincott Williams & Wilkins, 2007:286 –298.
3. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 1999;130:461– 470.
4. National Kidney Foundation: K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002;39(suppl):S1–S266.
5. Ruggenenti P, Remuzzi G. Nephropathy of type-2 diabetes mellitus. *J Am Soc Nephrol* 1998;9:2157–2169.
6. Stehouwer CD, Nauta JJ, Zeldenrust GC, Hackeng WH, Donker AJ, den Ottolander GJ. Urinary albumin excretion, cardiovascular disease, and endothelial dysfunction in non-insulin-dependent diabetes mellitus. *Lancet* 1992;340:319 –323.
7. Pedrinelli R, Giampietro O, Carmassi F, Melillo E, Dell'Omo G, Catapano G, Matteucci E, Talarico L, Morale M, De Negri F. Microalbuminuria and endothelial dysfunction in essential hypertension. *Lancet* 1994;344:14 –18.
8. Brugts JJ, Knetsch AMK, Mattace-Raso FUS, Hofman A, Witteman JCM. Renal function and risk of myocardial infarction in an elderly opulation: the Rotterdam Study. *Arch Intern Med* 2005;165:2659–2665.
9. Clausen P, Jensen JS, Jensen G, Borch-Johnsen K, Feldt-Rasmussen B. Elevated urinary albumin excretion is associated with impaired arterial dilatory capacity in clinically healthy subjects. *Circulation* 2001;103:1869 –1874.
10. Cosson E, Pham I, Valensi P, Paries J, Attali JR, Nitenberg A. Impaired coronary endothelium-dependent vasodilation is associated with microalbuminuria in patients with type 2 diabetes and angiographically normal coronary arteries. *Diabetes Care* 2006;29:107–112.
11. Berton G, Cordian RO, Palmieri R, Cucchini F, de Toni R, Palatini P. Microalbuminuria during acute myocardial infarction; a strong predictor for 1-year mortality. *Eur Heart J* 2001;22:1466 –1475.
12. Gerstein HC, Mann JF, Yi Q, Zinman B, Dinneen SF, Hoogwerf B, Hallé JP, Young J, Rashkow A, Joyce C, Nawaz S, Yusuf S; HOPE Study Investigators. Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. *JAMA* 2001;286:421– 426.
13. Hillege HL, Fidler V, Diercks GF, van Gilst WH, de Zeeuw D, van Veldhuisen DJ, Gans RO, Janssen WM, Grobbee DE, de Jong PE; Prevention of Renal and Vascular End Stage Disease (PREVEND) Study Group. Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in general population. *Circulation* 2002; 106:1777–1782.
14. Wachtell K, Ibsen H, Olsen MH, Borch-Johnsen K, Lindholm LH, Mogensen CE, Dahlöf B, Devereux RB, Beevers G, de Faire U, et al. Albuminuria and cardiovascular risk in hypertensive patients with left ventricular hypertrophy: the LIFE study. *Ann Intern Med* 2003;139: 901–906.
15. Marso SP, Ellis SG, Tuzcu M, Whitlow PL, Franco I, Raymond RE, Topol EJ. The importance of proteinuria as a determinant of mortality following percutaneous coronary revascularization in diabetics. *J Am Coll Cardiol* 1999;33:1269 –1277.
16. Reeder GS, Holmes DR, Lennon RJ, Larson TS, Frye RL. Proteinuria, serum creatinine, and outcome of percutaneous coronary intervention in patients with diabetes mellitus. *Am J Cardiol* 2002;89:760 –764.
17. Ibsen H, Olsen MH, Wachtell K, Borch-Johnsen K, Lindholm LH, Mogensen CE, Dahlöf B, Devereux RB, de Faire U, Fyhrquist F, et al. Reduction in albuminuria translates to reduction in cardiovascular events in hypertensive patients: Losartan Intervention For Endpoint Reduction In Hypertension Study. *Hypertension* 2005;45:198 –202.

18. Ix JH, Shlipak MG, Chertow GM, Ali S, Schiller NB, Whooley MA. Cystatin C, left ventricular hypertrophy, and diastolic dysfunction: data from the Heart and Soul Study. *J Card Fail* 2006;12:601– 607.
19. Bruch C, Rothenburger M, Gotzmann M, Wichter T, Scheld HH, Breithardt G, Gradaus R. Chronic kidney disease in patients with chronic heart failure—impact on intracardiac conduction, diastolic function and prognosis. *Int J Cardiol* 2007;118:375–380.

# Chapter 6

# The cardioprotective effects of the ACE-inhibitor perindopril are not modified by renal insufficiency: Results from the EUROPA trial.

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## ABSTRACT

**Objectives:** This study sought to examine whether the cardioprotective effects of angiotensin-converting enzyme (ACE) inhibitor therapy by perindopril are modified by renal function in patients with stable coronary artery disease.

**Background:** A recent study reported that an impaired renal function identified a subgroup of patients with stable coronary artery disease more likely to benefit from ACE inhibition therapy. In light of the growing interest in tailored therapy for targeting medications to specific subgroups, remarks on the consistency of the treatment effect by ACE inhibitors are highly important.

**Methods**: The present study involved 12.056 patients with stable coronary artery disease without heart failure randomized to perindopril or placebo. Estimated glomerular filtration rate (eGFR) was calculated using the abbreviated Modification of Diet in Renal Disease equation. Cox regression analysis was used to estimate multivariable-adjusted hazard ratios.

**Results**: The mean eGFR was 76.2 (18.1) ml/min/1.73 $m^2$. During follow-up, the primary end point (cardiovascular death, nonfatal myocardial infarction, or resuscitated cardiac arrest) occurred in 454 of 5,761 patients (7.9%) with eGFR ≥75 and in 631 of 6.295 patients (10.0%) with eGFR <75. Treatment benefits of perindopril were apparent in both patient groups either with eGFR ≥75 (hazard ratio 0.77; 95% CI 0.64 to 0.93) or eGFR <75 (hazard ratio 0.84; 95% CI 0.72 to 0.98). We observed no significant interaction between renal function and treatment benefit (p = 0.47). Using different cutoff points of eGFR at the level of 60 or 90 resulted in similar trends.

**Conclusions:** The treatment benefit of perindopril is consistent and not modified by mild to moderate renal insufficiency.

## INTRODUCTION

Several clinical trials in patients with stable coronary artery disease (CAD) have shown that inhibitors of the angiotensin-converting enzyme (ACE) reduce the incidence of cardiovascular events during long-term follow-up [1–4]. Because these effects are apparent in both low- and high-risk populations, as well as in those with and without preserved left ventricular function, clinical treatment guidelines argue that ACE inhibitors should be used as routine secondary prevention for the broad group of patients with known CAD [5]. Still, it should be realized that absolute treatment effects in low-risk patients are modest. Because the cost effectiveness of medications is of increasing importance, there is a rapidly growing interest in tailored therapy. In cardiovascular disease, targeting ACE inhibitor therapy to specific patient groups that are most likely to benefit is of high clinical relevance. Patients with impaired renal function are a potential target because renal function is independently associated with adverse clinical outcome in cardiovascular disease [6,7].

In a recent substudy of the PEACE (Prevention of Events With ACE Inhibition) trial, a significant heterogeneity in treatment effect with trandolapril was observed in relation to renal function [8]. In patients with poor renal function, defined as an estimated glomerular filtration rate (eGFR) below 60 ml/min/1.73 $m^2$, trandolapril was associated with a significant reduction in all-cause mortality (27% relative risk reduction) as compared with placebo. In contrast, no risk reduction was observed in patients with higher eGFR levels. The PEACE Investigators concluded that, in a stable CAD population, ACE inhibition offered the best cardiovascular protection in patients with poor renal function, which could be used as a subgroup to target therapy [8,9]. As the treatment effect of trandolapril in the entire PEACE study was neutral [10], retrospective analyses to define patient populations with positive ACE inhibitor effects should be regarded cautiously and verified in comparable patient populations.

The EUROPA (European Trial on Reduction of Cardiac Events With Perindopril) study examined the preventive effects of ACE inhibition in a large population of patients with stable CAD and preserved left ventricular function. In light of the growing interest in tailored therapy and the recent results of the PEACE trial, we examined whether renal function modified the cardioprotective benefits of ACE inhibition therapy by perindopril in the EUROPA study.

## METHODS

### Study population

The design and principal results of the EUROPA study have been reported elsewhere [2,11]. In short, the EUROPA study was a randomized, double-blind, multicenter study of 12,218 patients with stable CAD without overt heart failure designed to assess the effect of 8 mg perindopril (n = 6,110) versus placebo (n = 6,108) on the combined end point of cardiovascular death, non

fatal myocardial infarction (MI), and resuscitated cardiac arrest. After a mean follow-up of 4.2 years, 8.0% of patients randomized to perindopril and 9.9% of those randomized to placebo reached the primary end point, which yields a 20% relative risk reduction with perindopril (hazard ratio [HR] 0.80; 95% confidence interval [CI] 0.71 to 0.91). In the EUROPA study, a serum creatinine level >1.7 mg/dl was an exclusion criteria; however, 30 patients (0.02%) enrolled with serum creatinine between 1.7 and 2.2 mg/dl. Baseline blood samples with standardized measurements of serum creatinine levels according to protocol were available in 12.056 patients. Written informed consent was obtained from all patients.

### Assessment of renal function

Renal function was assessed by eGFR using the abbreviated 4-variable Modification of Diet in Renal Disease equation [12]. The dimension of all mentioned eGFR levels is in ml/min/1.73 $m^2$.

### Outcome measures

The primary end point was a composite of cardiovascular death, non fatal MI, and resuscitated cardiac arrest. Secondary end points were the composite of total mortality, non fatal MI, hospital admission for unstable angina, and cardiac arrest with successful resuscitation; cardiovascular mortality, non fatal MI, and stroke or unstable angina; fatal and non fatal MI and unstable angina; stroke; and admission for heart failure. In addition, we assessed total mortality and cardiovascular mortality as individual end points. The diagnosis of MI was based on the recommendations of the European Society of Cardiology and the American College of Cardiology [13].



### Statistical analysis

Summary statistics for continuous variables are presented as mean ± standard deviation. Categorical data are summarized as frequencies and percentages. One-way analysis of variance and Pearson chi square tests were used to calculate p values. We examined eGFR as a categorical variable for the association of renal function and clinical outcome (<45, 45 to 59.9, 60 to 74.9, 75 to 89.9, and ≥90 ml/min/1.73 $m^2$). In our initial analyses for the relation between renal function and clinical outcome, we confined ourselves to this clinically relevant classification. Still, we realize that dichotomization of a continuous measure may result in loss of information. Therefore, all analyses were repeated with eGFR as a continuous variable. Because both approaches showed similar results (we found no evidence of heterogeneity in treatment effect in relation to renal function), we present our findings of the analysis of renal function and treatment benefit by perindopril according to a binary classification. To systematically test the consistency of perindopril in relation to renal function, we have chosen 2 approaches. First, because there is a continuous relation between eGFR and cardiovascular risk, we divided the study population according to the median eGFR in our study. This resulted in 2 groups of comparable size, which we defined as relatively preserved (eGFR ≥75) versus impaired (eGFR <75) renal function. Second, from a clinical point of view, we have chosen a cutoff (also dichotomous) at an eGFR ≥60 or an eGFR <60 and at an eGFR

≥90 or an eGFR <90, corresponding to the presence of chronic kidney disease or a normal renal function at baseline, respectively. In the literature, there is an ongoing debate regarding which cutoff point to use. For completeness and comparability, we present all treatment effects on all cardiovascular end points at different cutoff points of eGFR, namely 60, 75, and 90 ml/min/1.73 $m^2$. Because of numerous studies reporting that the increased risk of cardiovascular events is already apparent at the earliest stages of renal insufficiency, well above 60 ml/min/1.73 $m^2$, we confined ourselves to the cutoff at 75 ml/min/1.73 $m^2$ for our main analyses [14–16].

The incidence of the primary and secondary end points over time was studied using the Kaplan-Meier method. Differences in incidence according to renal function were evaluated by log-rank tests. Absolute risk differences were calculated until 4 years of follow-up; after that Kaplan-Meier estimates became increasingly unstable because of the small number of patients at risk.

Univariable and multivariable Cox proportional hazards regression analyses were applied to examine the association between renal function and study end points. In multivariable analysis, we adjusted for the following (potentially) confounding baseline characteristics: age, gender, systolic blood pressure, diastolic blood pressure, presence of diabetes mellitus, hypercholesterolemia, current smoking, history of CAD (MI, percutaneous coronary intervention, coronary artery bypass graft surgery). Interaction between renal function and treatment effect was analyzed in a continuous as well as a categorical model for eGFR. Each model was tested for interaction and included an [renal function * treatment group] interaction term. The assumption of proportional hazards was assessed by visual judgment of the log-minus-log survival plots. All measures of association are presented as multivariable-adjusted HRs together with 95% CIs. All analyses were based on intention to treat. Statistical tests were 2-sided, and a value of $p < 0.05$ was considered significant. We used SPSS statistical software (version 12.01 for Windows, SPSS Inc., Chicago, Illinois) for our calculations.

## RESULTS

### Patients

The distribution of eGFR in the EUROPA trial is presented in Figure 1. The mean eGFR in our study population was 76.2 ± 18.1 (median 74.2, interquartile range 64.6 to 85.2) ml/min/1.73 $m^2$, corresponding to a mean serum creatinine of 1.1 ± 0.2 mg/dl. A total of 6,295 (52.1%) patients had impaired renal function (eGFR <75). Baseline characteristics are shown in Table 1. Patients with lower eGFR were older and more often were female. Furthermore, patients with impaired renal function were more likely to have a higher frequency of comorbidities including hypertension and diabetes mellitus, but less often reported current smoking. Baseline characteristics for patients randomized for treatment with perindopril or placebo were in balance in the subjects considered in the analysis of treatment benefits and yielded no clinically relevant differences.

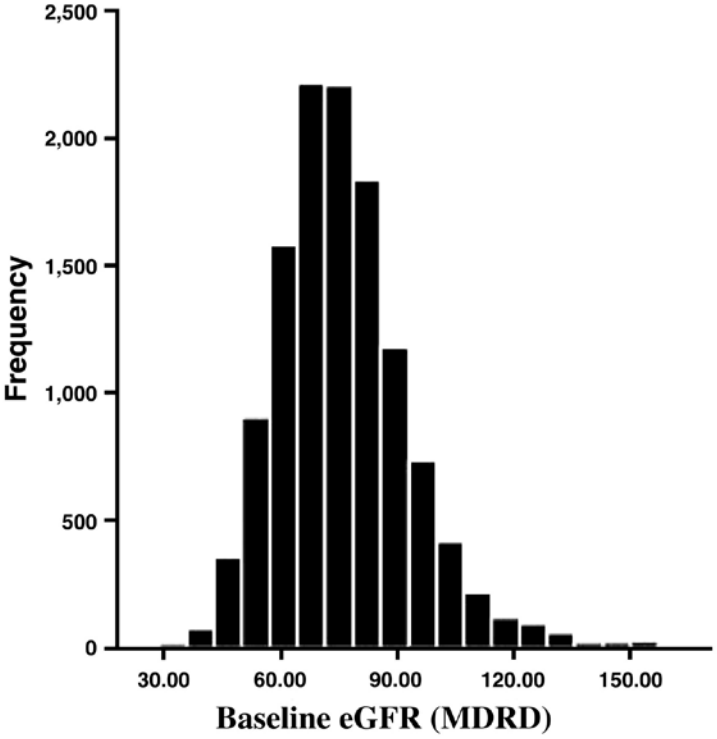





**Figure 1** Distribution of estimated glomerular filtration rate (eGFR) in the EUROPA trial (n = 12,056). MDRD = Modification of Diet in Renal Disease equation.

## Renal function and clinical outcome

Regardless of allocated treatment, renal function was significantly associated with clinical outcome. In patients allocated to perindopril, each 10 ml/min/1.73 $m^2$ decrease in eGFR was related to an 8.7% (HR 1.09, 95% CI 1.03 to 1.15, p = 0.005) increased risk in the primary end point. A similar increased risk was found in those allocated to placebo: 6.5% (HR 1.06, 95% CI 1.02 to 1.12, p = 0.015). With worsening eGFR categories, the associated HRs increased considerably for all end points in both treatment groups (Table 2). Kaplan-Meier estimates of the primary end point with perindopril and placebo for the 2 different categories of eGFR are presented in Figure 2. Log-rank tests were performed for perindopril versus placebo in patients with eGFR ≥75 and <75, which resulted in p values of 0.005 and 0.023, respectively.

## Renal function and treatment effects by perindopril

In patients with a relatively preserved renal function, perindopril was associated with a 23% (HR 0.77, 95% CI 0.64 to 0.93) relative reduction in the incidence of the primary end point as compared with placebo. For patients with impaired renal function, perindopril was associated

**Table 1.** Baseline characteristics of study population (n=12,056).

| Characteristic | Estimated GFR (ml/min per 1.73 m²) | | | | | |
|---|---|---|---|---|---|---|
| | ≥90 | 75-89.9 | 60 - 74.9 | 45.0 - 59.9 | <45 | Pv |
| | (n=2131) | (n=3630) | (n=4378) | (n=1756) | (n=161) | |
| Mean (Sd) age (years) | 55.2 (9.3) | 58.3 (9.1) | 61.5 (8.5) | 65.1 (7.9) | 69.1 (6.7) | <0.01 |
| Gender, female | 155 (7.3) | 322 (8.9) | 678 (15.5) | 516 (29.4) | 94 (58.4) | <0.01 |
| Hypertension * | 472 (22.1) | 926 (25.5) | 1218 (27.8) | 610 (34.7) | 64 (39.8) | <0.01 |
| Hypercholesterolemia † | 1288 (60.4) | 2312 (63.6) | 2815 (64.2) | 1134 (64.5) | 94 (58.4) | 0.02 |
| Diabetes Mellitus | 232 (10.9) | 411 (11.3) | 529 (12.1) | 277 (15.8) | 31 (19.3) | <0.01 |
| Current smoking | 469 (22.0) | 626 (17.2) | 538 (12.3) | 180 (10.2) | 17 (10.6) | <0.01 |
| Peripheral vessel disease | 130 (6.1) | 236 (6.5) | 328 (7.5) | 161 (9.2) | 18 (11.2) | <0.01 |
| Previous stroke/TIA | 47 (2.2) | 91 (2.5) | 165 (3.8) | 96 (5.5) | 10 (6.2) | <0.01 |
| History of CAD | | | | | | |
| MI | 1444 (67.8) | 2359 (64.9) | 2771 (63.3) | 1130 (64.3) | 113 (70.2) | 0.01 |
| PCI | 637 (29.9) | 1107 (30.5) | 1259 (28.8) | 475 (27.0) | 37 (23.0) | 0.03 |
| CABG | 514 (24.1) | 1011 (27.8) | 1408 (32.2) | 556 (31.6) | 55 (34.2) | <0.01 |
| Medication | | | | | | |
| Platelet inhibitors | 1965 (92.2) | 3386 (93.2) | 4039 (92.3) | 1589 (90.4) | 140 (87.0) | <0.01 |
| Statins | 1134 (53.2) | 2040 (56.2) | 2492 (56.9) | 1002 (57.0) | 79 (49.1) | 0.02 |
| β-blockers | 1299 (60.9) | 2269 (62.5) | 2781 (63.5) | 1128 (64.2) | 96 (59.6) | 0.18 |
| Calcium-antagonists | 624 (29.3) | 1130 (31.1) | 1428 (32.6) | 647 (36.8) | 73 (45.3) | <0.01 |
| Nitrates | 960 (45.0) | 1553 (42.8) | 1917 (43.8) | 846 (48.1) | 82 (50.9) | <0.01 |
| Diuretics | 139 (6.5) | 237 (6.5) | 467 (10.6) | 325 (18.5) | 49 (30.4) | <0.01 |
| Mean (Sd) SBP, mmHg | 134.5(14.2) | 136.3(15.2) | 138.0(15.8) | 139.5(15.8) | 143.5(17.5) | <0.01 |
| Mean (Sd) DBP, mmHg | 82.0 (8.1) | 81.8 (8.2) | 81.8 (8.1) | 81.4 (8.4) | 80.5 (9.0) | 0.08 |
| Mean (Sd) eGFR | 104.1(18.3) | 81.7 (4.2) | 68.0 (4.2) | 54.5 (3.9) | 40.9 (4.4) | <0.01 |
| Randomized,perindopril | 1060 (49.7) | 1809 (49.8) | 2189 (50.0) | 906 (51.6) | 72 (44.7) | 0.46 |

Values are n (%) unless marked otherwise. MI= myocardial infarction. PCI= percutaneous coronary-intervention. CABG= coronary bypass surgery. TIA= transient ischemic attack. * Blood pressure > 160/95 mm Hg or receiving antihypertensive treatment. † Cholesterol > 6.5 mmol/L or receiving lipid-lowering treatment.

with a 16% reduction (HR 0.84, 95% CI 0.72 to 0.98). There was no evidence of heterogeneity in the cardioprotective effect of perindopril in relation to eGFR, when assessed as a categorical ($p = 0.47$) or as a continuous variable ($p = 0.37$). Similar consistencies were found for all other end points considered. The treatment effects of perindopril at the other cutoff levels of 60 and 90 ml/min/1.73 $m^2$ are presented in Table 3. The results were similar, and no significant heterogeneity in treatment effect of perindopril was observed over the whole range of eGFR on all cardiovascular end points.

## Absolute risks during follow-up

The absolute risk of the primary end point was highest in patients with impaired renal function using placebo (10.3%). The absolute risk reduction of the primary end point by perindopril at 4 years of follow-up was 1.90% in patients with an eGFR ≥75 and 1.77% in patients with eGFR <75 (Table 4). For comparability, we present the number of events of total mortality, cardiovascular mortality, and nonfatal MI in the PEACE and EUROPA trials in Table 5.

**Table 2.** Multivariable-adjusted hazard ratios for baseline renal function and clinical outcome (n=12,056)

| | | Placebo (n=6,027) | | Perindopril (n=6,029) | |
|---|---|---|---|---|---|
| Endpoints | eGFR | Events /total | HR 95% CI | Events /total | HR 95% CI |
| **Primary endpoint** | Reference | 8.6 | 1.00 | 6.7 | 1.00 |
| (cardiovascular death, non-fatal | 75-89.9 | 9.1 | 1.03 (0.80-1.33) | 6.9 | 1.00 (0.74-1.34) |
| MI, resuscitated CA) | 60-74.9 | 10.1 | 1.13 (0.88-1.46) | 8.0 | 1.10 (0.83-1.46) |
| | 45-59.9 | 12.3 | 1.31 (0.97-1.76) | 11.4 | 1.53 (1.10-2.11) |
| | <45 | 15.7 | 1.59 (0.88-2.86) | 15.3 | 1.86 (0.96-3.60) |
| **Total mortality, AMI, UAP or** | Reference | 16.0 | 1.00 | 12.5 | 1.00 |
| **cardiac arrest** | 75-89.9 | 15.4 | 0.92 (0.76-1.11) | 13.1 | 1.00 (0.80-1.23) |
| | 60-74.9 | 17.3 | 1.00 (0.83-1.20) | 15.1 | 1.09 (0.88-1.34) |
| | 45-59.9 | 20.7 | 1.15 (0.92-1.43) | 18.8 | 1.31 (1.03-1.66) |
| | <45 | 29.2 | 1.57 (1.01-2.42) | 29.2 | 1.86 (1.15-3.01) |
| **Cardiovascular mortality, AMI** | Reference | 9.2 | 1.00 | 7.1 | 1.00 |
| **and stroke** | 75-89.9 | 10.0 | 1.04 (0.82-1.33) | 8.0 | 1.08 (0.81-1.43) |
| | 60-74.9 | 11.4 | 1.18 (0.93-1.50) | 9.1 | 1.16 (0.89-1.53) |
| | 45-59.9 | 13.3 | 1.30 (0.98-1.73) | 12.9 | 1.61 (1.18-2.19) |
| | <45 | 19.1 | 1.77 (1.03-3.04) | 18.1 | 1.99 (1.08-3.69) |
| **Cardiovascular mortality, AMI** | Reference | 8.4 | 1.00 | 6.7 | 1.00 |
| | 75-89.9 | 9.0 | 1.05 (0.81-1.35) | 6.9 | 0.99 (0.74-1.33) |
| | 60-74.9 | 10.0 | 1.15 (0.89-1.48) | 8.0 | 1.10 (0.82-1.46) |
| | 45-59.9 | 12.1 | 1.31 (0.97-1.77) | 11.2 | 1.50 (1.09-2.08) |
| | <45 | 15.7 | 1.62 (0.90-2.93) | 15.3 | 1.86 (0.96-3.60) |
| **Cardiovascular mortality, AMI** | Reference | 13.4 | 1.00 | 10.5 | 1.00 |
| **and UAP** | 75-89.9 | 12.9 | 0.93 (0.76-1.15) | 11.2 | 1.04 (0.83-1.32) |
| | 60-74.9 | 14.8 | 1.06 (0.86-1.30) | 12.6 | 1.13 (0.90-1.42) |
| | 45-59.9 | 18.1 | 1.26 (0.99-1.60) | 15.5 | 1.38 (1.06-1.80) |
| | <45 | 23.6 | 1.58 (0.98-2.56) | 23.6 | 2.00 (1.18-3.41) |
| **Fatal and non fatal AMI** | Reference | 4.9 | 1.00 | 4.4 | 1.00 |
| | 75-89.9 | 7.2 | 1.47 (1.07-2.03) | 5.4 | 1.20 (0.84-1.70) |
| | 60-74.9 | 7.3 | 1.54 (1.12-2.12) | 4.7 | 1.03 (0.72-1.47) |
| | 45-59.9 | 7.1 | 1.46 (0.99-2.16) | 7.3 | 1.65 (1.11-2.45) |
| | <45 | 9.0 | 1.82 (0.84-3.94) | 6.9 | 1.56 (0.60-4.03) |
| **Total mortality** | Reference | 6.6 | 1.00 | 4.8 | 1.00 |
| | 75-89.9 | 5.5 | 0.74 (0.54-0.99) | 4.8 | 0.86 (0.61-1.22) |
| | 60-74.9 | 6.7 | 0.80 (0.59-1.05) | 6.5 | 1.04 (0.75-1.45) |
| | 45-59.9 | 9.5 | 0.94 (0.67-1.31) | 8.8 | 1.23 (0.85-1.78) |
| | <45 | 14.6 | 1.28 (0.69-2.40) | 16,7 | 1.72 (0.88-2.33) |
| **Cardiovascular mortality** | Reference | 4.0 | 1.00 | 2.6 | 1.00 |
| | 75-89.9 | 2.8 | 0.64 (0.43-0.96) | 2.7 | 0.93 (0.58-1.47) |
| | 60-74.9 | 4.2 | 0.86 (0.59-1.24) | 3.8 | 1.22 (0.78-1.89) |
| | 45-59.9 | 6.2 | 1.06 (0.69-1.62) | 5.3 | 1.49 (0.91-2.45) |
| | <45 | 9.0 | 1.32 (0.60-2.94) | 9.7 | 2.11 (0.88-5.05) |
| **Stroke** | Reference | 1.3 | 1.00 | 0.7 | 1.00 |
| | 75-89.9 | 1.2 | 0.79 (0.40-1.54) | 1.4 | 1.74 (0.75-4.03) |
| | 60-74.9 | 2.1 | 1.16 (0.63-2.14) | 1.7 | 1.88 (0.83-4.26) |
| | 45-59.9 | 1.9 | 0.87 (0.41-1.85) | 2.8 | 2.45 (1.02-5.87) |
| | <45 | 5.6 | 2.13 (0.71-6.36) | 2.8 | 1.84 (0.36-9.29) |
| **Heart failure** | Reference | 1.2 | 1.00 | 0.6 | 1.00 |
| | 75-89.9 | 1.1 | 0.80 (0.40-1.62) | 0.9 | 1.28 (0.50-3.29) |
| | 60-74.9 | 1.9 | 1.20 (0.63-2.28) | 1.1 | 1.41 (0.57-3.50) |
| | 45-59.9 | 2.8 | 1.43 (0.70-2.91) | 1.3 | 1.29 (0.46-3.57) |
| | <45 | 4.5 | 1.75 (0.53-5.74) | 6.9 | 5.39 (1.52-19.1) |

Cox regression multivariable-adjusted hazard ratios adjusted for age, gender, systolic blood pressure, diastolic blood pressure, presence of diabetes mellitus, hypercholesterolemia, current smoking, history of coronary artery disease (MI, PCI, CABG). CA= cardiac arrest, AMI= acute myocardial infarction, UAP= unstable angina pectoris.

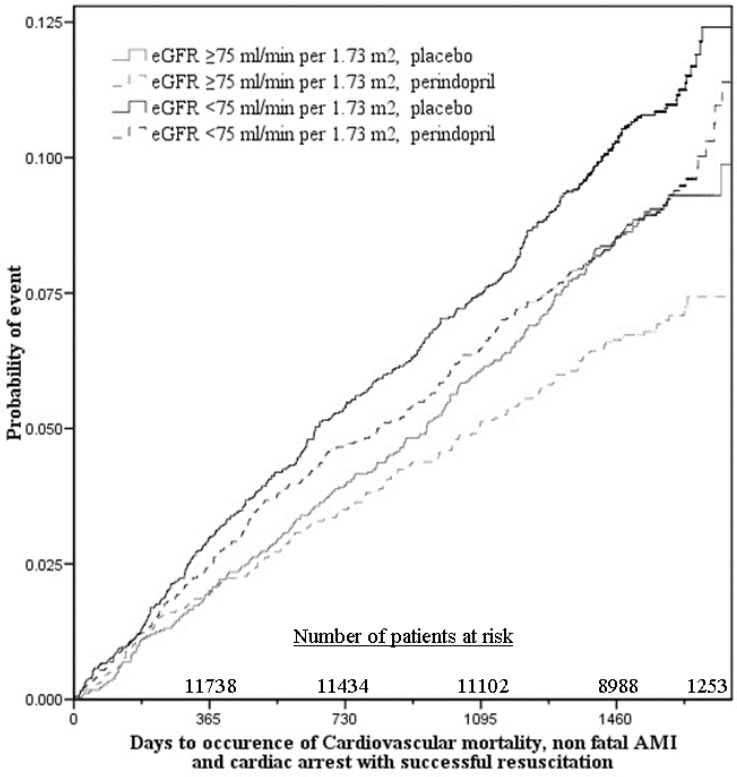



**Figure 2.** Kaplan-Meier Estimates of Primary End Point With Perindopril and Placebo According to Baseline eGFR. The **black line** corresponds to patients with an eGFR <75 using placebo, and the **dotted black line** corresponds to patients with an eGFR <75 using perindopril. The **red line** corresponds to patients with an eGFR ≥75 using placebo, and the **dotted red line** corresponds to patients with an eGFR ≥75 using perindopril. The X-axis represents the follow-up time in days. The Y-axis represents the risk of the primary end point. AMI = acute myocardial infarction.

## DISCUSSION

This analysis confirms that perindopril is effective in reducing cardiovascular events in patients with stable CAD irrespective of renal function. Treatment benefit by perindopril is substantial and consistent in patients with and without impaired renal function. Hence, renal function, as measured by eGFR, cannot be used to select a target population that will benefit most from ACE inhibition.

Regarding clinical outcome, we showed a significant relationship with renal function. With worsening eGFR, patients showed higher comorbidity and the associated HRs increased considerably for all end points. On a continuous scale, each 10 ml/min/1.73 $m^2$ decrease in eGFR was related to a 6.5% increase in risk of the primary end point in the placebo group. The relation between renal function and risk of cardiovascular events has been intensively investigated for

**Table 3.** Treatment benefit of perindopril at different levels of renal function (n=12,056)

| | **eGFR 60** | | **eGFR 75** | | **eGFR 90** | |
|---|---|---|---|---|---|---|
| | HR 95% CI | Pint | HR 95% CI | Pint | HR 95% CI | Pint |
| Primary endpoint | 0.77 (0.68-0.89) | 0.19 | 0.77 (0.64-0.93) | 0.47 | 0.76 (0.56-1.04) | 0.71 |
| | 0.96 (0.74-1.24) | | 0.84 (0.72-0.98) | | 0.82 (0.72-0.93) | |
| Total mortality, AMI, UAP or cardiac arrest | 0.84 (0.76-0.93) | 0.48 | 0.83 (0.72-0.95) | 0.44 | 0.76 (0.61-0.96) | 0.30 |
| | 0.92 (0.76-1.13) | | 0.89 (0.79-1.00) | | 0.88 (0.80-0.97) | |
| CV mortality, AMI and stroke | 0.79 (0.69-0.90) | 0.12 | 0.79 (0.66-0.94) | 0.48 | 0.75 (0.55-1.01) | 0.51 |
| | 1.00 (0.78-1.27) | | 0.86 (0.74-0.99) | | 0.84 0.74-0.95) | |
| CV mortality, AMI | 0.78 (0.68-0.89) | 0.20 | 0.77 (0.64-0.93) | 0.50 | 0.78 (0.57-1.07) | 0.81 |
| | 0.96 (0.74-1.24) | | 0.84 (0.72-0.99) | | 0.82 (0.72-0.93) | |
| CV mortality, AMI and UAP | 0.84 (0.75-0.93) | 0.76 | 0.83 (0.72-0.97) | 0.81 | 0.76 (0.59-0.97) | 0.41 |
| | 0.88 (0.71-1.09) | | 0.86 (0.75-0.97) | | 0.86 (0.77-0.95) | |
| Fatal and non fatal AMI, UAP | 0.82 (0.73-0.93) | 0.65 | 0.86 (0.73-1.01) | 0.62 | 0.80 (0.60-1.06) | 0.85 |
| | 0.89 (0.69-1.14) | | 0.82 (0.70-0.94) | | 0.84 (0.74-0.94) | |
| Fatal and non fatal AMI | 0.71 (0.61-0.84) | 0.06 | 0.79 (0.63-0.98) | 0.74 | 0.88 (0.59-1.30) | 0.45 |
| | 1.04 (0.75-1.46) | | 0.75 (0.62-0.92) | | 0.75 (0.64-0.88) | |
| Total mortality | 0.87 (0.74-1.03) | 0.72 | 0.80 (0.64-1.00) | 0.25 | 0.71 (0.49-1.02) | 0.24 |
| | 0.97 (0.72-1.29) | | 0.97 (0.81-1.16) | | 0.93 (0.80-1.08) | |
| CV mortality | 0.86 (0.69-1.06) | 0.94 | 0.81 (0.60-1.09) | 0.70 | 0.65 (0.59-0.94) | 0.32 |
| | 0.90 (0.62-1.30) | | 0.91 (0.72-1.14) | | 0.91 (0.74-1.11) | |
| Stroke | 0.86 (0.62-1.18) | 0.28 | 0.89 (0.55-1.44) | 0.79 | 0.50 (0.20-1.25) | 0.51 |
| | 1.24 (0.70-2.20) | | 0.96 (0.68-1.35) | | 1.00 (0.75-1.35) | |
| Heart failure | 0.64 (0.44-0.92) | 0.84 | 0.65 (0.38-1.12) | 0.80 | 0.46 (0.17-1.21) | 0.16 |
| | 0.59 (0.32-1.08) | | 0.61 (0.42-0.90) | | 0.65 (0.47-0.91) | |

Cox regression multivariable-adjusted hazard ratios adjusted for age, gender, systolic and diastolic BP, diabetes mellitus, hypercholesterolemia, current smoking, history of CAD (MI, PCI, CABG). Analysis of treatment effect by perindopril at different cut-off levels of eGFR: upper line corresponds to patients with an eGFR above the cut-off level and the lower line with an eGFR below the mentioned cut-off level for each endpoint (dichotomous ≥ or <60; ≥ or <75; ≥ or <90). CV = cardiovascular, AMI = acute myocardial infarction, UAP = unstable angina pectoris. Pint = p-value for testing interaction.





**Table 4.** Absolute risk reduction by perindopril for the primary endpoint (n=12,056).

| | **eGFR above 75** | | | **eGFR below 75** | | |
|---|---|---|---|---|---|---|
| Follow-up | Placebo | Perindopril | Risk Difference | Placebo | Perindopril | Risk Difference |
| 1 yr. | 2.01 % | 1.96 % | -0.05 % | 3.01 % | 2.44 % | -0.57 % |
| 2 yrs. | 3.95 % | 3.54 % | -0.41 % | 5.41 % | 4.72 % | -0.69 % |
| 3 yrs. | 6.08 % | 5.12 % | -0.96 % | 7.51 % | 6.49 % | -1.02 % |
| 4 yrs. | 8.54 % | 6.64 % | -1.90 % | 10.29 % | 8.52 % | -1.77 % |

Absolute risks during follow-up were calculated using Kaplan-Meier analysis.

**Table 5.** Comparing data from the EUROPA and PEACE-trials.

| | no. of events / no. of controls (%) | |
|---|---|---|
| | EUROPA | PEACE |
| Total mortality | 420/6108 (6.9) | 334/4132 (8.1) |
| Cardiovascular mortality | 249/6108 (4.1) | 152/4132 (3.7) |
| Non-fatal MI | 378/6108 (6.2) | 220/4132 (5.3) |

Mean follow-up EUROPA 4.2 years, PEACE 4.8 years.

several years. It has been suggested that the increased risk can be explained by the co-occurrence of a high prevalence of baseline risk factors [17,18]. However, in our study, the observed relationships remained significant after multivariable analysis including these factors. Another explanation may be that renal function is a marker of ongoing or pre-existing atherosclerosis starting in the smallest vessels at the glomerulus, explaining the increased risk of subjects with only mildly decreased renal function [16–18]. Regarding the observed treatment effect, we showed that perindopril reduced events in all patients with stable CAD regardless of the level of renal function. The relative reduction in the incidence of the primary end point by perindopril was somewhat better for patients with relatively preserved renal function. However, CIs were overlapping and we observed no significant interaction between treatment and renal function.

A recent substudy of the PEACE trial investigated the relationship between renal function and the effectiveness of ACE inhibition therapy in stable CAD [8]. In that study, patients with an eGFR <60 showed a significant treatment effect of trandolapril on total mortality, but not on the other studied end points nor in patients with better levels of renal function. The investigators observed a significant heterogeneity in treatment effect in relation to renal function. The inconsistency of the treatment effect of trandolapril was mainly related to the lack of benefit in patients with an eGFR >60. Therefore, they concluded that an impaired renal function defined a subset of CAD patients more likely to benefit from ACE inhibitor therapy for cardiovascular protection [8,9].

The results of the PEACE trial could not be confirmed by our analysis. Both trials studied a population of stable CAD patients with a similar cardiovascular risk profile and a similar eGFR and gender distribution. In contrast to the PEACE trial, we have shown considerable treatment benefits at different levels of renal function and no heterogeneity in the treatment effect of perindopril. In particular, no heterogeneity in the treatment effect was observed on total mortality and cardiovascular mortality in contrast to the PEACE trial analysis. The direction of the treatment benefit by ACE inhibition is different because point estimates were somewhat better at higher eGFR levels, implying that the treatment effect is also present in patients with relatively preserved renal function. The difference in direction must be considered against the background of the overall neutral results of the main PEACE trial. Our analysis confirms that the treatment benefit of ACE inhibition with perindopril is consistent within subgroups, which is in line with our prior subgroup analyses and risk models [2,19]. The HOPE (Heart Outcome and Prevention Evaluation) and SAVE (Survival and Ventricular Enlargement) trials studied the relationship between renal function and treatment effect of ACE inhibition, respectivelyramipril and captopril, in a high-risk population [20,21]. In these patients, no heterogeneity in treatment effect in relation to renal function was shown.

In the main study of the PEACE trial, the overall treatment effect of trandolapril was neutral [10]. The investigators performed subgroup analyses for possible explanations for this neutral finding. They stated that their study consisted of relatively few patients with poor renal function (16.3% eGFR <60). Trandolapril reduced the incidence of total mortality only in patients with

poor renal function. Because of this low prevalence in the PEACE trial, the investigators stated that this could potentially explain the overall neutral results. However, the distribution of eGFR in the EUROPA trial was similar (15.9% eGFR <60). Still, the overall effect of the main EUROPA study was in favor of ACE inhibition therapy [2]. The different result in the PEACE trial may be explained by the fact that the PEACE study potentially had the lowest-risk population.

Renal insufficiency could identify a higher risk subgroup and hence explain why the PEACE trial shows a benefit only in this subgroup in an otherwise low-risk population. However, subgroup analyses of the HOPE and EUROPA studies in low-risk groups showed similar event rates compared with those of the PEACE study, and in low-risk groups of the EUROPA study, perindopril reduced the risk of cardiovascular mortality and non fatal MI by 17%, contrasting with 3% in the PEACE trial. These analyses indicate that the apparent neutral results of the PEACE trial may not be attributable to the lower risk of these patients nor to the background therapies used, but rather are related to the reduced power of the PEACE trial caused by greater crossover and shorter follow-up than in the other studies [1,2,4,10,19]. In addition, the different results may be related to substance-specific or (target) dose-dependent differences between ACE inhibitors potentially in relation to the level of renal function, which may have resulted in suboptimal dosages [22]. In the EUROPA trial, patients were assigned to receive a relatively high dose of perindopril (8 mg), which was achieved rapidly and in a high proportion of patients, whereas in the PEACE trial, trandolapril was up-titrated to the target dose (4 mg) only 6 months after randomization. At 3 years, target dose was achieved in 57.8% of patients in the PEACE trial and 93.0% of patients in the EUROPA trial. Both agents are in a broadly similar ACE inhibitor subgroup, share chemical moieties, are lipophilic, and are mainly excreted from the kidney and were used in doses that showed important pharmacologic effects. Still, without head-to-head trials it cannot be excluded that there are pharmacologic differences between the agents, possibly in relation to renal insufficiency, that are important to their clinical efficacy to reduce cardiovascular end points [22].

In an additional analysis, we investigated whether the treatment effect of perindopril showed any differences between the renal groups in absolute risks during follow-up. Impaired renal function was associated with higher comorbidity, such as hypertension, and worse clinical outcome in our study. This may explain the small difference in absolute risk reduction in the beginning of follow-up, in which this group shows a direct benefit presumably from the blood pressure lowering effects. Still, at longer follow-up the absolute benefits of perindopril were the same in both groups, which may further be related to the additional effects of ACE inhibitors (beyond lowering blood pressure). The ACE inhibitors with high tissue affinity especially improve the angiotensin II– bradykinin balance, reduce remodeling, improve endothelial function, and may have antiatherosclerotic effects [23,24].

## Clinical perspective

Remarks on the consistency of thetreatment effect of ACE inhibition in patients with stable CAD are clinically relevant. In our study, in contrast to the PEACE trial, also patients with an eGFR >60 ml/min/ 1.73 $m^2$ benefited from ACE inhibition by perindopril (eGFR >60: 83.4% in the PEACE trial, 84.1% in the EUROPA trial). In patients with mild renal insufficiency, an increased risk of cardiovascular events was already apparent and perindopril significantly reduced cardiovascular events. Therefore, the earliest stages of renal insufficiency can be considered a key target for preventing the progression of renal disease and to decrease the risk of cardiovascular disease, especially when we also take into account the recent remarks on potential renoprotective effects of ACE inhibitors. We question the conclusion of the PEACE trial to specifically target therapy to patients with an eGFR <60 as the subgroup more likely to benefit from ACE inhibition.

Some limitations of this study can be noted. The generalizability of these findings to patients with severe renal insufficiency is limited because numbers in the lowest eGFR category were relatively small, which limits our statistical power to detect differences in treatment benefit in these patients. The current analysis mainly addresses patients with mild to moderate renal insufficiency according to the National Kidney Foundation Kidney Disease Outcomes Quality Initiative guidelines [25]. Furthermore, the eGFR levels calculated with the Modification of Diet in Renal Diseaseequation remain an estimate of the true GFR; however, it is superior to using the serum creatinine level or the Cockcroft-Gault equation [12]. Unfortunately, we did not have data on microalbuminuria [26].

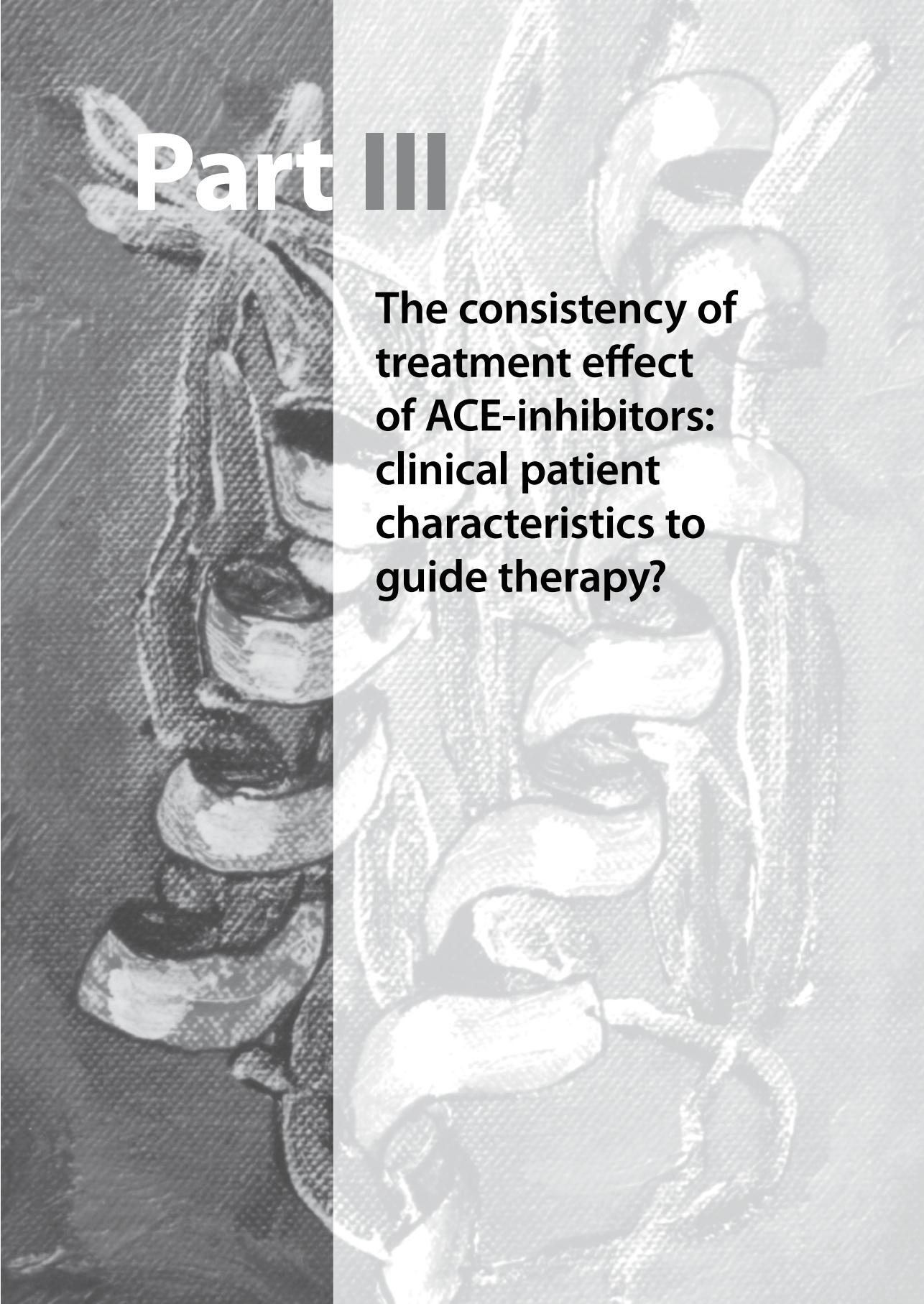
## Conclusions

The treatment benefit of perindopril is consistent and not modified by the level of renal function in patients with stable CAD. We observed no heterogeneity in the treatment effect of perindopril in relation to mild or moderate renal insufficiency.

## REFERENCES


1. Yusuf S, Sleight P, Pogue J, et al., the Heart Outcomes Prevention Evaluation Study Investigators. Effects of an angiotensin-converting enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. *N Engl J Med* 2000;342:145–53.
2. The European Trial on Reduction of Cardiac Events With Perindopril in Stable Coronary Artery Disease Investigators. Efficacy of perindopril in reduction of cardiovascular events among patients with stable coronary heart disease: randomized, double-blind, placebo-controlled, multicentre trial (the EUROPA study). *Lancet* 2003;362:782– 8.
3. Pfeffer MA, Braunwald E, Hawkins CM, for the SAVE Investigators. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction: results of the Survival and Ventricular Enlargement trial. *N Engl J Med* 1992;327: 669–77.
4. Dagenais GR, Pogue J, Fox K, Simoons ML, Yusuf S. Angiotensin converting-enzyme inhibitors in stable vascular disease without left ventricular systolic dysfunction or heart failure: a combined analysis of three trials. *Lancet* 2006;368:581– 8.
5. Smith SC, Allen J, Taubert KA, et al. AHA/ACC guidelines for secondary prevention for patients with coronary and other atherosclerotic vascular disease: 2006 update. *J Am Coll Cardiol* 2006; 47:2130 –9.
6. Sarnak MJ, Levey AS, Schoolwerth AC, et al. Kidney disease as a risk factor for development of cardiovascular disease. A statement from the AHA Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. *Circulation* 2003;108:2154–69.
7. Anavekar NS, McMurray JJ, Velazquez EJ, et al. Relation between renal dysfunction and cardiovascular outcomes after myocardial infarction. *N Engl J Med* 2004;351:1285–95.
8. Solomon SD, Rice MM, Braunwald E, et al. Renal function and the effectiveness of angiotensin-converting enzyme inhibitor therapy in patients with chronic stable coronary disease in the Prevention of Events With ACE Inhibition (PEACE) trial. *Circulation* 2006;114: 26–31.
9. McCullough PA. Chronic kidney disease: tipping the scale to the benefit of ACE inhibitors in patients with coronary artery disease. *Circulation* 2006;114:6 –7.
10. Braunwald E, Domanski MJ, Fowler SE, et al.; the PEACE Trial Investigators. Angiotensin-converting enzyme inhibition in stable coronary artery disease. *N Engl J Med* 2004;351:2058–68.
11. Gomma AH, Fox KM, for the EUROPA Investigators. The EUROPA trial: design, baseline demography and status of the substudies. *Cardiovasc Drugs Ther* 2001;15:169 –79.
12. Levey A, Bosch J, Lewis J, et al. A more accurate method to estimateglomerular filtration rate from serum creatinine: a new prediction equation. *Ann Intern Med* 1999;130:461–70.
13. The Joint European Society of Cardiology/American College of Cardiology Committee. Myocardial infarction redefined: a consensus document of The Joint European Society of Cardiology/ American College of Cardiology Committee for the redefinition of myocardial infarction. *Eur Heart J* 2000;21:1502–13.
14. Schrier RW. Role of diminished renal function in cardiovascular mortality: marker or pathogenetic factor? *J Am Coll Cardiol* 2006;47: 1–8.
15. Fried LF, Shlipak MG, Newman AB. Renal insufficiency as a predictor of cardiovascular outcomes and mortality in elderly individuals. *J Am Coll Cardiol* 2003;41:1364 –72.
16. Brugts JJ, Knetsch AM, Mattace-Raso FU, Hofman A, Witteman J.Renal function and risk of myocardial infarction in an elderly population: the Rotterdam Study. *Arch Intern Med* 2005;165:2659–65.
17. Manjunath G, Tighiouart H, Ibrahim H, et al. Level of kidney function as a risk factor for atherosclerotic cardiovascular outcomes in the community. *J Am Coll Cardiol* 2003;41:47–55.
18. Mann JFE, Dulau-Florea I, Franke J, et al. Looking for people at high cardiovascular risk? Look at serum creatinine. *Eur Heart J* 2003;24: 381–3.
19. Deckers JW, Fox K, Simoons ML. Treatment benefit by perindopril in patients with stable coronary artery disease at different levels of risk. *Eur Heart J* 2006;27:796–801.

20. Mann JF, Gerstein HC, Yusuf S, et al. Renal insufficiency as a predictor of cardiovascular outcomes and the impact of ramipril: the HOPE randomized trial. *Ann Intern Med* 2001;134:629 –36.
21. Tokmakova MP, Skali H, Solomon SD, et al. Chronic kidney disease, cardiovascular risk and response to angiotensin-converting enzyme inhibition after myocardial infarction. The Survival and Ventricular Enlargement (SAVE) study. *Circulation* 2004;110:3667–73.
22. Friedrich EB, Teo KK, Bohm M, et al. ACE-inhibition in secondary prevention: are the results controversial? *Clin Res Cardiol* 2006;95: 61–7.
23. Voors AA, van Veldhuisen DJ, van Gilst WH, et al. The current role of ACE inhibitors for secondary prevention in cardiovascular disease; from pathogenesis to clinical practice. *Cardiovasc Drugs Ther* 2006; 20:69 –73.
24. Ferrari R, Pasanisi G, Ceconi C, et al. Specific properties and effect of perindopril in controlling the renin-angiotensin system. *Am J Hypertens* 2005;18:142–54.
25. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification and stratification. *Am J Kidney Dis* 2002;39 Suppl 1:S1–266.
26. Knobler H, Zornitzki T, Vered S, et al. Reduced glomerular filtration rate in asymptomatic diabetic patients: predictor of increased risk for cardiac events independent of albuminuria. *J Am Coll Cardiol* 2004;44:2142– 8.



# Part III

## The consistency of treatment effect of ACE-inhibitors: clinical patient characteristics to guide therapy?

# Chapter 7

# The consistency of the treatment effect of ACE-inhibitor therapy in patients with vascular disease: A combined analysis of ADVANCE, EUROPA and PROGRESS trials.

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## ABSTRACT

**Aims:** Angiotensin-converting enzyme (ACE) inhibitors have been shown to reduce cardiovascular risk in different groups of patients. Whether these effects can be generalized to the broad group of patients with vascular disease is unknown. Therefore, we undertook a combined analysis using individual data from ADVANCE, EUROPA, and PROGRESS to determine the consistency of the treatment effect of perindopril-based regimen in patients with vascular disease or at high risk of vascular disease.

**Methods**: We studied all-cause mortality and major cardiovascular outcomes during a follow-up of about 4 years in the 29.463 patients randomly assigned a perindopril-based treatment regimen or placebo.

**Results:** The perindopril-based regimens were associated with a significant reduction in all-cause mortality [hazard ratio (HR) 0.89; 95% confidence interval (CI) 0.82–0.96; P = 0.006], cardiovascular mortality (HR 0.85; 95% CI 0.76–0.95; P = 0.004), non-fatal myocardial infarction (HR 0.80; 95% CI 0.71–0.90; P < 0.001), stroke (HR 0.82; 95% CI 0.74–0.92; P = 0.002), and heart failure (HR 0.84; 95% CI 0.72–0.96; P = 0.015). Results were consistent in subgroups with different clinical characteristics, concomitant medication use, and across all strata of baseline blood pressure.

**Conclusion:** This study provides strong evidence for a consistent cardiovascular protection with an ACE-inhibitor treatment regimen (perindopril–indapamide) by improving survival and reducing the risk of major cardiovascular events across a broad spectrum of patients with vascular disease.

## INTRODUCTION

Clinical trials have demonstrated the efficacy of angiotensin-converting enzyme (ACE)-inhibitors in specific groups of patients at risk of cardiovascular events[1–9]. Nowadays, the use of ACE-inhibitors is recommended in guidelines on the management of hypertension, stable coronary artery disease (CAD), myocardial infarction (MI), and heart failure[10-15]. The beneficial effect of ACE-inhibitors is related, at least in part, to the blood pressure (BP)-lowering effects. However, ACE-inhibitors may have many valuable protective properties quite apart from BP lowering. Especially, perindopril has been extensively investigated in this regard and has been shown to improve endothelial function and neurohumoral balance, and inhibit remodelling of the coronary arteries[16-19]. Since, the BPLTTC has demonstrated that BP-independent effects of ACE-inhibitor-based regimens do contribute to the reduction in CAD[20,21], it becomes more important to base decisions on the treatment of individual patients, on the assessment of total cardiovascular risk, rather than on arbitrary cut-off points for single risk factors such as BP. Most of the evidence currently available has been obtained from studies conducted in patient populations with vascular disease in a single vascular territory or with a metabolic disorder such as diabetes. There is, therefore, a need for a broadly based study pooling individual patient data from patient populations with a wider spectrum of vascular disease.

For this purpose, we undertook a combined analysis of the individual data from trials of perindopril-based regimens in patients with diabetes, CAD, and cerebrovascular disease[7–9]. We investigated the treatment effect on clinical endpoints and their consistency in patient subgroups.

## METHODS

The methodological principles that lie behind a combined analysis of randomized clinical trials based on data from individual patients have been described in detail. [22] We therefore only briefly describe the applied methods of trial selection, data-management, endpoint definitions, and statistical analysis.

### Trial selection

We obtained data from the ADVANCE, EUROPA, and PROGRESS studies that are the three main large trials with a regimen based primarily on the ACE-inhibitor perindopril. The ASCOT-BPLA was not selected because the combined treatment regimen was based on amlodipine with the addition of perindopril so that it was impossible to make unbiased estimates for the treatment effect of perindopril[23]. Since all trials studied a regimen based on the same agent, perindopril, we had the opportunity to include individual data in this combined analysis, which made important subgroup analyses possible at the patient level. The types of patients included in

these studies were different in their primary diagnoses, but since atherosclerosis and vascular disease is not restricted to a single vascular bed, we conclude that these patients are at least homogenous in having vascular disease or being at a high risk of vascular disease. The combined data set consisted of 29.463 patients, who were followed for on average 4 years. We had full access to all individual data of the trials. After data merging, data were tested carefully for completeness, internal consistency of patients' records, and consistency with the published reports. Table 1 shows the main inclusion and exclusion criteria, outcomes, recruitment, and follow-up of the three trials.

**Table 1** Characteristics of clinical trials

| | **ADVANCE** (N=11.140) | **EUROPA** (n=12.218) | **PROGRESS** (n=6.105) |
|---|---|---|---|
| **Main inclusion criteria** | | | |
| Age at entry (years) | ≥ 55 | ≥ 18 | Not specified |
| Type of patients (entry) | Diabetes Mellitus | Stable CAD | Stroke or TIA |
| **Main exclusion criteria** | | | |
| Known CHF | No | No | No |
| Patients with LVEF <40% | Not assessed | 18 / 7096 assessed | Not assessed |
| Myocardial Infarction | --- | Within 3 months | --- |
| Stroke | --- | --- | Within 5 years |
| PCI | --- | Within 6 months | --- |
| CABG | --- | Within 6 months | --- |
| **ACE-inhibitor and target daily dose** | Perindopril 4 mg / Indapamide 1.25 mg | Perindopril 8 mg | Perindopril 4 mg / Indapamide 2.5 mg |
| **Main outcomes** | | | |
| Primary (composite) | Major macro- or microvascular events | Cardiac death, MI, or cardiac arrest. | Fatal or non-fatal stroke. |
| **Recruitment** | | | |
| Recruitment period | June 2001, to March 2003 | October 1997, to June 2000 | May 1995, to November 1997 |
| Mean follow-up duration | 4.3 years | 4.2 years | 3.9 years |

MI= myocardial infarction, UAP= unstable angina pectoris, CHF = congestive heart failure, LVEF = left ventricular ejection fraction, TIA= transient ischaemic attack, Sd =standard deviation, HF = heart failure, ACE= angiotensin-converting enzyme.



## Outcomes

In this analysis, we assessed all-cause mortality, cardiovascular mortality, fatal and non-fatal stroke, revascularization, non-fatal MI, hospital admission for heart failure, and major cardiovascular endpoints as a composite of cardiovascular mortality, MI, and stroke. The definitions of endpoints in these trials were carefully checked and were not essentially different across the trials. Where there were slight differences in endpoints or thresholds, we did not try to match everything, since that this is often impossible retrospectively. Moreover, this is not necessary

since in each study the treatment effects were compared within rigorously randomized and well-balanced treatment groups. Since we know that heterogeneity in an endpoint definition will not lead to invalid results, we applied the trial-specific definition of MI for practical reasons[24]. Several endpoints were not presented in the main papers of the ADVANCE and PROGRESS trials, for example, the data for revascularization. For a comprehensive and comparable analysis, these endpoints were included.

## Subgroups

We tested the consistency of the treatment effect of perindopril-based regimens in relation to baseline clinical characteristics [gender, age categories (<60; 60–70; >70 years), mean age (<63; >63 years) hypertension, diabetes, cerebrovascular accident (CVA) / transient ischaemic attack (TIA), and revascularization] and in relation to concomitant medication (anti-platelet agents, lipid-lowering agents, b-blockers, diuretics, and calcium antagonists). We investigated the treatment effect of perindopril-based regimen on the outcomes according to BP levels at baseline (first screening visit). Ordinal categories of systolic BP and diastolic BP were defined as follows: systolic BP ,120, 120–139, 140–159, and ≥160 mmHg; diastolic BP <80, 80–90, 90–100, and ≥100 mmHg. Further, we investigated whether the treatment effect was independent of the level of BP-reduction by active treatment. All patients were treated during run-in period of 4 weeks. Blood pressure reduction was calculated as the difference between the baseline measurement and the end of the run-in period. We accounted for the difference in dosage of perindopril (EUROPA 4–8 mg, ADVANCE and PROGRESS 2–4 mg) and combination with indapamide (ADVANCE and PROGRESS 2.5 mg) in all analyses. We emphasize that for analyses of BP reduction during run-in, baseline BP was added as continuous variable in the model to adjust for the starting level of BP before initiation of ACE-inhibitor treatment (avoids regression to the mean).

## Statistical analysis

The statistical principles used in these analyses have been described previously in the main papers of the trials for the assessment of reduction in outcomes. Although baseline characteristics are well balanced between the randomized groups for each trial, we choose to perform a multivariate Cox regression analysis because of the difference between the studies themselves and because adjustment for baseline characteristics, even when randomized, is recommended in clinical trials[25]. By multivariate analysis, confidence intervals (CIs) will widen slightly (although compensated by the large number of patients) but the validity will increase which is most important. In multivariate Cox regression analysis, we adjusted for age, gender, hypertension, diabetes, smoking, prior MI, prior percutaneous coronary intervention / coronary artery bypass grafting, prior CVA/TIA, medication use (anti-platelet agents, b-blockers, diuretics, calcium-antagonists, and lipid-lowering agents), indapamide use, and active treatment (perindopril) dosage. We further adjusted for differences in baseline risk across trials by adding dummy study variables to the model. Tests for heterogeneity in the treatment effects were

performed by including interaction terms [treatment * characteristic] in the multivariate model for each covariate separately. Test for heterogeneity among trials was by including interaction terms of dummy study variables with treatment, and we calculated the difference in -2 log likelihood of two models. The difference chi-square (Q-test) between the two -2 log likelihoods follows chi-square distribution. If P <0.05, then there is evidence of heterogeneity between trials. Survival analysis was performed by the Kaplan–Meier analysis. Hazard ratios (HR) and 95% CIs are presented with corresponding two-sided P-values. A p-value <0.05 was considered significant. In all trials, analysis was by intention-to-treat principle.

## RESULTS

The baseline characteristics of the total study population (n = 29.463) are summarized in Table 2. The mean (SD) age was 63.0 (8.8) years, 28.4% were female, 54.1% hypertensives, 45.5% diabetics, and 32.8% experienced a previous MI. Mean BP was 142/82 mmHg, 70.8% were

**Table 2.** Baseline characteristics patients in the ADVANCE, EUROPA and PROGRESS trials.

| | **ADVANCE (n=11.140)** | **EUROPA (n=12.218)** | **PROGRESS (n=6.105)** | **Combined (n=29.463)** |
|---|---|---|---|---|
| Characteristics | | | | |
| Age (years), mean (Sd) | 66 (6) | 60 (9) | 64 (10) | 63 (9) |
| Female (%) | 42.5 | 14.6 | 30.3 | 28.4 |
| Previous MI (%) | 12.0 | 64.8 | 7.0 | 32.8 |
| Previous PCI/CABG (%) | 8.5 | 54.9 | 2.7 | 26.6 |
| Previous CVA/TIA (%) | 12.9 | 3.4 | 99.9* | 27.0 |
| Previous PAD (%) | 2.4 | 7.3 | 4.1 | 4.8 |
| Current smokers (%) | 15.1 | 15.2 | 20.0 | 16.2 |
| Diabetes (%) | 100.0 | 12.3 | 12.5 | 45.5 |
| Hypertension (%) | 68.7 | 27.1 | 47.8 | 54.1 |
| Hypercholesterolemia (%) | 58.9 | 63.3 | --- | 61.2** |
| Systolic blood pressure (mmHg) | 145 (21) | 137 (15) | 147 (9) | 142 (19) |
| Diastolic blood pressure (mmHg) | 81 (11) | 82 (8) | 86 (11) | 82 (10) |
| | | | | |
| Medications | | | | |
| Antiplatelet agents (%) | 46.7 | 92.3 | 72.3 | 70.8 |
| β-blockers (%) | 24.5 | 61.7 | 17.0 | 38.8 |
| Lipid-lowering agents (%) | 35.3 | 55.9 | 14.1 | 39.5 |
| Calcium antagonists (%) | 30.8 | 31.4 | 39.9 | 33.3 |
| Diuretics use (%) † | 9.2 | 23.7 | 11.5 | 15.1 |

Summary statistics for continuous variables are presented as mean (standard deviation (SD).) Categorical data are summarized as percentages. * Protocol violation for 7/6105 patients. ** Hypercholesterolemia data was not present in PROGRESS, percentage was 61.2% (14294) based on 23358 patients (advance and europa). † diuretics use, not indapamide study medication. MI= myocardial infarction, PCI= percutaneous coronary intervention, CABG = coronary artery bypass grafting, CVA = cerebrovascular disease, TIA = transient ischemic attack, PAD = peripheral arterial disease.

taking anti-platelet agents, 38.8% beta-blockers, 39.5% lipid-lowering agents, and 33.3% calcium antagonists. Of the 29463 patients, 14730 (50.0%) were randomized to active treatment (perindopril-based regimen) and 14733 (50.0%) received matched placebo. Indapamide was used in 14684 patients (49.8%), and other diuretics in 15.1%.

## Clinical endpoints

The perindopril-based regimen was associated with a significantly lower cumulative incidence of all-cause mortality as well as major cardiovascular events when compared with placebo in the Kaplan–Meier analysis (both log ranks P-value <0.001) as presented in Figure 1a and 1b. During a mean follow-up of 4.0 years (SD 0.8), there were 1089 deaths (7.4%) in the active treatment group and 1210 (8.2%) in the placebo group (HR 0.89; 95% CI 0.82–0.96; P-value = 0.006). No heterogeneity between the studies was observed (P for interaction 0.56). Results for the other endpoints were comparable and are presented in Table 3. Significant heterogeneity in the treatment effect between the studies was observed for heart failure admission (no effect in ADVANCE) and for fatal and non-fatal stroke (no effect in ADVANCE or EUROPA). The perindopril-based regimen did not significantly affect subsequent revascularizations (HR 0.92; 95% CI 0.84–1.01; P-value = 0.092), with 852 revascularizations (5.8%) in treatment group and 920 revascularizations (6.2%) in placebo group.

## Blood pressure

The perindopril-based treatment regimen was associated with lower major cardiovascular event rates across all strata of systolic BP as well as diastolic BP, although in the lower systolic BP categories, the treatment effect estimates did not reach significance. Also, the relative treatment benefit was somewhat larger at higher diastolic BP levels (P for interaction <0.001) (Figure 2a). During the run-in period of four weeks, all patients were treated with perindopril (n = 29463) and BP decreased from 142/82 to 134/78 mmHg. The average BP reduction during run-in was 8.3/3.8 mmHg and during follow-up 5.4/2.3 mmHg. The BP reduction during run-in was comparable in each of the three trials (ADVANCE: 8/3 mmHg; EUROPA 9/4 mmHg; and PROGRESS 9/5 mmHg). The perindopril-based regimen reduced the incidence of major cardiovascular events in all categories of BP reduction during run-in (Figure 2b). However, the relative treatment benefit was somewhat larger in patients with a higher diastolic BP reduction during run-in (P for interaction 0.05).

## Subgroups

The treatment effect of the perindopril-based regimen was independent of baseline clinical characteristics and concomitant medication (Figure 3). In subgroups of age and diabetes mellitus, P for interaction was, respectively, 0.07 and 0.06, but the perindopril-based regimen was associated with a significant positive treatment effect in patients with or without diabetes, and in patients aged <60, 60–70, or >70 years. A dichotomized age variable, at the median age of the

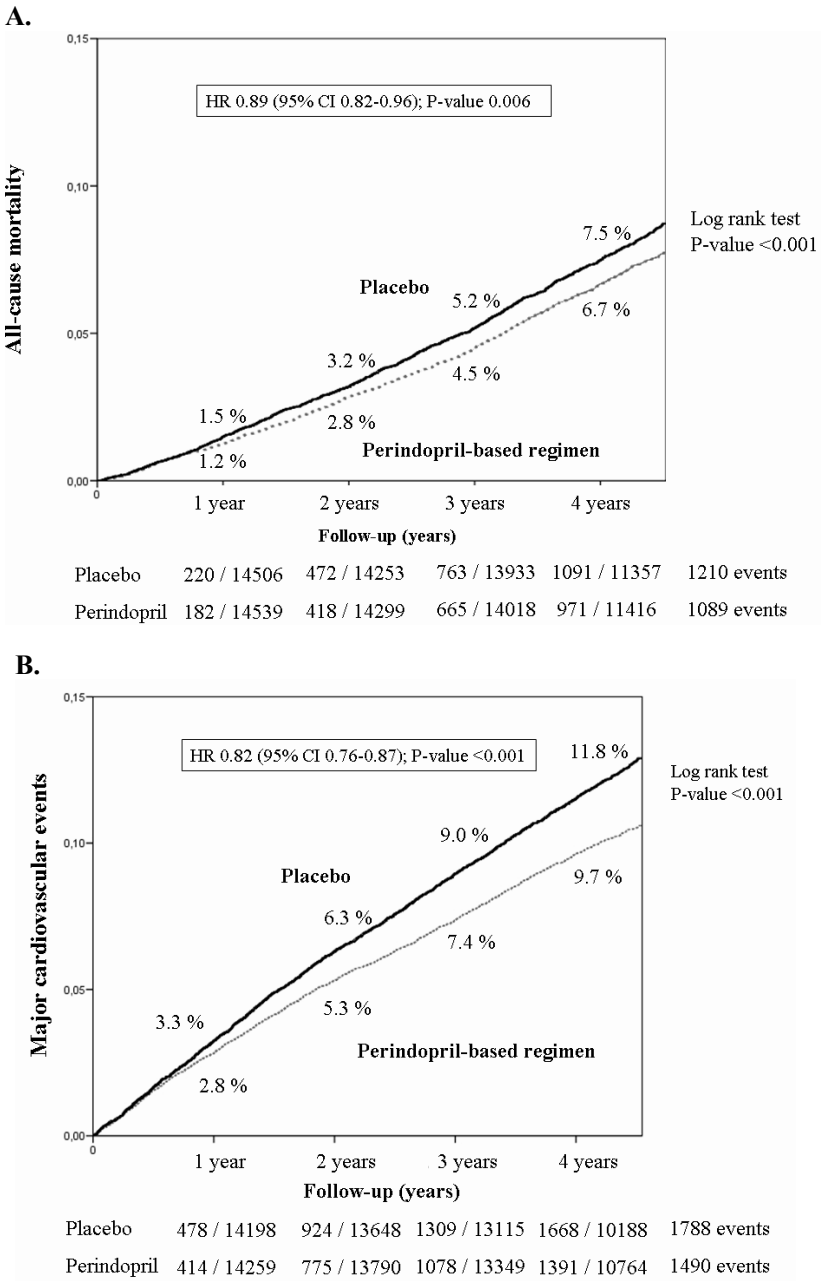


**Figure 1a** and b Kaplan Meier analysis of cumulative incidence of all-cause mortality (A) and major cardiovascular events (cardiovascular mortality, MI and stroke) (B) in 29.463 patients. The x-axis corresponds to the duration of follow-up in years. The y-axis to the cumulative incidence of all-cause mortality **(A)** and major cardiovascular events in percentages (%) **(B)**. The percentages along the lines correspond to the Kaplan–Meier estimates at 1, 2, 3, and 4 years of follow-up in patients allocated the perindopril-based regimen (red line) or placebo (black line). Below the graph, the number of events and the number of patients at risk during follow-up (per year) are presented.

**Table 3.** Data for total mortality and cardiovascular events.

| | Active treatment Events/total (%) | Placebo Events/total (%) | HR 95% CI | P | Pint |
|---|---|---|---|---|---|
| **All-cause mortality** | | | | | |
| ADVANCE (n=11.140) | 408/5569 (7.3) | 471/5571 (8.5) | 0.86 (0.75-0.98) | 0.026 | 0.56 |
| EUROPA (n=12.218) | 375/6110 (6.1) | 420/6108 (6.9) | 0.89 (0.77-1.02) | 0.098 | |
| PROGRESS (n=6105) | 306/3051 (10.0) | 319/3054 (10.4) | 0.96 (0.82-1.12) | 0.596 | |
| Total (n= 29.463) | 1089/14730 (7.4) | 1210/14733 (8.2) | 0.89 (0.82-0.96) | 0.006 | |
| **Cardiovascular mortality** | | | | | |
| ADVANCE (n=11.140) | 211/5569 (3.8) | 257/5571 (4.6) | 0.82 (0.68-0.98) | 0.028 | 0.58 |
| EUROPA (n=12.218) | 215/6110 (3.5) | 249/6108 (4.1) | 0.86 (0.72-1.03) | 0.001 | |
| PROGRESS (n=6105) | 181/3051 (5.9) | 198/3054 (6.5) | 0.91 (0.75-1.12) | 0.380 | |
| Total (n= 29.463) | 607/14730 (4.1) | 704/14733 (4.8) | 0.85 (0.76-0.95) | 0.004 | |
| **Cardiovascular mortality, MI** | | | | | |
| ADVANCE (n=11.140) | 320/5569 (5.7) | 370/5571 (6.6) | 0.86 (0.74-1.00) | 0.045 | 0.82 |
| EUROPA (n=12.218) | 484/6110 (7.9) | 596/6108 (9.8) | 0.80 (0.71-0.91) | <0.001 | |
| PROGRESS (n=6105) | 230/3051 (7.5) | 279/3054 (9.1) | 0.82 (0.69-0.96) | 0.025 | |
| Total (n= 29.463) | 1034/14730 (7.0) | 1245/14733 (8.5) | 0.82 (0.76-0.88) | <0.001 | |
| **Cardiovascular mortality, MI, Stroke** | | | | | |
| ADVANCE (n=11.140) | 480/5569 (8.6) | 520/5571 (9.3) | 0.91 (0.80-1.03) | 0.135 | 0.10 |
| EUROPA (n=12.218) | 552/6110 (9.0) | 664/6108 (10.9) | 0.83 (0.74-0.92) | 0.001 | |
| PROGRESS (n=6105) | 458/3051 (15.0) | 604/3054 (19.8) | 0.74 (0.66-0.84) | <0.001 | |
| Total (n= 29.463) | 1490/14730(10.1) | 1788/14733(12.1) | 0.82 (0.76-0.87) | <0.001 | |
| **Fatal and non-fatal stroke** | | | | | |
| ADVANCE (n=11.140) | 215/5569 (3.9) | 218/5571 (3.9) | 0.98 (0.82-1.19) | 0.755 | 0.02* |
| EUROPA (n=12.218) | 98/6110 (1.6) | 102/6108 (1.7) | 0.96 (0.73-1.27) | 0.774 | |
| PROGRESS (n=6105) | 307/3051 (10.1) | 420/3054 (13.8) | 0.72 (0.62-0.83) | <0.001 | |
| Total (n= 29.463) | 620/14730 (4.2) | 740/14733 (5.0) | 0.82 (0.74-0.92) | 0.002 | |
| **Non-fatal MI** | | | | | |
| ADVANCE (n=11.140) | 136/5569 (2.4) | 135/5571 (2.4) | 1.01 (0.80-1.29) | 0.913 | 0.06 |
| EUROPA (n=12.218) | 295/6110 (4.8) | 378/6108 (6.2) | 0.77 (0.66-0.90) | 0.001 | |
| PROGRESS (n=6105) | 60/3051 (2.0) | 96/3054 (3.1) | 0.62 (0.44-0.86) | 0.003 | |
| Total (n= 29.463) | 491/14730 (3.3) | 609/14733 (4.1) | 0.80 (0.71-0.90) | <0.001 | |
| **Hospitalization for heart failure** | | | | | |
| ADVANCE (n=11.140) | 197/5569 (3.5) | 199/5571 (3.6) | 0.98 (0.81-1.20) | 0.856 | 0.04 |
| EUROPA (n=12.218) | 63/6110 (1.0) | 103/6108 (1.7) | 0.61 (0.44-0.83) | 0.002 | |
| PROGRESS (n=6105) | 75/3051 (2.5) | 93/3054 (3.1) | 0.80 (0.59-1.09) | 0.159 | |
| Total (n= 29.463) | 335/14730 (2.3) | 395/14733 (2.7) | 0.84 (0.72-0.96) | 0.015 | |

Cox regression multivariate analysis was used to calculate hazard ratio's and 95% CI adjusted for age, gender, hypertension, diabetes mellitus, smoking, history of MI, prior revascularization, prior CVA/TIA, use of beta-blockers, lipid-lowering agents, anti-platelet agents, diuretics, calcium antagonists, indapamide, and perindopril dosage. HR=hazard ratio; CI=confidence interval. MI= myocardial infarction, HF= heart failure, Pint = p-value for testing interaction. * In single therapy analysis, P for heterogeneity >0.10.

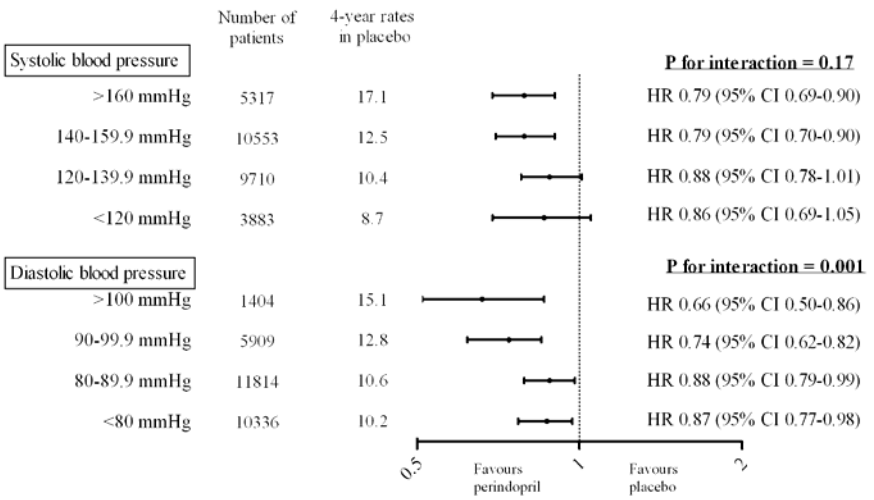


**Figure 2a.** Treatment effect of perindopril-based regimen in relation to major cardiovascular disease (Cardiovascular mortality, MI, stroke) according to baseline blood pressure strata. **(A)** Cox regression multivariate analysis was used to calculate HRs and 95% CI adjusted the full model. Additionally, P-values for interaction were calculated by including an interaction term in the model using systolic and diastolic BP levels as continuous variable * treatment. HR, hazard ratio; CI, confidence interval.



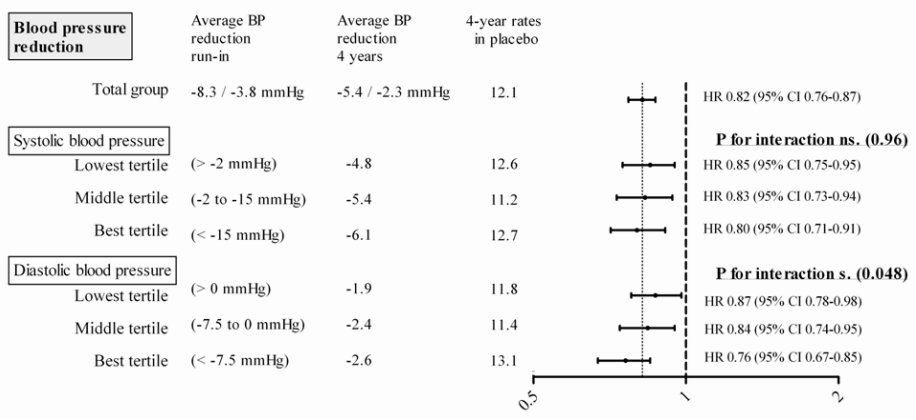


**Figure 2b.** Treatment effect of perindopril-based regimen in relation to major cardiovascular disease (Cardiovascular mortality, MI, stroke) according to blood pressure reduction levels. **(B)** Cox regression multivariate analysis was used to calculate HRs and 95% CI. These analyses were also corrected for the starting systolic and diastolic BP values before application of treatment in all patients. Additionally, P-values for interaction were calculated by including an interaction term in the model using systolic and diastolic BP reduction levels as continuous variable * treatment. MI, myocardial infarction; HR, hazard ratio; CI, confidence interval.

study population (63.0 years), resulted in similar treatment effect estimates (age <63: HR 0.78; 95% CI 0.69–0.87 and age >63: HR 0.85; 95% CI 0.78–0.93). For combinations of concomitant medication use compromising platelet-inhibitors, lipid-lowering agents, calcium antagonists,

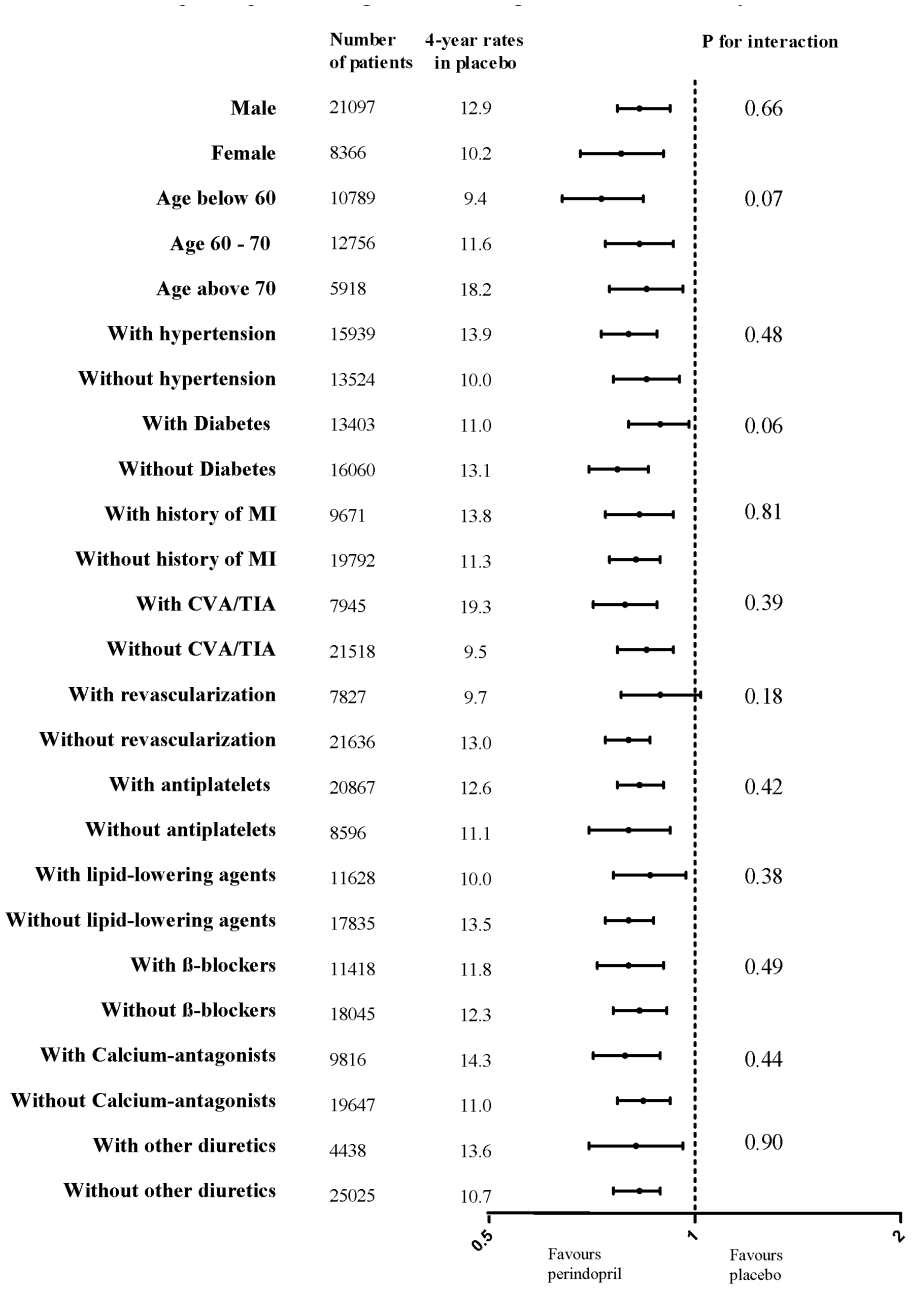


**Figure 3.** Treatment effect of perindopril-based regimen in reducing cardiovascular mortality, MI and stroke according to baseline characteristics. For combinations of concomitant medication use (none, one, two , three, four, all) results were similar (data not shown)

b-blockers, and diuretics use (none, one, two, three, four, or all of the mentioned medication use), HRs were similar as shown for the individual medications and no heterogeneity in the treatment effect was observed (Figure 3, data not shown). Results for other study endpoints were similar and no heterogeneity was observed. Significant heterogeneity between the studies was observed for fatal and non-fatal stroke, non-fatal MI, and hospital admission for heart failure (Table 3). In a separate analysis of single therapy by perindopril (n = 14.779), by excluding all indapamide use, heterogeneity vanished (P = 0.10). Single therapy was no longer associated with a significant treatment benefit on fatal and non-fatal stroke (HR 0.96; 95% CI 0.82–1.12). In contrast, the effect on non-fatal MI and heart failure admissions increased to, respectively, HR 0.76 (95% CI 0.66–0.89) and HR 0.71 (95% CI 0.55–0.92).

## DISCUSSION

The present analysis, based on individual data from 29.463 patients, demonstrates that a perindopril-based regimen reduces the risk of mortality and major cardiovascular events among patients with various levels of cardiovascular risk. Furthermore, the benefit was observed across a wide spectrum of baseline BP levels. Therefore, the use of ACE-inhibitors should be considered in all patients with established vascular disease or at high-risk of vascular disease even in patients with normal BP levels.



A prior study, a meta-analysis, which studied three trials with similar type of patients with CAD (HOPE, EUROPA, and PEACE) showed a consistent treatment effect of ACE-inhibitors in reducing cardiovascular events[26]. The current analysis demonstrates clear evidence for a consistent cardiovascular protection in a broader range of patients with vascular disease including clear benefits in reducing death. These results confirm the generalizability of prior studies in separate patients' groups and support the provision of treatment, not on the basis of arbitrary cut-off points for BP, but rather on the assessment of absolute or total vascular risk, which is raised in patients with stable CAD, diabetes, and stroke as shown in EUROPA, ADVANCE, and PROGRESS. These results are consistent with the findings of the HOPE trial which studied the ACE-inhibitor ramipril[5]. Significant interaction was observed for fatal and non-fatal stroke as a separate outcome (Table 3). Such heterogeneity can be related to differences across trials in the study medication, patient selection, or concomitant medication (Table 2). While ADVANCE and EUROPA showed low stroke rates and no treatment benefit for stroke, PROGRESS showed a significant reduction of 28% in stroke in patients with cerebrovascular disease. Therefore, the ACE-inhibitor-based treatment regimen was more effective in reducing recurrent stroke (PROGRESS) than in reducing incident stroke in patients without a history of cerebrovascular disease (EUROPA and ADVANCE). These benefits were contingent on the BP-lowering effect with additional indapamide use. Single therapy with perindopril was not associated with a significant reduction in the risk of stroke. Additional interaction was observed on the separate outcome of non fatal MI and hospitalization for heart failure (Table 3), mainly caused by the lack of benefit in ADVANCE conducted in patients with DM but most without a history of

macrovascular disease. In both cases, interaction was observed for cause-specific outcomes; however, there was no heterogeneity in reducing the over-arching endpoints of all cause mortality or the composite endpoint of cardiovascular mortality, MI, and stroke. In the analysis of single perindopril therapy, heterogeneity vanished and the risk reduction by single therapy improved for both outcomes.

Regarding BP, the findings from the BPLTTC already suggested some BP-dependent and some BP-independent effects of ACE-inhibitors on the risk of major coronary disease events by using tabular data[20,21,27,28]. Now, we can confirm these findings in a large combined patient-level data analysis with access to individual BP measurements. We showed a lower major cardiovascular event rate across all strata of systolic and diastolic BPs, although not statistically significant for lower systolic BP strata, as well as across the tertiles of BP reduction during run-in. Patients with the highest diastolic BP or highest diastolic BP reduction experienced a somewhat larger relative treatment effect. Also the patients with the highest BP reduction remained on average also at lower BP during follow-up.

The current study is unique and important because it studies the individual data of large trials across a broad range of vascular risk and is focused on one ACE-inhibitor. The use of individual data makes the results robust and provides a unique opportunity to detect the subgroups of patients who might (or might not) benefit the most from treatment. Furthermore, the ACE-inhibitor perindopril has several properties different from other agents in its class, being a long-acting (24 h), once-daily lipophilic ACE-inhibitor with high affinity for both tissue and circulating ACE. Tissue ACE affinity is related to specific anti-atherosclerotic and anti-thrombotic effects, as well as improvements in endothelial function[29]. However, we acknowledge that the exact clinical meaning of these properties remains unknown, since ACE-inhibitors have not been prospectively compared with each other in studies.

Several limitations of this analysis can be noted. The trials differed in the patient selection, adjunctive therapy, and drug dosage. Therefore, we adjusted for perindopril dosage and indapamide use in all analyses and checked for interaction or heterogeneity among trials. This is unlikely to have affected our conclusions as discussed above. Furthermore, we acknowledge that the accuracy of the BP measurements in large trials and different hospital settings may be questioned. However, any inaccuracy would be equally distributed among randomized treatment groups and unlikely to affect our conclusions. Although, the patient populations in the three trials studied were different, it is well established that atherosclerotic vascular disease is a generalized process not restricted to a single vascular bed. Therefore, we believe that our pooled patient sample is broadly representative of patients with established vascular disease at high risk of vascular disease across the world. Despite the differences between the trials, the outcomes are consistent with findings from other different meta-analyses on patients without heart failure[26,28,30,31] and patients with heart failure[32].

The currents study demonstrates that perindopril-based regimens (perindopril in part of the patients combined with indapamide) reduce death in patients with vascular disease.

The consistency of the relative effects across subgroups indicates that the absolute benefits conferred by treatment will be determined mainly by each patient's future risk of vascular complications, rather than their initial level of BP alone or other risk factors.

These results support the provision of ACE-inhibitor-based treatment, not on the basis of arbitrary cut-off points for BP, but rather on the assessment of vascular risk, which is raised in patients with stable CAD, diabetes, and stroke. This approach, based on total cardiovascular risk, should form the basis for the recommendations for treatment in the revision of the major national and international guidelines.

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This combined analysis was initiated by the authors and was designed, conducted, interpreted, and reported independently of the original sponsor. The current study had no funding source or any with a participating role in data collection, outcome assessment, or writing of the manuscript. All authors had joint responsibility for the decision to submit for publication.



*Conflict of interest*

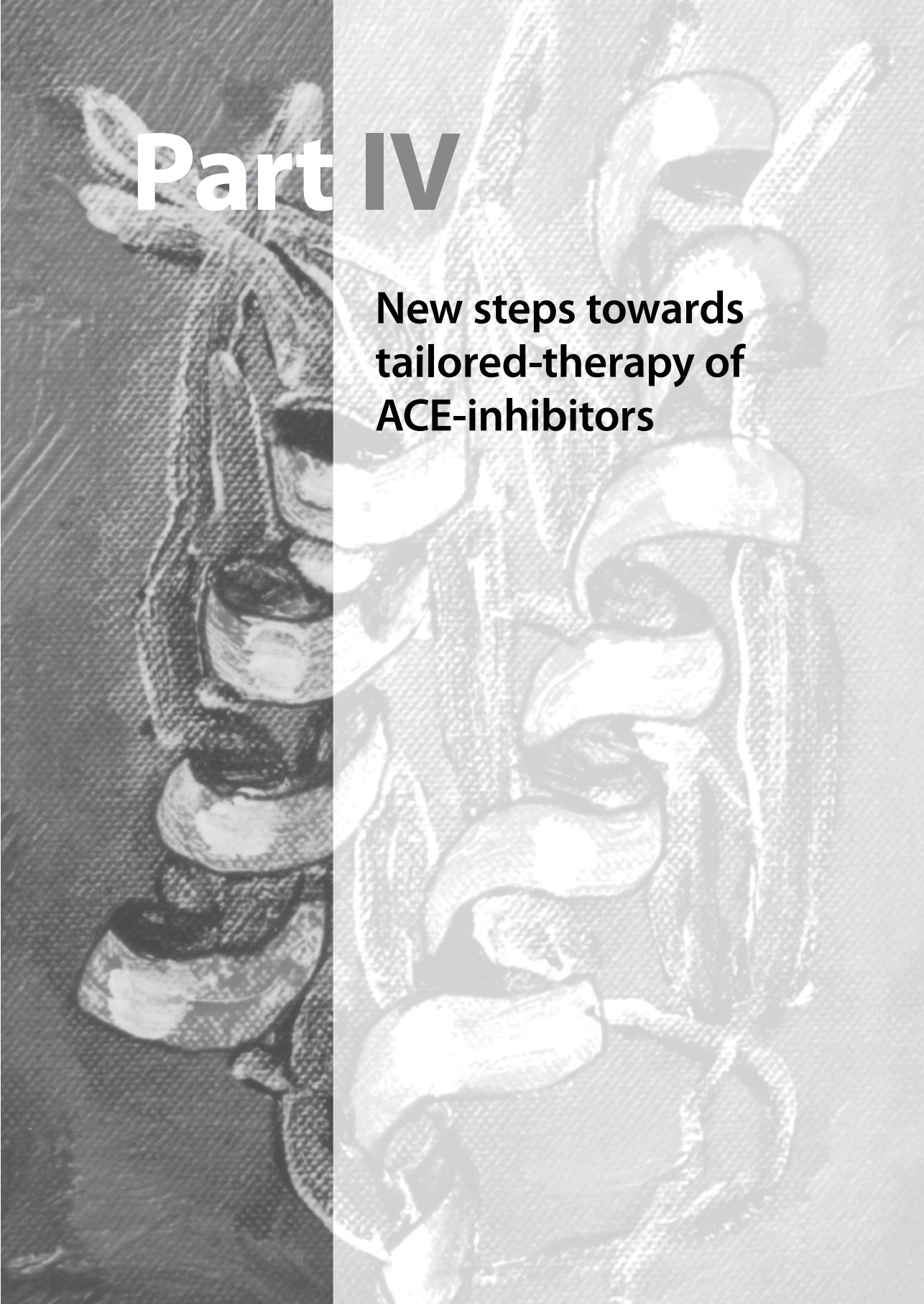
J.J.B., T.N., and E.B. have declared no conflict of interest. J.C., S.M., K.F., R.F., and M.L.S. have received research grants and fees from Servier.

## REFERENCES


1. Pfeffer MA, Braunwald E, Moye LA, Basta L, Brown EJ Jr, Cuddy TE, Davis BR, Geltman EM, Goldman S, Flaker G, for the SAVE Investigators. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction and myocardial infarction: results of the survival and ventricular enlargement trial. *N Engl J Med* 1992;327:669–677.
2. The SOLVD investigators. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. *N Engl J Med* 1991; 325:293–302.
3. The Acute Infarction Ramipril Efficacy (AIRE) study investigators. Effect of ramipril on mortality and morbidity of survivors of acute myocardial infarction with clinical evidence of heart failure. *Lancet* 1993;342:821–828.
4. Torp-Pedersen C, Kober L, for the TRACE Study Group. Effect of ACE inhibitor trandolapril on life expectancy of patients with reduced left-ventricular function after acute myocardial infarction. *Lancet* 1999;354:9–12.
5. The Heart Outcomes Prevention Evaluation study investigators. Effects of an angiotensin-converting enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. *N Engl J Med* 2000;342:145–153.
6. The PEACE Trial Investigators. Angiotensin-converting-enzyme inhibition in stable coronary artery disease. *N Engl J Med* 2004;351:2058–2068.
7. The European trial on reduction of cardiac events with perindopril in patients with stable coronary artery disease investigators. Efficacy of perindopril in reduction of cardiovascular events in stable coronary artery disease: randomised, double-blind, placebo-controlled, multicentre trial (the EUROPA study). *Lancet* 2003;362:782–788.
8. ADVANCE Collaborative Group. Effects of a fixed combination of perindopril and indapamide on macrovascular and microvascular outcomes in patients with type 2 diabetes mellitus (the ADVANCE trial): a randomised controlled trial. *Lancet* 2007;370:829–840.
9. PROGRESS Collaborative Group. Randomised trial of a perindopril-based blood-pressure-lowering regimen among 6105 individuals with previous stroke or transient ischaemic attack. *Lancet* 2001;358:1033–1041.
10. The Task Force on the Management of Stable Angina Pectoris of the European Society of Cardiology. Guidelines on the management of stable angina pectoris. *Eur Heart J* 2006;27:1341–1381.
11. Wood D, DeBacker G, Faergam O, Graham I, Mancia G, Pyo¨ra¨la¨ K, and the task force. Prevention of coronary disease in clinical practice: recommendation of the second Joint Task Force of European and other societies on Coronary Prevention. *Atherosclerosis* 1998;140:99–270.
12. Authors/Task Force Members, Ryde´n L, Standl E, Bartnik M, Van den Berghe G, Betteridge J, de Boer M-J, Cosentino F, Jo¨nsson B, Laakso M, Malmberg K, Priori S, O'Stergren J, Tuomilehto J, Thrainsdottir I, Other Contributors, Vanhorebeek I, Stramba-Badiale M, Lindgren P, Qiao Q, ESC Committee for Practice Guidelines (CPG), Priori SG, Blanc J-J, Budaj A, Camm J, Dean V, Deckers J, Dickstein K, Lekakis J, McGregor K, Metra M, Morais J, Osterspey A, Tamargo J, Zamorano JL, Document Reviewers, Deckers JW, Bertrand M, Charbonnel B, Erdmann E, Ferrannini E, Flyvbjerg A, Gohlke H, Gonzalez Juanatey JR, Graham I, Monteiro PF, Parhofer K, Pyo¨ra¨la¨ K, Raz I, Schernthaner G, Volpe M, Wood D Guidelines on diabetes, pre-diabetes, and cardiovascular diseases: executive summary: The Task Force on Diabetes and Cardiovascular Diseases of the European Society of Cardiology (ESC) and of the European Association for the Study of Diabetes (EASD). *Eur Heart J* 2007;28:88–136.
13. Task Force Members, Lo´pez-Sendo´n J, Swedberg K, McMurray J, Tamargo J, Maggioni AP, Dargie H, Tendera M, Waagstein F, Kjekshus J, Lechat P, Torp Pedersen C. Expert consensus document on angiotensin converting enzyme inhibitors in cardiovascular disease: The Task Force on ACE-inhibitors of the European Society of Cardiology. *Eur Heart J* 2004;25:1454–1470.
14. Redon J, Ruschitzka F, Tamargo J, van Zwieten P, Viigimaa M, Waeber B, Williams B, Luis Zamorano J. 2007 Guidelines for the management of arterial hypertension: The Task Force for the Management of

Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur Heart J* 2007;28:1462–1536.
15. Gibbons RJ, Abrams J, Chatterjee K, Daley J, Deedwania PC, Douglas JS, Ferguson TB Jr, Fihn SD, Fraker TD Jr, Gardin JM, O'Rourke RA, Pasternak RC, Williams SV; American College of Cardiology; American Heart Association Task Force on practice guidelines (Committee on the Management of Patients With Chronic Stable Angina). ACC/AHA 2002 guideline update for the management of patients with chronic stable angina—summary article: a report of the American College of Cardiology/American Heart Association Task Force on practice guidelines (Committee on the Management of Patients With Chronic Stable Angina). *J Am Coll Cardiol* 2003;41:159–168.
16. Bots ML, Remme WJ, Lu¨scher TF, Fox KM, Bertrand M, Ferrari R, Simoons ML, Grobbee DE. ACE Inhibition and Endothelial Function: Main Findings of PERFECT, a Sub-Study of the EUROPA Trial. *Cardiovasc Drugs Ther* 2007;21: 269–279.
17. Rodriguez-Granillo GA, Vos J, De Feyter PJ. Long-term effect of perindopril on coronary atherosclerosis progression (from the PERindopril's Prospective Effect on Coronary aTherosclerosis by Angiography and IntraVascular Ultrasound Evaluation [PERSPECTIVE] Study). *Am J Cardiol* 2007;100:159–163.
18. Ceconi C, Fox KM, Remme WJ, Simoons ML, Bertrand M, Parrinello G, Kluft C, Blann A, Cokkinos D, Ferrari R, EUROPA Investigators; PERTINENT Investigators the Statistical Committee. ACE inhibition with perindopril and endothelial function. Results of a substudy of the EUROPA study: PERTINENT. *Cardiovasc Res* 2007;73:237–246.
19. Rodriguez-Granillo GA, de Winter S, Bruining N, Ligthart JMR, Garcı´a-Garcıa HM, Valgimigli M, Pim J, de Feyter on behalf of the EUROPA/PERSPECTIVE Investigators. Effect of perindopril on coronary remodelling: insights from a multicentre, randomized study. *Eur Heart J* 2007;28:2326–2331.
20. Blood Pressure Lowering Treatment Trialists' Collaboration. Blood pressure dependent and independent effects of agents that inhibit renin-angiotensin system. *J Hypertens* 2007;25:951–958.
21. Blood Pressure Lowering Treatment Trialists' Collaboration. Effects of different regimens to lower blood pressure on major cardiovascular events in older and younger adults: meta-analysis of randomised trials. *BMJ* 2008;336:1121–1123.
22. Direct Thrombin Inhibitors Trialists' Collaborative Group. Direct thrombin inhibitors in acute coronary syndromes and during percutaneous coronary intervention: design of a meta-analysis based on individual patient data. *Am Heart J* 2001;141:e2.
23. Poulter NR, Wedel H, Dahlo¨ f B, Sever PS, Beevers DG, Caulfield M, Kjeldsen SE, Kristinsson A, McInnes GT, Mehlsen J, Nieminen M, O´ Brien E, O'Stergren J, Pocock S, for the ASCOT investigators. Role of blood pressure and other variables in the differential cardiovascular event rates noted in the Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm (ASCOT-BPLA). *Lancet* 2005;366:907–913.
24. Early Breast Cancer Trialists' Collaborative Group. Treatment of Early Breast Cancer, Vol 1: Worldwide Evidence 1985–1990. Oxford: Oxford University Press; 1990. p12–18.
25. Steyerberg EW, Bossuyt PM, Lee KL. Clinical trials in acute myocardial infarction: should we adjust for baseline characteristics? *Am Heart J* 2000;139:761–763.
26. Dagenais GR, Pogue J, Fox K, Simoons ML, Yusuf S. Angiotensin-converting-enzyme inhibitors in stable vascular disease without left ventricular systolic dysfunction or heart failure: a combined analysis of three trials. *Lancet* 2006;368:581–588.
27. MacMahon S, Chalmers J. Blood pressure lowering and ACE inhibition for the avoidance of cardiac and cerebral events. *Eur Heart J* 2003;24:391–393.
28. Turnbull F, Woodward M, Neal B, Barzi F, Ninomiya T, Chalmers J, Perkovic V, Li N, MacMahon S, and the Blood Pressure Lowering Treatment Trialists' Collaboration. Do men and women respond differently to blood pressure-lowering treatment? Results of prospectively designed overviews of randomized trials. *Eur Heart J* 2008;29:2669–2680.
29. Ferrari R, Pasanisi G, Notarstefano P, Campo G, Gardini E, Ceconi C. Specific properties and effect of perindopril in controlling the renin-angiotensin system. *Am J Hypertens* 2005;18:142S–154S.

30. Danchin N, Cucherat M, Thuillez C, Durand E, Kadri Z, Steg PG. Angiotensin-converting enzyme inhibitors in patients with coronary artery disease and absence of heart failure or left ventricular systolic dysfunction: an overview of long-term randomized controlled trials. *Arch Intern Med* 2006;166:787–796.
31. Al-Mallah MH, Tleyjeh IM, Abdel-Latif AA, Weaver WD. Angiotensin-converting enzyme inhibitors in coronary artery disease and preserved left ventricular systolic function: a systematic review and meta-analysis of randomized controlled trials. *J Am Coll Cardiol* 2006;47:1576–1583.
32. Flather MD, Yusuf S, Køber L, Pfeffer M, Hall A, Murray G, Torp-Pedersen C, Ball S, Pogue J, Moye´ L, Braunwald E. Long-term ACE-inhibitor therapy in patients with heart failure or left-ventricular dysfunction: a systematic overview of data from individual patients. ACE-Inhibitor Myocardial Infarction Collaborative Group. *Lancet* 2000;355:1575–1581.



# Part IV

## New steps towards tailored-therapy of ACE-inhibitors

# Chapter 8

## The Renin Angiotensin Aldosteron System: Approaches to guide ACE-inhibition in patients with stable coronary artery disease

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## ABSTRACT

Drugs that modulate the renin-angiotensin-aldosterone system (RAAS) play an important role in modern cardiovascular prevention strategies. Inhibitors of the RAAS, in particular angiotensin-converting enzyme (ACE) inhibitors, have been proven to be beneficial in specific patient groups, including patients with hypertension, heart failure, diabetes mellitus and stable coronary artery disease. Although clinical trials demonstrated a rather consistent beneficial effect of ACE inhibitors across groups of patients based on clinical characteristics, the variability in treatment response on the individual patient level is extensive. Recent publications suggest that genetic polymorphisms in the RAAS are related to cardiovascular risk. Genetic variability also seems associated with the response to ACE inhibitor therapy, and can probably be used to tailor treatment. This review discusses several approaches to guide ACE inhibitor therapy in patients with coronary artery disease. In addition, the potential impact of pharmacogenetics regarding this particular topic is highlighted.

## INTRODUCTION

The renin-angiotensin-aldosterone system (RAAS) plays an integral role in the preservation of hemodynamic stability in humans. The RAAS accomplishes this action by regulation of extracellular fluid volume, sodium balance and cardiovascular function through direct and indirect effects on multiple organ systems[1]. The RAAS complements and interacts with other vasomotor systems, such as the autonomic nervous system and several vasoactive hormones. The RAAS is stimulated in response to threats that compromise blood pressure stability and extracellular fluid volume homeostasis.

### Components of the RAAS

The RAAS is composed of a cascade of hormones initially triggered by the release of renin from the kidney (figure 1) [2,3]. The production of renin is raised by a decrease in perfusion of the juxtaglomerular apparatus. Renin is a proteolytic enzyme that has a local action in the kidney as well as in the circulation upon the substrate angiotensinogen, which is a protein precursor that is produced in and secreted by the liver. Angiotensinogen is cleaved by renin to form the biologically inactive peptide angiotensin I (A-I). This circulating decapeptide is then efficiently converted to the active octapeptide angiotensin II (A-II) by angiotensin-converting enzyme (ACE). ACE is a largely tissue-based zinc metalloprotease, mainly generated by the lungs, the cell membranes of the kidneys and the endothelial cells of the vasculature. A-II is produced in a number of organs, largely locally from locally generated A-I. This local production of A-I

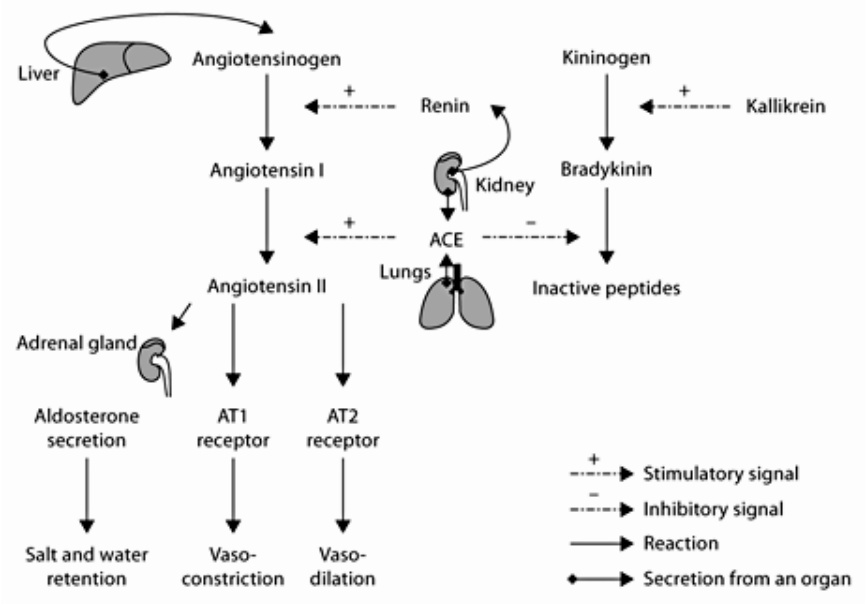


**Figure 1** The RAAS cascade.

involves renal renin taken up at tissue sites, possibly involving a renin receptor [4]. This allows the tissue renin levels to be higher than expected on the basis of simple diffusion from blood. Consequently, A-II levels are often much higher in tissues than in the circulation. Two well-characterized subtypes of A-II receptors mediate the major physiologic actions of A-II in humans: these have been termed angiotensin type 1 (AT1) and angiotensin type 2 (AT2) receptors. Both receptors are G-coupled polypeptides, containing approximately 360 amino acids, and have 7 cell membrane-spanning regions [5,6]. In the human body, the AT1 receptor is more widely distributed and thus more important than the AT2 receptor. Genetically, both receptor subtypes share a sequence homology of only 30%. Their genes reside on different chromosomes: the AT1 receptor on chromosome 3 and the AT2 receptor on the X chromosome [7,9]. Stimulating either the AT1 or the AT2 receptor results in activation of different signal transduction pathways, which results in antagonizing effects (table 1 ). For example, A-II, stimulating the AT1 receptor, is a potent vasoconstrictor, whereas AT2 receptor stimulation by A-II results in vasodilation. In addition to its vasoconstricting and other effects, A-II can activate AT1 receptors in the adrenal gland, which results in synthesis of the steroid hormone aldosterone [10]. It is generally accepted that excessive stimulation of the AT1 receptor by A-II results in unfavourable effects, whereas stimulation of the AT2 receptor is responsible for the beneficial, however in humans less important, effects of A-II. The overall effect of activation of the RAAS is an increase in effective circulating volume, resulting in an increase in perfusion of the juxtaglomerular apparatus. Through this phenomenon, the release of renin by the kidney is inhibited: a feedback mechanism.



**Table 1.** Effects of stimulation of the most important angiotensin II receptor subtypes: AT1 and AT2

| Receptor subtypes | Effects of receptor stimulation |
|---|---|
| **AT1 receptor** | Arteriolar vasoconstriction |
| | Aldosterone synthesis |
| | Increased tubular $Na^+$ and $Cl^-$ re-absorption and $K^+$ excretion |
| | Increased plasminogen activator inhibitor 1 activity |
| | Increased sympathetic activity |
| | ADH secretion ® $H_2O$ absorption by collecting duct |
| | Vascular smooth muscle growth |
| | Endothelial dysfunction |
| | Increased connective tissue and LDL accumulation in vascular media |
| | Activation of adhesion molecules and monocyte chemo-attractants |
| | Activation of inflammatory cytokines |
| | Oxidative stress |
| | Enhanced expression of matrix metalloproteinases |
| **AT2 receptor** | Vasodilation |
| | Anti-proliferation |
| | Decreased renal sodium re-absorption |
| | Decreased myocyte hypertrophy |
| | Decreased cardiac fibrosis |

*ADH*, antidiuretic hormone

## The RAAS: an important drug target

As mentioned above, the RAAS has an important function in maintaining normal hemodynamics and electrolyte balances. However, excessive or maladaptive stimulation of this hormonal cascade can cause pathologic changes in a wide variety of organ systems. For example, A-II-induced vasoconstriction of renal efferent arterioles increases glomerular filtration rate, which is a protective response to maintain glomerular filtration rate in states of renal hypoperfusion. However, an overactive RAAS and thus overproduction of A-II results in extravagant vasoconstriction, which is associated with hypertension, renal injury, atherosclerosis and left ventricular dysfunction. Understandably, an activated RAAS has become a key therapeutic target in patients with cardiovascular disease. The clinically most important examples of pharmacologic agents that block the RAAS include ACE inhibitors, AT1 receptor blockers (ARBs) and aldosterone antagonists. This review focuses on ACE inhibitors.

## ACE Inhibitors – Chemical Structure

Although all ACE inhibitors are 2-methylpropionyl- L - proline analogues, they differ from each other by their individual chemical structure [11]. ACE inhibitors exert their effect on ACE by chelating $Zn^{2+}$ in the active center. The functional ($Zn^{2+}$ -chelating) group binding to ACE is the primary structural difference among these agents. Most ACE inhibitors have a carboxyl functional group. On the other hand, captopril contains a sulfhydryl group and fosinopril has a phosphinyl group [12]. Some of the characteristics of these agents may be linked to these different binding groups.

## ACE Inhibitors – Pharmacokinetic Profile

The absorption of ACE inhibitors varies from 25 to 75%, depending on lipophilicity and molecular size of the specific agent. Food either has no effect or reduces the rate of absorption. Several ACE inhibitors are prodrugs: they remain inactive until they are converted into active metabolites in the liver or in the gastrointestinal tissue [12]. The peak plasma drug concentrations are reached 1 – 4 h after ingestion. Most ACE inhibitors and their metabolites are excreted by the renal route, whereas fosinopril, zofenopril, trandolapril and spirapril are eliminated through hepatic and renal routes [13]. Captopril is eliminated rapidly from the body, which accounts for its brief duration of action: less than 6 h. Ramiprilat, which is the active metabolite of ramipril, and especially trandolaprilat are eliminated more slowly than other ACE inhibitors. For most ACE inhibitors, dose reductions are required in the presence of impaired renal function [13]. Dose reductions are not necessary in case of use of the aforementioned ACE inhibitors that are excreted in both the urine and the bile.

## ACE Inhibitors – Pharmacodynamics

ACE inhibitors competitively block the conversion of A-I into A-II. This blockade results in a decrease in circulating and local levels of A-II, thus inhibiting the effects of A-II. It is important

to realize that ACE inhibitors do not antagonize the AT1 receptor and thus do not inhibit the unfavourable effects of A-II completely. In addition to this, it needs to be addressed that when ACE is inhibited, formation of A-II is restored, at least in part, due to the reactive renin rise that occurs when blocking the A-II-induced negative feedback on renin release. A second beneficial effect of ACE inhibitors, in contrast to ARBs, is the increase in bradykinin levels by a decrease in transformation of bradykinin in inactive peptides [14]. The increase in bradykinin levels induced by ACE inhibitors leads to the release of nitric oxide and prostaglandins, resulting in vasodilation [15].

## Clinical Effects of ACE Inhibitors

As indicated above, ACE inhibitors exert their pharmacologic effect through a decrease in production of A-II and an increase in bradykinin levels. The clinically most important short-term effect of ACE inhibitors is the decrease in blood pressure. In the long term, ACE inhibitor use has antiproliferative effects, resulting in a reduction of vascular and cardiac hypertrophy, which is an important aspect in the pathophysiology of chronic hypertension and heart failure. The remodelling effects of ACE inhibitors take place at the level of the small blood vessels, the large arteries and the heart [16]. These beneficial effects of ACE inhibitors are an important reason to administer these agents routinely to patients with heart failure. It is generally believed that ACE activity in the heart and the blood vessels contributes both to the development and the progression of atherosclerotic vascular disease. It has been presumed for years that ACE inhibitors can improve endothelial function and stabilize atherosclerotic plaques. Evidence that ACE inhibitors indeed can delay the development of atherosclerosis first originated from research in laboratory animals [17]. Recently, 3 large randomized controlled trials have addressed whether patients with chronic ischemic heart disease, but withou t heart failure, would benefit from ACE inhibitor therapy [18]. The first trial was called Heart Outcomes Prevention Evaluation (HOPE) and in this trial ramipril reduced both mortality and cardiovascular events in coronary artery disease (CAD) patients [19]. Second, the European Trial on Reduction of Cardiac Events with Perindopril in Stable Coronary Artery Disease (EUROPA) demonstrated that perindopril significantly lowered the incidence of a combined cardiovascular endpoint, consisting of death, myocardial infarction or cardiac arrest [20]. Third, the Prevention of Events with Angiotensin-Converting Enzyme Inhibition (PEACE) trial concluded that trandolapril did not influence cardiovascular outcome or mortality [21]. The negative results from the PEACE trial were most likely due to a lack of statistical power. A meta-analysis of the HOPE, EUROPA and PEACE studies by Dagenais et al. [22] assessed cardiovascular outcomes and total mortality in the 29.805 patients of these 3 trials, randomly assigned an ACE inhibitor or placebo and followed for a mean of about 4.5 years. This analysis demonstrated that ACE inhibitors significantly reduced the incidences of all-cause mortality (7.8 vs. 8.9%, $p = 0.0004$), cardiovascular mortality (4.3 vs. 5.2%, $p = 0.0002$), non fatal myocardial infarction (5.3 vs. 6.4%, $p = 0.0001$), all stroke (2.2 vs. 2.8%, $p = 0.0004$), heart failure (2.1 vs. 2.7%, $p = 0.0007$) and coronary-artery bypass surgery (6.0 vs. 6.9%, $p = 0.0036$) [22]. Based on these findings, the authors of the meta-analysis advised to consider the use of ACE inhibitors in all patients with atherosclerotic vascular disease.

## ACE Inhibitors versus ARBs

ACE inhibitors have been shown to reduce mortality and cardiovascular morbidity among patients with myocardial infarction complicated by left ventricular systolic dysfunction, heart failure or both. The VALIANT trial compared the effect of the ACE inhibitor captopril versus the ARB valsartan in this population of patients and showed that valsartan is as effective as captopril in patients who are at high risk for cardiovascular events after myocardial infarction [23]. In patients with vascular disease or high-risk diabetes without heart failure, ACE inhibitors reduce mortality and morbidity from cardiovascular causes, but the role of ARBs in such patients is unknown. The ONTARGET study compared the ACE inhibitor ramipril, the ARB telmisartan and the combination of the 2 drugs [24]. The investigators concluded that telmisartan was equivalent to ramipril in patients with vascular disease or high-risk diabetes. Telmisartan was as effective as captopril in reducing the rates of death and other cardiovascular outcomes (including the prevention of myocardial infarction). The combination of the two drugs was associated with more adverse events without an increase in benefit.

## ACE Inhibition – Clinical Applications and Guidelines

It has been suggested that ACE inhibition has the broadest impact of any class of drugs in cardiovascular medicine [25]. The use of ACE inhibitors is beneficial for patients with heart failure, left ventricular dysfunction, after myocardial infarction, hypertension, nephropathy, peripheral vascular disease, diabetes mellitus and stroke. Current European Society of Cardiology recommendations on the administration of ACE inhibitors in specific patient categories are listed in table 2 [26]. These recommendations overlap to a large extent with guidelines for secondary prevention for patients with coronary vascular disease, as published by the American Heart Association/ American College of Cardiology [27]. The latter guidelines advice that ACE inhibitors should be given to all patients with a left ventricular ejection fraction < 40% and in those with hypertension, diabetes mellitus or chronic kidney disease, unless contraindicated (class I-A recommendation; for an explanation of classes and levels of evidence please see footnote of table 2). In addition, administration of an ACE inhibitor should be considered in all other patients with coronary and other atherosclerotic vascular diseases (class I-B recommendation). Finally, the American guidelines state that the use of ACE inhibitors may be considered among lower-risk patients with normal left ventricular ejection fraction in whom cardiovascular risk factors are well controlled and revascularization has been performed (class IIa-B recommendation).

## Attempts to Guide ACE Inhibition

Clinical guidelines are helpful tools to standardize medical healthcare. However, it often remains difficult in the clinic to predict cardiovascular risk and the response to a certain ACE inhibitor in the individual patient. Nowadays, beneficial effects of medications are frequently evaluated in subgroups of patients in large clinical trials. One important research question behind these subgroup analyses is whether a possible heterogeneity in treatment effect in patient

**Table 2.** ESC Guidelines on administration of ACE inhibitors in specific patient categories

| Indication | Specified | Level |
|---|---|---|
| ***I. Heart failure*** | - Symptomatic heart failure and reduced LVEF | I-A |
| | - LVSD after AMI | I-A |
| | - Reduced LVEF (40-45%) without symptoms, no previous MI | I-A |
| | - Diastolic heart failure | IIa-C |
| ***IIa. AMI, first 24 h*** | - High risk patients (heart failure, LVD, no reperfusion, large infarcts) | I-A |
| | - All patients | IIa-A |
| ***IIb. Evolving AMI (>24 h), post MI*** | - Clinical heart failure or asymptomatic LVD (LVEF<45%) | I-A |
| | - Diabetes mellitus or other high risk patients | I-A |
| ***III. Hypertension*** | - To control blood pressure | I-A |
| | - Patients with heart failure, systolic LVD, diabetics, previous MI or stroke, high coronary disease risk | I-A |
| ***IV. Secondary prevention*** | - High-risk patients (evidence of cardiovascular disease or diabetes and one other risk factor) | I-A |
| ***V. To prevent sudden cardiac death*** | - Patients with heart failure | I-A |
| | - Patients with previous MI | I-A |
| | - Patients with dilated cardiomyopathy | I-B |

LVEF, left ventricular ejection fraction; LVSD, left ventricular systolic dysfunction; AMI, acute myocardial infarction; LVD, left ventricular dysfunction; MI, myocardial infarction. Class I: Evidence and/or general agreement that a given procedure/treatment is beneficial, useful and effective. Class II: Conflicting evidence about the efficacy of the treatment. Class IIa: Weight of evidence/opinion is in favor of efficacy. Level of Evidence A: Data derived from multiple randomised clinical trials or meta-analyses. Level of Evidence B: Data derived from a single randomised clinical trial or non-randomised studies. Level of Evidence C: Consensus of opinion of the experts and/or small studies.



subgroups could result in a more specified indication of a certain agent, which may enhance the cost effectiveness of drug administration. In CAD patients, the heterogeneity in treatment effect by ACE inhibition has been extensively investigated. In the EUROPA trial, the beneficial effect of perindopril on the primary endpoint was consistent across predefined subgroups of patients with and without hypertension, diabetes mellitus or previous myocardial infarction [20]. In addition, the treatment benefit was independent of (and not affected by) treatment with other cardiovascular agents, including statins, platelet inhibitors and _ -blockers. Based on the EUROPA study population, Deckers et al. [28] demonstrated that the relative treatment benefit of perindopril was independent of baseline risk levels (high, intermediate and low risk) based on clinical characteristics. Some investigators have argued that markers of an activated RAAS, such as impaired renal function, may be used as a target for ACE inhibition therapy [29]. A subgroup analysis of the negative PEACE trial indeed showed that there was heterogeneity in the treatment effect of trandolapril with regard to renal insufficiency [30]. However, Brugts et al. [31,32] recently demonstrated that the treatment benefit of perindopril was consistent in subgroups

of patients with mild to moderate renal insufficiency with stable CAD is rather consistent across various subgroups. Until now, based on prior analyses, no adequate way to guide ACE inhibition has been demonstrated. Therefore, simple clinical characteristics are not useful to select those patients who are most likely to benefit from ACE inhibition in CAD. As an alternative way, the relatively new field of pharmacogenetics seems promising to reach a more individualized way of treatment.

## GENETICS: AN EVOLVING FIELD

CAD is a complex, multifactorial disease, influenced by pathophysiologic conditions as well as by genetic and environmental factors [33]. Genetic differences are supposed to be an explanation for the fact that some people, irrespective of lifestyle and common classical cardiovascular risk factors, are more prone to the development of CAD than others. A large number of researchers are currently investigating the most simple and common forms of variation in the human genome. These variations are termed single nucleotide polymorphisms (SNPs) and insertion / deletion polymorphisms. SNPs represent single base pair substitutions along a DNA sequence of a candidate gene. SNPs occur when a single nucleotide (that is, adinine, thymine, cytosine or guanine) in the gene is altered. These alterations arise, by definition, in more than 1% of the population. Some polymorphisms may play a role in causing diseases, whereas others do not seem to be of real significance. Apart from their relation with the genesis and development of diseases, it has been hypothesized that SNPs may predict a patient's response to medicine as well.

### RAAS Polymorphisms and CAD

Polymorphisms have been associated with varying RAAS activity, for example variance in plasma ACE levels, indicative for an increased activity of the RAAS [34]. The rationale of intensive investigation of these polymorphisms was that an enhanced activity of the RAAS, associated with these polymorphisms, might subsequently lead to adverse physiologic consequences such as an increased cardiovascular risk. The AT1 receptor A1166C and AGT M235T polymorphisms have been associated with increased RAAS activity and CAD risk [35-39], although we have to take into account that negative results have been published as well [40-43]. The most comprehensively studied RAAS polymorphism in relationship with CAD is the ACE insertion/deletion (ACE I/D) polymorphism. This polymorphism is based on the presence (I) or absence (D) of a 287-bp Alu repeat sequence within intron 16 of the ACE gene [44]. The D allele has been associated with increasing plasma and tissue concentrations of ACE [44,45]. A number of studies have investigated the influence of the ACE I/D polymorphism on the development of CAD, resulting in inconsistent results: several research groups have reported an association between the D allele and CAD [46-50], whereas others could not confirm this finding [40,51,52]. In order to interpret these conflicting results more comprehensively, 3 meta-analyses have

been performed. First, Samani et al. [53] carried out a meta-analysis of 15 studies containing 3,394 patients with myocardial infarction and 5,479 control subjects. The mean odds ratio (OR) for myocardial infarction for DD versus ID/II genotypes across all studies was 1.26 (95% CI 1.11–1.38). The relative risk appeared to be even higher in the Japanese population. The second meta-analysis analyzed 46 studies with a total number of 32,715 white individuals [54]. Five of these 46 studies were regarded as large, since they included more than 600 patients. Although the overall results from this meta-analysis were positive (pooled OR = 1.21, 95% CI 1.11–1.32, for DD vs. ID/II genotypes), the increased risk was only found in small but not in larger studies (small vs. large: p = 0.001 for risk of myocardial infarction). In the meta-analysis by Keavney et al. [55], 4.629 myocardial infarction patients from the United Kingdom were compared to 5.934 controls. The ACE DD genotype was found in 29.4% of the myocardial infarction cases and in 27.6% of the controls (risk ratio of 1.10, 95% CI 1.00–1.21). In addition to these meta-analyses, cardiovascular risk associated with the ACE I/D polymorphism was evaluated in the Genetics of Hypertension-Associated Treatment (GenHAT) study [56]. This study was a trial in 37.939 American hypertensive patients who were randomized to antihypertensive treatment, that is, chlorthalidone, amlodipine, lisinopril or doxazosin. Primary outcome measures used were fatal coronary heart disease and/or nonfatal myocardial infarction. In this large trial, the ACE DD genotype was not associated with an increased risk of fatal or nonfatal coronary heart disease events (relative risk of DD vs. ID and II: 0.99, 95% CI 0.91–1.07). In conclusion, it is currently doubted whether the ACE I/D polymorphism is linked to clinical events [57].



## PHARMACOGENETICS OF THE RAAS: TOWARDS TAILORED THERAPY

Pharmacogenetic profiling may enable physicians: (1) to predict the individual risk of disease and (2) to predict a patient's response to drugs. Pharmacogenetics of the RAAS in relationship with ACE inhibition is a novel research field and its clinical value still has to be proven. If indeed useful, genetic profiling might become a cost-effective tool for routine use in future clinical practice. The clinically most relevant studies are those that investigate ACE inhibitor pharmacogenetics in relationship to reduction of clinical endpoints. Several studies have investigated ACE inhibitor pharmacogenetics using common clinical endpoints of atherosclerotic disease, such as myocardial infarction, ischemic stroke or mortality, as a primary outcome measure. The polymorphisms that were most extensively investigated in prior studies were the AGT M235T polymorphism and, once again, the ACE I/D polymorphism. Regarding these polymorphisms, we highlight the largest studies that investigated the modifying effect of genotype on outcome, related to the use of an ACE inhibitor. First, Bis et al. [58] investigated the relationship between AGT M235T polymorphism and ACE inhibitor therapy. In this study of treated hypertension patients from the United States of America, genotyping was performed in survivors of either stroke or myocardial infarction (total n = 324). Compared with nonuse, ACE inhibitor use was

associated with a lower risk of stroke among AGT TT homozygotes relative to AGT M carriers [OR 0.37 (0.14–0.99) vs. 1.4 (0.88–2.4)]. In this study, genotype did not modify the association of ACE inhibitor use with risk of myocardial infarction. Unfortunately, the relevance of this particular study is limited by its sample size, its retrospective nature and the fact that duration, dose and agent choice were not clearly defined. Another study that investigated major clinical endpoints was a substudy of the Perindopril Protection against Recurrent Stroke Study (PROGRESS) [59] . PROGRESS was a large trial that evaluated the effects of a perindopril-based blood pressure-lowering regimen on the risks of major vascular events in patients with a history of stroke. In the original study, perindopril provided significant benefit for stroke reduction when used in combination with indapamide, but not when used as a single agent [60. The PROGRESS substudy investigated the association between ACE I/D polymorphism and blood pressure as well as the effects of ACE inhibitor treatment on the risk of stroke, cardiac events and mortality. The ACE I/D polymorphism was determined in 5.688 patients. All patients had a previous ischemic stroke or transient ischemic attack and were enrolled in various countries in Europe, Australia and Asia. When evaluating blood pressure response to perindopril use, there was no ACE genotype-specific benefit compared with placebo. In addition to this finding, beneficial effects of the study treatment were not associated with a specific genotype. The ACE I/D polymorphism was also investigated in the Dutch Rotterdam study. This population-based study of primarily hypertensive patients (n = 3.365) investigated the association of ACE genotype, ACE inhibitor treatment and total and cardiovascular mortality during a mean follow-up of 7.8 years [61]. Various ACE inhibitor agents were used in this study. ACE inhibitor use was associated with increased total mortality in subjects with the DD genotype. The authors explained their finding as a relative resistance to ACE inhibitor therapy in patients with the DD genotype. Finally, cardiovascular risk associated with the ACE I/D polymorphism was evaluated in the aforementioned GenHAT study, which included 37.939 American hypertensive patients [56]. In this study, the 6-year hazard rate for fatal and nonfatal coronary heart disease in the DD genotype group was not different from the ID and II genotype group stratified by type of treatment.

To summarize, there are only a few large studies that investigated whether the effects of ACE inhibition were modified by different RAAS genotypes. Several potential limitations should be mentioned when considering the inconsistent results of the aforementioned studies. One of the most important limitations is the lack of statistical power to reveal clinically relevant differences. One should realize that most studies that have been performed thus far were not designed as pharmacogenetics studies. Second, the interpretation of the observed results is complicated by the broad variety in specific medication and doses studied. Finally, we have to take into account that CAD is likely to be a polygenetic disease state. All pharmacogenetic studies on the complicated RAAS have focused on 1 or 2 polymorphisms, with the rare exception of a few research groups that investigated multiple SNPs [62,63]. It seems reasonable to state that the likelihood that a single polymorphism accounts for a substantial ACE inhibitor-related response is small. Therefore, future pharmacogenetic analyses should ideally be performed in

larger studies of ACE inhibitor treatment that focus on multiple polymorphisms in multiple RAAS genes. These studies should aim at integrating information by complete haplotype analysis, which is a more comprehensive method of genetic profiling. Haplotypes are a combination of alleles at different markers along the same chromosome that are inherited as a unit. Although each marker can be analyzed independently of the other marker, it is much more informative to analyze markers in a region of interest simultaneously which can explore genetic variants underlying various human traits. In this way, a more comprehensive in-depth analysis of RAAS polymorphisms in relationship to ACE inhibition can be established, which is more likely to discover true positive pharmacogenetic associations. In addition, it is important to emphasize that when this new approach to guide ACE inhibitor treatment proves to be successful, it will need additional validation in prospective trials before it can truly be accepted as guides to clinical practice.

## CONCLUSIONS

ACE inhibitors belong to a drug class that is frequently administered to patients with cardiovascular disease. The beneficial effects of ACE inhibition have been demonstrated in various subgroups of patients. It remains difficult to guide ACE inhibition to those patients who will benefit most. Subgroup analyses in ACE inhibitor trials could be used as a way to target therapy in patients with CAD. However, due to the shown consistency of the beneficial effect of ACE inhibition across various subgroups of CAD patients, this approach has not been useful in guiding therapy. Still, it would be of outmost importance in clinical practice to distinguish those patients who will have the highest risk of cardiovascular events. This concept could be used to decrease the number needed to treat and thus enhance the cost-effectiveness of drug administration. Pharmacogenetics is regarded as a novel promising way to enable tailored treatment. So far, results from pharmacogenetic studies investigating the RAAS are inconclusive. The additional value of pharmacogenetics of the RAAS has still to be shown. Large pharmacogenetic studies in this field, using an adequate methodology, are therefore necessary.



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## REFERENCES


1 Skeggs LT, Dorer RE, Kahn JR, Lentz KE, Levine M: The biochemistry of the renin-angiotensin system and its role in hypertension. *Am J Med* 1976; 60: 737–748.
2 Erdos EG: Angiotensin I converting enzyme and the changes in our concepts through the years. *Hypertension* 1990; 16: 363–370.
3 Perazella MA, Setaro JF: Renin-angiotensin-aldosterone system: fundamental aspects and clinical implications in renal and cardiovascular disorders. *J Nucl Cardiol* 2003; 10: 184–194.
4 Batenburg WW, Krop M, Garrelds IM, de Vries R, de Bruin RJ, Burcklé CA, Müller DN, Bader M, Nguyen G, Danser AH: Prorenin is the endogenous agonist of the (pro)renin receptor. Binding kinetics of renin and prorenin in rat vascular smooth muscle cells overexpressing the human (pro)renin receptor. *J Hypertens* 2007; 25: 2441–2453.
5 Goodfriend TL, Elliott ME, Catt KJ: Angiotensin receptors and their antagonists. *N Engl J Med* 1996; 334: 1649–1654.
6 Unger T, Culman J, Gohlke P: Angiotensin II receptor blockade and end-organ protection: pharmacological rationale and evidence. *J Hypertens* Suppl 1998; 16:S3–S9.
7 Szpirer C, Riviere M, Szpirer J, Levan G, Guo DF, Iwai N, Inagami T: Chromosomal assignment of human and rat hypertension candidate genes: type 1 angiotensin II receptor genes and the SA gene. *J Hypertens* 1993; 11: 919–925.
8 Duncan JA, Scholey JW, Miller JA: Angiotensin type 1-receptor gene polymorphisms in humans: physiology and pathophysiology of the genotypes. *Curr Opin Nephrol Hypertens* 2001; 10: 111–116.
9 Koike G, Horiuchi M, Yamada T, Szpirer C, Jacob HJ, Dzau VJ: Human type 2 angiotensin II receptor gene: cloned, mapped to the X-chromosome, and its mRNA is expressed in the human lung. *Biochem Biophys Res Commun* 1994; 203: 1842–1850.
10 Laragh JH, Angers M, Kelly WG, Lieberman S: Hypotensive agents and pressor substances: the effect of epinephrine, norepinephrine, angiotensin II, and others on the secretory rate of aldosterone in man. *JAMA* 1960; 174: 234–240.
11 Piepho RW: Overview of the angiotensin converting- enzyme inhibitors. *Am J Health Syst Pharm* 2000; 57:S3–S7.
12 Brown NJ, Vaughan DE: Angiotensin-converting enzyme inhibitors. *Circulation* 1998; 97: 1411–1420.
13 Hoyer J, Schulte KL, Lenz T: Clinical pharmacokinetics of angiotensin converting enzyme (AC) inhibitors in renal failure. *Clin Pharmacokinet* 1993; 24: 230–254.
14 Pellacani A, Brunner HR, Nussberger J: Plasma kinins increase after angiotensin enzyme inhibition in human subjects. *Clin Sci* (Lond) 1994; 87: 567–574.
15 Ferrari R: Angiotensin-converting enzyme inhibition in cardiovascular disease: evidence with perindopril. *Expert Rev Cardiovasc Ther* 2005; 3: 15–29.
16 Cohn JN: Left ventricle and arteries: structure, function, hormones, and disease. *Hypertension* 2001; 37: 346–349.
17 Pitt B: Potential role of angiotensin converting enzyme inhibitors in the treatment of atherosclerosis. *Eur Heart J* 1995; 16: 49–54.
18 Kober L, Torp-Pedersen C: Do ACE inhibitors improve outcome in patients with stable vascular disease? *Nat Clin Pract Cardiovasc Med* 2007; 4: 124–125.
19 Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G: Effects of an angiotensin-converting- enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. *N Engl J Med* 2000; 342: 145–153.
20 Fox KM: Efficacy of perindopril in reduction of cardiovascular events among patients with stable coronary artery disease: randomised, double-blind, placebo-controlled, multicentre trial (the EUROPA study). *Lancet* 2003; 362: 782–788.

21 Braunwald E, Domanski MJ, Fowler SE, Geller NL, Gersh BJ, Hsia J, Pfeffer MA, Rice MM, Rosenberg YD, Rouleau JL: Angiotensin- converting-enzyme inhibition in stable coronary artery disease. *N Engl J Med* 2004; 351: 2058–2068.
22 Dagenais GR, Pogue J, Fox K, Simoons ML, Yusuf S: Angiotensin-converting-enzyme inhibitors in stable vascular disease without left ventricular systolic dysfunction or heart failure: a combined analysis of three trials. *Lancet* 2006; 368: 581–588.
23 Pfeffer MA, McMurray JJ, Velazquez EJ, Rouleau JL, Køber L, Maggioni AP, Solomon SD, Swedberg K, Van de Werf F, White H, Leimberger JD, Henis M, Edwards S, Zelenkofske S, Sellers MA, Califf RM: Valsartan, captopril, or both in myocardial infarction complicated by heart failure, left ventricular dysfunction, or both. *N Engl J Med* 2003; 349: 1893–1906.
24 Yusuf S, Teo KK, Pogue J, Dyal L, Copland I, Schumacher H, Dagenais G, Sleight P, Anderson C: Telmisartan, ramipril, or both in patients at high risk for vascular events. *N Engl J Med* 2008; 358: 1547–1559.
25 White HD: Should all patients with coronary disease receive angiotensin-converting-enzyme inhibitors? *Lancet* 2003; 362: 755–757.
26 Lopez-Sendon J, Swedberg K, McMurray J, Tamargo J, Maggioni AP, Dargie H, Tendera M, Waagstein F, Kjekshus J, Lechat P, Torp- Pedersen C: Expert consensus document on angiotensin converting enzyme inhibitors in cardiovascular disease. The Task Force on ACE-inhibitors of the European Society of Cardiology. *Eur Heart J* 2004; 25: 1454–1470.
27 Smith SC, Allen J, Blair SN, Bonow RO, Brass LM, Fonarow GC, Pfeffer MA, Taubert KA: AHA/ACC guidelines for secondary prevention for patients with coronary and other atherosclerotic vascular disease: 2006 update. *Circulation* 2006; 113: 2363–2372.
28 Deckers JW, Goedhart DM, Boersma E, Briggs A, Bertrand M, Ferrari R, Remme WJ, Fox K, Simoons ML: Treatment benefit by perindopril in patients with stable coronary artery disease at different levels of risk. *Eur Heart J* 2006; 27: 796–801.
29 Asselbergs FW, van Gilst WH: Angiotensin converting enzyme inhibition in cardiovascular risk populations: a practical approach to identify the patient who will benefit most. *Curr Opin Cardiol* 2007; 22: 267–272.
30 Solomon SD, Rice MM, Jablonski K, Jose P, Domanski M, Sabatine M, Gersh BJ, Rouleau J, Pfeffer MA, Braunwald E, Prevention of Events with ACE inhibition (PEACE) Investigators: Renal function and effectiveness of angiotensin-converting enzyme inhibitor therapy in patients with chronic stable coronary disease in the Prevention of Events with ACE inhibition (PEACE) trial. *Circulation* 2006; 114: 26–31.
31 Brugts JJ, Boersma E, Chonchol M, Deckers JW, Bertrand M, Remme WJ, Ferrari R, Fox K, Simoons ML: The cardioprotective effects of the angiotensin-converting enzyme inhibitor perindopril in patients with stable coronary artery disease are not modified by mild to moderate renal insufficiency: insights from the EUROPA trial. *J Am Coll Cardiol* 2007; 50: 2148–2155.
32 Brugts JJ, Knetsch AM, Mattace-Raso FU, Hofman A, Witteman JC: Renal function and risk of myocardial infarction in an elderly population: the Rotterdam study. *Arch Intern Med* 2005; 165: 2659–2665.
33 McPherson R, Pertsemlidis A, Kavaslar N, Stewart A, Roberts R, Cox DR, Tybjaerg-Hansen A, Folsom AR, Boerwinkle E, Hobbs HH, Cohen JC: A common allele on chromosome 9 associated with coronary heart disease. *Science* 2007;316: 1488–1491.
34 Cambien F, Alhenc-Gelas F, Herbeth B, Andre JL, Rakotovao R, Gonzales MF, Allegrini J, Bloch C: Familial resemblance of plasma angiotensin-converting enzyme level: the Nancy study. *Am J Hum Genet* 1988; 43: 774–780.
35 Tiret L, Bonnardeaux A, Poirier O, Ricard S, Marques-Vidal P, Evans A, Arveiler D, Luc G, Kee F, Ducimetière P, et al.: Synergistic effects of angiotensin-converting enzyme and angiotensin-II type 1 receptor gene polymorphisms on risk of myocardial infarction. *Lancet* 1994; 344: 910–913.
36 Kamitani A, Rakugi H, Higaki J, Ohishi M, Shi SJ, Takami S, Nakata Y, Higashino Y, Fujii K, Mikami H, et al.: Enhanced predictability of myocardial infarction in Japanese by combined genotype analysis. *Hypertension* 1995; 25: 950–953.

37 Katsuya T, Koike G, Yee TW, Sharpe N, Jackson R, Norton R, Horiuchi M, Pratt RE, Dzau VJ, MacMahon S: Association of angiotensinogen gene T235 variant with increased risk of coronary heart disease. *Lancet* 1995;345: 1600–1603.
38 Winkelmann BR, Russ AP, Nauck M, Klein B, Bohm BO, Wolf A, Wieland H, Gross W, Galton DJ, März W: Angiotensinogen M235T polymorphism is associated with plasma angiotensinogen and cardiovascular disease. *Am Heart J* 1999; 137: 698–705.
39 Rodríguez-Pérez JC, Rodríguez-Esparragón F, Hernández-Perera O, Anabitarte A, Losada A, Medina A, Hernández E, Fiuza D, Avalos O, Yunis C, Ferrario CM: Association of angiotensinogen M235T and A(–6)G gene polymorphisms with coronary heart disease with independence of essential hypertension: the PROCAGENE study. Prospective Cardiac Gene. *J Am Coll Cardiol* 2001; 37: 1536–1542.
40 Jeunemaitre F, Ledru F, Battaglia S, Guillanneuf MT, Courbon D, Dumont C, Darmon O, Guize L, Guermonprez JL, Diebold B, Ducimetière P: Genetic polymorphisms of the renin angiotensin system and angiographic extent and severity of coronary artery disease: the CORGENE study. *Hum Genet* 1997; 99: 66–73.
41 Kee F, Morrison C, Poirier O, McCrum E, Mallet C, Dallongeville J, Fruchart JC, Evans AE: Angiotensin II type-I receptor and ACE polymorphisms and risk of myocardial infarction in men and women. *Eur J Clin Invest* 2000; 30:1076–1082.
42 Renner W, Nauck M, Winkelmann BR, Hoffmann MM, Scharnagl H, Mayer V, Boehm BO, März W: Association of angiotensinogen haplotypes with angiotensinogen levels but not with blood pressure or coronary artery disease: the Ludwigshafen Risk and Cardiovascular Health Study. *J Mol Med* 2005; 83: 235–239.
43 Sethi AA, Nordestgaard BG, Tybjaerg-Hansen A: Angiotensinogen gene polymorphism, plasma angiotensinogen, and risk of hypertension and ischemic heart disease. *Arterioscler Thromb Vasc Biol* 2003; 23:1269–1275.
44 Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F: An insertion/deletion polymorphism on the angiotensin I converting enzyme gene accouting for half the variance of serum enzyme levels. *J Clin Invest* 1990; 86: 1343–1346.
45 Danser AH, Schalekamp MA, Bax WA, van den Brink AM, Saxena PR, Riegger GA: Angiotensin converting enzyme in the human heart: effect of the deletion/insertion polymorphism. *Circulation* 1995; 92: 1387–1388.
46 Cambien F, Poirier O, Lecerf L, Evans A, Cambou JP, Arveiler D: Deletion polymorphism in the gene for angiotensin converting enzyme is a potent risk factor for myocardial infarction. *Nature* 1992; 359: 641–644.
47 Nakai K, Itoh C, Miura Y, Hotta K, Musha T, TI, Miyakawa T, Iwasaki R, Hiramori K: Deletion polymorphism of the angiotensin-1 converting enzyme gene is associated with serum ACE concentrations and increased risk for CAD in the Japanese. *Circulation* 1994; 90: 2199–2202.
48 Alvarez R, Reguero JR, Batalia A, Iglesias-Cubero G, Cortina A, Alvarez V: Angiotensin-converting enzyme and angiotensin II receptor 1 polymorphism: association with early coronary disease. *Cardiovasc Res* 1998; 40: 375–379.
49 Amant C, Bauters C, Bodart JC, Lablanche JM, Grollier G, Danchin N, Hamon M, Richard F, Helbecque N, McFadden EP, Amouyel P, Bertrand ME: D allele of the angiotensin I converting enzyme is a major risk factor for restenosis after coronary stenting. *Circulation* 1997; 96: 56–60.
50 Ribichini F, Steffenino G, Dellavalle A, Matullo G, Colajanni E, Camilla T, Vado A, Benetton G, Uslenghi E, Piazza A: Plasma activity and insertion/deletion polymorphism of angiotensin I-converting enzyme: a major risk factor and a marker of risk for coronary stent restenosis. *Circulation* 1998; 97: 147–154.
51 Lindpaintner K, Pfeffer MA, Kreutz R, Stampfer MJ, Goldstein F, LaMotte F, Buring J, Hennekens CH: A prospective evaluation of an angiotensin-converting-enzyme gene polymorphism and the risk of ischemic heart disease. *N Engl J Med* 1995; 332: 706–711.
52 Agerholm-Larsen B, Nordestgaard BG, Steffensen R, Sorensen TI, Jensen G, Tybjaerg-Hansen A: ACE gene polymorphism: ischemic heart disease and longevity in 10,150 individuals: a case referent

and retrospective cohort study based on the Copenhagen City Heart Study. *Circulation* 1997; 95: 2358–2367.
53 Samani NJ, Thompson JR, O'Toole L, Channer K, Woods KL: A meta-analysis of the deletion allele of the angiotensin converting enzyme gene with myocardial infarction. *Circulation* 1996; 94: 708–712.
54 Agerholm-Larsen B, Nordestgaard BG, Tybjaerg- Hansen A: ACE gene polymorphism in cardiovascular disease: meta-analysis of small and large studies in whites. *Arterioscler Thromb Vasc Biol* 2000; 20: 484–492.
55 Keavney B, McKenzie C, Parish S, Delépine M, Lathrop M, Peto R, Collins R: Large-scale test of hypothesised associations between the ACE insertion/deletion polymorphism and myocardial infarction in about 5,000 cases and 6,000 controls. International Studies of Infarct Survival (ISIS) Collaborators. *Lancet* 2000; 355: 434–442.
56 Arnett DK, Davis BR, Ford CE, Boerwinkle E, Leiendecker-Foster C, Miller MB, Black H, Eckfeldt JH: Pharmacogenetic association of the angiotensin-converting enzyme insertion/deletion polymorphism on blood pressure and cardiovascular risk in relation to antihypertensive treatment. *Circulation* 2005; 111: 3374–3384.
57 Brugts JJ, Den Uil CA: ACE insertion/deletion polymorphism in sepsis and acute respiratory distress syndrome. *Intensive Care Med* 2008;34:1733.
58 Bis JC, Smith NL, Psaty BM, Heckbert SR, Edwards KL, Letmaitre RN, Lumley T, Rosendaal FR: Angiotensinogen Met235Thr polymorphism, angiotensin-converting enzyme inhibitor therapy, and the risk of nonfatal stroke or myocardial infarction in hypertensive patients. *Am J Hypertens* 2003; 16: 1011–1017.
59 Harrap SB, Tzourio C, Cambien F, Poirier O, Raoux S, Chalmers J, Chapman N, Colman S, Leguennec S, MacMahon S, Neal B, Ohkubo T, Woodward MPCG: The ACE gene I/D polymorphism is not associated with the blood pressure and cardiovascular benefits of ACE inhibition. *Hypertension* 2003; 42: 297–303.
60 PROGRESS Collaborative Group: Randomised trial of a perindopril-based bloodpressure- lowering regimen among 6,105 individuals with previous stroke or transient ischaemic attack. *Lancet* 2001; 358: 1033–1041.
61 Bleumink GS, Schut AF, Sturkenboom MC, van Duijn CM, Deckers JW, Hofman A, Kingma JH, Witteman JC, Stricker BH: Mortality in patients with hypertension on ACE-inhibitor treatment is influenced by the ACE insertion/deletion polymorphism. P*harmacogenet Genomics* 2005; 15: 75–81.
62 Katzov H, Bennet AM, Kehoe P, Wiman B, Gatz M, Blennow K, Lenhard B, Pedersen NL, de Faire U, Prince JA: A cladistic model of ACE sequence variation with implications for myocardial infarction, Alzheimer disease and obesity. *Hum Mol Genet* 2004; 13: 2647–2657.
63 Marciante KD, Bis JC, Rieder MJ, Reiner AP, Lumley T, Monks SA, Kooperberg C, Carlson C, Heckbert SR, Psaty BM: Renin-angiotensin system haplotypes and the risk of myocardial infarction and stroke in pharmacologically treated hypertensive patients. *Am J Epidemiol* 2007; 166: 19–27.

# Chapter 9

# Pharmacogenetics of ACE-inhibition in stable coronary artery disease: steps towards tailored-drug therapy.

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## ABSTRACT

**Purpose of review:** Several trials demonstrated that angiotensin-converting enzyme inhibitors reduce the incidence of cardiovascular events during long-term follow-up in high-risk and low-risk patients. Clinical treatment guidelines propose that angiotensin-converting enzyme inhibitors should be considered in the routine secondary prevention in the broad group of coronary artery disease patients. This review discusses several approaches to guide angiotensin-converting enzyme-inhibition therapy to more specific groups of patients that are most likely to benefit.

**Recent findings**: The beneficial effect of angiotensin-converting enzyme inhibition has been shown to be consistent across subgroups in stable coronary artery disease. Still, large inter-individual variability in blood pressure response is well documented. It should also be realized that the absolute treatment effects are modest. The efficiency and cost-effectiveness of this prolonged prophylactic treatment would be significantly enhanced if those patients can be distinguished who benefit most. Recently, it was suggested that markers of an activated renin–angiotensin–aldosterone system might be used to guide angiotensin-converting enzyme-inhibition therapy.

**Summary:** At the start of treatment, clinical characteristics are not sufficient to distinguish between patients who will and will not benefit from angiotensin-converting enzyme inhibitors. Although pharmacogenetic research in coronary artery disease is still in a premature stage, it may be expected to provide a useful tool in optimizing and individualizing the management of angiotensin-converting enzyme-inhibitor therapy in coronary artery disease patients.

## INTRODUCTION

Clinical treatment guidelines recommend the use of angiotensin-converting enzyme (ACE) inhibitors as routine secondary prevention for the broad group of coronary artery disease (CAD) patients. As the cost-effectiveness of medications is of increasing importance, there is a rapidly growing interest in targeting ACE-inhibitor therapy to specific patient groups that are most likely to benefit. Subgroup analyses of large ACE-inhibitor trials have shown a strong consistency across subgroups. Simple clinical patient characteristics may therefore be insufficient to guide ACE-inhibition therapy and new approaches that integrate more patient specific characteristics are needed. Pharmacogenetic profiling might be a new promising tool to select patients for prolonged prophylactic treatment with ACE inhibitors.

## TEXT OF REVIEW

The beneficial effect of ACE inhibition on the incidence of cardiovascular events has been examined in three large clinical trials (HOPE, PEACE, EUROPA) consisting of patients with stable vascular disease without left ventricular systolic dysfunction or heart failure [1-3]. HOPE demonstrated that ramipril reduced the incidence of cardiovascular events during long-term follow-up in high-risk patients [1]. EUROPA demonstrated a significant reduction in the incidence of cardiovascular events by perindopril during long-term follow-up in low-risk patients with stable CAD [2]. In a comparable patient population, PEACE did not show a significant reduction in cardiovascular events and all-cause mortality with trandolapril [3]. The PEACE investigators suggested that the absence of any treatment benefit was related to a lower baseline risk for cardiovascular events of their patients as compared with HOPE and EUROPA, in particular, because of the high proportion of patients who had previously undergone revascularization or were using more concomitant medication. However, subgroup analyses in EUROPA showed that the beneficial treatment effect of perindopril was independent of baseline risk levels but also of the use of concomitant therapy (beta-blockers, statins, and platelet-inhibitors) [2,4]. Thus, the absence of a treatment benefit in PEACE is more likely related to the reduced power caused by greater crossover and shorter follow-up.

A recent article proposed an alternative explanation for the observed treatment benefit differences between PEACE and EUROPA by arguing that ACE inhibitors are most effective in patients with an increased cardiovascular risk and an activated rennin–angiotensin–aldosterone system (RAAS) [5]. The investigators argued that markers of an activated RAAS, such as left ventricular dysfunction, left ventricular hypertrophy (LVH), and renal function as assessed by eGFR or urinary albumin excretion, might be used to target ACE-inhibitor therapy in stable CAD. This argument was, in part, based on a recent substudy of the PEACE trial showing heterogeneity in the treatment effect of trandolapril [6]. Trandolapril was effective in reducing mortality in patients with an eGFR below 60 ml/min per 1.73 $m^2$. The authors state that an impaired

renal function could therefore be used to target ACE-inhibitor therapy. Although we share the interest in analyses of subgroups to gain insight into the consistency of the treatment effect of ACE inhibitors, we are reluctant to draw conclusions from subgroup analyses of negative trials. A recent substudy of the EUROPA trial examined whether the cardio protective effects of perindopril were indeed modified by renal function [7,8]. The authors conclude that the treatment benefit of perindopril remained consistent and was not modified by renal insufficiency. According to this analysis, renal insufficiency could not be used to guide ACE-inhibitor therapy in stable CAD.

## Consistency of treatment effect of ACE-inhibitors in stable CAD

Several subgroup analyses have shown that the beneficial treatment effect of ACE inhibitors is consistent among subgroups of diabetes mellitus, hypertension, and renal insufficiency, and is independent of baseline risk [2,4,7-9]. Also when the three ACE-inhibitor trials in stable CAD [Heart Outcomes Prevention Evaluation (HOPE), EUropean trial on Reduction Of cardiac events with Perindopril in stable coronary Artery disease (EUROPA), Prevention of Events with Angiotensin Converting Enzyme (PEACE)] were combined, the results show significant reductions in total and cardiovascular mortality, recurrent or new nonfatal myocardial infarction, fatal and nonfatal strokes, heart failure requiring hospitalization, and coronary artery bypass graft (CABG) surgery with no significant differences between the studies [9]. In our opinion, this consistency makes it impossible to guide ACE-inhibitor therapy based on simple clinical characteristics.



## From guidelines towards tailored therapy

Currently, ESC and AHA/ACC clinical treatment guidelines recommend to consider the use of ACE inhibitors in the routine secondary prevention measures for the broad group of CAD patients with, respectively, a class I (level of evidence A) recommendation for ACE-inhibitor therapy in CAD patients with coincident indications for ACE inhibition such as hypertension, heart failure, left ventricle (LV) dysfunction, prior myocardial infarction (MI) with LV dysfunction or diabetes, and a class IIa recommendation of ACE-inhibitor therapy in all patients with angina and proven coronary disease (level of evidence B) in angina pectoris without coexisting indication for ACE-inhibitor treatment [10,11]. The anticipated benefit should be weighed against the costs and risks of side-effects and the dose and agent used of proven efficacy for this indication.

The recommendations that are made in treatment guidelines are usually based on clinical trials providing consistent evidence for the prescription of a certain drug to a selected group of patients. However, a problem clinicians encounter is that the individual patient in the ward or outpatient clinic often does not necessarily fit the exact profile (inclusion criteria and exclusion criteria) that was used in those trials. Also, large inter-individual variability in the response to treatment is well documented for many drugs. These factors may lead to uncertainty over how to decide what is best for the individual patient, and explain the inadequate lower adherence to guidelines as demonstrated by the findings of the Euro Heart Survey [12].

In light of the growing interest in targeting medications to specific subgroups, knowledge of the consistency of the treatment effect by ACE inhibitors is highly important. The ACE inhibitors comprise some of the most commonly used drugs in stable CAD. The discovery of the determinants of treatment effect may lead to a more specified indication, prescription or dosage of a certain drug, which will enhance its cost-effectiveness. The number of patients needed to treat could be significantly reduced if we targeted therapy to those patients with the highest risk, or those with the highest benefit from therapy. Also, patients are likely to differ in the needed dosage or type of drug. As discussed earlier, prior subgroup analyses have not resulted in adequate means to target ACE-inhibition therapy in stable CAD. As a better method, we propose a new strategy to guide ACE-inhibition therapy by integrating patients' genetic information into treatment decisions.

## Pharmacogenetics of ACE-inhibition in stable coronary artery disease

The new field of pharmacogenetics involves examining the genetic determinants of patients' responses to drugs; in other words, understanding why some drugs work better for some people than others and why some people are more likely than others to experience side-effects. In our opinion, pharmacogenetic profiling might be a new way to reach significant advances in individualized cardiovascular medicine. A priori, it is expected for several types of factors that they play a role in determining the response of a patient to therapy. Genetic factors causing differences in drug absorption and metabolic clearance are also relevant; however; there is yet a relatively unexplored field. Genetic factors within the pathway that is directly affected by the drug, the renin–angiotensin–aldosterone–system, can affect the efficiency of ACE inhibitors. With regard to the RAAS genes, several polymorphisms have been shown to contribute to the risk of cardiovascular events. Because of this relationship with cardiovascular risk, these genetic variants are now also of utmost interest in pharmacogenetic analyses of ACE-inhibitor drug response. We will discuss the most extensively studied RAAS genes, the ACE gene and the angiotensinogen (AGT) gene, first with regard to cardiovascular risk associations and then following the first pharmacogenetic investigations with ACE-inhibitor drug response.

### *Angiotensin-converting enzyme gene*

ACE generates angiotensin II from angiotensin I and degrades bradykinin. Several studies have demonstrated that genetic polymorphisms in the ACE gene influence plasma and tissue ACE levels [13,14]. Reasonably, ACE gene polymorphisms could therefore also be related to clinical outcomes. This has already been investigated extensively, although with inconclusive results. The most frequently studied genetic polymorphism for the association with CAD is the ACE insertion/deletion genotype, which is based on the presence (I) or absence (D) of a 287-base pair Alu repeat sequence within intron 16. In 1992, the first report of a significant association with MI for the ACE DD genotype (OR 1.34) was presented [15]. This resulted in an enormous boost of research, in which many groups reported positive associations, but others did not. The great

inconsistency of the results was mainly related to study sample size and ethnicity. In addition, the percentage of ACE variability that can be accounted for by the I/D polymorphism turned out to be much lower (<20%) than originally described, and ACE I/D polymorphism-related differences in angiotensin I–II conversion could not be observed in vivo [16,17]. In a recent large meta-analysis, the existence of any substantial association with MI could not be confirmed [18]. The risk ratio of ACE I/D with MI seems to lie in the range of a 10 % increased risk. Another large meta-analysis, in 32.715 patients, concluded that the ACE I/D polymorphism affects plasma ACE activity but not blood pressure or the risk of MI [19]. Smaller studies tended to show a more favourable result for the ACE I/D polymorphism (small versus large studies: P for heterogeneity <0.001) [19]. The contribution of the ACE I/D polymorphism may therefore be much smaller than frequently thought and is not likely to be an important causative factor in CAD.

With regard to the relation with the response to ACE inhibitors, pharmacogenetic knowledge is limited. In hypertensive patients, studies focused mainly on the relation between ACE I/D genotype and the response to ACE inhibitors [20-23]. Again the smaller studies tended to be favourable for an association of the ACE genotype with response to ACE inhibitors. More recent larger studies reported no effect of the ACE I/D genotype on the response of ACE inhibitors [24,25]. Of special interest are two relatively large pharmacogenetic association analyses. The GenHAT study consisted of hypertensive patients aged 55 years or over and concluded that ACE I/D genotype group (n=7528, lisinopril) was not a predictor of coronary heart disease (CHD), nor did it modify the response to antihypertensive treatment [25]. The AASK trial (n=342), however, showed that the ACE I/D polymorphism predicted the time-course of blood pressure reduction in response to ACE inhibition in African–American patients [26]. Regrettably, all studies investigated the ACE I/D polymorphism only. To date, we have to conclude that, due to inconsistent results, we do not know whether there is a relation of the ACE I/D polymorphism with CAD or response to ACE inhibitors. At least, the initial enthusiasm has to be tempered. But we also have to realize the enormous gap in information, as nearly all studies focused on only one polymorphism in the ACE gene, which determines less than 20 % of ACE variability. The ACE I/D polymorphism is just one out of several hundred polymorphisms in the ACE gene and many more need to be investigated comprehensively before we can generalize these results to the entire ACE gene. Many other polymorphisms are expected to be more important based on functionality, location in the gene (promoter, exon) or larger effects on plasma ACE levels as compared with the ACE I/D polymorphism, which is located in an intronic region of the ACE gene.



### *Angiotensinogen gene*

More promising results were obtained when studying the AGT gene. Polymorphisms in the angiotensinogen (AGT) gene, one of the major structural genes in the RAAS pathway, have been associated with hypertension [27-31]. The AGT single-nucleotide polymorphisms (SNPs) M235T (rs699), M174T (rs4762), and A-6G (rs5051) have been associated with increased serum AGT levels [28-31]. The AGT SNPs have also been associated with BP-related phenotypes within

different ethnic groups [29]. The most extensively studied polymorphism is the M235T polymorphism, a non synonymous SNP with a functional amino acid change (Met-Thr) located in exon 2 of the AGT gene [32]. Of special interest is the PROCAGENE study (n=615), which showed that genetic variation of the AGT (M235T polymorphism) contributed significantly to the presence of CHD independently of blood pressure profile in a subset of the Spanish population with a high prevalence of CHD [33]. In multiple logistic regression analysis, the odds ratio for CHD associated with 235T was 1.7 (1.1–2.6). The Group Health study (n=1412) used a haplotype approach in the AGT gene to assess the risk of MI [34]. The authors did find a significant association of AGT haplotypes with the risk of MI; however, results were not statistically significant given the number of tests performed. In this study, the haplotype approach was demonstrated as a promising tool in pharmacogenetic research. AGT gene polymorphisms have also been studied for associations with the response to ACE inhibitors. Two studies with unspecified ACE inhibitors found a similar pharmacogenetic association between treatment and AGT M235T (although outcomes were different – stroke risk vs. BP lowering, respectively) [35,36]. However, in studies of AGT M235T with lisinopril and captopril, no pharmacogenetic effect was observed [37,38]. In a population-based study, Schelleman et al. [40] studied the M235T polymorphism in relation to risk of CVD and drug–gene interaction with antihypertensive treatment but no strong associations were found. Of special interest is a pharmacogenetic study that originated from the Chinese Community-Based Comprehensive Prevention and Control of Hypertension project [40]. In this substudy, 1447 hypertensive patients from a 3-year benazepril postmarket surveillance trial were genotyped for 14 SNPs in the AGT, and AGT-receptor type 1 genes. The AGT rs7079 (C/T) SNP (30-untranslated region) polymorphism was significantly associated with the response of diastolic blood pressure (BP) to benazepril (diastolic BP response: 7.4 mmHg for CC genotype, 8.9 mmHg for CA, and 10.1 mmHg for AA; $P<0.001$). The authors concluded that variants of the AGT gene are associated with BP response to ACE-inhibition therapy. If confirmed in other populations, they will be useful for predicting blood pressure response to ACE-inhibition treatment.

The enormous open area for the pharmacogenetics of the RAAS polymorphisms and ACE-inhibitor treatment effects suggests that, although considerable work with ACE inhibitors has been done, potentially important pharmacogenetic combinations remain unexplored. Most studies were small and therefore limited by statistical power, which explains a large part of the inconsistency in the current literature. Also, inconsistent results appear due to methodological limitations (e.g., genotyping or phenotyping errors), study design (lack of haplotype approach), and differences between study subjects (ethnicity) or applied medications. Regrettably, nearly all studies have investigated only one polymorphism, mainly the ACE I/D or the M235T polymorphism, in relation to CAD risk or drug response to ACE inhibitors. Combining information from multiple SNPs in the RAAS genes, preferably using the haplotype approach, will give much more insight in future pharmacogenetic analyses. Haplotypes are a combination

of alleles at different markers along the same chromosome that are inherited as a unit (linkage disequilibrium patterns). If a disease causal SNP is not in linkage disequilibrium with the marker / analyzed SNP, association may be missed by single SNP association tests. It is more informative to simultaneously analyze markers in a region of interest that identifies genetic variants underlying various human traits. In this way, a more comprehensive in-depth analysis of RAAS polymorphisms in relation to ACE-inhibitor therapy is performed, which is more likely to unravel a pharmacogenetic association.

## CONCLUSION

The ACE-inhibitors comprise some of the most commonly used drugs in stable CAD. Several approaches to target ACE-inhibition therapy to those patients who are most likely to benefit have not resulted in adequate ways to guide the prescription of ACE inhibitors. Such 'tailored therapy' may be achieved through the integration of genetic information of patients. Pharmacogenetic research of ACE inhibitors in CAD patients is still in a premature stage, but, if it were feasible to construct a pharmacogenetic profile related to cardiovascular risk as well as to drug response and side effects, it would be the way to help clinicians to target therapy to those patients who will benefit most. Until now, pharmacogenetic results have been largely inconsistent and we do not know yet whether this new approach will have an impact on clinical practice.



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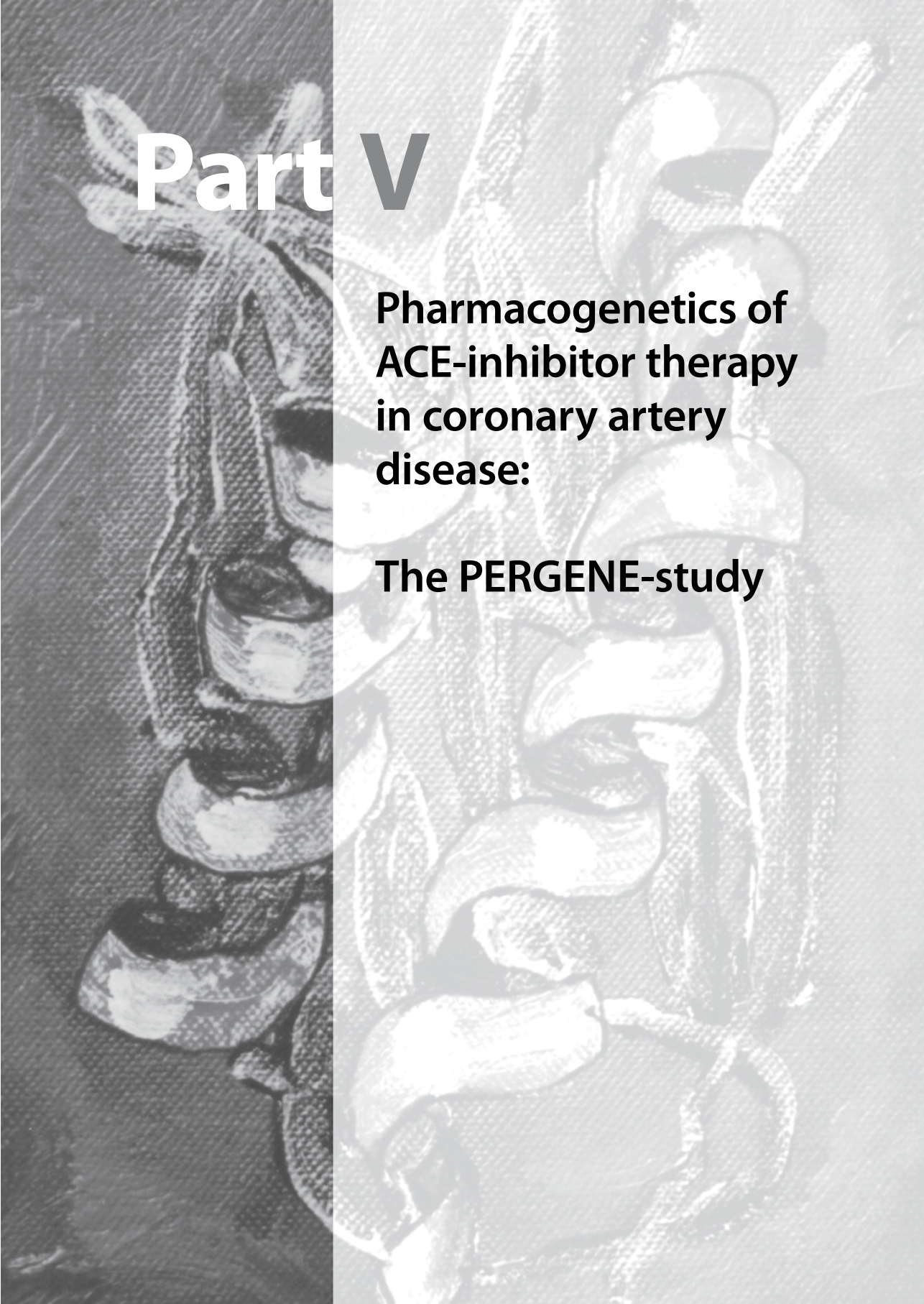
## REFERENCES


1. The Heart Outcomes Prevention Evaluation Study Investigators. Effects of an angiotensin-converting enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. *N Engl J Med* 2000; 342:145–153.
2. The European Trial on Reduction of Cardiac Events with Perindopril in Patients with Stable Coronary Artery Disease Investigators. Efficacy of perindopril in reduction of cardiovascular events in stable coronary artery disease: randomised, double-blind, placebo-controlled, multicentre trial (the EUROPA study). *Lancet* 2003; 362:782–788.
3. The PEACE Trial Investigators. Angiotensin-converting-enzyme inhibition in stable coronary artery disease. *N Engl J Med* 2004; 351:2058–2068.
4. Deckers JW, Goedhart DM, Boersma E, et al. Treatment benefit by perindopril in patients with stable coronary artery disease at different levels of risk. *Eur Heart J* 2006; 27:796–801.
5. Asselbergs FW, van Gilst WH. Angiotensin converting enzyme inhibition in cardiovascular risk populations: a practical approach to identify the patient who will benefit most. *Curr Opin Cardiol* 2007; 22:267–272.
6. Solomon SD, Rice MM, Braunwald E, et al. Renal function and the effectiveness of angiotensin-converting enzyme inhibitor therapy in patients with chronic stable coronary disease in the Prevention of Events With ACE Inhibition (PEACE) trial. *Circulation* 2006; 114:26–31.
7. Brugts JJ, Boersma E, Simoons ML, et al. The cardioprotective effects of the angiotensin-converting enzyme inhibitor perindopril in patients with stable coronary artery disease are not modified by mild to moderate renal insufficiency: insights from the EUROPA trial. *J Am Coll Cardiol* 2007; 50:2148–2155.
8. Brugts JJ, Knetsch A, Hofman A, et al. Renal function and risk of myocardial infarction in an elderly population: the Rotterdam Study. *Arch Intern Med* 2005; 165:2659–2665.
9. Dagenais GR, Pogue J, Yusuf S. Angiotensin-converting-enzyme inhibitors in stable vascular disease without left ventricular systolic dysfunction or heart failure: a combined analysis of three trials. *Lancet* 2006; 368:581–588.
10. The Task Force on the Management of Stable Angina Pectoris of the European Society of Cardiology. Guidelines on the management of stable angina pectoris. *Eur Heart J* 2006; 27:1341–1381.
11. Gibbons RJ, Abrams J, Chatterjee K, et al. ACC/AHA 2002 guideline update for the management of patients with chronic stable angina–summary article: a report of the American College of Cardiology/American Heart Association Task Force on practice guidelines (Committee on the Management of Patients With Chronic Stable Angina). *J Am Coll Cardiol* 2003; 41:159–168.
12. Lenzen MJ, Boersma E, Reimer WJ, et al. Under-utilization of evidence-based drug treatment in patients with heart failure is only partially explained by dissimilarity to patients enrolled in landmark trials: a report from the Euro Heart Survey on Heart Failure. *Eur Heart J* 2005; 26:2706–2713.
13. Villard E, Tiret L, Soubrier F, et al. Identification of new polymorphisms of the angiotensin I-converting enzyme (ACE) gene, and study of their relationship to plasma ACE levels by two-QTL segregation-linkage analysis. *Am J Hum Genet* 1996; 58:1268–1278.
14. Danser AH, Schalekamp MA, Schunkert H, et al. Angiotensin-converting enzyme in the human heart. Effect of the deletion/insertion polymorphism. *Circulation* 1995; 92:1387–1388.
15. Cambien F, Poirier O, Lecerf L, et al. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature* 1992; 359:641–644.
16. Danser AH, Batenburg WW, Danilov SM, et al. ACE phenotyping as a first step toward personalized medicine for ACE inhibitors. *Pharmacol Ther* 2007; 113:607–618.
17. Danser AH, Deinum J, Schalekamp MA, et al. Angiotensin I to angiotensin II conversion in the human forearm and leg. Effect of the angiotensin converting enzyme gene insertion/deletion polymorphism. *J Hypertens* 1999; 17:1867–1872.

18. Keavney B. Large-scale test of hypothesised associations between the ACE insertion/deletion polymorphism and myocardial infarction in about 5000 cases and 6000 controls. International Studies of Infarct Survival (ISIS) Collaborators. *Lancet* 2000; 355:434-442.
19. Agerholm-Larsen B, Nordestgaard BG, Tybjærg-Hansen A. ACE gene polymorphism in cardiovascular disease: meta-analyses of small and large studies in whites. *Arterioscler Thromb Vasc Biol* 2000; 20:484–492.
20. Stavroulakis GA, Makris TK, Kyriakidis M, et al. Predicting response to chronic antihypertensive treatment with fosinopril: the role of angiotensin-converting enzyme gene polymorphism. *Cardiovasc Drugs Ther* 2000; 14:427–432.
21. Harrap SB, Tzourio C, Woodward M, et al. The ACE gene I/D polymorphism is not associated with the blood pressure and cardiovascular benefits of ACE inhibition. *Hypertension* 2003; 42:297–303.
22. Li X, Du Y, Huang X, et al. Correlation of angiotensin-converting enzyme gene polymorphism with effect of antihypertensive therapy by angiotensin-converting enzyme inhibitor. *J Cardiovasc Pharmacol Ther* 2003; 8:25–30.
23. Yu H, Zhang Y, Liu G, et al. Relationship between polymorphism of the angiotensin-converting enzyme gene and the response to angiotensin-converting enzyme inhibition in hypertensive patients. *Hypertens Res* 2003; 26:881–886.
24. Voors AA, van Geel PP, van Gilst WH, et al. Vascular effects of quinapril completely depend on ACE insertion/deletion polymorphism. *J Renin Angiotensin Aldosterone Syst* 2004; 5:130–134.
25. Arnett DK, Davis BR, Eckfeldt JH, et al. Pharmacogenetic association of the angiotensin-converting enzyme insertion/deletion polymorphism on blood pressure and cardiovascular risk in relation to antihypertensive treatment. The Genetics of Hypertension-Associated Treatment (GenHAT) Study. *Circulation* 2005; 111:3374–3383.
26. Bhatnagar V, O'Connor DT, Lipkowitz MS, et al. Angiotensin-converting enzyme gene polymorphism predicts the time-course of blood pressure response to angiotensin converting enzyme inhibition in the AASK trial. *J Hypertens* 2007; 25:2082–2092.
27. Jeunemaitre X, Soubrier F, Corvol P, et al. Molecular basis of human hypertension: role of angiotensinogen. *Cell* 1992; 71:169–180.
28. Pereira AC, Mota GF, Krieger JE, et al. Angiotensinogen 235T allele 'dosage' is associated with blood pressure phenotypes. *Hypertension* 2003; 41:25–30.
29. Markovic D, Tang X, Ott J, et al. Association of angiotensinogen gene polymorphisms with essential hypertension in African-Americans and Caucasians. *Hum Hered* 2005; 60:89–96.
30. Brand-Herrmann SM, Ko¨ pke K, Brand E, et al. Angiotensinogen promoter haplotypes are associated with blood pressure in untreated hypertensives. *J Hypertens* 2004; 22:1289–1297.
31. Gu CC, Chang YP, Rao DC, et al. Haplotype association analysis of AGT variants with hypertension-related traits: the HyperGEN Study. *Hum Hered* 2005; 60:164–176.
32. Sethi AA, Nordestgaard BG, Tybjærg-Hansen A, et al. Angiotensinogen gene polymorphism, plasma angiotensinogen, and risk of hypertension and ischemic heart disease: a meta-analysis. *Arterioscler Thromb Vasc Biol* 2003; 23:1269–1275.
33. Rodriguez-Perez JC, Rodriguez-Esparragon F, Ferrario CM, et al. Association of angiotensinogen M235T and A(-6)G gene polymorphisms with coronary heart disease and with independence of essential hypertension: The PROCAGENE Study. *J Am Coll Cardiol* 2001; 37:1536–1542.
34. Marciante KD, Bis JC, Psaty BM, et al. Renin-angiotensin system haplotypes and the risk of myocardial infarction and stroke in pharmacologically treated hypertensive patients. *Am J Epidemiol* 2007; 166:19–27.
35. Bis JC, Smith NL, Rosendaal FR, et al. Angiotensinogen Met235Thr polymorphism, angiotensin-converting enzyme inhibitor therapy, and the risk of nonfatal stroke or myocardial infarction in hypertensive patients. *Am J Hypertens* 2003; 16:1011–1017.

36. Hingorani AD, Jia H, Brown MJ, et al. Renin–angiotensin system gene polymorphisms influence blood pressure and the response to angiotensin converting enzyme inhibition. *J Hypertens* 1995; 13:1602–1609.
37. Dudley C, Keavney B, Ratcliffe P, et al. Prediction of patient responses to antihypertensive drugs using genetic polymorphisms: investigation of renin-angiotensin system genes. *J Hypertens* 1996; 14:259–262.
38. Mondorf UF, Russ A, Lenz T, et al. Contribution of angiotensin I converting enzyme gene polymorphism and angiotensinogen gene polymorphism to blood pressure regulation in essential hypertension. *Am J Hypertens* 1998; 11:174–183.
39. Schelleman H, Klungel OH, Stricker BH, et al. Angiotensinogen M235T polymorphism and the risk of myocardial infarction and stroke among hypertensive patients on ACE-inhibitors or beta-blockers. *Eur J Hum Genet* 2007; 15:478–484.
40. Xiaowen S, Lee L, Yang H, et al. Association between angiotensinogen, angiotensin ii receptor genes, and blood pressure response to an angiotensin converting enzyme inhibitor. *Circulation* 2007; 115:725–732.



# Part V

## Pharmacogenetics of ACE-inhibitor therapy in coronary artery disease:

## The PERGENE-study

# Chapter 10

# The design and rationale of the PERindopril GENEtic Association Study (PERGENE): a pharmacogenetic analysis of treatment benefit of ACE-inhibitor therapy in patients with stable CAD

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## ABSTRACT

**Background:** Angiotensin-converting enzyme (ACE) inhibitors reduce clinical symptoms and improve outcome in patients with hypertension, heart failure, and stable coronary artery disease (CAD) and are among the most frequently used drugs in these patient groups. For hypertension, treatment is guided by the level of blood pressure. In the secondary prevention setting, there are no means of guiding therapy. Prior attempts to target ACE-inhibitors to those patients that are most likely to benefit have not been successful, mainly due to the consistency in the treatment effect in clinical subgroups. Still, for prolonged prophylactic treatment with ACE-inhibitors it would be best to target treatment to only those patients most likely to benefit, which would considerably lower the number needed to treat and increase cost-effectiveness. A new approach for such "tailored-therapy" may be to integrate information on the genetic variation between patients. Until now, pharmacogenetic research of the efficacy of ACE-inhibitor therapy in CAD patients is still in a preliminary stage.

**Methods**: The PERindopril GENEtic association study (PERGENE) is a substudy of the EUROPA trial, a randomized double-blind placebo-controlled multicentre clinical trial which demonstrated a beneficial effect of the ACE-inhibitor perindopril in reducing cardiovascular morbidity and mortality in 12.218 patients with stable coronary artery disease (mean follow-up 4.2 years). Blood tubes were received from patients at the beginning of the EUROPA trial and buffy coats were stored at −40°C at the central core laboratory. Candidate genes were selected in the renin–angiotensin-system and bradykinin pathways. Polymorphisms were selected based on haplotype tagging principles using the HapMap genome project, Seattle and other up-to-date genetic database platforms to comprehensively cover all common genetic variation within the genes. Selection also took into consideration the functionality of SNP's, location within the gene (promoter) and existing relevant literature. The main outcome measure of PERGENE is the effect of genetic factors on the treatment benefit with ACE-inhibitors. The size of this pharmacogenetic substudy allows detection with a statistical power of 98 % to detect a difference in hazard ratios (treatment effect) of 20 % between genotypes with minor allele frequency of 0.20 (two-sided alpha 0.05).

**Conclusion**: The PERGENE study is a large cardiovascular pharmacogenetic study aimed to assess the feasibility of pharmacogenetic profiling of the treatment effect of ACE-inhibitor use with the perspective to individualize treatment in patients with stable coronary artery disease.

## INTRODUCTION

The efficacy of ACE-inhibitors to improve outcome has been demonstrated by several large clinical trials in patients with cardiovascular disease. These include post-myocardial infarction (MI) patients, patients with asymptomatic left ventricular systolic dysfunction, heart failure or a history of cerebrovascular disease, and stable CAD patients with preserved left ventricular function [1-7]. Currently, the use of ACE inhibitors is recommended in guidelines on the management of hypertension, stable CAD, MI, heart failure, and in the prevention of the progression of renal insufficiency in diabetes mellitus related kidney disease [8-10].

The ACE-inhibitor perindopril has been extensively studied in several large, controlled trials in a variety of patient groups with different etiologies [7,11-14]. Of these, the EUROPA trial is noteworthy, as it is the only secondary prevention study with perindopril in a stable CAD population. ACE inhibitor research has also led to a better understanding of pathophysiological processes and maladaptive responses in the renin–angiotensin aldosterone system (RAAS). In particular, several sub-studies of EUROPA (including PERFECT, PERSPECTIVE and PERTINENT), have established that ACE inhibitors have additional effects beyond the blood pressure reduction alone such as the improvement of endothelial function, improvement of the neurohumoral balance, and reduction of unfavorable remodeling of the coronary arteries [15-18].

For hypertension, treatment is guided by the level of blood pressure. In the secondary prevention setting, there are no means by which we can guide therapy. Many patients need to be treated, and absolute benefits are modest. It is not yet possible to predict in advance which patients are to benefit most from treatment. Prior attempts to target ACE-inhibitors to those patients who are most likely to benefit have not been successful, mainly because of the consistency in the treatment effect in clinically relevant subgroups based on simple clinical characteristics [19-21]. Treating only those patients who are most likely to benefit would considerably lower the number needed to treat and increase cost-effectiveness.

A new approach to "tailored-therapy" concerns cardiovascular pharmacogenetics which examine the genetic determinants of patients' responses to cardiovascular drugs; in other words, understanding why some drugs work better for some patients than others and why some patients are more likely to experience serious side-effects than others. Many (if not all) aspects of human physiology have genetic determinants and could therefore be subject to pharmacogenetic studies. Several factors may be expected to play a dominant role in determining the response of a patient to therapy. In particular, in the case of ACE inhibition, genetic factors within the RAS pathway are likely to affect its pharmacodynamics and clinical efficacy. Genetic factors causing differences in drug absorption and metabolic clearance are also relevant. However, until now there are no strong leads to explore this pharmacogenetically since there are no metabolic (CYP) genes linked specifically to ACE-inhibitors, although this might also be a relevant pathway to investigate in future research. In previous studies, several genetic polymorphisms in RAAS genes have been associated with high blood pressure levels or an

increased cardiovascular risk [22,23]. Nearly all these studies focused at two polymorphisms, the ACE I/D polymorphism and the M235T polymorphism in the angiotensinogen gene. Because of limited power, due to limited study sample size, results have been inconsistent and the underlying questions not answered adequately.

With regard to interaction between genetic factors and treatment response, the results are scarce. No prior studies have been performed yet with ACE-inhibitors at a large scale neither in a randomized setting nor in stable CAD (one of the major indications of ACE-inhibitors). Another important limitation of prior research in cardiovascular pharmacogenetics is the investigation of one or two polymorphisms within only one gene, thereby ignoring the well documented feedback mechanisms within the RAAS and the fact that there are two angiotensin II receptors (AT1 and AT2), which have counteracting effects also in humans. We suggest that a more comprehensive coverage of genetic variation in multiple RAAS genes is needed, by using a correct haplotype approach. This is achieved by using the latest information on genetic variation and linkage disequilibrium patterns in the selection of haplotype-tagging SNP's.

The PERGENE study aims at assessing the feasibility of pharmacogenetic profiling of ACE-inhibitor therapy in patients with stable CAD. We hypothesized that genetic polymorphism in the RAAS and kininogen–kallikrein–bradykinin pathways may influence the treatment effect of ACE-inhibitors in patients with stable CAD. The PERGENE study is unique in the field of pharmacogenetic studies because of the large sample size, a randomized and placebo-controlled design, and the availability of extensive and accurate phenotypic data. Also, the extensive selection of tagging SNP's in multiple genes in both pathways ensures a new and comprehensive coverage of common genetic variation in the candidate genes. A detection of heterogeneity in the treatment benefit according to genetic determinants may lead to significant advances in tailored therapy and personalized medicine.



## METHODS

### *Study population and design*

The PERGENE study is a substudy of the EUROPA-trial that will investigate genetic determinants of the treatment effect of ACE-inhibition in all subjects. The study design of the EUROPA trial has been described in detail elsewhere [24]. In short, the EUROPA-trial was a randomized, double-blind, placebo-controlled clinical trial, with 12.218 patients who were randomized after a 4-week run-in period. Mean follow-up was 4.2 years. The study recruited men and women aged ≥ 18 years without clinical evidence of heart failure and with evidence of coronary artery disease documented by either previous MI, percutaneous or surgical coronary revascularization or angiographic evidence of ≥ 70% narrowing of ≥ 1major coronary artery. Men were also recruited if they had a history of chest pain and a positive exercise test or regional wall motion abnormalities during stress echocardiography or nuclear scintigraphy or with transient perfusion defects during scintigraphy perfusion imaging.

The EUROPA study comprised a run-in period of 2 weeks during which patients received perindopril 4 mg/day, followed by 2 weeks during which patients received perindopril 8 mg/day provided that the 4 mg/day of perindopril was well tolerated. At the end of the run-in period, a double-blind treatment period of at least 36 months started during which patients received either perindopril 8 mg/day or placebo. Patients continued in the study until the last patient included completed the follow-up period. Following randomization, patients were seen at 3, 6 and 12 months and thereafter at six monthly intervals. Written informed consent was obtained from all patients for performing genetic association analyses.

### *Outcome measures*

The main outcome measure of PERGENE is to assess (1) the effect of RAAS and bradykinin polymorphisms on the risk reduction of the primary endpoint of EUROPA (cardiovascular mortality, non-fatal MI, or successful resuscitated cardiac arrest) by perindopril. Other outcome measures are: (2) the effect of RAAS- and bradykinin polymorphisms on the amount of blood pressure reduction by perindopril treatment during the run-in period (in which all patient were treated with perindopril), (3) the effect of RAAS- and bradykinin polymorphisms on blood pressure, (4) the effect of RAAS- and bradykinin polymorphisms on incident cardiovascular risk during 4 years follow-up, and (5) the effect of RAAS- and bradykinin polymorphisms in relation to intolerance of ACE-inhibitors (Table 1). Other relevant hypotheses can be investigated in the future.

**Table 1.** Outcome measures of the PERGENE study

| **Endpoint definitions of PERGENE** |
|---|
| - The effect of genetic factors on the treatment effect by perindopril * |
| - The effect of genetic factors on blood pressure reduction levels by ACE-inhibition. |
| - The effect of genetic factors in relation to baseline hypertension ** |
| - The effect of genetic factors on incident cardiovascular risk during 4 years of follow-up. |
| - The effect of genetic factors on side-effects or intolerance during ACE-inhibitor treatment. |

* Defined as the reduction in the primary endpoint of the EUROPA-trial (cardiovascular mortality, non-fatal MI, or successfully resuscitated cardiac arrest). ** pre-defined in the EUROPA study protocol as 160 mmHg and/or use of antihypertensives.

### *Data collection*

A logistic procedure and a high-quality program for the creation of a DNA bio-bank were established within the EUROPA trial (Figure 1) [25]. Our group defined a successful protocol for large-scale blood samples collection in a large multicentre clinical trial. Blood sample were sent to a central laboratory (TNO Leiden, the Netherlands), registered, labelled and erythrocytes were lyzed. Buffy coats were frozen at –40°C in three aliquots. A total of 10.497 blood tubes of participants of the EUROPA-trial were received at the central core laboratory for storage. The average time between blood collection and processing was 10.1 (SD 8.4) days. The effect of transport time in the EUROPA trial was validated and shown to have no major effect on the DNA quality and quantity in a selection of 61 blood samples representing a wide range of transport

times and countries. In all cases, sufficient amounts of DNA could be isolated and obtained from all samples; there was no relation with the region of origin, or with varying environmental temperatures, and the amount of DNA isolated. The quality and quantity of the DNA was high and well suited for performing genetic association studies [25].

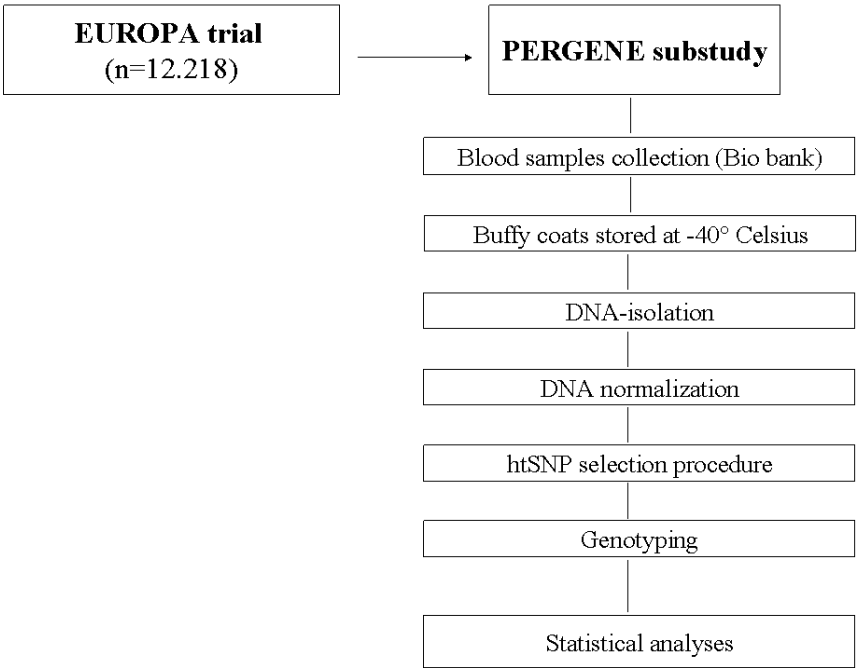


**Figure 1.** Flow diagram of PERGENE study design. htSNP= haplotype-tagging SNP

*DNA isolation*

DNA was isolated from the stored white blood cells at the Genetic Laboratory of the department of Internal Medicine at the Erasmus MC using an automated isolation process (Hamilton liquid handler coupled with Magnetic separator for automated DNA extraction). The isolated DNA was stored in matrix 2D tubes and normalized and reformatted, using a pipetting robot, and dispensed into 384-well PCR plates using a Caliper Sciclone ALH3000 pipetting robot (Caliper LS, Mountain View, CA).

*Candidate genes*

The candidate genes to be studied are located in the RAAS and bradykinin pathways as presented in Figure 2. ACE-inhibitors inhibit the angiotensin-converting enzyme, which is a central component of the RAAS and genes in this pathway are therefore highly relevant to be studied with respect to treatment effect of ACE-inhibitors. Candidate genes are ACE, angiotensinogen

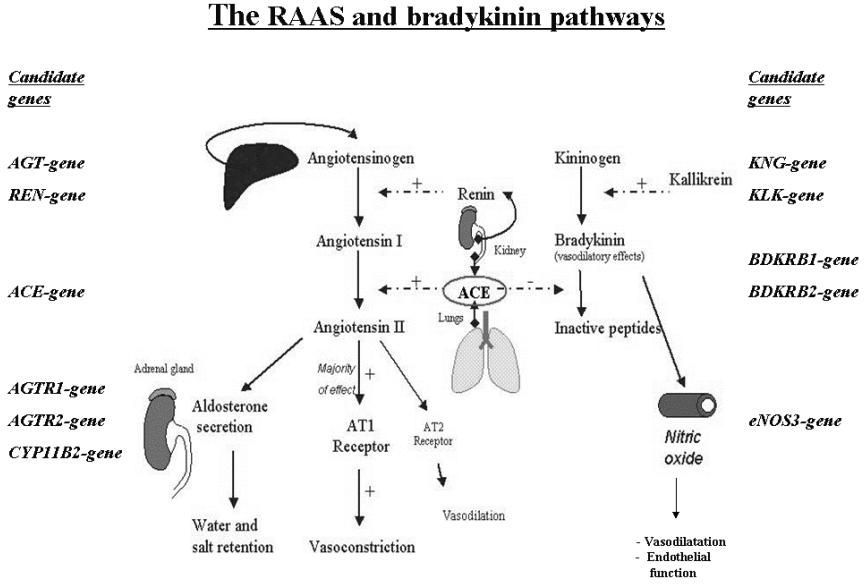


**Figure 2.** Selected candidate genes in the renin-angiotensin-aldosteron-system and bradykinin pathways. Abbreviations: AGT (angiotensinogen), REN (renin), ACE (angiotensin-converting enzyme), AGTR1, (angiotensin receptor type 1), AGTR2 (angiotensin receptor type 2), CYP11B (aldosteron synthase), KLK (kallikrein), KNG (kininogen), BDKR1 (bradykinin receptor type 1), BDKR2 (bradykinin receptor type 2), eNOS3 (nitric oxide synthase).

(AGT) and the angiotensin II receptor type 1 and type 2 gene (AGTR1, AGTR2). Also, several new and relatively unexplored genes in this pathway are of interest to investigate pharmacogenetically, such as the renin (REN) and aldosterone synthase genes (CYP11b2). Moreover, as ACE cleaves bradykinin into inactive peptides, ACE-inhibitors increase bradykinin, which, amongst others, results in anti-remodeling, anti-atherosclerotic and anti-thrombotic effects, improves endothelial function and is also a strong vasodilator. As such, bradykinin counteracts the effect of angiotensin II in many ways. This pathway has not been explored yet with respect to pharmacogenetics, but may be very interesting, especially since the blood pressure effect and clinical effect of ACE-inhibitors are likely to be caused by bradykinin [26]. Relevant genes in this pathway are kallikrein (KLK), kininogen (KNG), and bradykinin-receptors (BDKRB 1 and 2), and the resultant endothelial nitric oxide synthase genes (eNOS3). This list of candidate genes ensures a comprehensive coverage of relevant genes in the RAAS and bradykinin pathways, which have not been investigated to this extent in prior studies.

### *Selection of single nucleotide polymorphisms (SNPs)*

To cover the genetic variation in these candidate genes more comprehensively, the list of selected polymorphisms in the candidate genes were selected using the latest genetic information

from available databases (such as dbSNP: http://www.ncbi.nlm.nih.gov/SNP and Celera http://www.celeradiscoverysystem.com) and our own research (WAVE dHPLC; sequencing). The final selection will also take into account the haplotype block structure of these SNPs by using HapMap data (HapMap Release 23a/ Phase II Mar 08/on NCBI B36 assembly/ DbSNP b126), and SeattleSNP databases (see: http://hapmap.jst.go.jp/index.html and http://pga.gs.washington.edu), as well as Ensemble, PARC and OMIM. Tagging SNP's are representative SNP's in a region of the gene with high linkage disequilibrium, which makes it possible to identify genetic variation without genotyping every SNP within the gene and reach a high coverage of common variation within the gene with a limited number of tagging SNP's. Linkage disequilibrium, as provided by the HapMap project, is a measure of the non-random association between polymorphisms at different loci and it describes a situation in which some combinations of alleles or genetic markers occur with a higher or lower frequency in a population than would be expected from a random formation of haplotypes from alleles based on their frequencies. We selected tagging SNP's are within the candidate genes using Caucasian subjects as reference population since in the EUROPA-trial more than 99 % of the patients were of Caucasian origin. In Haploview, we will use a cut-off of minor allele frequency of 5 % and haplotype frequency of 5% with $r^2$ of 0.80 to select the haplotype tagging SNP's within the candidate genes and aim to achieve more than 90 % coverage of common genetic variation. The selected area of the gene always should contain about 2 kb at the 5′ and 3′ ends of the gene to ensure maximum coverage of functionally relevant genetic areas. In selecting these haplotype-tagging SNP's, we prefer to use functional SNP's or SNP's located in regulatory or promoter regions of the gene. In addition, we will add several relevant SNP's to our list based on prior literature.



*Genotyping*

We will use a high-throughput genotyping facility including a Caliper Sciclone ALH 3000 pipetting robot (including a TwisterII, and integrated plate sealer, plate reader OD260/ 280)), and polymerase-chain-reaction (PCR) machine (ABI 9700, 2×384)), an ABI7900HT Taqman (running 2 ng gDNA in 2 µL reactions). The most commonly used genotyping techniques are Taqman (for one to ten SNP's) and Sequenom (for five to 40 SNP's). Allelic discrimination with Taqman Genotypes will be determined in genomic DNA with the Taqman allelic discrimination assay (Applied Biosystems, Foster City, CA, USA). Assay-by-Design service (http://www.appliedbiosystems.com) will be used to set up a TaqMan allelic discrimination assay for the selected SNP's; primer designs are readily available at ABI. The PCR mixture includes 1–2 ng genomic DNA in a 2-µl volume and the following reagents: probes (200 nM), primers (0.9 µM), 2× Taqman PCR master mix (AB gene, or ABI). Reagents are dispensed in a 384-well plate using the Deerac Equator NS808 (Deerac Fluidics, Dublin, Ireland). PCR cycling reactions will be performed in 384-well PCR plates in an ABI 9700 PCR system (Applied Biosystems Inc., Foster City, CA). These consist of initial denaturation for 15 min at 95 °C and 40 cycles with denaturation for 15 s at 95 °C and annealing and extension for 60 s at 60 °C. Allele specific fluorescence was then analyzed

on an ABI Prism 7900HT Sequence Detection System with SDS v 2.1 (Applied Biosystems) and results analyzed by the ABI TaqMan 7900HT using the sequence detection system 2.22 software (Applied Biosystems). To confirm the accuracy of the genotyping, 5 % randomly selected samples and duplicates will be included and genotyped additionally in the same procedure.

### *Mass-spectrometric genotyping*

Polymerase-chain-reaction (PCR) assays and extension primers for these SNP's are designed with the use of MassARRAY software, version 3.0 (Sequenom). The MassARRAY Designer software can automatically design both PCR and MassEXTEND primers for multiplexed assays. MassEXTEND is a primer extension process designed to detect sequence differences at the single nucleotide level. The primer is extended, dependent upon the template sequence, resulting in an allele-specific difference in mass between extension products. This mass difference allows the data analysis software to differentiate between SNP alleles. PCR and extension reactions will be performed according to the manufacturer's instructions, and extension product sizes determined by mass spectrometry (Sequenom). The iPLEX Gold assay uses a single termination mix and universal reaction conditions for all SNP's. The SpectroCHIP arrays are placed into the MALDI-TOF mass spectrometer and the mass correlating genotype is determined in real time. Duplicate test samples (control plates, 5 %) and six water samples per plate (PCR-negative controls), of which the technician is unaware, are included in each 96-well plate. The rate of concordant results between duplicate samples will be checked.

Quality control for the genotyping will further involve testing for Hardy–Weinberg equilibrium and repeated laboratory analyses in a random group of samples. To ensure high-quality output of SNP, eg. if the call rate or clustering was difficult, the SNP can be tested with alternative approaches (e.g. Taqman reaction mixtures) or on the other genotyping platform (Sequenom) likewise.

### *Data analysis*

#### **Statistical analysis and statistical power**

In the EUROPA study, 9 % of patients (n=1091) had a major cardiovascular event during follow-up (cardiovascular death, MI, cardiac arrest: 603 (10 %) placebo and 488 (8 %), 20 % relative risk reduction (95 % CI 9–29, p=0.0003). A secondary endpoint (total mortality, non-fatal MI, hospital admission for UAP, cardiac arrest) occurred in 16 % of patients (n = 1.947). Event rates will be compared between treatment groups and the treatment effect will be compared between genotype strata (for each gene). Gene–drug interaction for relative risk reductions will be assessed using Cox proportional hazards regression analysis. Hardy–Weinberg equilibrium of each polymorphism will be tested using Chi-square analysis. All analyses will be based on intention to treat. A p-value of 0.05 or less will be deemed significant.

*Haplotype analysis*

Haplotypes will be inferred with use of the program Haplo. Stats and R. Haplotype alleles present in the patient population were inferred by means of the haplo.em function of the program Haplo Stats (http://cran.r-project.org/src/contrib/Descriptions/haplo.stats.html), which computes maximum likelihood estimates of haplotype probabilities.

*Sample size considerations*

The large study size and large number of events (n>1,000) will provide sufficient power for the detection of interaction effects. The size of this pharmacogenetic substudy allows detection with a power of 98 % to detect a difference in hazard ratios (treatment effect for the primary endpoint) of 20 % between genotypes with minor allele frequency of 0.20, based upon ten-thousand patients (two-sided alpha 0.05). For other genotype distributions, power will be less but for most comparisons still above 80%. For a minor allele frequency of 0.10, statistical power is 88 % to detect a difference in treatment effect of 20 % with a two-sided alpha 0.05 (Figure 3).

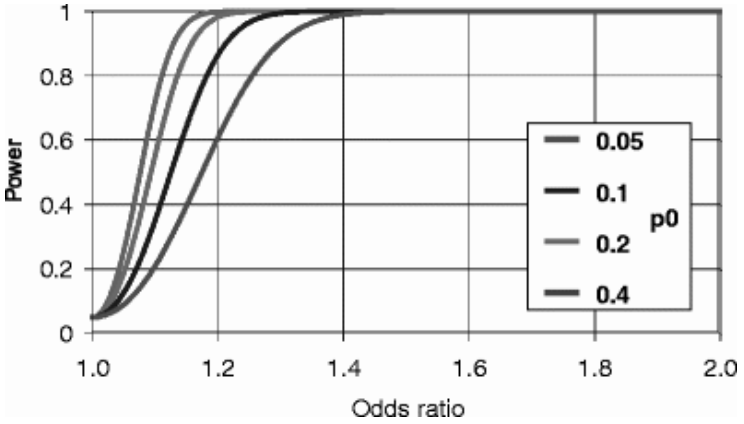


**Figure 3.** Power estimations for PERGENE

## DISCUSSION

In European countries and in other countries worldwide, millions of patients have coronary artery disease, and are at risk of (recurrent) events, particularly cardiovascular death and myocardial infarction. The ACE-inhibitors are among one of the most frequently prescribed drugs for secondary prevention in patients with stable coronary artery disease. Still, in the secondary prevention setting, there are no means of guiding therapy to those patients who are most likely to benefit. Prior attempts to target ACE-inhibitors have not been successful, largely due to because of the consistency in the treatment effect in clinical subgroups [19-21].

In the EUROPA study, treatment with perindopril in 50 patients for a period of 4 years (200 patient years) was required to prevent one major cardiovascular event. The efficiency and the cost-effectiveness of such prolonged prophylactic treatment would be significantly enhanced if patients who do benefit and those who do not benefit from an ACE-inhibitor could be distinguished prior to the start of treatment. For this purpose, it is important to study possible heterogeneities in the treatment effect to assess whether a variation in the treatment effect does exist which then can be used to guide ACE-inhibitor therapy to the patients most likely to benefit of treatment. There are several arguments that this variation in treatment effect may indeed exist. For instance, the activity of ACE and angiotensinogen (plasma levels) have been shown to vary widely between patients, also in the response to an ACE-inhibitor [17]. Also, a large inter-individual variability in blood pressure response to ACE inhibition is well documented. Whether the treatment effect on outcome reduction varies in a similar way is unknown. Until now subgroup analysis based on clinical characteristics has resulted in consistent treatment effects [19-21]. Still, relative and absolute risk reductions with ACE inhibitor therapy vary. A larger risk reduction is seen in heart failure patients as compared to coronary artery disease patients [19]. Therefore it is likely that the treatment effect of ACE-inhibitor use on outcome (in CAD patients) will also differ, some patients will benefit others will not.

The integration of genetic information, which is highly specific for each individual patient, can be a new way to identify a true heterogeneity in the treatment effect of ACE-inhibitors. A pharmacogenetic profile related to drug response can be used to target ACE-inhibitors to those patients most likely to benefit of treatment. It has been suggested that the response to drug therapy may be influenced by genetic polymorphisms in different ways. Firstly, variations within genes of the RAAS and related systems may influence the disease process (atherosclerosis) and inherent differences in accessibility to therapeutic agents such as ACE-inhibitors. Secondly, pharmacodynamics may be affected by polymorphisms in the genes of all proteins involved in the RAAS and related systems, including receptors and signal transduction molecules. Thirdly, variations in drug absorption and metabolic clearance may cause inter-individual variation in pharmacokinetics.

Genetic polymorphisms in the ACE and AGT genes have been shown to influence plasma levels of these enzymes, mainly M235T and T174M in AGT and ACE I/D in ACE [27]. For example with the M235T polymorphism, the TT-allele results in higher levels of angiotensinogen. Regarding intermediate endpoints, a series of relatively small studies (34 to 345 patients) reported that polymorphisms in the ACE, angiotensinogen and AGTR1 and AGTR2 genes modulated the effects of ACE-inhibitors [28]. Such interactions were shown for specific alleles in relation with blood pressure reduction, regression of left ventricular hypertrophy, diastolic cardiac function and restenosis after percutaneous coronary intervention.

Regarding outcomes, pharmacogenetic data is very scarce. The PROGRESS study, which included 5,688 patients with history of stroke, did not show an association of the ACE I/D polymorphism and ACE-inhibition on risk reduction of cerebrovascular events [29]. The inconsistency

in these studies could be explained by the fact that in virtually all association studies only one RAAS gene was taken into consideration, thereby ignoring the well-documented feedback mechanisms within the RAAS [30]. For instance, the elevated angiotensinogen levels which are found in Thr235 homozygotes are accompanied by reduced renin levels, so that the angiotensin I-generating capacity returns to normal in those with high angiotensinogen concentrations. In addition, the two angiotensin II receptors (AT1 and AT2) that exist in humans have counteracting effects [31,32]. Thus, future studies should preferably investigate more than one RAAS gene (and ideally all genes: renin, angiotensinogen, ACE, AGTR1, AGTR2, aldosterone synthase).

The DNA samples collected in the EUROPA study offer a unique opportunity to investigate the relations between polymorphisms in genes of the RAAS with the treatment benefit of an ACE inhibitor on cardiovascular events in a sufficiently large population and in a randomized double-blind setting. As mentioned, the available studies of this subject have been of small size, not randomized and therefore, reported relationships may have been due to chance findings. Furthermore, the majority of studies so far included only one polymorphism or one gene of the RAAS. In contrast, the PERGENE study uses a haplotype tagging selection procedure to comprehensively cover all common genetic variations (> 90 %) in the relevant genes within the RAAS and bradykinin pathways. We will use the latest information from HapMap Genome project, SEATTLE and other up to date genetic information platforms as well as sophisticated software packages as Haplostats and R for these haplotype analyses. Haplotypes are a combination of alleles at different markers along the same chromosome that are inherited as a unit (linkage disequilibrium-patterns). The determination of haplotypes is essential for understanding genetic variation and the inheritance of complex diseases. An analysis based on haplotypes is advantageous over an analysis based on individual SNPs, especially in the presence of multiple susceptibility alleles, and when linkage disequilibria between SNPs are weak. With a single SNP approach, associations may be missed when the causal SNP is not in linkage disequilibrium with the single analyzed SNP. It is more informative to simultaneously analyze multiple markers in a region of interest that identifies genetic variants underlying various human traits; also, these markers should be selected based on tagging principles and linkage disequilibrium. By combining information from multiple SNP's in the RAAS and bradykinin pathway genes, a more efficient and comprehensive in-depth analysis of common genetic variation in relation to ACE-inhibitor therapy is performed, which is more likely to unravel any important pharmacogenetic associations.

In summary, this project is unique because of its size, design (randomized-setting), accurate phenotypic data, complete coverage of two pathways (RAAS and bradykinin), but also because of the extensive and comprehensive SNP selection procedure which involves multiple SNP in multiple genes of both pathways with integrating information on the haplotype structure of RAAS and bradykinin genes. Until now, attempts to target therapy using simple clinical patient characteristics have been insufficient to guide ACE-inhibition therapy and it is not yet possible to say in advance who to treat or not [19-21]. New and improved approaches that integrate more

patient-specific characteristics are needed to target ACE-inhibitor therapy. We will investigate whether specific genetic polymorphisms in RAAS genes modify the treatment effect of ACE-inhibitor therapy. Our aim is to develop a pharmacogenetic profile associated with the benefit of ACE inhibitor therapy in patients with stable coronary artery disease. If it is possible to construct a pharmacogenetic profile related to treatment benefit, this could lead to a significant reduction of the number of patients needed to treat. It should be realized in this regard that the absolute treatment effects are only modest (about 2 % absolute risk reduction) in stable CAD patients. In the EUROPA trial, 200 patient years of treatment with perindopril 8 mg was necessary to prevent one event in the primary endpoint (number needed to treat 50 for 4 years) [7,33]. A pharmacogenetic profile related to the benefit of perindopril may enable the selection of those patients advance of treatment. Likewise, targeting therapy to only those patients that are to benefit will considerably increase the cost-effectiveness of treatment. Until now, cardiovascular pharmacogenetic research is still in a premature stage but has the potential to enhance personalized medicine and tailored-therapy in cardiovascular medicine.

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## REFERENCES


1. Pfeffer MA, Braunwald E, Moye LA, SAVE Investigators, et al. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction and myocardial infarction: results of the survival and ventricular enlargement trial. *N Engl J Med* 1992;327:669–77.
2. The SOLVD investigators. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. *N Engl J Med* 1991;325:293–302.
3. The Acute Infarction Ramipril Efficacy (AIRE) study investigators. Effect of ramipril on mortality and morbidity of survivors of acute myocardial infarction with clinical evidence of heart failure. *Lancet* 1993;342:821–8.
4. Torp-Pedersen C, Kober L, TRACE Study Group. Effect of ACE inhibitor trandolapril on life expectancy of patients with reduced left-ventricular function after acute myocardial infarction. *Lancet* 1999;354:9–12.
5. The Heart Outcomes Prevention Evaluation study investigators. Effects of an angiotensin-converting enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. *N Engl J Med* 2000;342:145–53.
6. The PEACE Trial Investigators. Angiotensin-converting-enzyme inhibition in stable coronary artery disease. *N Engl J Med* 2004;351:2058–68.
7. The European Trial on Reduction of Cardiac Events with Perindopril in Patients with Stable Coronary Artery Disease investigators. Efficacy of perindopril in reduction of cardiovascular events in stable coronary artery disease: randomized, double-blind, placebo controlled, multicentre trial (the EUROPA study). *Lancet* 2003;362:782–8.
8. The Task Force on the Management of Stable Angina Pectoris of the European Society of Cardiology. Guidelines on the management of stable angina pectoris. *Eur Heart J* 2006 ;27:1341–81.
9. Wood D, DeBacker G, Faergam O, Graham I, Mancia G, Pyörälä K, and the task force. Prevention of coronary disease in clinical practice: recommendation of the second Joint Task Force of European and other societies on Coronary Prevention. *Atherosclerosis* 1998;140:99–270.
10. Gibbons RJ, Abrams J, Chatterjee K, et al. ACC/AHA 2002 guideline update for the management of patients with chronic stable angina—summary article: a report of the American College of Cardiology/American Heart Association Task Force on practice guidelines (Committee on the Management of Patients With Chronic Stable Angina). *J Am Coll Cardiol* 2003;41:159–68.
11. Dahlöf B, Sever PS, Östergren J. Prevention of cardiovascular events with an antihypertensive regimen of amlodipine adding perindopril as required versus atenolol adding bendroflumethiazide as required, in the Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm (ASCOT-BPLA): a multicentre randomized controlled trial. *Lancet* 2005;366:895–906.
12. PROGRESS Collaborative Group. Randomised trial of a perindopril based blood pressure-lowering regimen among 6105 individuals with previous stroke or transient ischemic attack. *Lancet*. 2001;200(358):1033–41.
13. The PREAMI Investigators. Effects of angiotensin-converting enzyme inhibition with perindopril on left ventricular remodeling and clinical outcome. Results of the Randomized Perindopril and Remodeling in Elderly with Acute Myocardial Infarction (PREAMI) Study. *Arch Intern Med* 2006;166:659–66.
14. ADVANCE Collaborative Group. Effects of a fixed combination of perindopril and indapamide on macrovascular and microvascular outcomes in patients with type 2 diabetes mellitus (the ADVANCE trial): a randomized controlled trial. *Lancet* 2007;370:829–40.
15. Bots ML, Remme WJ, Lüscher TF, et al. ACE inhibition and endothelial function: main findings of PERFECT, a sub-study of the EUROPA trial. *Cardiovasc Drugs Ther* 2007;21:269–79.
16. Rodriguez-Granillo GA, Vos J, De Feyter PJ. Long-term effect of perindopril on coronary atherosclerosis progression (from the PERindopril's Prospective Effect on Coronary aTherosclerosis by Angiography and IntraVascular Ultrasound Evaluation [PERSPECTIVE] Study). *Am J Cardiol* 2007;100:159–63.
17. Ceconi C, Fox KM, Ferrari R, et al. ACE inhibition with perindopril and endothelial function. Results of a substudy of the EUROPA study: PERTINENT. *Cardiovasc Res* 2007;73:237–46.

18. Remme WJ, Deckers JW, Fox KM, Ferrari R, Bertrand M, Simoons ML. for the EUROPA investigators. Secondary prevention of coronary disease with ACE inhibition—does blood pressure reduction with perindopril explain the benefits in EUROPA? *Cardiovasc Drugs Ther* 2009;23.
19. Dagenais GR, Pogue J, Fox K, Simoons ML, Yusuf S. Angiotensin converting-enzyme inhibitors in stable vascular disease without left ventricular systolic dysfunction or heart failure: a combined analysis of three trials. *Lancet* 2006;368:581–8.
20. Deckers JW, Goedhart DM, Boersma E, et al. Treatment benefit by perindopril in patients with stable coronary artery disease at different levels of risk. *Eur Heart J* 2006;27:796–801.
21. Brugts JJ, Boersma E, Chonchol M, et al. The cardioprotective effects of the angiotensin-converting enzyme inhibitor perindopril in patients with stable coronary artery disease are not modified by mild to moderate renal insufficiency: insights from the EUROPA trial. *J Am Coll Cardiol* 2007;50:2148–55.
22. Brugts JJ, de Maat M, den Uil CA, et al. Pharmacogenetics of ACE inhibition in stable coronary artery disease: steps towards tailored drug therapy. *Curr Opin Cardiol* 2008;23:296–301.
23. Brugts JJ, den Uil CA, de Maat M, Danser AHJ. The renin–angiotensin–aldosteron system: approaches to guide angiotensin converting enzyme inhibition in patients with coronary artery disease. *Cardiology* 2008;112:303–12.
24. Gomma AH, Fox KM, for the EUROPA investigators. The EUROPA trial: design, baseline demography and status of the substudies. *Cardiovasc Drugs Ther* 2001;15:169–79.
25. Nederhand RJ, Droog S, Kluft C, Simoons ML, de Maat MP. Logistics and quality control for DNA sampling in large multicenter studies. *J Thromb Haemost* 2003;1:987–91.
26. Gainer JV, Morrow JD, Loveland A, King DJ, Brown NJ. Effect of bradykinin-receptor blockade on the response to angiotensinconverting-enzyme inhibitor in normotensive and hypertensive subjects. *N Engl J Med* 1998;339:1285–92.
27. Danser AHJ, Schunkert H. Renin–angiotensin system gene polymorphisms: potential mechanisms for their association with cardiovascular diseases. *Eur J Pharmacol* 2000;410:303–16.
28. Schelleman H, Stricker BH, De Boer A, et al. Drug–gene interactions between genetic polymorphisms and antihypertensive therapy. *Drugs* 2004;64:1801–16.
29. Harrap SB, Tzourio C, Cambien F. The ACE gene I/D polymorphism is not associated with the blood pressure and cardiovascular benefits of ACE inhibition. *Hypertension* 2003;42:297.
30. Danser AH, Derkx FH, Hense HW, Jeunemaitre X, Riegger GA, Schunkert H. Angiotensinogen (M235T) and angiotensin converting enzyme (I/D) polymorphisms in association with plasma renin and prorenin levels. *J Hypertens* 1998;16:1879–83.
31. Deinum J, van Gool JM, Kofflard MJ, ten Cate FJ, Danser AH. Angiotensin II type 2 receptors and cardiac hypertrophy in women with hypertrophic cardiomyopathy. *Hypertension* 2001;38:1278–81.
32. Batenburg WW, Garrelds IM, Bernasconi CC, et al. Angiotensin II type 2 receptor-mediated vasodilation in human coronary microarteries. *Circulation* 2004;109:2296–301.
33. Briggs A, Mihaylova B, Sculpher M, EUROPA-trial investigators, et al. Cost effectiveness of perindopril in reducing cardiovascular events in patients with stable coronary artery disease using data from the EUROPA study. *Heart* 2007;93:1081–6.

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Rondepierre D, Roynard JL, Van Belle E, Veyrat A, Germany, Gaudron P, Karsch KR, Lauer B, Retti St˘srmer G, Riessen R, RutschW, Sechtem U, Sigel HA, Simon R, Stork S, Von Schacky C, Winkelmann BR, Greece, Christakos S, Cokkinos D, Feggos S, Geleris P, Georgiadis S, Gialafos J, Goudevenos I, Kardara D, Kardaras F, Karidis C, Kelesides C, Kyriakidis M, Koliopoulos N, Kremastinos D, Liberi S, Manolis AN, Pyrgakis V, Papasteriadis E, Papazoglou N, Skoufas P, Stamatelopoulos S, Stambola S, Stavridis A, Syribeis S, Vardas P, Vassiliadis I, Voudris V, Zacharoulis A, Zobolos S, Zouras C, Hungary, Berenyi I, Bocsa Z, Csendes E, Edes I, Gelesz E, Janosi A, Kalo E, Karpati P, Kornel S, Pap I, Pinter I, Polak G, Reiber I, Rusznak M, Simon A, Tarjan J, Tihanyi L, Timar S, Toth K, Veress G, Ireland, Barton J, Crean P, Daly K, Kearney P, Meany TB, Mulcahy D, Quigley P, Italy, Azzolini P, Barone G, Barsotti A, Bellone E, Borghetti A, Branzi A, Brunelli C, Capponi E, Capucci A, Casaccia M, Casali G, Cecchetti E, Ceci V, Celegon L, Chimini C, Colombo A, Corsini G, Cucchini F, Dalla Volta S, De Luca I, De Servi S, Delise P, Di Donato M, Di Giacomo U, Di Pasquale G, Fiorentini C, Gaddi O, Giannetto M, Giannuzzi P, Giordano A, Giovannini E, Iacono A, Inama G, Ippoliti G, Leghissa R, Lorusso R, Marzilli M, Minutiello L, Moretti G, Mosele GM, Pasotti C, Pettinati G, Pezzano A, Polimeni MR, Portaluppi F, Proto C, Riva S, Sanguinetti M, Santini M, Severi S, Sinagra G, Tantalo L, Tavazzi L, Vajola SF, Vincenzi M, Volterrani M, Zavatteri G, Zogno M, Latvia, Gailiss E, Gersamija A, Kalnins U, Ozolina MA, Skards J, Lithuania, Baubiniene A, Berukstis E, Grigoniene L, Grybauskas, Kibarskis A, Kirkutis A, Marcinkus R, Milvidaite I, Vasiliauskas D, Netherlands, Aalders JCA, Bruggeling WAJ, Bucx JJJ, De Feyter PJ, De Leeuw MJ, De Waard DEP, De Weerd GJ, De Zwaan C, Dijkgraaf R, Droste HT, Freericks MP, Hagoort Kok AW, Jap WTJ, Jochemsen GM, Kiemeney K, Kuijer PJP, Mannaerts HFJ, Piek JJ, Saelman JPM, Simoons ML, Slob FD, Smits WCG, Spierenburg HAM, Suttorp MJ, Tan TB, Van Beek GJ, Van Daele MERM, Van Den Merkhof LFM, Van Den Toren EW, Van Hessen MWJ, Van Langeveld RAM, Van Loo LWH, Van Nierop PR, Van Rey FJW, Van Straalen MJ, Vos J, Werner HA, Westendorp JJC, Zwiers G, Norway, Erikssen J, Poland, Achremczyk P, Adamus J, Baska J, Bolinska Soltysiak H, Bubinski R, Ceremuzynski L, Cieslinski A, Dariusz D, Deptulski T, Drewla P, Drozdowski P, Dubiel JS, Dudek D, Galewicz M, Ghlebus K, Halawa B, Jakubowska Majnigier M, Janion M, Jaworska K, Kaszewska I, Kleinrok A, Kornacewicz Jach Z, Krawczyk W, Krynicki R, Krzciuk M, Krzeminska Pakula M, Kuch J, Kuzniar J, Legutko J, Liszewska Pfejfer D, Loboz Grudzien K, MusialW, Opolski G, Pasyk S, Piwowarska W, Pulkowski G, Radziszewski B, Romanski B, Ruzyllo W, Rynkiewicz A, Skura M, Slowinski S, Smielak Korombel W, Targonski R, Templin W, Tendera M, Tracz W, Trusz Gluza M, Turek P, Tyminska Sedek K, Wodniecki J, Zalewski M, Zinka E, Portugal, Carrageta M, Coelho Gil J, Ferreira R, Leitao Marques A, Santos Andrade CM, Seabra Gomes R, Slovakia, Bada V, Belicova M, Buksarova E, Dukat A, Gonsorcik J, Jonas P, Kamensky G, Karvaj M, Micko K, Mikes Z, Palinsky M, Pella D, Renker B, Riecansky I, Sefara P, Sojka G, Sulej P, Szakacs M, Szentivanyi M, Spain, Aguirre Salcedo JM, Alonso Orcajo N, Ancillo Garcia P, Auge Sanpera JM, Ayuela Azcarate J, Bardaji Mayor JL, Bertomeu Martinez V, Blanco Coronado JL, Bros Caimari R, Bruguera Cortada J, CaparrosValderrama J, DeArmas Trujillo D, Del Rio Ligorit A, Espinosa Caliani JS, Fernandez Aviles F, Garcia Guerrero JJ, Garcia Lopez D, Gonzalez Cocina E, Guallar Urena C, Jodar Lorente L, Lopez Garcia ArandaV, Macaya

De Miguel C, Maroto Montero J, Martinez Romero P, Navarro Lopez F, Noriega Peiro F, Olague De Ros J, Orellana Mas J, Paz Bermejo MA, Placer Peralta LJ, Rodriguez Padial L, Salvador Sanz A, Segui Bonnin J, Simarro Martin E, Sobrino Daza J, Valles Belsue F, Sweden, Ekdahl S, Erhardt L, Forslund L, Ohlin H, Persson S, Switzerland, Pieper M, Moccetti T, Turkey, Acartˇsrk E, Guzelsoy D, Oto A, TMZsaruhan O, Ozturk M, SansoyV, Turkoglu C, Yˇsksel H, United Kingdom, Adgey AAJ, Ahsan A, Al Khafaji M, Ball SG, Birkhead J, Boon N, Bowker T, Brack M, Bridges A, Buchalter M, Buchalter B, Calder B, Cooke RA, Corr L, Cowell R, Curzen NP, Davidson C, Davies E, Davies J, De Belder M, Dhiya L, Doig JC, Findlay IN, Fox K, Francis CM, Glancy JM, Glen S, GreenwoodTW, Groves P, Hall AS, Hamilton G, Hillman R, Holdright D, HubbardW, Travill C, Hutton I, Ilsley C, Innes M, James M, Jennings K, Jones CJH, Joy M, Keeling P, Kooner J, Lawson C, Levy RD, Lip G, Lorimer AR, Marshall H, Mclachlan B, Montgomery H, Morley C, Murdoch DL, Muthusamy R, Oakley GD, PennyW, Pohl JEF, Purvis J, Pye M, Ramsdale D, Reid D, Roberts DH, Rowlands D, Rozkovec A, Saltissi S, Schofield PM, Scott M, Shapiro LM, Silke B, Stephens J, Sutherland S, Swan JW, Shakespeare C, Tildesley G, Travill C, Watson RDS, Wilkinson P.

# Chapter 11

# Genetic determinants of treatment benefit of ACE-inhibitor therapy in patients with stable coronary artery disease: Results of the PERGENE study.

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## ABSTRACT

**Aims:** The efficacy of angiotensin-converting enzyme (ACE) inhibitors in stable coronary artery disease (CAD) may be increased by targeting therapy to those patients most likely to benefit. However, these patients cannot be identified by specific clinical characteristics. We investigated whether genetic determinants of treatment benefit of ACE-inhibitors exist which could be used to tailor-therapy of ACE-inhibitors by pharmacogenetic profiling.

**Methods:** In 8907 stable CAD patients participating in the randomized placebo-controlled EUROPA-trial, we analyzed 52 haplotype-tagging single nucleotide polymorphisms (SNPs) in 12 candidate genes within the pharmacodynamic pathway of ACE-inhibitors. The primary outcome was the reduction in cardiovascular mortality, non-fatal myocardial infarction and resuscitated cardiac arrest during 4.2 years of follow-up. Multivariate Cox regression was performed with multiple-testing corrections using permutation analysis.

**Results:** In unadjusted analysis, 7 polymorphisms were significantly associated with the treatment benefit of perindopril. After multivariate adjustment for confounders and correction for multiple testing three SNPs, located in the angiotensin-II type I receptor and bradykinin type I receptor genes, remained significant. The pharmacogenetic profile, combining these 3 SNPs, demonstrated a stepwise decrease in treatment benefit of perindopril with increasing number of unfavourable alleles (interaction $p<0.0001$). A pronounced treatment benefit was observed in a subgroup of 73.5% of the patients (HR 0.67; 95% CI 0.56-0.79), without any benefit in the remaining 26.5% (HR 1.26; 95% CI 0.97-1.67). An interaction effect of similar direction and magnitude, though not significant, was observed in a confirmatory analysis of 1051 patients with cerebrovascular disease from the PROGRESS-trial.

**Conclusion:** The current study identified genetic determinants of treatment benefit of ACE-inhibitor therapy. Our findings support the emerging concept of individualized-therapy to optimize patients' benefit by pharmacogenetic profiling.

## INTRODUCTION

ACE-inhibitors improve outcome in patients with stable CAD and are recommended in clinical guidelines on secondary prevention of patients with stable CAD [1-6]. Accordingly, ACE-inhibitors are among the most frequently used drugs in these patients. However, in a relatively low-risk population of stable CAD patients, the absolute treatment benefits are modest (2 % reduction of cardiovascular death or myocardial infarction at follow-up) and, therefore, the number of patients needed to be treated (50 patients treated for 4 years to prevent 1 event in the EUROPA trial) remains relatively high [2].

To optimally treat patients and to develop ways to guide ACE-inhibitor treatment, it is necessary to identify those patients who are most likely to benefit from therapy. In secondary prevention trials, however, the treatment effect was consistent among all clinical subgroups, and no intermediate parameter could be identified to assess the efficacy of ACE-inhibitor therapy [1,7-10]. Also blood pressure, which guides hypertension treatment, did not predict treatment efficacy [10]. Thus, it is not feasible to base the selection of patients who respond or not respond to treatment upon clinical characteristics. A new approach may be to integrate information on genetic variation in patients. This approach could have a large impact on clinical practice by increasing the patient's chances to benefit from specific therapies and by reducing healthcare costs.

The direct pharmacodynamic pathways affected by ACE-inhibitors are the renin-angiotensin aldosterone system (RAAS) which converts angiotensin-I into angiotensin-II, and the kallikrein-bradykinin (KB) pathway, which degrades bradykinin into inactive peptides [11-13]. We hypothesized that genetic variation in these pathways is associated with the treatment benefit of ACE-inhibitors. The PERGENE substudy of the EUROPA-trial provides the opportunity to evaluate this hypothesis, since the EUROPA trial is a large randomized double-blind placebo-controlled clinical trial with complete phenotypic data [2,14,15]. We applied a haplotype tagging-SNP procedure in 12 candidate genes to ensure comprehensive coverage of genetic variation in both pathways [15], and replicated our findings in another randomized clinical trial with the same ACE-inhibitor (PROGRESS) [16].

## METHODS

### Study populations and design

The PERindopril GENEtic association study (PERGENE) is a sub-study of the EUROPA-trial. The designs of both studies were previously described in detail [14,15]. In short, the EUROPA-trial randomized 12.218 stable CAD patients to perindopril (8 mg/day) or placebo. Perindopril was associated with a 20% reduction (HR 0.80; 95% CI 0.71-0.91) in the rate of the primary endpoint (composite of cardiovascular mortality, non-fatal MI, or resuscitated cardiac arrest) during a mean follow-up of 4.2 years [2]. The PERGENE study investigates whether common

genetic variation modifies the treatment effect of perindopril [15]. Written informed consent for performing genetic association analyses was obtained from all patients. The confirmation study, the PROGRESS-trial, is a randomized, double-blind, placebo-controlled clinical trial of a perindopril based-regimen (perindopril 4 mg or perindopril 4 mg + indapamide 2.5 mg) versus placebo in 6105 patients with cerebrovascular disease [16]. The PROGRESS study demonstrated a reduction in stroke in patients receiving perindopril-based therapy (HR 0.72; 95%CI 0.62-0.83). In PROGRESS the observed benefits were contingent on indapamide use.

### Data collection

A DNA bio-bank was established within the EUROPA trial for the PERGENE substudy [17]. Blood samples were received from 10.060 patients and DNA from 9454 patients was successfully isolated using an automated isolation process (Hamilton liquid handler coupled with Magnetic separator for automated DNA extraction; Nevada, USA). Similarly DNA was isolated from 5.600 patients participating in PROGRESS at the INSERM laboratory in Paris, of which 1051 samples from Caucasian patients using perindopril alone (single therapy) or placebo were used as replication cohort.

### Candidate genes and selection of tagging-SNPs

Genes that play an important role in pharmacodynamic pathway of ACE-inhibitors, the RAAS and KB systems were selected for this analysis (supplement table 1). The candidate genes were: the renin (REN), prorenin receptor, angiotensinogen, angiotensin-converting enzyme, angiotensin-II receptor type 1 (AT1) and 2, aldosteron synthase, endothelial nitric oxide synthase, kininogen, kallikrein, and bradykinin type 1 (BK1) and 2 receptor genes. To cover common variation in these 12 candidate genes comprehensively, haplotype-tagging SNPs (ht-SNP) were selected based on the linkage disequilibrium (LD) structure as provided by the HapMap (http://www.hapmap.org) and SeattleSNPs (http://pga.mbt.washington.edu) databases [18]. Within these genes, plus their flanking regions, a total of 52 ht-SNP's were identified. The haplotype-tagging approach was used because within the genes there is a high level of linkage disequilibrium, and this approach allowed us to combine minimal genotyping with comprehensive coverage of the genetic variation in the genes [19]. The selection criteria of the ht-SNPs also included: minor allele frequency ≥5%, $r^2 < 0.80$, haplotype frequency ≥ 1% (HapMap Release 23a/Phase II Mar 08/on NCBI B36 assembly/ DbSNP b126). In the process of selecting tagging SNPs our aim was to include, when available, SNPs for which functionality has previously been described, SNPs that gave an amino acid change or SNPs that were located in regulatory regions or intron-exon boundaries. Further details of this methodology can be found elsewhere [15]. In our population, several SNPs were in stronger LD than suggested by the HapMap data, and we defined our set of tagging-SNPs by excluding one of the SNPs if there was a pairwise $r^2 > 0.95$.

## Genotyping

Taqman allelic discrimination assays (Applied Biosystems, Foster City, CA, USA) and Sequenom (San Diego, CA, USA) mass-spectrometric genotyping were used to genotype the selected SNPs, according to the manufacturer's protocols. The assays, primers, and probes for these assays are readily available from the Assay-by-Design service (www.appliedbio-systems.com) or can be requested from Sequenom for all mentioned rs-numbers (supplementary table 1). Quality control for the accuracy of genotyping involved testing duplicates from a randomly selected group of samples (5%) for concordance between samples (>99%). Individual SNP call rates ranged between 95% and 98%. To ensure DNA quality, only patients who were successfully genotyped for more than 90% of the 52 SNPs were included in the analyses (n=8907).

## Statistical analysis

We tested whether genotypes and allele frequencies were distributed according to Hardy-Weinberg equilibrium using a $\chi^2$ test. The treatment effect of perindopril was defined as the reduction in the event rate of the primary endpoint of the EUROPA-trial (composite of cardiovascular mortality, non-fatal MI, and resuscitated cardiac arrest) and compared between genotype strata for each SNP (additive model assumption). Genotype-treatment interactions were assessed with Cox proportional hazards regression models. Two models were fitted: one included genotype, treatment, and treatment * genotype interaction, with adjustments for age and gender; the second model additionally included all covariates that were related to the incidence of the primary endpoint in the EUROPA trial [7]. The results for the full model are presented in all analyses, which are concordant with the age/gender model.

Multiple testing corrections of treatment interaction terms, and estimation of empirical p-values, were implemented using Monte Carlo permutation analysis (10,000 permutations) on a per gene basis [20]. Permutation was chosen as a method of multiple testing correction, because, due to the linkage disequilibrium between the SNPs and the fact that the genes are located within a common pathway, Bonferoni adjustment would be too conservative. As we corrected for the number of tagging-SNPs within each of the 12 candidate genes, the expected number of "chance" findings is correctly calculated as 12 * 0.05 = 0.6 SNPs . Permutated p-values below 0.05 were considered to be statistically significant.

Haplotypes were inferred using the estimation-maximization algorithm implemented in haplo.stats [21]. The associations between the estimated haplotypes and risk of the primary endpoint, taking into account the posterior probabilities of the haplotype estimates, were assessed with the GLM function in haplo.stats. The haplotype analysis used the same models as the Cox analysis. Global p-values for treatment * haplotype interaction were estimated with a likelihood ratio test, comparing models with and without the interaction terms.

A pharmacogenetic profile based on SNPs that modified the treatment effect was constructed by counting the number of unfavourable alleles present. Using these risk profile categories, both the relative and absolute risk of events were estimated to assess the treatment

benefit according to the number of unfavourable alleles. Baseline clinical characteristics and intermediate phenotypes, such as blood pressure at baseline were compared between the pharmacogenetic profile categories. In an additional analysis, we assessed the relation between patients with <3 and with ≥3 unfavourable alleles and the incidence of the primary endpoint during 4 years of follow-up using multivariate Cox regression analysis (full model).

All genetic polymorphisms which modified the treatment effect of perindopril (permutated p-value <0.10) in the EUROPA-trial were tested on the corresponding endpoint (cardiovascular mortality, MI) in the European subjects from the PROGRESS trial. As the treatment effect in PROGRESS was contingent on the combination of perindopril with indapamide (duo-therapy), we studied 1051 patients who received perindopril alone (as single therapy) or placebo [16]. The interaction effects on treatment of the 3 individual SNPs were further verified in a combined meta-analysis of the two studies. Results from the two studies were combined using an inverse variance method in a random effects model [22,23]. Additionally, an analysis of treatment effect relative to the overall study effect (as a % change in treatment effect according to genotype) was performed to study the modification of treatment benefit in both studies.

All analyses were conducted using R software. Meta-analyses were conducted using RevMan 5.0 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2008). Analyses are based on intention-to-treat principle. In statistical analyses, a p-value of <0.05 (two-sided) was considered significant.



## RESULTS

Baseline characteristics of the PERGENE study population were similar to those of the total EUROPA trial and are shown in table 1. All genetic variants were in Hardy-Weinberg equilibrium. Complete data on follow-up and covariates was obtained for 8746 patients from the EUROPA trial. The mean age was 59.9 (9.3) years and 85.7 % were male. Median follow-up was 4.2 years.

### Genetic determinants of treatment benefit of perindopril

In the study, 785 events (9.0%) occurred, 342 in patients with perindopril (8.0 %) and 443 in patients receiving placebo (10.2%), and the overall treatment effect was HR 0.80 (95% CI 0.68-0.92). In unadjusted analysis (without adjustment for confounders or correction for multiple testing), 7 SNPs in 4 genes significantly modified the treatment effect of perindopril: AT1 rs275651 and rs5182; REN rs2887284, rs10900555 and rs11571082; BK1 rs12050217; AGT rs4762 (table 2). In the multivariate model with correction for multiple testing, 3 SNPs in 2 genes remained significant (AT1 rs275651 and rs5182; BK1 rs12050217). In the bradykinin type I (BK1) receptor gene; rs12050217 was a strong modifier of the treatment benefit of perindopril. The hazard ratio (95% CI) for the reduction in the event rate of the primary endpoint for AA (62.1%) genotypes was 0.64 (0.55-0.78), for AG (33.2%) genotypes 1.02 (0.79-1.29) and for GG (4.7%) genotypes 1.10

**Table 1.** Baseline characteristics of the PERGENE study population (n=8907).

| Characteristics | Total | <3 unfavourable alleles | ≥ 3 unfavourable alleles |
|---|---|---|---|
| Age, years | 59.9 (9.3) | 59.8 (9.3) | 60.0 (9.3) |
| Gender, % female | 14.5 | 14.5 | 14.5 |
| Hypertension, % | 28.5 | 28.2 | 29.1 |
| Diabetes, % | 12.7 | 12.9 | 12.4 |
| Hypercholesterolemia, % | 62.8 | 63.2 | 62.2 |
| Smoking, % | 14.8 | 14.4 | 15.4 |
| Body mass index (>30 kg/m$^2$) , % | 21.3 | 21.4 | 21.3 |
| Symptomatic CAD, % | 25.3 | 25.4 | 25.3 |
| Family history of CAD, % | 27.2 | 27.3 | 27.1 |
| Prior myocardial infarction, % | 65.0 | 65.1 | 65.0 |
| Prior revascularization, % | 54.6 | 54.9 | 53.8 |
| Prior CVA or PVD, % | 8.9 | 8.7 | 9.4 |
| Medication use | | | |
| Platelet-inhibitors, % | 92.2 | 92.3 | 92.0 |
| Beta-blockers, % | 63.2 | 63.2 | 63.4 |
| Lipid-lowering agents, % | 55.3 | 55.9 | 54.4 |
| Calcium antagonists, % | 31.7 | 31.3 | 32.5 |
| Total cholesterol, mg/dl | 5.4 (1.1) | 5.4 (1.0) | 5.4 (1.1) |
| Creatinine clearance, µmol/l | 86.5 (25.7) | 86.7 (26.0) | 86.1 (25.1) |
| Randomization, perindopril, % | 49.9 | 49.7 | 50.3 |
| Systolic BP, mmHg | 136.9 (15.2) | 136.9 (15.3) | 136.8 (15.1) |
| Diastolic BP, mmHg | 81.8 (8.1) | 81.8 (8.2) | 81.8 (8.1) |
| BP reduction, mmHg* | 8.6 / 4.0 | 8.6 / 4.0 | 8.6 / 4.0 |

Summary statistics for continuous variables are presented as mean (standard deviation (sd)). Categorical data are summarized as percentages. * Blood pressure reduction was calculated as the mean difference in blood pressure from screening visit 1 to randomization after the 4 week run-in period of the EUROPA-trial in which all patients were treated with the ACE-inhibitor perindopril. BP = blood pressure.

(0.56-2.19), respectively (Table 2). The p-values for interaction were 0.004 (empirical) and 0.012 (permuted). In the angiotensin-II type I (AT1) receptor gene, rs275651 and rs5182 significantly modified the treatment benefit of perindopril, with empirical p-values of 0.008 and 0.011, and permutated p-values of 0.049 and 0.054, respectively. No further associations of treatment interaction were observed for the other genes (supplementary table 1a and 1b).

Haplotype analysis confirmed the association between the identified SNPs and treatment effect modification observed in single SNP analysis, as presented in supplement tables 2a and 2b. In both genes, the haplotype carriers of the unfavourable alleles of the identified SNPs significantly modified the treatment benefit of perindopril. No association between these individual SNPs and the rate of the primary endpoint in either the placebo or perindopril-treated group in separate analysis.

**Table 2.** Modification of the treatment benefit of ACE-inhibitor therapy in renin-angiotensin-aldosterone and kallikrein-bradykinin system genes.

| Gene | Allele | Genotype frequencies | | | Location | Homozygous common allele | Heterozygous | Homozygous minor allele | Interaction Effect | Pemp | Pperm |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | % | % | % | | | | | | | |
| **Angiotensin-II type I receptor** | | | | | | HR 95% CI | HR 95% CI | HR 95% CI | HR 95% CI | | |
| rs275651 | A > T | 67.4 | 29.4 | 3.3 | Promoter | 0.65 (0.53-0.81) | 1.07 (0.81-1.41) | 0.97 (0.46-1.92) | 1.42 (1.09-1.85) | 0.008 | 0.049 |
| rs10935724 | A > C | 44.3 | 44.2 | 11.5 | Intron | 0.83 (0.68-1.03) | 0.73 (0.59-0.91) | 0.71 (0.47-1.07) | 0.88 (0.71-1.09) | 0.23 | 0.80 |
| rs931490 | A > G | 66.6 | 30.0 | 3.4 | Intron | 0.73 (0.61-0.86) | 1.03 (0.78-1.35) | 0.84 (0.39-1.82) | 1.29 (0.99-1.68) | 0.05 | 0.29 |
| rs4681440 | C > T | 68.6 | 28.3 | 3.2 | Intron | 0.79 (0.67-0.94) | 0.79 (0.60-1.04) | 0.62 (0.26-1.42) | 0.93 (0.71-1.21) | 0.56 | 0.99 |
| rs5182 | C > T | 27.3 | 49.9 | 22.8 | Exon | 0.99 (0.74-1.27) | 0.84 (0.67-1.02) | 0.59 (0.44-0.80) | 0.77 (0.63-0.94) | 0.011 | 0.054 |
| rs5186 | A > C | 51.9 | 40.6 | 7.5 | Exon | 0.70 (0.58-0.85) | 0.88 (0.70-1.11) | 0.93 (0.55-1.57) | 1.23 (0.98-1.54) | 0.068 | 0.35 |
| **Bradykinin type I receptor** | | | | | | | | | | | |
| rs4905475 | G > C | 81.2 | 17.7 | 1.1 | Promoter | 0.77 (0.66-0.90) | 0.90 (0.63-1.25) | 0.94 (0.23-4.90) | 1.13 (0.81-1.58) | 0.46 | 0.92 |
| rs12050217 | A > G | 62.1 | 33.2 | 4.7 | Intron | 0.64 (0.55-0.78) | 1.02 (0.79-1.29) | 1.10 (0.56-2.19) | 1.44 (1.13-1.83) | 0.004 | 0.012 |
| rs885845 | C > T | 41.7 | 45.2 | 13.1 | Intron | 0.66 (0.53-0.82) | 0.95 (0.76-1.15) | 0.80 (0.51-1.18) | 1.16 (0.94-1.43) | 0.16 | 0.50 |
| rs2071084 | G > A | 68.4 | 28.3 | 3.2 | Exon | 0.83 (0.70-0.99) | 0.71 (0.53-0.90) | 0.82 (0.41-1.63) | 0.89 (0.69-1.15) | 0.37 | 0.85 |

* The treatment effect refers to the reduction in risk of the primary endpoint by perindopril as compared to placebo during 4 years of follow-up. The treatment effect of perindopril in the main EUROPA trial was HR 0.80 (95% CI 0.71-0.91). Cox proportional hazard regression analysis was used to estimate treatment effects across genotype strata adjusted for age, gender, systolic BP, diabetes mellitus, smoking, BMI >30, creatinine clearance, prior MI, prior stroke or peripheral vascular disease, symptomatic CAD, and family history of CAD. Complete data on follow-up and covariates in 8746 patients. LD between rs275651 and rs5182 r2 0.1. Empirical and permutation p-values based on 10,000 permutations ( p-values for interaction). Pemp = multivariate adjusted empirical p-value. Pperm = multivariate adjusted permutated p-value (including correction for multiple testing).

## Pharmacogenetic profile of treatment benefit

When we combined the 3 SNPs in a pharmacogenetic profile (composed of rs12050217, rs5182 and rs275651) the event rate decreased with an increasing number of unfavourable alleles in patients allocated placebo (from 12.2% to 8.1%), while the event rate increased in patients allocated perindopril (from 6.3% to 10.4%) (figure 1). A stepwise decrease in treatment benefit of perindopril was observed with increasing number of unfavourable alleles (p-value for interaction <0.0001). As presented in figure 1, treatment benefit was pronounced in patients with 0, 1 or 2 unfavourable alleles, while absent in patients with ≥3 unfavorable alleles. Integrating these findings in a pharmacogenetic profile composed of the 3 SNPs, we identified 73.5% of the population with a more pronounced treatment effect (<3 unfavourable alleles; HR 0.67; 95% CI 0.56-0.79) and 26.5% of the population not benefiting from treatment (≥3 unfavorable alleles; HR 1.26; 95% CI 0.97-1.67) as presented in figure 2.

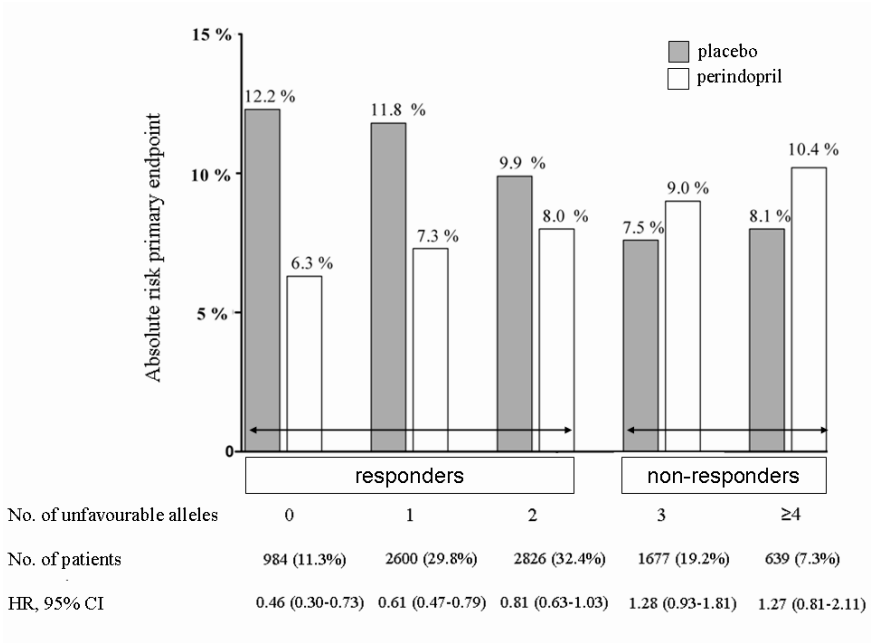


**Figure 1.** Pharmacogenetic profile of identified SNPs and the treatment effect of perindopril in stable CAD patients from the EUROPA trial. Multivariate Cox proportional hazard regression analysis was adjusted for age, gender, systolic BP, total cholesterol, diabetes mellitus, smoking, BMI, creatinine clearance, history of MI, history of stroke or peripheral vascular disease, prior revascularization, symptomatic CAD, and family history of CAD. 8726 out of 8746 patients have complete genotype data on rs275651, rs5182 and rs12050217. Patient with 0-2 (<3) unfavourable alleles experienced a treatment benefit of perindopril (called responders) and patients with 3 or more unfavourable alleles experienced no treatment benefit of perindopril (non-responders) as reflected in the HR and 95% CI of treatment benefit. P for interaction <0.0001. HR= hazard ratio; CI= confidence interval.

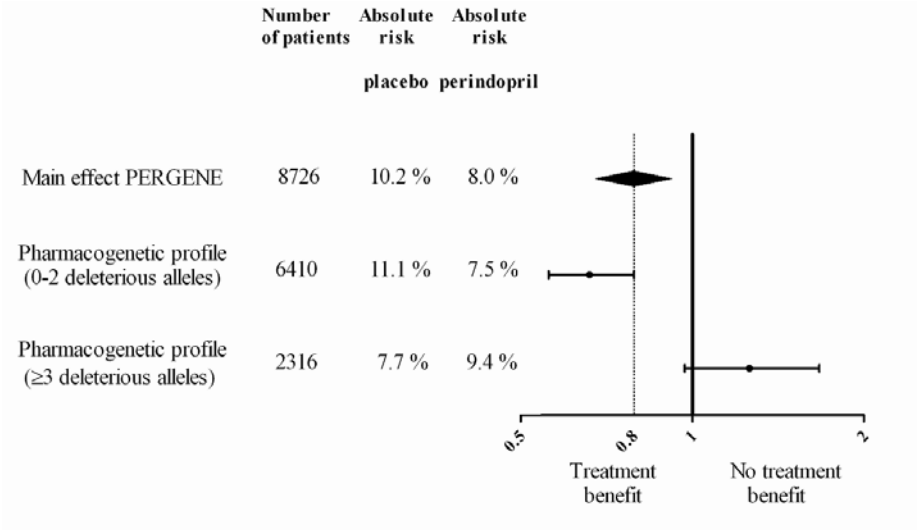


**Figure 2.** Pharmacogenetic profile to predict the treatment effect of perindopril in patients with stable coronary artery disease. Multivariate Cox proportional hazard regression analysis. The pharmacogenetic profile combined the patients with <3 unfavourable alleles (responders) and patients with ≥3 unfavourable alleles (non-responders) as one group (based upon the findings of figure 1). The hazard ratios were HR 0.67 (95% CI 0.56-0.79) and HR 1.26 (95% CI 0.97-1.67) for responders and non-responders, respectively.



In the overall study population, patients with ≥3 unfavorable alleles had a slightly lower risk compared to patients with <3 unfavorable alleles, although this difference was not statistically significant: HR 0.88 (95% CI 0.76-1.04). In patients allocated placebo, HR was 0.68 (95% CI 0.53-0.84) comparing those with ≥3 versus those with <3 unfavorable alleles, in patients allocated perindopril HR was 1.18 (95% CI 0.94-1.49), demonstrating the interaction effect.

No differences in clinical characteristics, including blood pressure, were observed between patients with ≥3 and <3 unfavorable alleles (table 1; all p-values = ns). Furthermore, no differences in intermediate phenotypes were observed in terms of blood pressure and blood pressure reduction to during the run-in period of the EUROPA-trial (table 1). Thus, the observed treatment interaction cannot be explained by clinical differences between the genotypes.

## Confirmation analysis in PROGRESS

In European patients from PROGRESS (n=1051) receiving perindopril as single therapy or placebo, no benefit of perindopril was apparent in the overall study group: HR 1.19 (95% CI 0.83 – 1.71). Minor allele frequencies of the 3 SNPs were similar to those in the PERGENE population.

The treatment effects for the individual SNPs are presented in table 3. The estimates of interaction effect on treatment in PROGRESS were of similar direction and magnitude as observed in the PERGENE study for all 3 individual SNPs (figure 3), although confidence intervals were wide

**Table 3.** Treatment effect of perindopril in Caucasian subjects of the PROGRESS trial (n=1051)

| Gene | SNP | Allele | Genotype frequencies (%) | | | Single therapy (perindopril only) | |
|---|---|---|---|---|---|---|---|
| | | | 1/1 | 1/2 | 2/2 | Common allele | Minor allele* |
| AT1 receptor | Rs275651 | A>T | 66.2 | 29.6 | 4.2 | 1.04 (0.64-1.70) | 1.46 (0.70-3.30) |
| | Rs5182 | C>T | 29.5 | 50.8 | 19.7 | 1.57 (0.68-3.56) | 1.03 (0.64-1.67) |
| BK1 receptor | Rs12050217 | A>G | 62.6 | 33.5 | 3.8 | 0.95 (0.57-1.61) | 1.55 (0.78-3.04) |

Overall study effect in PROGRESS (n=1051) was HR 1.19 (0.78-1.79), 526 patients allocated perindopril and 525 placebo. Treatment effect analyses are adjusted for full model variables. * heterozygous and homozygous minor allele groups combined due to low sample size in the homozygous minor allele group.

and statistical interaction terms were not significant in the relatively small PROGRESS cohort (n=1051; 526 perindopril vs 525 placebo). In a combined analysis of the interaction effects on treatment, the initially observed p-values from EUROPA improved by adding the PROGRESS data in meta-analysis for each of the 3 individual SNPs. The combined interaction effects were

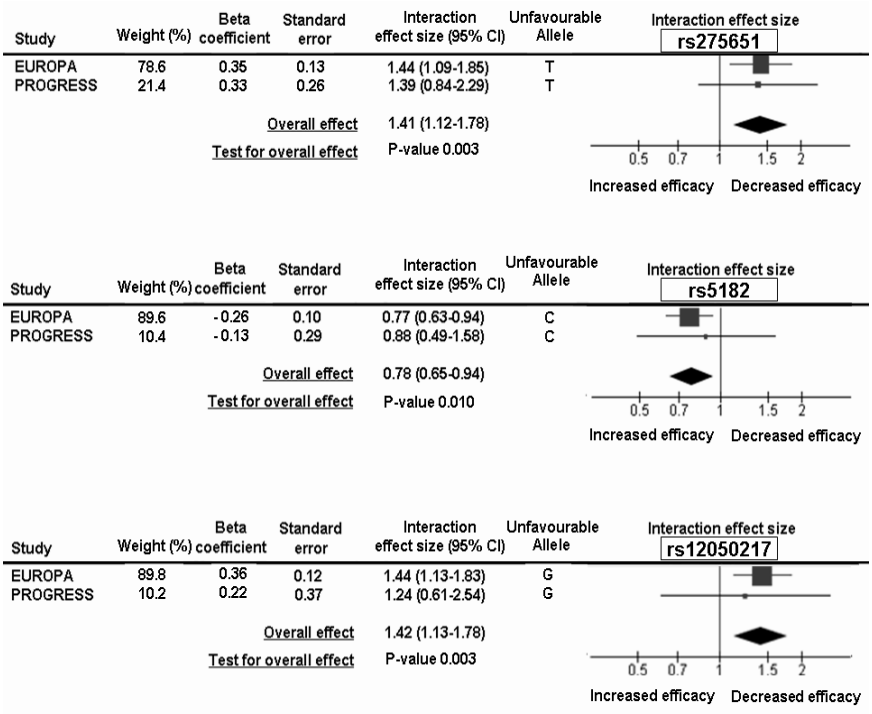


**Figure 3.** Replication of findings: meta-analysis of treatment interaction effects. The interaction effect sizes, standard errors and 95% CI were estimated using Cox proportional hazard regression analysis. Results from the two studies were combined in meta-analyses using an inverse variance weighted method in a random effects model. Heterogeneity tests for the meta-analysis for all three SNPs were non-significant (all $I^2 = 0$ for all three SNPs). Meta-analysis of the interaction effects improved the initially observed P-values for all 3 SNPs (p-values 0.003; 0.010; 0.003, respectively).

HR 1.41; 95% CI 1.12-1.78 (p= 0.003) ; HR 0.78; 95% CI 0.65-0.94 (p= 0.010) ; and HR 1.42; 95% CI 1.13-1.78 (p= 0.003) for rs275651, rs5182 and rs12050217, respectively. In figure 4 the treatment effects relative to the overall study (as a % change in treatment benefit) are presented for both studies, demonstrating the strong concordance of the effect modification in both independent populations.

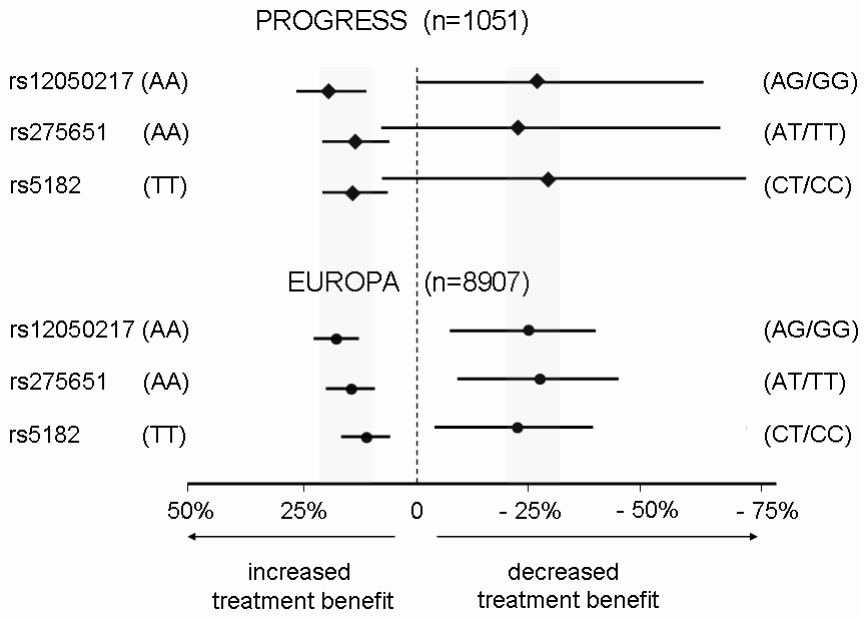


**Figure 4.** Relative treatment effects of perindopril in EUROPA and PROGRESS (% change in treatment effect)

## DISCUSSION

The current study demonstrates that the treatment benefit of ACE-inhibitor therapy by perindopril is modified by variation in 2 genes in the renin-angiotensin-aldosterone and kallikrein-bradykinin systems: the AT1 receptor gene and the BK1 receptor gene. Based on the pharmacogenetic profile, consisting of these variants, both patients with an enhanced treatment benefit (73.5% of the PERGENE population), and patients with a diminished, if not absent, treatment effect (26.5% of the PERGENE population) could be identified. A similar interaction of this profile with the treatment effect was observed in the replication cohort from PROGRESS.

The concept of pharmacogenetics to individualize medicine is emerging rapidly and clinically highly relevant as it has the potential to revolutionize future clinical practice. Large randomized clinical trials that have DNA available, provide the opportunity to study this concept of individualized therapy and several successes of this approach have recently been

demonstrated for different cardiovascular agents, such as the activation of clopidogrel [24], the risk of rhabdomyolysis associated with statin therapy [25], and anti-coagulation therapy by warfarin which prescription is already guided by pharmacogenetics [26]. Our study is the first large-scale pharmacogenetic analysis of patients with stable CAD randomized to ACE-inhibitor therapy versus placebo. The proposed pharmacogenetic profile was directly associated with the treatment benefit of ACE-inhibitors. This association was independent of baseline clinical characteristics and blood pressure, which is in accordance with previous studies in which clinical patient characteristics or subgroup analyses did not reveal any treatment heterogeneity [1,2,8-10,27].

The genetic basis of the observed interaction with the treatment response of perindopril in stable CAD has not yet been investigated comprehensively. Pharmacogenetic data in this area are scarce, mainly because only a few large randomized clinical trials with ACE-inhibitors have been conducted in these patients, often without systematic collection of DNA. Yet, solid conclusions on modification of the treatment effect can only be drawn from such studies. The few previous studies on this subject were mostly small, not randomized or lacking a placebo-control group and, therefore, the reported relations were largely inconclusive [12,15,27,28]. Virtually all of these studies focused on the ACE insertion/deletion (I/D) polymorphism, and two large studies found no association of this polymorphism with treatment response [29,30]. The GenHAT study was the first to study the concept of pharmacogenetics of the ACE I/D in combination with different anti-hypertensive drugs [30]. In our study, the optimal proxy (high LD; D' 1.0; $r^2$ 0.9) of the ACE I/D polymorphism, rs4343, also was not related with the treatment benefit of perindopril [31]. Another limitation of the previous studies was that they focussed on one single polymorphism. This, however, does not justify the complexity of the RAAS and KB system. Comprehensive coverage of all RAAS and KB system genes, with multiple tagging SNPs within multiple candidate genes in a common pathway, is necessary to allow truly meaningful conclusions.

In the main analysis of the EUROPA-trial, treatment with perindopril resulted in a relative risk reduction of 20% for the primary endpoint, which was consistent across all clinical subgroups [7]. In contrast, the subgroups based on the proposed genetic profile have a wide range of treatment effects, from patients without unfavourable alleles (11.3% of all patients) with a 54% reduction in the primary endpoint during follow-up, via patients with one unfavourable allele (29.8%) who experienced a 39% relative risk reduction and patients with two unfavourable alleles (32.4%) with a 19% relative risk reduction to which is more comparable to the overall study effect in these patients. Patients with <3 unfavourable alleles experience a positive treatment effect (responders). At the other end of the spectrum, patients with ≥3 unfavourable alleles experienced no benefit (26.5%) from perindopril treatment during four years of follow-up (non-responders). Refraining from treatment with perindopril in this group of patients, which were relatively insensitive or resistant to ACE-inhibitor therapy, may considerably reduce healthcare cost and increase overall efficacy of the drug.

Our findings suggest that the genetic variants modifying the treatment effect of perindopril are particularly located in the AT1 and BK1 receptor genes. The SNPs in the AT1 receptor were located in the promoter (rs275651) and exon (rs5182), the SNP in the BK1 receptor was located in an intron. These three SNPs were tagging SNPs and may either be functional themselves or in linkage equilibrium with functional SNPs. So far, functionality of these three SNPs is unknown. The AT1 receptor does mediate all the well-known effects of angiotensin II, including vasoconstriction, water and salt retention, aldosterone synthesis and hypertrophy. The role of the $B_1$ receptor, on the other hand, is less well established. Bradykinin is a potent vasodilator that also induces anti-atherosclerotic and anti-thrombotic effects, which are mediated by bradykinin type II ($B_2$) receptors. Previous studies indicated that the clinical benefit of ACE-inhibitors depends, at least in part, on $B_2$ receptor activation [32]. $B_1$ receptors are weakly expressed under physiological conditions, but are strongly induced in response to pathological conditions and/or RAAS blockade [33,34]. Interestingly, it has been suggested that $B_1$ receptors are directly activated by ACE-inhibitors (thus resulting in an increase in endothelial NO release, for instance in the heart [35,36]), by which they contribute to the cardioprotective effects of ACE-inhibitors, but this has not been uniformly confirmed by others [37]. Therefore, a more likely possibility is that the up-regulated $B_1$ receptors are activated by their endogenous ligand during ACE-inhibition. Given the hypotensive [38], cardioprotective [33] and cerebro-protective [39] effects of such activation, as observed in animals, one might speculate that patients with genetic defects in their $B_1$ receptor display a diminished response to ACE-inhibition. Clearly, more work is needed to support this concept.



In our study, the combination of ≥3 unfavourable alleles of the 3 SNPs was associated with a lower risk of CVD events in placebo patients, while the risk increased in patients receiving perindopril independent of clinical characteristics. It may be suggested that the absolute risk of events in these patients was very low, preventing any benefit of the addition of an ACE-inhibitor. However, the absolute risk for cardiovascular death or myocardial infarction in these patients was 7.7 % at 4.2 years follow-up. In an earlier analysis of the EUROPA trial a consistent treatment benefit was observed in the lower risk tertile (based on assessment of clinical characteristics) with a risk of only 5.3% in the placebo group as well as in the higher risk tertiles [7]. We observed no significant differences in clinical characteristics and intermediate phenotypes (blood pressure and blood-pressure reduction during the run-in period) between patients with <3 and with ≥3 unfavourable alleles. The mechanism underlying the association between the proposed genetic profile and treatment response is not explained by clinical characteristics and is elucidated by the pharmacogenetic basis of drug response. Unfortunately, no serum or plasma was available to measure levels of RAAS factors. Future studies will have to be designed to allow such mechanistic studies.

The current study has some limitations that should be noted. The EUROPA-trial consisted of predominantly male Caucasian subjects with stable CAD, which were treated with the ACE-inhibitor perindopril, which limits the generalizability of the results regarding type of patients

and type of agent. New pharmacogenetic studies in different patient populations and with different ACE-inhibitors as well as angiotensinogen II receptor blockers are warranted. In EUROPA we studied the ACE-inhibitor perindopril at a dose of 8 mg/day. One could argue that patients not benefiting from treatment were undertreated; however, 8 mg/day is a relatively high dose, and the effect on blood pressure was similar among patients with <3 and with ≥3 unfavourable alleles. The generalizability of our results to other ACE-inhibitors is unknown. Although differences in pharmacological properties do exist between ACE-inhibitors [40], the clinical relevance of these differences is uncertain and different ACE-inhibitors consistently improve outcome in trials of patients with CAD or heart failure [1]. Although we analysed a large group of patients, testing of multiple genes and SNPs might result in chance findings. Correction for multiple testing and confirmation in other cohorts is, therefore, necessary, which was performed in the current analysis. We have chosen to use a gene-based permutation. Another option would have been Bonferonni correction, but it is know that this is an overly conservative method because of the strength of our a priori study hypothesis and the linkage disequilibrium between the SNPs located within genes in a common pathway. Unfortunately, a replication cohort of similar size and design as EUROPA is not available and no other randomized placebo controlled trials with DNA are available for replication of our findings in patients with stable coronary artery disease. For an initial replication of our findings, we had the opportunity to use data of 1051 European patients of PROGRESS studying the same ACE-inhibitor, perindopril, albeit in lower dose of 4 mg. PROGRESS enrolled patients with cerebrovascular disease. Because the treatment benefit in PROGRESS was contingent on the combination with indapamide (2,5 mg) [16], we studied patients receiving single therapy with perindopril in the European subjects of PROGRESS, which ensures comparability with the EUROPA-trial subjects. Although the PROGRESS sample was underpowered, a similar direction and magnitude of the pharmacogenetic interaction was observed and the combined p-values were effected by adding the PROGRESS data despite smaller size and even improved for all 3 SNP's, which lends additional support to our findings. Still, the interaction terms in PROGRESS were not statistically significant which is related to the relatively small number of patients (n=1051, 526 perindopril, 525 placebo) as well as statistical confront of replicating interaction terms in general. The current study also has several strengths to be noted, as it is unique because of its sample size, design (randomization and a placebo group), replication cohort (perindopril), prospective follow-up, accurate phenotypic data and comprehensive coverage of multiple genes in the pathway of ACE-inhibitors.

In conclusion, our finding show that three out of four patients had an enhanced benefit of ACE-inhibitor therapy (33% reduction of cardiovascular death of myocardial infarction, up to 54% in patients without any unfavourable alleles) and one out of four patients experienced no, or a markedly diminished, benefit of long term perindopril treatment. By developing a pharmacogenetic profile related to treatment response, patients can be selected who are most likely to benefit from such treatment in advance. When the feasibility of pharmacogenetic profiling of

ACE-inhibitor therapy is confirmed, physicians will be able to predict the response to treatment (the exciting concept of responders and non-responders) before the start of prescription. Taken together, these pharmacogenetic analyses of clinical trials open up a perspective to individualise preventive therapy in patients with stable CAD [24-26] which may avoid unnecessary treatment, and considerably reduce health care costs by the concept of "individualizing therapy" based on genetic data. Moreover, the combination of these trials can be used to identify a genetic profile for cardiovascular drugs at large. To further explore this concept, we suggest that future randomized clinical trials should integrate a pharmacogenetic approach in the trial design to optimize patients' benefit.

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*Conflict of interest & financial disclosures:*

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| | |
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| Data collection: | J.J.Brugts; M.P.M. de Maat; T. Ninomiya; M.L.Simoons; A. Uitterlinden. |
| Statistical assessment | E.Boersma; J.J.Brugts; C.M. van Duijn; A.Isaacs; J.C.M. Witteman. |
| Outcome assessment | E.Boersma; J.J.Brugts; C.M. van Duijn; A.H.J.Danser; R.Ferrari, M.L.Simoons |
| Writing: | All authors contributed in writing the manuscript. |

**Supplement table 1a.** Renin-angiotensin-aldosteron pathway gene polymorphisms in relation to treatment effect of perindopril in patients with stable CAD.

| Gene | rs-number | | Location | Allele | Genotypes (%) | | | Treatment effect (HR 95% CI) | | | Interaction effect | | $P_{emp}$ | $P_{perm}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1/1 | 1/2 | 2/2 | 1/1 | 1/2 | 2/2 | HR | 95% CI | | |
| **Angiotensin-converting enzyme** | rs4291 | Chr 17 | Promoter | A > T | 37.0 | 47.5 | 15.5 | 0.85 (0.67-1.08) | 0.75 (0.61-0.92) | 0.80 (0.57-1.13) | 0.97 (0.79-1.18) | | 0.75 | 1.00 |
| | rs4293 | Chr 17 | Intron | T > C | 30.1 | 49.3 | 20.6 | 0.86 (0.66-1.12) | 0.75 (0.61-0.93) | 0.77 (0.56-1.04) | 0.95 (0.77-1.15) | | 0.57 | 0.99 |
| | rs4309 | Chr 17 | Exon | C > T | 33.0 | 47.8 | 19.3 | 0.82 (0.64-1.04) | 0.78 (0.64-0.96) | 0.72 (0.51-1.00) | 0.94 (0.77-1.15) | | 0.59 | 0.99 |
| | rs4311 | Chr 17 | Intron | C > T | 26.9 | 49.6 | 23.5 | 0.81 (0.61-1.08) | 0.75 (0.61-0.92) | 0.86 (0.64-1.15) | 1.04 (0.85-1.27) | | 0.70 | 1.00 |
| | rs4343 | Chr 17 | Exon | G > A | 28.3 | 49.3 | 22.4 | 0.77 (0.59-1.01) | 0.79 (0.65-0.97) | 0.76 (0.55-1.03) | 0.98 (0.81-1.20) | | 0.88 | 1.00 |
| **Angiotensinogen** | rs5049 | Chr 1 | romoter | G > A | 77.0 | 21.4 | 1.6 | 0.75 (0.64-0.89) | 0.84 (0.62-1.13) | 1.18 (0.30-4.62) | 1.13 (0.84-1.52) | | 0.43 | 1.00 |
| | rs5051 | Chr 1 | Promoter | C > T | 30.4 | 49.2 | 20.4 | 0.77 (0.59-1.00) | 0.75 (0.61-0.93) | 0.87 (0.65-1.16) | 1.06 (0.87-1.30) | | 0.54 | 1.00 |
| | rs4762 | Chr 1 | Exon | G > A | 74.2 | 23.9 | 1.9 | 0.75 (0.63-0.88) | 0.91 (0.69-1.20) | 1.37 (0.54-3.52) | 1.22 (0.93-1.61) | | 0.14 | 0.72 |
| | rs699 | Chr 1 | Exon | G > A | 30.2 | 49.5 | 20.3 | 0.77 (0.60-1.01) | 0.76 (0.62-0.93) | 0.85 (0.64-1.13) | 1.04 (0.86-1.27) | | 0.67 | 1.00 |
| | rs2478545 | Chr 1 | Intron | G > A | 56.4 | 37.8 | 5.8 | 0.74 (0.61-0.89) | 0.86 (0.68-1.08) | 0.94 (0.57-1.64) | 1.16 (0.92-1.45) | | 0.19 | 0.83 |
| | rs7079 | Chr 1 | 3′ UTR | T > G | 45.7 | 43.6 | 10.5 | 0.79 (0.64-0.97) | 0.77 (0.61-0.96) | 0.97 (0.62-1.52) | 1.03 (0.83-1.28) | | 0.75 | 1.00 |
| | rs10864770 | Chr 1 | 3′ UTR | G > A | 86.3 | 13.0 | 0.7 | 0.81 (0.70-0.95) | 0.66 (0.44-0.98) | 0.69 (0.14-3.84) | 0.76 (0.52-1.10) | | 0.13 | 0.69 |
| | rs943580 | Chr 1 | 3′ UTR | G > A | 30.5 | 49.4 | 20.1 | 0.79 (0.61-1.03) | 0.75 (0.61-0.92) | 0.85 (0.63-1.14) | 1.02 (0.83-1.24) | | 0.86 | 1.00 |
| **Angiotensin-II type 1 receptor** | rs275651 | Chr 3 | Promoter | A > T | 67.4 | 29.4 | 3.3 | 0.65 (0.53-0.81) | 1.07 (0.81-1.41) | 0.97 (0.46-1.92) | 1.42 (1.09-1.85) | | 0.008 | 0.049 |
| | rs10935724 | Chr 3 | Intron | A > C | 44.3 | 44.2 | 11.5 | 0.83 (0.68-1.03) | 0.73 (0.59-0.91) | 0.71 (0.47-1.07) | 0.88 (0.71-1.09) | | 0.23 | 0.80 |
| | rs931490 | Chr 3 | Intron | A > G | 66.6 | 30.0 | 3.4 | 0.73 (0.61-0.86) | 1.03 (0.78-1.35) | 0.84 (0.39-1.82) | 1.29 (0.99-1.68) | | 0.054 | 0.29 |
| | rs4681440 | Chr 3 | Intron | C > T | 68.6 | 28.3 | 3.2 | 0.79 (0.67-0.94) | 0.79 (0.60-1.04) | 0.62 (0.26-1.42) | 0.93 (0.71-1.21) | | 0.56 | 0.99 |
| | rs5182 | Chr 3 | Exon | C > T | 27.3 | 49.9 | 22.8 | 0.99 (0.74-1.27) | 0.84 (0.67-1.02) | 0.59 (0.44-0.80) | 0.77 (0.63-0.94) | | 0.011 | 0.054 |
| | rs5186 | Chr 3 | Exon | A > C | 51.9 | 40.6 | 7.5 | 0.70 (0.58-0.85) | 0.88 (0.70-1.11) | 0.93 (0.55-1.57) | 1.23 (0.98-1.54) | | 0.068 | 0.35 |
| **Angiotensin-II type 2 receptor** | rs3736556 | Chr X | Intron | A > T | 70.6 | 29.4 | -- | 0.75 (0.63-0.89) | 0.85 (0.65-1.11) | -- | 1.11 (0.94-1.31) | | 0.21 | 0.62 |
| | rs5193 | Chr X | 3′ UTR | G > T | 70.2 | 29.8 | -- | 0.77 (0.65-0.92) | 0.83 (0.64-1.08) | -- | 1.03 (0.87-1.21) | | 0.72 | 1.00 |
| | rs5194 | Chr X | 3′ UTR | A > G | 50.1 | 49.9 | -- | 0.73 (0.60-0.89) | 0.83 (0.68-1.02) | -- | 1.12 (0.96-1.29) | | 0.14 | 0.44 |
| | rs12840631 | Chr X | 3′ UTR | C > G | 95.3 | 4.7 | -- | 0.80 (0.69-0.92) | 0.68 (0.32-1.33) | -- | 0.85 (0.58-1.25) | | 0.41 | 0.88 |

**Supplement table 1a** continued

| Gene | rs-number | | Location | Allele | Genotypes (%) | | | Treatment effect (HR 95% CI) | | | Interaction effect | | $P_{emp}$ | $P_{perm}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1/1 | 1/2 | 2/2 | 1/1 | 1/2 | 2/2 | HR | 95% CI | | |
| **Renin** | rs2887284 | Chr 1 | Intron | C > A | 61.5 | 34.1 | 4.4 | 0.84 (0.70-1.00) | 0.75 (0.60-0.95) | 0.37 (0.17-0.82) | 0.83 | (0.65-1.06) | 0.13 | 0.41 |
| | rs1464816 | Chr 1 | Intron | G > T | 43.0 | 44.8 | 12.2 | 0.76 (0.62-0.95) | 0.69 (0.55-0.86) | 1.23 (0.86-1.83) | 1.19 | (0.97-1.47) | 0.10 | 0.46 |
| | rs5707 | Chr 1 | Exon | T > G | 59.7 | 35.3 | 5.0 | 0.81 (0.68-0.97) | 0.80 (0.63-1.02) | 0.37 (0.18-0.76) | 0.88 | (0.69-1.12) | 0.31 | 0.89 |
| | rs11571082 | Chr 1 | Intron | G > A | 74.8 | 23.4 | 1.8 | 0.84 (0.72-0.99) | 0.65 (0.49-0.88) | 0.47 (0.14-1.51) | 0.76 | (0.57-1.02) | 0.07 | 0.35 |
| | rs10900555 | Chr 1 | Intron | T > C | 42.5 | 44.9 | 12.6 | 0.92 (0.74-1.14) | 0.72 (0.58-0.88) | 0.60 (0.40-0.91) | 0.81 | (0.66-1.00) | 0.05 | 0.27 |
| | rs11571078 | Chr 1 | Intron | C > T | 76.4 | 21.8 | 1.8 | 0.80 (0.68-0.95) | 0.70 (0.52-0.94) | 0.82 (0.18-3.64) | 0.95 | (0.71-1.26) | 0.70 | 1.00 |
| **Aldosteron synthase** | rs11781082 | Chr 8 | Promoter | G > A | 58.9 | 35.9 | 5.2 | 0.78 (0.64-0.90) | 0.80 (0.64-1.02) | 0.77 (0.38-1.57) | 1.03 | (0.81-1.31) | 0.80 | 1.00 |
| | rs1799998 | Chr 8 | Promoter | T > C | 28.9 | 49.7 | 21.4 | 0.77 (0.59-1.00) | 0.79 (0.65-0.96) | 0.81 (0.59-1.12) | 1.04 | (0.85-1.26) | 0.74 | 1.00 |
| | rs3097 | Chr 8 | 3′ UTR | G > A | 51.1 | 41.3 | 7.6 | 0.82 (0.67-1.000 | 0.77 (0.62-0.95) | 0.70 (0.41-1.17) | 0.94 | (0.75-1.17) | 0.60 | 0.99 |
| | rs6433 | Chr 8 | Intron | A > G | 34.7 | 48.8 | 16.5 | 0.79 (0.62-1.01) | 0.80 (0.66-0.97) | 0.74 (0.52-1.07) | 0.98 | (0.80-1.20) | 0.86 | 1.00 |
| | rs4543 | Chr 8 | Exon | G > A | 82.4 | 16.6 | 1.0 | 0.77 (0.66-0.90) | 0.86 (0.61-1.22) | 0.92 (0.29-3.11) | 1.08 | (0.77-1.52) | 0.65 | 1.00 |
| **Prorenin receptor** | | | | | | | | | | | | | | |
| | rs2968915 | Chr X | Promoter | A > G | 88.1 | 11.8 | -- | 0.76 (0.6-0.89) | 0.95 (0.62-1.45) | -- | 1.16 | (0.92-1.47) | 0.20 | 0.37 |
| | rs5918008 | Chr X | Intron | A > C | 88.9 | 11.0 | -- | 0.76 (0.66-0.88) | 1.09 (0.71-1.70) | -- | 1.20 | (0.94-1.54) | 0.14 | 0.25 |

Treatment effect were calculated using multivariate Cox regression analysis adjusted for age, gender, systolic blood pressure, diabetes mellitus, total cholesterol, smoking, body mass index, creatinine clearance, prior myocardial infarction, prior revascularization, history of stroke/peripheral vascular disease, symptomatic CAD, and family history of CAD. For testing treatment interaction, the model included SNP, treatment and SNP*treatment. P-values were corrected for multiple testing by permutations analysis (based upon 10,000 permutations). HR= hazard ratio; CI= confidence interval. 1/1 = homozygous common allele; 1/2 = heterozygous; 2/2 = homozygous minor allele. * P-value of interaction term

**Supplement table 1b.**

| Gene | rs-number | | Location | Allele | Genotypes (%) | | | Treatment effect (HR 95% CI) | | | Interaction term | Pemp | Pperm |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1/1 | 1/2 | 2/2 | 1/1 | 1/2 | 2/2 | HR 95% CI | * | * |
| **Kininogen** | | | | | | | | | | | | | |
| | rs1050274 | Chr 3 | Exon | G > A | 45.6 | 43.5 | 10.9 | 0.78 (0.64-0.97) | 0.79 (0.63-0.97) | 0.80 (0.52-1.23) | 1.01 (0.82-1.25) | 0.91 | 0.99 |
| | rs1656922 | Chr 3 | Exon | C > T | 28.6 | 50.5 | 20.9 | 0.81 (0.63-1.05) | 0.78 (0.64-0.96) | 0.74 (0.55-1.00) | 0.97 (0.80-1.19) | 0.79 | 1.00 |
| | rs1469859 | Chr 3 | Exon | G > A | 42.4 | 45.8 | 11.7 | 0.76 (0.61-0.94) | 0.84 (0.68-1.04) | 0.72 (0.47-1.10) | 0.99 (0.80-1.23) | 0.94 | 1.00 |
| | rs1621816 | Chr 3 | Intron | A > G | 51.4 | 40.7 | 8.0 | 0.76 (0.62-0.92) | 0.76 (0.61-0.95) | 1.08 (0.67-1.78) | 1.11 (0.89-1.39) | 0.34 | 0.80 |
| **Kallikrein** | | | | | | | | | | | | | . |
| | rs5517 | Chr 19 | Exon | A > G | 53.7 | 38.9 | 7.4 | 0.77 (0.64-0.94) | 0.78 (0.62-0.98) | 0.87 (0.56-1.36) | 1.04 (0.84-1.30) | 0.69 | 0.92 |
| | rs1054713 | Chr 19 | Exon | C > T | 43.8 | 44.5 | 11.6 | 0.83 (0.67-1.02) | 0.78 (0.64-0.97) | 0.67 (0.43-1.04) | 0.94 (0.76-1.16) | 0.54 | 0.79 |
| **Bradykinin type 1 receptor** | rs4905475 | Chr 14 | Promoter | G > C | 81.2 | 17.7 | 1.1 | 0.77 (0.66-0.90) | 0.90 (0.63-1.25) | 0.94 (0.23-4.90) | 1.13 (0.81-1.58) | 0.46 | 0.92 |
| | rs12050217 | Chr 14 | Intron | A > G | 62.1 | 33.2 | 4.7 | 0.64 (0.55-0.78) | 1.02 (0.79-1.29) | 1.10 (0.56-2.19) | 1.44 (1.13-1.83) | 0.004 | 0.012 |
| | rs885845 | Chr 14 | Intron | C > T | 41.7 | 45.2 | 13.1 | 0.66 (0.53-0.82) | 0.95 (0.76-1.15) | 0.80 (0.51-1.18) | 1.16 (0.94-1.43) | 0.16 | 0.50 |
| | rs2071084 | Chr 14 | Exon | G > A | 68.4 | 28.3 | 3.2 | 0.83 (0.70-0.99) | 0.71 (0.53-0.90) | 0.82 (0.41-1.63) | 0.89 (0.69-1.15) | 0.37 | 0.85 |
| **Bradykinin type 2 receptor** | rs1799722 | Chr 14 | Promoter | G > A | 32.2 | 49.4 | 18.4 | 0.82 (0.63-1.05) | 0.72 (0.59-0.88) | 0.92 (0.68-1.31) | 1.03 (0.84-1.26) | 0.75 | 1.00 |
| | rs1046248 | Chr 14 | Exon | G > A | 83.4 | 15.8 | 0.7 | 0.82 (0.70-0.96) | 0.65 (0.45-0.94) | 0.41 (0.04-2.10) | 0.74 (0.52-1.06) | 0.10 | 0.34 |
| | rs5224 | Chr 14 | Exon | G > A | 65.9 | 30.2 | 3.9 | 0.84 (0.71-1.01) | 0.70 (0.54-0.90) | 0.69 (0.36-1.36) | 0.86 (0.68-1.10) | 0.24 | 0.65 |
| | rs5225 | Chr 14 | Intron | T > C | 79.5 | 19.1 | 1.4 | 0.76 (0.65-0.89) | 0.88 (0.63-1.20) | 0.70 (0.21-2.65) | 1.12 (0.82-1.53) | 0.48 | 0.92 |
| **Endothelial nitric oxide synthase** | rs1800779 | Chr 7 | Intron | A > G | 38.0 | 46.7 | 15.3 | 0.88 (0.69-1.12) | 0.72 (0.58-0.88) | 0.73 (0.52-1.02) | 0.90 (0.74-1.10) | 0.32 | 0.54 |
| | rs3918188 | Chr 7 | Intron | A > C | 41.1 | 46.2 | 12.7 | 0.74 (0.60-0.91) | 0.82 (0.66-1.02) | 0.86 (0.57-1.30) | 1.10 (0.89-1.36) | 0.36 | 0.59 |

Kallikrein-bradykinin pathway gene polymorphisms in relation to treatment effect of perindopril in patients with stable CAD. Treatment effect were calculated using multivariate Cox regression analysis adjusted for age, gender, systolic blood pressure, diabetes mellitus, total cholesterol, smoking, body mass index, creatinine clearance, prior myocardial infarction, prior revascularization, history of stroke/peripheral vascular disease, symptomatic CAD, and family history of CAD. For testing treatment interaction, the model included SNP, treatment and SNP*treatment. P-values were corrected for multiple testing by permutations analysis (based upon 10,000 permutations). HR= hazard ratio; CI= confidence interval. 1/1 = homozygous common allele; 1/2 = heterozygous; 2/2 = homozygous minor allele. * P-value of interaction term

**Supplement table 2a.** Haplotype analysis of the angiotensin-II type 1 receptor gene for heterogeneity in treatment effect.

| | Rs275651 | Rs10935724 | Rs931490 | Rs4681440 | Rs5182 | Rs5186 | Frequency (%) | Interaction effect size OR 95% CI | $P_{int}$ | Global P |
|---|---|---|---|---|---|---|---|---|---|---|
| Angiotensin-II type 1 receptor | A | A | A | C | T | A | 24.3 | Base | -- | 0.11 |
| | A | A | A | C | C | C | 15.0 | 1.42 (0.95-2.12) | 0.09 | |
| | A | C | A | C | T | A | 10.4 | 1.05 (0.66-1.65) | 0.85 | |
| | A | A | A | C | C | A | 8.2 | 1.22 (0.72-2.07) | 0.46 | |
| | A | C | A | T | T | A | 7.5 | 0.82 (0.46-1.44) | 0.48 | |
| | T | A | G | C | C | A | 6.6 | 1.57 (0.93-2.66) | 0.92 | |
| | A | C | A | T | C | A | 5.9 | 1.55 (0.92-2.61) | 0.10 | |
| | T | A | G | C | C | C | 5.8 | 1.94 (1.05-3.59) | *0.034* | |
| | T | A | G | C | T | A | 4.8 | 1.32 (0.71-2.46) | 0.37 | |

Multivariate model adjusted for age, gender, systolic blood pressure, diabetes mellitus, smoking, body mass index > 30, creatinine clearance, prior MI, prior stroke or peripheral vascular disease, symptomatic CAD, and family history of CAD. OR = odds ratio, CI = confidence interval.

**Supplement table 2b.** Haplotype analysis of the bradykinin type I receptor gene for heterogeneity in treatment effect.

| | Rs4905475 | Rs12050217 | Rs885845 | Rs2071084 | Frequency (%) | Interaction effect size OR 95% CI | $P_{int}$ | Global P |
|---|---|---|---|---|---|---|---|---|
| Bradykinin type 1 receptor | G | A | C | G | 36.8 | Base | --- | 0.03 |
| | G | G | T | G | 21.3 | 1.48 (1.11-1.97) | *0.008* | |
| | G | A | C | A | 17.5 | 1.00 (0.74-1.35) | 0.98 | |
| | G | A | T | G | 14.3 | 0.88 (0.62-1.24) | 0.46 | |
| | C | A | C | G | 10.0 | 1.23 (0.84-1.79) | 0.29 | |

Multivariate model adjusted for age, gender, systolic blood pressure, diabetes mellitus, smoking, body mass index > 30, creatinine clearance, prior MI, prior stroke or peripheral vascular disease, symptomatic CAD, and family history of CAD. The model included a genotype*treatment interaction term. OR = odds ratio, CI = confidence interval.

## REFERENCES


1. Dagenais GR, Pogue J, Fox K, Simoons ML, Yusuf S. Angiotensin-converting-enzyme inhibitors in stable vascular disease without left ventricular systolic dysfunction or heart failure: a combined analysis of three trials. *Lancet* 2006; 368: 581–88
2. The European trial on reduction of cardiac events with perindopril in patients with stable coronary artery disease investigators. Efficacy of perindopril in reduction of cardiovascular events in stable coronary artery disease: randomised, double-blind, placebo-controlled, multicentre trial (the EUROPA study). *Lancet* 2003; 362: 782–88.
3. The Heart Outcomes Prevention Evaluation study investigators. Effects of an angiotensin-converting enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. *N Engl J Med* 2000;342:145–53.
4. The PEACE Trial Investigators. Angiotensin-converting-enzyme inhibition in stable coronary artery disease. *N Engl J Med* 2004; 351: 2058–68.
5. The Task Force on the Management of Stable Angina Pectoris of the European Society of Cardiology. Guidelines on the management of stable angina pectoris. *Eur Heart J* 2006 27, 1341–81.
6. Gibbons RJ, Abrams J, Chatterjee K, Daley J, Deedwania PC, Douglas JS, Ferguson TB Jr, Fihn SD, Fraker TD Jr, Gardin JM, O'Rourke RA, Pasternak RC, Williams SV; American College of Cardiology; American Heart Association Task Force on practice guidelines (Committee on the Management of Patients With Chronic Stable Angina). ACC/AHA 2002 guideline update for the management of patients with chronic stable angina—summary article: a report of the American College of Cardiology/American Heart Association Task Force on practice guidelines (Committee on the Management of Patients With Chronic Stable Angina). *J Am Coll Cardiol* 2003;41:159-168.
7. Deckers JW, Goedhart DM, Boersma E, Briggs A, Bertrand M, Ferrari R, Remme WJ, Fox K, Simoons ML. Treatment benefit by perindopril in patients with stable coronary artery disease at different levels of risk. *Eur Heart J* 2006; 27: 796–801.
8. Brugts JJ, Boersma E, Chonchol M, Deckers JW, Bertrand M, Remme WJ, Ferrari R, Fox K, Simoons ML. The cardioprotective effects of the angiotensin-converting enzyme inhibitor perindopril in patients with stable coronary artery disease are not modified by mild to moderate renal insufficiency: insights from the EUROPA trial. *J Am Coll Cardiol.* 2007;50:2148-55.
9. Remme WJ, Deckers JW, Fox KM, Ferrari R, Bertrand M, Simoons ML; EUROPA Investigators. Secondary prevention of coronary disease with ACE inhibition--does blood pressure reduction with perindopril explain the benefits in EUROPA? *Cardiovasc Drugs Ther.* 2009;23(2):161-70.
10. Brugts JJ, Ninomiya T, Boersma E, Remme WJ, Bertrand M, Ferrari R, Fox K, MacMahon S, Chalmers J, Simoons ML. The consistency of the treatment effect of an ACE-inhibitor based treatment regimen in patients with vascular disease or high risk of vascular disease: a combined analysis of individual data of ADVANCE, EUROPA, and PROGRESS trials. *Eur Heart J.* 2009;30(11):1385-94.
11. Danser AHJ, Schunkert H. Renin–angiotensin system gene polymorphisms: potential mechanisms for their association with cardiovascular diseases. *Eur J Pharmacology* 2000.303–316.
12. Brugts JJ, den Uil CA, de Maat M, Danser AHJ. The renin-angiotensin-aldosterone system: Approaches to Guide Angiotensin-Converting Enzyme Inhibition in Patients with Coronary Artery Disease. *Cardiology*. 2008 Oct 2;112(4):303-312.
13. Gainer JV, Morrow JD, Loveland A, King DJ, Brown NJ. Effect of bradykinin-receptor blockade on the response to angiotensin-converting-enzyme inhibitor in normotensive and hypertensive subjects. *N Engl J Med* 1998;339:1285-1292.
14. Gomma AH, Fox KM, for the EUROPA investigators. The EUROPA trial: design, baseline demography and status of the substudies. C*ardiovasc Drugs Ther* 2001;15:169-179
15. Brugts JJ, de Maat MPM, Boersma E, Witteman JCM, van Duijn C, Uitterlinden AG, Bertrand M, Remme W, Fox K, Ferrrari R, Danser AHJ, Simoons ML. The Rationale and Design of the Perindopril Genetic Association Study: A Pharmacogenetic analysis of ACE-inhibitor therapy in patients with stable coronary artery disease. *Cardiovasc Drugs Ther*. 2009 Apr;23(2):171-81.

16. *PROGRESS Collaborative Group* Randomised trial of a perindopril-based blood-pressure-lowering regimen among 6105 individuals with previous stroke or transient ischaemic attack. *Lancet* 2001; 358: 1033–41.
17. Nederhand RJ, Droog S, Kluft C, Simoons ML, de Maat MP. Logistics and quality control for DNA sampling in large mult-center studies. *J Thromb Haemost* 2003;1:987–91.
18. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005 Jan 15 [PubMed ID: 15297300])
19. P.I.W. de Bakker, R. Yelensky, I. Pe'er, S.B. Gabriel, M.J. Daly, D. Altshuler. Efficiency and power in genetic association studies. *Nature Genetics* 2005; 37: 1217-1223
20. Churchill GA, Doerge RW. Empirical threshold values for quantitative trait mapping. *Genetics* 1994;138:963–971.
21. Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. *Am J Hum Genet*. 2002;70:425-434.
22. Kavvoura FK, Ioannidis JP. Methods for meta-analysis in genetic association studies: a review of their potential and pitfalls. *Hum Genet*. 2008;123(1):1-14
23. Trikalins TA, Salanti G, Zintzaras E, Ioannidis JP. Meta-analysis methods. *Advances in Genetics* 2008 ;60 :311-334.
24. Simon T, Verstuyft C, Mary-Kraue M, Quteineh L, Drouet E, Meneveau N, Steg PG, Ferrieres J, Danchin N, Becquemont L, for the FAST-MI Investigators. Genetic Determinants of Response to Clopidogrel and Cardiovascular Events. *New Engl J Med* 2009;360:363-375
25. The SEARCH collaborative group, SLCO1B1 variants and statin-induced myopathy, a genome wide study, *N Engl J Med* 2008, 359:789-799
26. International Warfarin Pharmacogenetics Consortium, Klein TE, Altman RB, Eriksson N, Gage BF, Kimmel SE, Lee MT, Limdi NA, Page D, Roden DM, Wagner MJ, Caldwell MD, Johnson JA. Estimation of the warfarin dose with clinical and pharmacogenetic data. *N Engl J Med.* 2009 Feb 19;360(8):753-64.
27. Brugts JJ, de Maat M, den Uil CA, Danser AHJ. Ferrari R, Simoons ML. Pharmacogenetics of ACE inhibition in stable coronary artery disease: steps towards tailored drug therapy. *Curr Opin Cardiol.* 2008;23(4):296-301.
28. Danser AH, Batenburg WW, van den Meiracker AH, Danilov SM. ACE phenotyping as a first step toward personalized medicine for ACE inhibitors. Why does ACE genotyping not predict the therapeutic efficacy of ACE inhibition? *Pharmacol Ther.* 2007;113:607-18.
29. *PROGRESS Collaborative Group.* The ACE gene I/D polymorphism is not associated with the blood pressure and cardiovascular benefits of ACE inhibition. *Hypertension* 2003;42:297-303.
30. Arnett DK, Davis BR, Ford CE, Boerwinkle E, Leiendecker-Foster C, Miller MB, Black H, Eckfeldt JH. Pharmacogenetic association of the angiotensin-converting enzyme insertion/deletion polymorphism on blood pressure and cardiovascular risk in relation to antihypertensive treatment: the Genetics of Hypertension-Associated Treatment (GenHAT) study. *Circulation* 2005;28;111:3374-83.
31. Abdollahi MR, Huang S, Rodriguez S, Guthrie PA, Smith GD, Ebrahim S, Lawlor DA, Day IN, Gaunt TR. Homogeneous assay of rs4343, an ACE I/D proxy, and an analysis in the British Women's Heart and Health Study (BWHHS). *Dis Markers*. 2008;24:11-7.
32. Gainer JV, Morrow JD, Loveland A, King DJ, Brown NJ. Effect of bradykinin-receptor blockade on the response to angiotensin converting-enzyme inhibitor in normotensive and hypertensive subjects. *N Engl J Med* 1998;339:1285–92.
33. Xu J, Carretero OA, Shesely EG, Rhaleb NE, Yang JJ, Bader M, Yang XP. The Kinin B1 Receptor Contributes to the Cardioprotective Effect of ACE Inhibitors and Angiotensin Receptor Blockers. *Exp Physiol.* 2009 Mar;94(3):322-9.
34. Marin-Castaño ME, Schanstra JP, Neau E, Praddaude F, Pecher C, Ader JL, Girolami JP, Bascands JL. Induction of functional bradykinin b(1)-receptors in normotensive rats and mice under chronic Angiotensin-converting enzyme inhibitor treatment. *Circulation.* 2002;5;627-32

35. Ignatovic T, Tan F, Brovkovych V, Skidgel RA, Erdos GE. Novel Mode of Action of Angiotensin I Converting Enzyme Inhibitors: Direct activation of the Bradykinin Receptor type I. *The Journal of Biological Chemistry* 2002; 277, 16847–16852.
36. Ignjatovic T, Stanisavljevic S, Brovkovych V, Skidgel RA, Erdos EG. Kinin B1 Receptors Stimulate Nitric Oxide Production in Endothelial Cells: Signaling Pathways Activated by Angiotensin I-Converting Enzyme Inhibitors and Peptide Ligands. *Mol. Pharmacol*. 2004; 66:1310-16
37. Forten JP, Gobeil F Jr, Adam A, Regoli D, Marceau F. Do angiotensin-converting enzyme inhibitors directly stimulate the kinin B1 receptor? *Am J Physiol Heart Circ Physiol*. 2003;285:H277-82.
38. Su JB, Hoüel R, Héloire F, Barbe F, Beverelli F, Sambin L, Castaigne A, Berdeaux A, Crozatier B, Hittinger L. Stimulation of Bradykinin $B_1$ Receptors Induces Vasodilation in Conductance and Resistance Coronary Vessels in Conscious Dogs. *Circulation* 2000; 101: 1848 - 1853.
39. Austinat M, Braeuninger S, Pesquero JB, Brede M, Bader M, Stoll G, Renné T, Kleinschnitz C. Blockade of bradykinin receptor B1 but not bradykinin receptor B2 provides protection from cerebral infarction and brain edema. *Stroke* 2009;40:285-93.
40. Ferrari R. Angiotensin-converting enzyme inhibition in cardiovascular disease: evidence with perindopril. *Expert Rev Cardiovasc Ther.* 2005;3:15-29.

# Chapter 12

# Genetic determinants of blood pressure and blood pressure response to ACE-inhibitor therapy in patients with cardiovascular disease: Results of the PERGENE study.

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## ABSTRACT

**Aims:** To investigate whether genetic variation in the renin-angiotensin-aldosterone system (RAAS) and kallikrein-bradykinin pathways are related to blood pressure (BP) and BP-response to ACE-inhibitor therapy in stable coronary artery disease (CAD) patients.

**Methods:** In 8907 stable CAD patients from the EUROPA-trial, 52 haplotype-tagging SNP's in 12 candidate genes within the RAAS and kallikrein-bradykinin pathways were investigated for association with hypertension (defined as BP≥160/95 mmHg or use of anti-hypertensives) and BP-response to ACE-inhibitors, during a 4 week run-in period. All analyses were adjusted for age, gender, body mass index and creatinine clearance and further corrected for multiple testing. Significant SNPs were verified in the PROGRESS-trial (n=3571).

**Results:** Hypertension was present in 28.3% of the patients (n=2526); median BP-reduction after perindopril was 10/4 mmHg. Four polymorphisms, located in the ACE (rs4291), angiotensinogen (rs5049) and prorenin receptor (rs2968915; rs5981008) genes were significantly related to hypertension. A cumulative profile demonstrated a stepwise increase in the prevalence of hypertension, mounting to a two-to-three fold increase in both populations (P for trend <0.001). In addition, genetic polymorphisms were identified that significantly modified the BP-reduction by ACE-inhibitor therapy, however, the observed BP-differences were small and did not remain significant after permutation analysis.

**Conclusion:** This large genetic association study identified genetic determinants of hypertension in patients with (cardio-)vascular disease. Genetic variation in the RAAS and kallikrein-bradykinin pathway did not modify the blood pressure response to ACE-inhibitors.

## INTRODUCTION

Hypertension is a global public health problem, affecting more than 20% of the adult population in western societies and one of the leading causes of cardiovascular, cerebrovascular and renal disease [1]. It is a multifactorial disease for which several important environmental factors have been elucidated as well as several genetic factors [1,2].

The renin-angiotensin-aldosterone system (RAAS) is an important regulator of blood pressure [3,4]. An activated RAAS leads to excessive production of angiotensin-II, resulting in vasoconstriction, increased sodium and water retention and elevated BP. The various genes coding for components of the RAAS are likely candidates that may predispose an individual to hypertension. Prior genetic research on the RAAS and hypertension has mainly focused on the ACE I/D polymorphism and the M235T polymorphism in the angiotensinogen (AGT) gene [5-10]. However, the investigation of only one or two polymorphisms within a single gene ignores the well-documented feedback mechanisms within the RAAS and the presence of two angiotensin II receptors (AT1 and AT2) with counteracting effects [4]. A further limitation of most prior studies is their limited sample size. This probably underlies the many inconsistencies in current literature [4-10].

RAAS activity may determine, at least in part, the response to blockers of this system, i.e., the renin inhibitors, the ACE inhibitors and the AT1 receptor antagonists. Clearly, not all patients respond equal to RAAS blockade. For instance, patients with high renin levels are likely to respond more strongly to renin inhibitors than patients with low renin levels [11,12]. Furthermore, since ACE degrades bradykinin, the activity of the kallikrein-bradykinin system is also likely to contribute to the effect of ACE inhibitors [4]. As the levels and functions of these factors are influenced by genetic factors, a relationship between genetics and response to ACE inhibitors may exist, and could have clinical impact.

Currently, pharmacogenetic research in randomized clinical trials of ACE-inhibitors for their BP-lowering effect is scarce [13]. Therefore, we conducted a large-scale pharmacogenetic association analysis in patients of the EUROPA trial randomized to the ACE-inhibitor perindopril versus placebo [14,15]. To ensure comprehensive coverage of genetic variation in the related hormonal systems, a haplotype-tagging approach was used to select single nucleotide polymorphisms (SNP's) in multiple RAAS and kallikrein-bradykinin system genes together [15]. As hypertension is a strong intermediate phenotype of (cardio)-vascular disease, we investigated two issues: whether genetic variation in the cascade of RAAS / bradykinin system genes 1) is related to the level of blood pressure and 2) determines the BP-response to ACE-inhibitor therapy. In the future, such information could be used to tailor ACE-inhibitor therapy, i.e., to select the patient that may respond well to ACE-inhibition.

## METHODS

### Study population and design

The PERindopril GENEtic association study (PERGENE) is a sub-study of the EUROPA-trial; the designs of both studies were previously described in detail [15,16]. In short, the EUROPA-trial randomized 12.218 stable CAD patients to perindopril (8 mg/day) or placebo. During the 4 week run-in period, all patients were treated with perindopril after which randomization started. Written informed consent was obtained from all patients for performing genetic association analyses. Data from the PROGRESS study were used to verify genetic associations observed in PERGENE. In brief, the PROGRESS trial is a randomized, double-blind, placebo controlled clinical trial of a perindopril based-regimen (perindopril 4 mg / indapamide 2.5 mg) versus placebo in patients with cerebrovascular disease. [17]

### Data collection

A DNA bio-bank was established within the EUROPA trial [18]. In total, 10060 blood samples from EUROPA participants were received at the central laboratory; DNA from 9454 patients was successfully isolated using an automated isolation process (Hamilton liquid handler coupled with Magnetic separator for automated DNA extraction, Nevada, USA).



#### *Candidate genes and selection of tagging-SNP's*

Candidate genes were selected from genes in the RAAS and kallikrein-bradykinin systems. The list of candidate genes ensured extensive coverage of relevant genetic targets in both pharmacodynamic pathways affected by ACE-inhibitors (supplementary table 1). We selected the genes encoding for renin, the (pro)renin receptor, angiotensinogen, angiotensin-converting enzyme, angiotensin-II receptor type 1 and 2, aldosterone synthase, endothelial nitric oxide synthase, kininogen, kallikrein, and bradykinin type 1 and 2 receptor. To cover common variation in these candidate genes, linkage disequilibrium (LD) structure was estimated in Haploview using the data in the HapMap (http://www.hapmap.org) and SeattleSNPs (http://pga.mbt.washington.edu) databases [19]. SNPs were then selected utilizing an accepted haplotype-tagging method [20]. We have applied the following criteria in the initial selection using HapMap: minor allele frequency 0.05; $r^2$ 0.80; haplotype frequency 1% with the aim to cover at least 90% of the common variation within all candidate genes (HapMap Release 23a/Phase II Mar 08/on NCBI B36 assembly/ DbSNP b126). Functionality, and the location of SNPs in a regulatory or promoter region of the gene, was also taken into consideration. Further details of this methodology can be found elsewhere [15]. Based on our dataset, several SNPs were in stronger LD than suggested by HapMap, and we defined our own final set of tagging SNPs by excluding SNPs with pairwise $r^2 > 0.95$.

*Genotyping*

Taqman allelic discrimination assays (Applied Biosystems, Foster City, CA, USA) and Sequenom (San Diego, CA, USA) mass-spectrometric genotyping were used to genotype the selected SNPs, according to the manufacturer's protocols. The assays, primers, and probes for these assays are readily available from the Assay-by-Design service (www.appliedbio-systems.com) or can be requested from Sequenom for all mentioned rs-numbers (supplementary table 1). Quality control for the accuracy of genotyping involved testing duplicates from a randomly selected group of samples (5%) for concordance between samples (>99%). Individual SNP call rates ranged between 95% and 98%. To ensure DNA quality, only patients who were successfully genotyped for more than 90% of the 52 SNPs were included in the analyses (n=8907). Taqman allelic discrimination assays of the PROGRESS samples (n=3571) were performed in the same laboratory using identical methodology.

## Statistical analysis

We tested whether genotypes and allele frequencies were distributed according to Hardy-Weinberg equilibrium (HWE) using a $\chi^2$ test. Hypertension was defined per original study protocol of EUROPA as blood pressure ≥160 mmHg systolic or 95 mmHg diastolic or the use of anti-hypertensives at baseline (screening visit 1). This will be referred to as "hypertension" throughout the manuscript while it refers to stage 2 (moderate-to-severe hypertension) according to JCN-7 criteria [21]. The association of the polymorphisms and hypertension was analyzed using logistic regression analysis adjusted for age, gender, body mass index and creatinine clearance (multivariate model). The BP-reduction by ACE-inhibitor therapy was calculated as the BP difference measured at randomization (after 4 weeks of treatment with perindopril in all patients) minus BP at screening visit 1 (before treatment). BP-reduction was analyzed using z-scores adjusted for age and gender.

By performing 52 tests, 2.6 SNP's could be significant due to mere chance. However, most likely, the SNPs and genes within the RAAS are not completely independent and the actual number of chance findings will be lower. In our study, we present uncorrected, empirical P-values and in addition, we present multivariate adjusted p-values which were further corrected for multiple testing by Monte Carlo permutation analysis (n = 10,000 permutations) on a per gene basis [22]. As we corrected for the number of tagging-SNP's (tests) within each gene, the expected number of false positive "chance" findings is calculated as 12 * 0.05 = 0.6 SNP's in our overall study.

Haplotype analyses were performed for all genes. Haplotypes were inferred using the estimation-maximization algorithm implemented in haplo.stats [23]. The associations between the estimated haplotypes and hypertension, taking into account the posterior probabilities of the haplotype estimates, were assessed with the GLM function in haplo.stats. Global p-values were estimated using haplo.score function. The same models used in the logistic regression

analysis were also utilized in the haplotype analysis (adjustment for age, gender, body mass index and creatinine clearance).

All genetic polymorphisms significantly associated with hypertension and delta blood pressure during the run-in period (permuted p-value <0.05) in PERGENE were tested on the corresponding endpoint in the Caucasian subjects (n=3571) of PROGRESS (58.4% of total study population). Results from the two studies on hypertension were combined in a meta-analysis using the RevMan software v5.0 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2008). As the sample size of the replication cohort was smaller as compared to EUROPA, which limits statistical power to reach significance, we tested the SNPs for significance in PROGRESS, and additionally, used as confirmation of the initial findings in EUROPA the concordance of the direction and magnitude of the effect for the SNP's in PROGRESS. As a main criteria of confirmation of our initial findings in PERGENE we tested whether the combined p-values improved by adding the PROGRESS data (as compared to the initial p-value observed in EUROPA), weighted for standard error.

We tested the cumulative effect of these identified SNPs by counting the number of unfavorable genotypes (associated with hypertension) present. Using this cumulative profile, we assessed the prevalence of hypertension according to the number of unfavorable genotypes of identified SNPs as a trend in both cohorts. All analyses were conducted using R-software. Analyses were based on intention-to-treat principle. A p-value of <0.05 (two-sided) was considered significant.



## RESULTS

Baseline characteristics of the study population are presented in table 1. Hypertension was present in 28.3% (n=2526) patients of the PERGENE study. Information on SNP's and genotype frequencies is presented in supplementary table 1. All genetic variants were in HWE.

### Hypertension

In unadjusted analysis, that was not corrected for multiple testing 11 polymorphisms were significantly related to hypertension. In multivariate analysis 9 polymorphisms, and after the correction for multiple testing by permutation analysis, 7 polymorphisms remained significantly associated with an increased prevalence of hypertension in PERGENE (supplementary tables 1a, 1b). The individual results for these SNP's, which were located in the ACE (rs4291), angiotensinogen (AGT) (rs5049, rs5051, rs699 and rs943580) and (pro)renin-receptor genes (ATP6A2) (rs2968915, rs5981008), are presented in table 2. No further associations were observed for the other genes (supplementary tables 1a,b).

Stratified analysis (86.7% males, 14.3% females) according to gender revealed no relevant new associations other than presented above. However, the effect of the (pro)renin receptor

**Table 1** Baseline characteristics of the PERGENE study population (n=8907).

| Characteristics | |
|---|---|
| Age, years | 59.9 (9.3) |
| Gender, % female | 14.5 |
| Hypertension, % | 28.5 |
| Diabetes, % | 12.7 |
| Hypercholesterolemia, % | 62.8 |
| Smoking, % | 14.8 |
| Body mass index, >30 kg/m2 | 21.3 |
| Symptomatic CAD, % | 25.3 |
| Family history of CAD, % | 27.2 |
| Prior myocardial infarction, % | 65.0 |
| Prior revascularization, % | 29.3 |
| Prior CVA or PVD, % | 29.1 |
| Medication use | |
| Platelet-inhibitors, % | 92.2 |
| Beta-blockers, % | 63.2 |
| Lipid-lowering agents, % | 55.3 |
| Calcium antagonists, % | 31.7 |
| Systolic blood pressure, mmHg | 136.9 (15.2) |
| Diastolic blood pressure, mmHg | 81.8 (8.2) |
| Total cholesterol, mg/dl | 5.4 (1.1) |
| Creatinine clearance, umol/l | 86.5 (25.7) |
| Randomization, perindopril, % | 49.9 |

Summary statistics for continuous variables are presented as mean (standard deviation).
Categorical data are summarized as percentage.

gene polymorphisms (located on the X-chromosome) was limited to males (rs2968915: males OR 1.14 (95% CI 1.05-1.22); females OR 1.01 (95% CI 0.79-1.29) and rs5981008 males OR 1.12 (95% CI 1.03-1.22); females OR 0.99 (95% CI 0.77-1.27)).

Haplotype analysis of the ACE, AGT and (pro)renin receptor genes reinforced the associations between the individual SNP's and hypertension, as presented in table 3. Carriers of the haplotypes containing the unfavorable alleles of the associated SNPs had a higher prevalence of hypertension (Global haplotype p-values per gene: ACE = 0.009; AGT = 0.0004; ATP6A2 = 0.03, respectively). No further associations were observed for the other genes.

## Verification analysis in PROGRESS

In PROGRESS, hypertension was present in 2197 subjects (61.4 %). All replicated SNP's were in HWE and occurred with similar allele frequencies as compared to PERGENE. The results of the 7 individual SNPs in PROGRESS are presented in table 4. In the AGT gene, rs5049 replicated significantly (OR 1.15 (95% CI 1.00-1.32) P-value 0.04) and in the ATP6A2 gene, rs2968916 replicated borderline significantly (OR 1.13 (95% CI 0.99-1.30) P-value 0.06) (table 4). The meta-analysis of the associations with hypertension from the two populations (PERGENE and PROGRESS) improved the initially observed p-values (and narrowed confidence intervals) for 4

**Table 2** Genetic determinants of moderate to severe hypertension in the renin-angiotensin-system

| Gene | rs-number | Location | Allele | Genotypes (%) | | | Hypertension (OR 95% CI) | | | Trend | | Pemp | Pperm |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1/1 | 1/2 | 2/2 | 1/1 | 1/2 | 2/2 | OR | 95% CI | | |
| **Angiotensin-converting enzyme** | rs4291 | Promoter | A > T | 37.0 | 47.6 | 15.4 | 1.00 | 1.08 (0.98-1.20) | 1.28 (1.11-1.47) | 1.12 | (1.05-1.20) | 0.002 | 0.005 |
| | rs4293 | Intron | T > C | 30.1 | 49.3 | 20.6 | 1.00 | 1.04 (0.93-1.17) | 1.15 (1.01-1.32) | 1.07 | (1.00-1.15) | 0.039 | 0.18 |
| | rs4309 | Exon | C > T | 32.9 | 47.8 | 19.3 | 1.00 | 0.94 (0.84-1.04) | 0.89 (0.77-1.01) | 0.94 | (0.88-1.00) | 0.067 | 0.30 |
| | rs4311 | Intron | C > T | 27.0 | 49.5 | 23.4 | 1.00 | 1.05 (0.94-1.18) | 1.17 (1.03-1.34) | 1.08 | (1.01-1.16) | 0.019 | 0.10 |
| | rs4343 | Exon | G > A | 28.3 | 49.3 | 22.5 | 1.00 | 0.96 (0.85-1.06) | 0.91 (0.79-1.03) | 0.95 | (0.89-1.01) | 0.124 | 0.49 |
| **Angiotensinogen** | | | | | | | | | | | | | |
| | rs5049 | Promoter | G > A | 77.2 | 21.2 | 1.6 | 1.00 | 1.19 (1.06-1.33) | 1.14 (0.78-1.65) | 1.16 | (1.05-1.28) | 0.004 | 0.03 |
| | rs5051 | Promoter | C > T | 30.6 | 49.1 | 20.3 | 1.00 | 1.12 (1.00-1.25) | 1.25 (1.09-1.44) | 1.11 | (1.04-1.19) | 0.002 | 0.01 |
| | rs4762 | Exon | G > A | 74.2 | 23.9 | 1.9 | 1.00 | 1.12 (1.01-1.26) | 1.48 (1.07-2.05) | 1.14 | (1.04-1.25) | 0.007 | 0.06 |
| | rs699 | Exon | G > A | 30.2 | 49.4 | 20.4 | 1.00 | 1.08 (0.97-1.20) | 1.25 (1.09-1.43) | 1.11 | (1.04-1.18) | 0.003 | 0.02 |
| | rs2478545 | Intron | G > A | 56.4 | 37.8 | 5.8 | 1.00 | 1.05 (0.95-1.16) | 1.20 (0.98-1.47) | 1.07 | (0.99-1.15) | 0.095 | 0.56 |
| | rs7079 | 3' UTR | T > G | 45.9 | 43.6 | 10.5 | 1.00 | 1.00 (0.90-1.10) | 1.00 (0.85-1.17) | 1.00 | (0.93-1.07) | 0.981 | 1.00 |
| | rs10864770 | 3' UTR | G > A | 86.3 | 13.0 | 0.6 | 1.00 | 1.02 (0.89-1.18) | 0.52 (0.26-1.03) | 0.95 | (0.84-1.08) | 0.469 | 0.99 |
| | rs943580 | 3' UTR | G > A | 30.6 | 49.2 | 20.2 | 1.00 | 1.12 (1.01-1.26) | 1.30 (1.14-1.49) | 1.13 | (1.06-1.21) | <0.001 | 0.001 |
| **(Pro)renin receptor** | | | | | | | | | | | | | |
| | rs2968915 | Promoter | A > G | 88.2 | 11.8 | -- | 1.00 | 1.22 (1.06-1.40) | -- | 1.13 | (1.04-1.22) | 0.002 | 0.0046 |
| | rs5918008 | Intron | A > C | 89.0 | 11.0 | -- | 1.00 | 1.17 (1.01-1.35) | -- | 1.10 | (1.02-1.19) | 0.019 | 0.04 |

Logistic regression analysis adjusted for age, gender, body mass index and creatinine clearance. Univariate analyses data not shown. Empirical and permutation p-values based on 10,000 permutations. Pemp = multivariate adjusted empirical p-value. Pperm = multivariate adjusted permutated p-value (including correction for multiple testing). $R^2$ 0.95 rs5051 and rs699; $R^2$ 0.86 rs5051 and rs943580, r2 based on our study sample.

**Table 3.** Associations between haplotypes and moderate to severe hypertension

| Gene | Haplotype | Frequency | Hypertension | $P_{int}$ | Global P |
|---|---|---|---|---|---|
| | | | OR 95%CI | | |
| **Angiotensin Converting Enzyme** | 1 1 2 1 2 | 41.1 % | Reference | --- | 0.009 |
| | 2 2 1 2 1 | 37.6 % | 1.12 (1.04-1.21) | 0.002 | |
| | 1 1 1 2 1 | 8.1 % | 0.95 (0.83-1.08) | 0.42 | |
| | 1 2 1 1 1 | 4.9 % | 0.89 (0.76-1.05) | 0.18 | |
| | 1 1 1 1 2 | 3.4 % | 1.16 (0.97-1.39) | 0.11 | |
| | 1 1 2 2 1 | 1.3 % | 0.93 (0.68-1.27) | 0.64 | |
| | 1 2 1 1 2 | 0.7 % | 0.90 (0.58-1.38) | 0.62 | |
| | 2 2 1 1 2 | 0.6 % | 0.72 (0.44-1.19) | 0.40 | |
| **Angiotensinogen** | | | | | |
| | 1 1 1 1 1 2 1 1 | 30.3 % | Reference | --- | 0.0004 |
| | 1 1 1 1 1 1 1 1 | 22.4 % | 0.86 (0.79-0.95) | 0.002 | |
| | 1 2 2 2 2 1 1 2 | 12.9 % | 1.12 (1.01-1.25) | 0.034 | |
| | 2 2 1 2 1 1 1 2 | 12.1 % | 1.11 (0.99-1.24) | 0.06 | |
| | 1 2 1 2 2 1 1 2 | 9.9 % | 0.97 (0.86-1.10) | 0.62 | |
| | 1 2 1 2 1 1 2 2 | 7.0 % | 0.94 (0.82-1.09) | 0.43 | |
| | 1 1 1 1 1 1 1 2 | 1.0 % | 1.01 (0.72-1.61) | 0.94 | |
| | 1 2 1 1 1 2 1 1 | 0.8 % | 1.15 (0.79-1.68) | 0.46 | |
| | 1 2 1 2 1 1 1 2 | 0.7 % | 1.08 (0.73-1.61) | 0.70 | |
| | 1 2 2 2 2 2 1 1 | 0.7 % | 0.61 (0.39-0.97) | 0.04 | |
| | 1 1 1 2 2 1 1 2 | 0.7 % | 1.07 (0.71-1.61) | 0.73 | |
| **(Pro)renin receptor** | | | | | |
| | 1 1 | 88.8 % | Reference | --- | 0.028 |
| | 2 2 | 8.9 % | 1.12 (1.03-1.22) | 0.008 | |
| | 2 1 | 1.6 % | 1.14 (0.94-1.37) | 0.18 | |
| | 1 2 | 0.8 % | 0.92 (0.68-1.23) | 0.55 | |

Logistic regression analysis adjusted for age, gender, body mass index and creatinine clearance. Haplotype allele combinations are based upon the order of the SNPs within the gene as presented in supplementary table 1 for each gene.

of the 7 individual SNP's as presented in table 4 and figure 1. The combined odds ratios were OR 1.10 95% CI 1.04-1.17 (p-value 0.001); OR 1.16 95% CI 1.07-1.25 (p-value 0.002); OR 1.13 95% CI 1.06-1.21 (p-value 0.001); and OR 1.10 95% CI 1.03-1.17 (p-value 0.001), for rs4291, rs5049, rs2968915 and rs5981008, respectively. For these 4 SNPs, the risk estimates in PROGRESS (n=3571) were in same direction and of similar magnitude as those from PERGENE. The remaining, non-replicating 3 SNPs (rs699, rs5051 and rs943580) were all located in the AGT gene. Their genotype frequencies were virtually identical (Table 2) and there was high linking disequilibrium between these SNPs ($r^2$ >0.85 for all 3 SNP's).

## Cumulative effect of identified risk markers

Combining the unfavorable alleles of the 4 SNPs (rs4291; rs5049; rs2968915; rs5981008) resulted in a stepwise increase in the prevalence of hypertension in both populations. As compared to the reference category (no unfavorable alleles), each additional unfavorable allele

**Table 4** Meta-analyses of identified SNP's and effect on moderate to severe hypertension

| GENE | PERGENE (n=8907) | | PROGRESS (n=3571) | | Combined meta-analysis | |
|---|---|---|---|---|---|---|
| | OR 95% CI | P | OR 95% CI | P | OR 95% CI | P |
| **ACE** | | | | | | |
| Rs4291 | 1.12 (1.05-1.20) | 0.002 | 1.06 (0.96-1.18) | 0.286 | 1.10 (1.04-1.17) | 0.0006 |
| **AGT** | | | | | | |
| Rs5049 | 1.16 (1.05-1.28) | 0.004 | 1.15 (1.00-1.32) | 0.045 | 1.15 (1.06-1.25) | 0.0006 |
| Rs5051 | 1.11 (1.04-1.19) | 0.002 | 0.98 (0.87-1.08) | 0.570 | 1.04 (0.92-1.19) | Ns |
| Rs699 | 1.11 (1.04-1.18) | 0.003 | 0.99 (0.89-1.10) | 0.802 | 1.06 (0.95-1.18) | Ns |
| Rs943580 | 1.13 (1.06-1.21) | <0.001 | 0.96 (0.87-1.07) | 0.502 | 1.05 (0.89-1.23) | Ns |
| **ATP6A2** | | | | | | |
| Rs2968915 | 1.13 (1.04-1.23) | 0.002 | 1.13 (0.99-1.30) | 0.062 | 1.13 (1.05-1.21) | 0.0005 |
| Rs5981008 | 1.10 (1.02-1.19) | 0.019 | 1.08 (0.94-1.25) | 0.289 | 1.10 (1.02-1.18) | 0.009 |

Logistic regression analysis adjusted for age, gender, body mass index, and creatinine clearance. Meta-analyses were conducted using the RevMan 5.0.



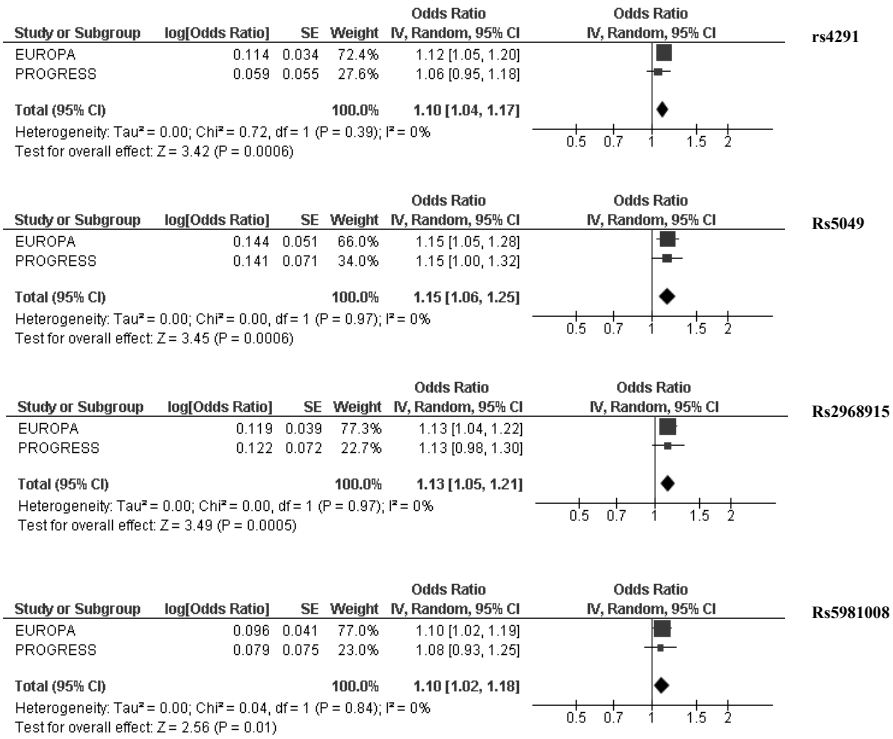


**Figure 1** Meta-analysis of identified SNP's and moderate to severe hypertension. Overall combined p-values: rs4291 (ACE) p = 0.0006 ; rs5049 (AGT) p = 0.0006 ; rs2968915 (ATP6A2) p = 0.0005 and rs5981008 p = 0.009 after adding the PROGRESS data in meta-analysis.

was associated with a 7-8% increase in the prevalence of hypertension (OR 95% CI for trend in PERGENE 1.08 (1.05-1.12) P<0.01) and in PROGRESS 1.07 (1.02-1.14) P<0.01). The cumulative effect of the 4 SNPs is presented in figure 2. Adding the 3 nonsignifant SNPs to this analysis did not alter the ORs (1.07 (95% CI 1.02 – 1.11; P<0.01) in PERGENE and 1.05 (95% CI 1.00-1.11; P 0.03) in PROGRESS).

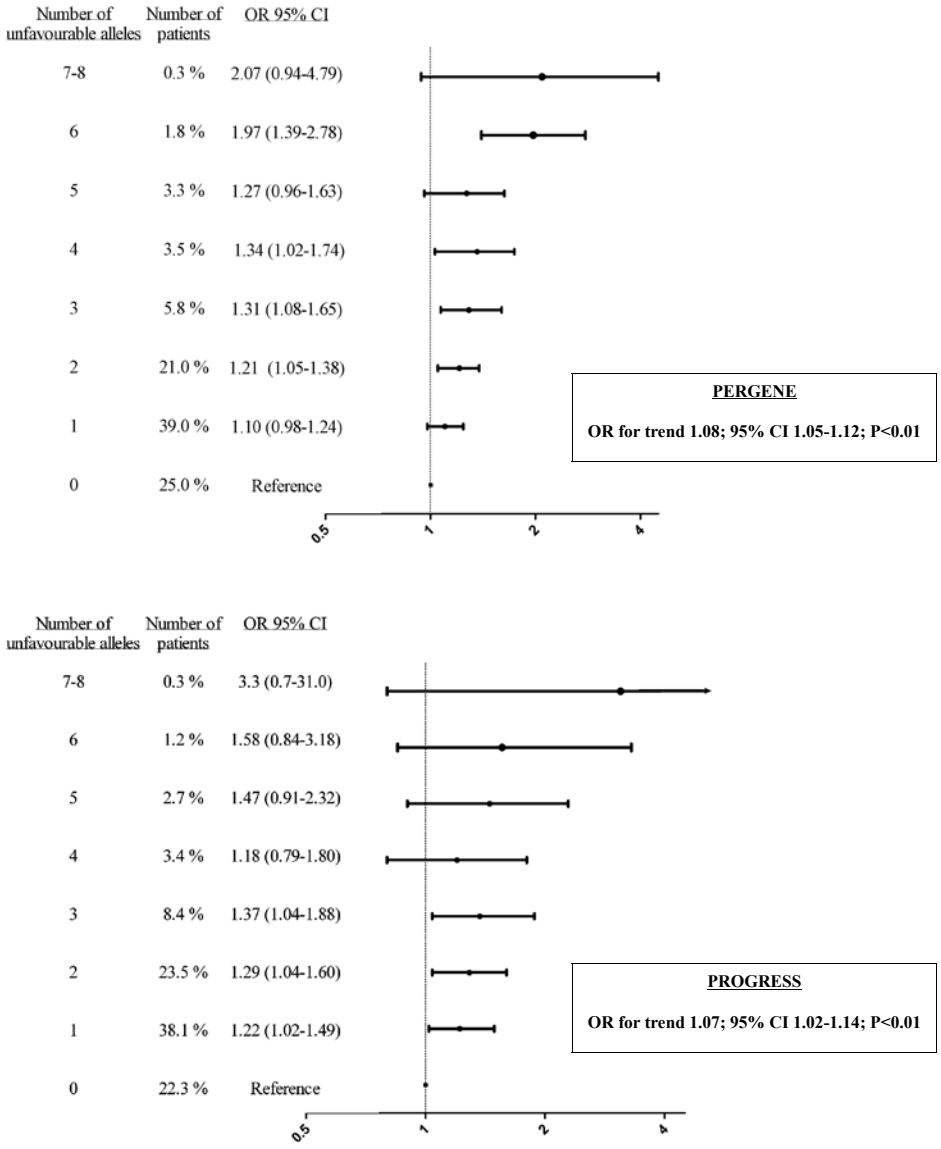


**Figure 2** Cumulative effects of the identified SNPs on the prevalence of moderate to severe hypertension. For the complete SNP profile, OR trend was 1.07 (95% CI 1.02 – 1.11; P<0.01) in EUROPA and OR trend 1.05 (95% CI 1.00-1.11; P 0.03) in PROGRESS. $R^2$ 0.95 rs5051 and rs699; $r^2$ rs5051 and rs943580 $r^2$ 0.86

## Blood pressure reduction

In PERGENE, median systolic and diastolic BP reduction during the run-in period was 10 / 4 mmHg (mean SBP reduction 8.6 (SD 14.6), mean DBP reduction 4.0 (SD 8.6). Several polymorphisms significantly modified the BP reduction by ACE-inhibitor therapy, either in terms of systolic or diastolic BP (table 5a, 5b respectively). However, the differences across genotypes were only modest (1-2 mmHg) and did not remain significant after permutation analysis. Haplotype analysis revealed no significant results for BP changes (P >0.05, for all genes) and no further verification was sought in the PROGRESS.

**Table 5a.** Genetic determinants of systolic blood pressure response to ACE-inhibitor therapy.

| Gene | SNP | Genotype frequencies (%) | Alleles 1 / 1 | 1 / 2 | 2 /2 | Empirical P-value* | Permuted P-value |
|---|---|---|---|---|---|---|---|
| AGTR1 | Rs275651 | 67.4 / 29.4 / 3.3 | 9.0 | 10.0 | 8.5 | 0.01 | 0.08 |
| AGTR1 | Rs931490 | 66.6 / 30.0 / 3.4 | 8.5 | 10.0 | 8.0 | 0.03 | 0.15 |
| BDKRB1 | Rs4905475 | 81.2 / 17.7 / 1.1 | 9.0 | 10.0 | 10.0 | 0.03 | 0.11 |
| BDKRB2 | Rs5225 | 79.5 / 19.1 / 1.4 | 9.0 | 10.0 | 10.0 | 0.04 | 0.14 |

Overall systolic blood pressure reduction by ACE-inhibitor therapy (perindopril) during the 4 weerk run-in period was: 10 mmHg. * z-score adjusted for age and gender.

**Table 5b.** Genetic determinants of diastolic blood pressure response to ACE-inhibitor therapy.

| Gene | SNP | Genotype frequencies (%) | Alleles 1 / 1 | 1 / 2 | 2 /2 | Empirical P-value* | Permuted P-value |
|---|---|---|---|---|---|---|---|
| AGT | Rs5051 | 30.7 / 49.0 / 20.3 | 5.0 | 4.0 | 2.5 | 0.03 | 0.23 |
| AGT | Rs699 | 30.3 / 49.4 / 20.3 | 5.0 | 4.0 | 2.5 | 0.04 | 0.28 |
| AGT | Rs10864770 | 86.3 / 13.0 / 0.6 | 4.0 | 4.0 | 0 * | 0.02 | 0.14 |
| AGT | Rs943580 | 30.7 / 49.2 / 20.1 | 5.0 | 4.0 | 2.5 | 0.05 | 0.32 |
| AGTR1 | Rs931490 | 66.6 / 30.0 / 3.4 | 3.5 | 5.0 | 4.0 | 0.03 | 0.19 |

Overall diastolic blood pressure reduction by ACE-inhibitor therapy (perindopril) during the 4 week run-in period was: 4 mmHg. * z-score adjusted for age and gender.



## DISCUSSION

The current pharmacogenetic study is the first to comprehensively investigate a large combination of genes within a common pathway, the renin-angiotensin-aldosterone and kallikrein-bradykinin systems, with the aim to elucidate candidate genes that determine baseline BP and/or the BP-response to ACE-inhibitor therapy in two large patient populations: 8907 patients with stable coronary artery disease and 3571 patients with cerebrovascular disease (totalling 12.478 patients). Our approach was meant to overcome the limitations of prior studies related to sample size, and the limited number of genetic targets studied within the complex cascade of counter-regulatory RAAS hormones. While we found several important genetic determinants of hypertension in the direct pharmacodynamic pathway of ACE-inhibitors in both populations,

which could be combined in an accumulating risk score, we found no genetic determinants of BP-response to perindopril therapy.

The most important genetic determinants of hypertension in our study were: rs4291 in the ACE gene (promoter region), rs5049 in the AGT gene (promoter region), and rs2968915 and rs5981008 in the (pro)renin receptor gene (exon, and intron, respectively). Polymorphisms within the ACE and AGT genes have been described before to be individually associated with BP, although several other studies were negative [4-9]. In our analysis particularly the promoter polymorphisms within the ACE and AGT genes were related to hypertension as opposed to the previously described polymorphisms. The promoter region of a gene determines its transcriptional activity. The strong relation between the (pro)renin-receptor gene and hypertension in vascular disease patients is a novel genetic target for hypertension research and our study is the first to demonstrate the combined effect of the identified SNPs.

SNP rs4291 in the ACE gene was related to 8 and 28% increases in the prevalence of hypertension in CAD patients with one or both minor allele(s), respectively (P for trend <0.001). The rs4291 polymorphisms is not frequently studied, but was an important haplotype tagging SNP in our study. The functional consequences of this polymorphism are currently unknown but could, due to its location in the promoter region, involve changes in ACE expression. Interestingly, rs4343, which was included in our study as a direct proxy of the ACE I/D polymorphism (high LD: $D^1$ 1.0; r2 0.9) [24], did not show any association with hypertension. This is most likely due to the fact that the effects of the ACE I/D polymorphism on ACE levels are relatively modest [25]. Such modest effects of one or more ACE SNPs, in combination with a limited sample size, might underlie the conflicting results in the literature with regard to the ACE gene [9]. In fact, although the effect of rs4291 was identical in the PROGRESS trial patients, it did not reach complete significance in that population. Since the PROGRESS population is 2-3 times smaller, this may have been a matter of power. Nevertheless, this implies that even the relation of rs4291 with hypertension in the 8907 patients of PERGENE needs to be interpreted with care.

The AGT gene appeared to be an important factor in the etiology of hypertension in CAD patients, as evidenced by the significant effect of 4 AGT SNPs in PERGENE. Yet, only one of these SNPs (rs5049) replicated significantly in PROGRESS. The rs5049 polymorphism is again located in the promoter region and associates with the plasma levels of AGT [8]. This is a logical explanation for its BP effect, assuming at least that the higher AGT levels are not counterbalanced by decreased renin levels [24]. Two more widely studied polymorphism in the AGT gene, rs699 (M235T) and rs5051 has also been associated with plasma AGT and hypertension [4-10]. Nevertheless, although the PERGENE study supported these observations, the PROGRESS data did not. Again, this could be a matter of power. Alternatively, BP regulation in patients with cerebrovascular disease may differ from patients with CAD or the promoter region of the AGT gene is more important than frequently assumed which is a novel finding.

Finally, our data suggest a novel association between the (pro)renin receptor gene and BP in Caucasians, both in CAD population as in patients with cerebrovascular disease. One previous

study in a Japanese population cohort also reported this association, albeit in men only [27]. The (pro)renin receptor is a recently discovered component of the RAAS [28,29]. It binds both renin and its inactive precursor, prorenin, and allows the latter to display enzymatic activity [30]. Prorenin is believed to be particularly important for angiotensin production at tissue sites [31], and the stronger effect of renin inhibitors on renal plasma flow as compared to other RAAS blockers may relate to their interference with prorenin-dependent tissue angiotensin production [32]. (Pro)renin receptors downregulate during RAAS blockade [33], and the current study now suggests that these receptors affect blood pressure, possibly via the above mechanism. Future studies should investigate the functional role of the 2 polymorphisms within the (pro) renin receptor gene that associate with hypertension.

Unexpectedly, no role for any of the kallikrein-bradykinin system genes was observed, suggesting that the contribution of bradykinin to BP regulation (and the BP response to ACE-inhibition), at least in this population, is modest or absent. This may of course be different in other patient groups, e.g. younger patients [34].

The cumulative effect of the various RAAS gene polymorphisms (Figure 2) is in agreement with the concept of an angiotensin-generating cascade involving the (pro)renin receptor, angiotensinogen and ACE. Clearly these 3 genetic components are major determinants of the activity of the system and our study is the first to demonstrate the cumulative effect of various RAAS components by combining the relevant genetic targets within these pathways.



The second endpoint of our study was the relation between genetic variation in RAAS and BP response to ACE-inhibitors. A large inter-individual variability in the BP response to ACE-inhibitors exists (as reflected in the standard deviations), which could be related to genetic differences between patients. Genetic factors that modify the response to ACE-inhibitors could potentially be used to target ACE-inhibitors to those patients most likely to benefit of such therapy. In fact, we identified several genetic polymorphisms, mainly in the ACE, AGT and AT1 receptor genes, that were related to a significantly different BP response to perindopril in PERGENE. However, the absolute differences (mmHg) were small and their clinical usefulness is therefore probably limited. Also, these SNP's did not remain significant after correction for multiple testing, in contrast to the hypertension results.

Pharmacogenetic data on the BP-effect of ACE-inhibitors in randomized placebo-controlled clinical trials are very scarce. So far, only 2 studies investigated the BP response in relationship with the ACE I/D polymorphism and found no association either [13,35]. No other genetic studies of the BP response to ACE-inhibitor therapy in a large randomized placebo-controlled setting exist to our knowledge. Considering our findings, genetic variation in the RAAS and kallikrein-bradykinin system genes is not likely to determine the large inter-individual differences in BP response. Thus, according to the current results, in the future, hypertensive patients are unlikely to be selected for BP response to the ACE-inhibitor treatment on the basis of their RAAS and kallikrein-bradykinin system genetic profile. In other studies, the level of BP reduction by ACE-inhibitor therapy was not related to treatment benefit [36].

Strengths of the current study are the number of patients, design (particularly randomization), extensive phenotypic data, and the comprehensive coverage of multiple genes in a common pathway. In both populations, the principal genetic influence on hypertension by the RAAS is through the ACE, AGT and prorenin genes. The prorenin receptor and its role in hypertension is a novel finding in Caucasians. However, several limitations should be noted. The study mainly consisted of male patients, predominantly of European Caucasian descent (>99%), with stable CAD. These factors may limit the extent to which these findings can be generalized, but they also reduce the chance of confounding due to population stratification. Accordingly, the statistical power in female subjects is limited. Furthermore, in both studies, blood pressure or blood pressure reduction were no primary outcome measures. BP response curves in EUROPA was 5 / 2 mmHg and in PROGRESS 5 / 3 mmHg during follow-up [14,17]. The BP cut-off of 160/95, by study protocol, refers to stage 2 of the JCN-7 criteria for hypertension [21]. Changing the definition of hypertension retrospectively would have introduced bias, and therefore we kept to the original definition. Additionally, multiple testing is an issue due to the possibility of chance findings. Correction is, therefore, necessary and we chose for a gene-based permutation analysis as we selected our candidate genes in the pharmacodynamic pathways of ACE-inhibitors based on our a priori study hypothesis and selected tagging-SNP's which are not entirely independent. Applying more conservative correction (i.e., Bonferroni) would be overly conservative due to the strength of the a priori study hypothesis and might lead to a failure to notice real existing differences. The initial analysis revealed 11 significant SNPs out of 52 SNPs. After multiple testing corrections, we identified 7 significant findings in PERGENE, which was much higher than the expected rate of a chance finding. A replication cohort of similar size, patients and design as EUROPA does not exist. For replication of our findings of hypertension as an intermediate phenotype in patients with cardiovascular disease, we could use data from the PROGRESS-trial which studied the same agent in a population of patient with cerebrovascular disease. Unfortunately, a replication cohort of healthy individuals was not available which would additionally require an even larger population sample to allow a meaningful statistical evaluation. We investigated the 3571 Caucasian subjects of PROGRESS, to ensure comparability with the EUROPA-trial subjects. Only the rs5049 (AGT) and rs2968915 (ATP6A2) replicated (borderline) significantly, probably related to the lower minor allele frequency and 2-3 times smaller sample size of PROGRESS. Nevertheless, a similar direction and magnitude of the interaction effect was observed for 4 SNP's and combined meta-analysis improved the initial p-values for the 4 SNPs. The combined profile of the identified SNP's supports the cumulative effect of various combinations of genetic determinants and hypertension in both populations. This strong concordance lends additional weight to our findings.

In conclusion, although genetic variation in the RAAS and kallikrein-bradykinin pathway does determine baseline BP, it does not modify the blood pressure response to ACE-inhibitors. Future studies should now investigate whether genetic variation, e.g. in pathways beyond the above systems, modifies the response to ACE inhibition.

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*Conflict of interest & financial disclosures:*

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**Supplementary table** 1a.

| Gene | rs-number | | Location | Allele | Genotypes (%) | | | Hypertension (OR 95% CI) | | | Trend | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1/1 | 1/2 | 2/2 | 1/1 | 1/2 | 2/2 | OR | 95% CI | Pemp | Pperm |
| **Angiotensin-converting enzyme** | Rs4291 | Chr 17 | Promoter | A > T | 37.0 | 47.6 | 15.4 | 1.00 | 1.08 (0.98-1.20) | 1.28 (1.11-1.47) | 1.12 | (1.05-1.20) | 0.002 | 0.005 |
| | Rs4293 | Chr 17 | Intron | T > C | 30.1 | 49.3 | 20.6 | 1.00 | 1.04 (0.93-1.17) | 1.15 (1.01-1.32) | 1.07 | (1.00-1.15) | 0.039 | 0.18 |
| | Rs4309 | Chr 17 | Exon | C > T | 32.9 | 47.8 | 19.3 | 1.00 | 0.94 (0.84-1.04) | 0.89 (0.77-1.01) | 0.94 | (0.88-1.00) | 0.067 | 0.30 |
| | Rs4311 | Chr 17 | Intron | C > T | 27.0 | 49.5 | 23.4 | 1.00 | 1.05 (0.94-1.18) | 1.17 (1.03-1.34) | 1.08 | (1.01-1.16) | 0.019 | 0.09 |
| | Rs4343 | Chr 17 | Exon | G > A | 28.3 | 49.3 | 22.5 | 1.00 | 0.96 (0.85-1.06) | 0.91 (0.79-1.03) | 0.95 | (0.89-1.01) | 0.124 | 0.49 |
| **Angiotensinogen** | | | | | | | | | | | | | | |
| | Rs5049 | Chr 1 | Promoter | G > A | 77.2 | 21.2 | 1.6 | 1.00 | 1.19 (1.06-1.33) | 1.14 (0.78-1.65) | 1.16 | (1.05-1.28) | 0.004 | 0.03 |
| | Rs5051 | Chr 1 | Promoter | C > T | 30.6 | 49.1 | 20.3 | 1.00 | 1.12 (1.00-1.25) | 1.25 (1.09-1.44) | 1.11 | (1.04-1.19) | 0.002 | 0.01 |
| | Rs4762 | Chr 1 | Exon | G > A | 74.2 | 23.9 | 1.9 | 1.00 | 1.12 (1.01-1.26) | 1.48 (1.07-2.05) | 1.14 | (1.04-1.25) | 0.007 | 0.055 |
| | Rs699 | Chr 1 | Exon | G > A | 30.2 | 49.4 | 20.4 | 1.00 | 1.08 (0.97-1.20) | 1.25 (1.09-1.43) | 1.11 | (1.04-1.18) | 0.003 | 0.02 |
| | Rs2478545 | Chr 1 | Intron | G > A | 56.4 | 37.8 | 5.8 | 1.00 | 1.05 (0.95-1.16) | 1.20 (0.98-1.47) | 1.07 | (0.99-1.15) | 0.095 | 0.56 |
| | Rs7079 | Chr 1 | 3' UTR | T > G | 45.9 | 43.6 | 10.5 | 1.00 | 1.00 (0.90-1.10) | 1.00 (0.85-1.17) | 1.00 | (0.93-1.07) | 0.981 | 1.00 |
| | Rs10864770 | Chr 1 | 3' UTR | G > A | 86.3 | 13.0 | 0.6 | 1.00 | 1.02 (0.89-1.18) | 0.52 (0.26-1.03) | 0.95 | (0.84-1.08) | 0.469 | 0.99 |
| | Rs943580 | Chr 1 | 3' UTR | G > A | 30.6 | 49.2 | 20.2 | 1.00 | 1.12 (1.01-1.26) | 1.30 (1.14-1.49) | 1.13 | (1.06-1.21) | <0.001 | 0.001 |
| **Angiotensin-II Type 1 receptor** | Rs275651 | Chr 3 | Promoter | A > T | 67.4 | 29.4 | 3.3 | 1.00 | 0.97 90.88-1.08) | 1.08 (0.83-1.40) | 0.99 | (0.91-1.08) | 0.792 | 1.00 |
| | Rs10935724 | Chr 3 | Intron | A > C | 44.3 | 44.2 | 11.5 | 1.00 | 1.08 (0.98-1.190 | 0.96 (0.82-1.13) | 1.01 | (0.94-1.08) | 0.773 | 1.00 |
| | Rs931490 | Chr 3 | Intron | A > G | 66.6 | 30.0 | 3.4 | 1.00 | 1.00 (0.89-1.10) | 1.20 (0.93-1.55) | 1.02 | (0.94-1.11) | 0.638 | 1.00 |
| | Rs4681440 | Chr 3 | Intron | C > T | 68.6 | 28.3 | 3.2 | 1.00 | 1.13 (1.01-1.25) | 0.92 (0.65-1.22) | 1.06 | (0.97-1.16) | 0.171 | 0.68 |
| | Rs5182 | Chr 3 | Exon | C > T | 27.3 | 49.9 | 22.8 | 1.00 | 0.99 (0.90-1.11) | 0.98 (0.86-1.12) | 0.98 | (0.92-1.05) | 0.562 | 0.99 |
| | Rs5186 | Chr 3 | Exon | A > C | 51.9 | 40.6 | 7.5 | 1.00 | 1.01 (0.92-1.12) | 1.14 (0.95-1.36) | 1.06 | (0.98-1.14) | 0.151 | 0.62 |
| **Angiotensin-II Type 2 receptor** | Rs3736556 | Chr X | Intron | A > T | 70.6 | 29.4 | -- | 1.00 | 0.97 (0.87-1.08) | -- | 0.97 | (0.92-1.03) | 0.345 | 0.81 |
| | Rs5193 | Chr X | 3' UTR | G > T | 70.2 | 29.9 | -- | 1.00 | 0.95 (0.85-1.05) | -- | 0.98 | (0.92-1.03) | 0.401 | 0.87 |
| | Rs5194 | Chr X | 3' UTR | G > A | 50.2 | 49.8 | -- | 1.00 | 1.10 (1.00-1.21) | -- | 1.03 | (0.99-1.09) | 0.165 | 0.52 |
| | Rs12840631 | Chr X | 3' UTR | C > G | 95.3 | 4.7 | -- | 1.00 | 1.10 (0.89-1.37) | -- | 1.06 | (0.93-1.19) | 0.383 | 0.86 |

**Supplementary table** 1a. continued

| Gene | rs-number | | Location | Allele | Genotypes (%) | | | Hypertension (OR 95% CI) | | | Trend | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1/1 | 1/2 | 2/2 | 1/1 | 1/2 | 2/2 | OR | 95% CI | Pemp | Pperm |
| **Renin** | | | | | | | | | | | | | | |
| | Rs2887284 | Chr 1 | Intron | C > A | 61.5 | 34.2 | 4.4 | 1.00 | 0.89 (0.80-0.98) | 0.99 (0.79-1.25) | 0.95 | (0.87-1.03) | 0.180 | 0.55 |
| | Rs1464816 | Chr 1 | Intron | G > T | 43.1 | 44.8 | 12.2 | 1.00 | 1.07 (0.97-1.19) | 1.06 (0.91-1.23) | 1.04 | (0.97-1.11) | 0.296 | 0.88 |
| | Rs5707 | Chr 1 | Exon | T > G | 59.6 | 35.5 | 5.0 | 1.00 | 0.96 (0.86-1.06) | 0.90 (0.72-1.13) | 0.95 | (0.88-1.03) | 0.244 | 0.80 |
| | Rs11571082 | Chr 1 | Intron | G > A | 74.8 | 23.4 | 1.8 | 1.00 | 0.95 (0.85-1.06) | 1.09 (0.78-1.55) | 0.98 | (0.89-1.08) | 0.701 | 1.00 |
| | Rs10900555 | Chr 1 | Intron | T > C | 42.3 | 45.1 | 12.6 | 1.00 | 0.98 (0.88-1.08) | 0.91 (0.78-1.06) | 0.96 | (0.90-1.03) | 0.278 | 0.86 |
| | Rs11571078 | Chr 1 | Intron | C > T | 76.3 | 21.9 | 1.8 | 1.00 | 0.92 (0.82-1.04) | 0.96 (0.67-1.38) | 0.94 | (0.85-1.04) | 0.257 | 0.82 |
| **Aldosterone synthase** | Rs11781082 | Chr 8 | Promoter | G > A | 58.9 | 35.9 | 5.2 | 1.00 | 0.94 (0.85-1.04) | 0.88 (0.70-1.09) | 0.94 | (0.86-1.01) | 0.091 | 0.40 |
| | Rs1799998* | Chr 8 | Promoter | T > C | 28.9 | 49.7 | 21.4 | 1.00 | 1.04 (0.94-1.17) | 1.09 (0.95-1.25) | 1.05 | (0.98-1.12) | 0.181 | 0.63 |
| | Rs6433 | Chr 8 | Intron | A > G | 34.7 | 48.8 | 16.5 | 1.00 | 0.84 (0.80-0.98) | 0.94 (0.81-1.08) | 0.95 | (0.89-1.02) | 0.163 | 0.57 |
| | Rs4543 | Chr 8 | Exon | G > A | 82.4 | 16.6 | 1.0 | 1.00 | 1.01 (0.89-1.15) | 0.79 (0.48-1.29) | 0.98 | (0.88-1.10) | 0.728 | 1.00 |
| | Rs3097 | Chr 8 | 3'UTR | G > A | 51.1 | 41.3 | 7.6 | 1.00 | 0.94 (0.85-1.04) | 1.04 (0.87-1.25) | 0.99 | (0.92-1.07) | 0.816 | 1.00 |
| **(Pro)renin receptor** | | | | | | | | | | | | | | |
| | Rs2968915 | Chr X | Promoter | A > G | 88.2 | 11.8 | -- | 1.00 | 1.22 (1.06-1.40) | -- | 1.13 | (1.04-1.22) | 0.002 | 0.0046 |
| | Rs5918008 | Chr X | Intron | A > C | 89.0 | 11.0 | -- | 1.00 | 1.17 (1.01-1.35) | -- | 1.10 | (1.02-1.19) | 0.019 | 0.04 |

Renin-angiotensin-aldosterone pathway genes in relation to moderate to severe hypertension.

**Supplementary table** 1b.

| Gene | rs-number | | Location | Allele | Genotypes (%) | | | Hypertension (OR 95% CI) | | | Trend | Pemp | Pperm |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1/1 | 1/2 | 2/2 | 1/1 | 1/2 | 2/2 | OR 95% CI | | |
| **Kininogen** | | | | | | | | | | | | | |
| | Rs1050274 | Chr 3 | Exon | G > A | 45.6 | 43.5 | 10.8 | 1.00 | 1.00 (0.90-1.10) | 1.02 (0.87-1.19) | 1.00 (0.93-1.07) | 0.954 | 0.999 |
| | Rs1656922 | Chr 3 | Exon | C > T | 28.6 | 50.5 | 20.9 | 1.00 | 1.04 (0.93-1.16) | 1.06 (0.93-1.22) | 1.03 (0.96-1.10) | 0.370 | 0.843 |
| | Rs1469859 | Chr 3 | Exon | G > A | 42.4 | 45.9 | 11.7 | 1.00 | 1.04 (0.94-1.15) | 0.93 (0.80-1.09) | 1.00 (0.93-1.07) | 0.952 | 1.000 |
| | Rs1621816 | Chr 3 | Intron | A > G | 51.4 | 40.7 | 7.9 | 1.00 | 0.98 (0.88-1.08) | 0.98 (0.82-1.17) | 0.97 (0.91-1.05) | 0.490 | 0.935 |
| **Kallikrein** | | | | | | | | | | | | | |
| | Rs5517 | Chr 19 | Exon | A > G | 53.9 | 38.8 | 7.3 | 1.00 | 1.04 (0.94-1.14) | 1.18 (0.99-1.42) | 1.07 (0.99-1.15) | 0.088 | 0.164 |
| | Rs1054713 | Chr 19 | Exon | C > T | 43.7 | 44.6 | 11.7 | 1.00 | 1.07 (0.96-1.18) | 1.02 (0.87-1.19) | 1.03 (0.96-1.11) | 0.360 | 0.591 |
| **Bradykinin** | | | | | | | | | | | | | |
| **Receptor type 1** | Rs4905475 | Chr 14 | Promotor | G > C | 81.2 | 17.7 | 1.1 | 1.00 | 0.96 (0.85-1.09) | 1.16 (0.76-1.79) | 0.99 (0.88-1.10) | 0.810 | 1.000 |
| | Rs12050217 | Chr 14 | Intron | A > G | 62.1 | 33.2 | 4.7 | 1.00 | 1.09 (0.98-1.20) | 1.10 (0.88-1.37) | 1.07 (0.98-1.15) | 0.113 | 0.387 |
| | Rs885845 | Chr 14 | Intron | C > T | 41.7 | 45.2 | 13.1 | 1.00 | 1.03 (0.94-1.15) | 1.03 (0.89-1.20) | 1.01 (0.95-1.09) | 0.672 | 0.989 |
| | Rs2071084 | Chr 14 | Exon | G > A | 68.4 | 28.3 | 3.2 | 1.00 | 1.06 (0.95-1.18) | 1.00 (0.76-1.31) | 1.04 (0.84-1.07) | 0.340 | 0.806 |
| **Bradykinin** | | | | | | | | | | | | | |
| **Receptor type 2** | Rs1046248 | Chr 14 | Exon | G > A | 83.4 | 15.8 | 0.7 | 1.00 | 0.94 (0.83-1.07) | 0.89 (0.50-1.58) | 0.95 (0.84-1.07) | 0.391 | 0.864 |
| | Rs5224 | Chr 14 | Exon | G > A | 65.9 | 30.2 | 3.9 | 1.00 | 1.04 (0.94-1.16) | 0.96 (0.75-1.23) | 1.03 (0.94-1.11) | 0.541 | 0.949 |
| | Rs5225 | Chr 14 | Intron | T > C | 79.5 | 19.1 | 1.4 | 1.00 | 0.97 (0.86-1.10) | 1.22 (0.83-1.79) | 1.01 (0.91-1.11) | 0.901 | 1.000 |
| | Rs1799722 | Chr 14 | Promotor | G > A | 32.2 | 49.4 | 18.4 | 1.00 | 0.92 (0.83-1.03) | 0.98 (0.86-1.13) | 0.97 (0.91-1.04) | 0.353 | 0.832 |
| **Endothelial Nitric** | | | | | | | | | | | | | |
| **Oxide synthase** | Rs1800779 | Chr 7 | Intron | A > G | 38.0 | 46.7 | 15.3 | 1.00 | 1.03 (0.93-1.15) | 1.12 (0.97-1.29) | 1.05 (0.98-1.12) | 0.169 | 0.303 |
| | Rs3918188 | Chr 7 | Intron | C > A | 41.1 | 46.2 | 12.7 | 1.00 | 0.97 (0.87-1.07) | 0.94 (0.80-1.09) | 0.97 (0.90-1.04) | 0.330 | 0.553 |

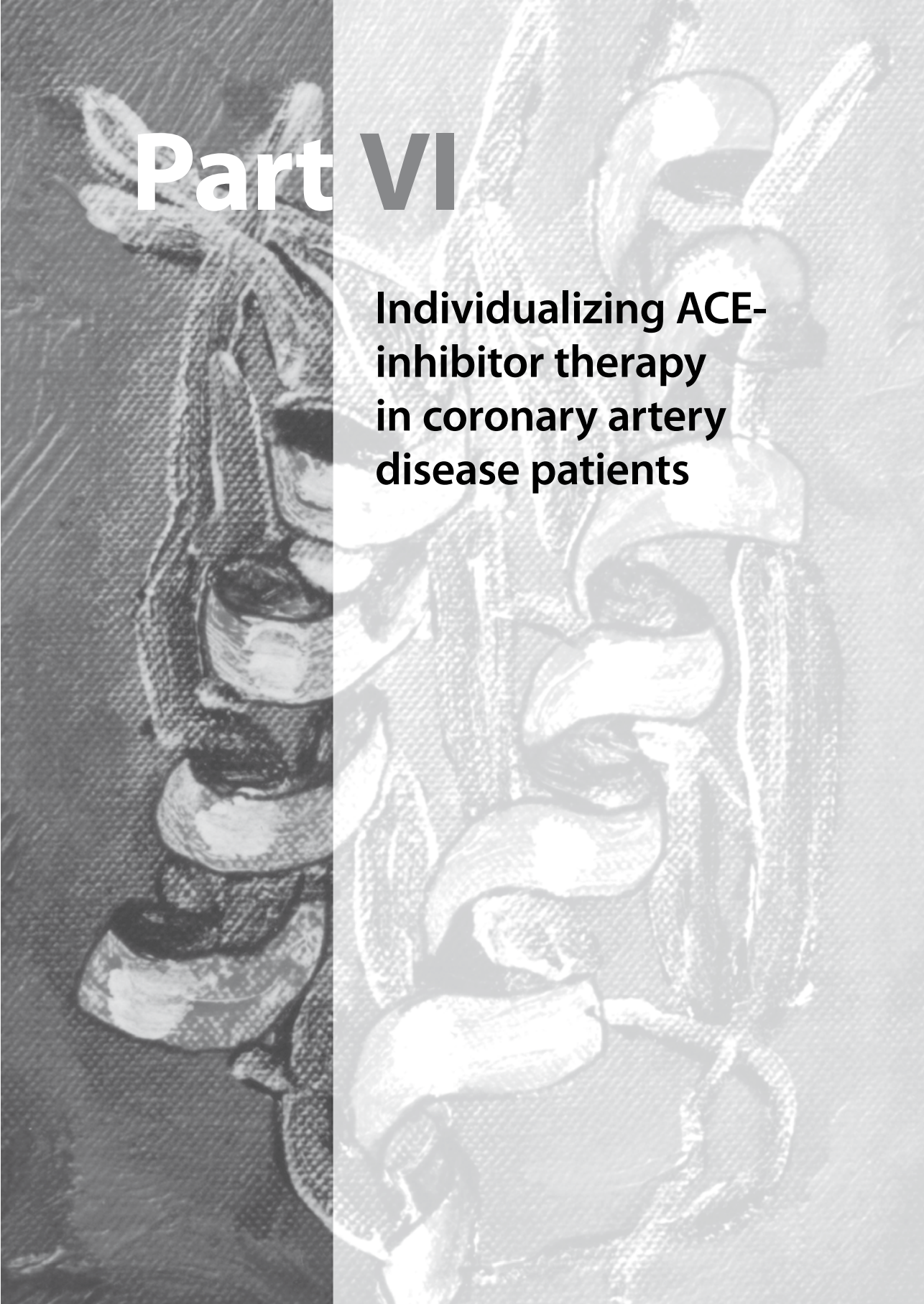
Kallikrein-bradykinin pathway genes in relation to moderate to severe hypertension

Empirical and permutation p-values based on 10,000 permutations. Pemp = multivariate adjusted empirical p-value. Pperm = multivariate adjusted permutated p-value (including correction for multiple testing). HR= hazard ratio; CI= confidence interval. 1/1 = homozygous common allele; 1/2 = heterozygous; 2/2 = homozygous minor allele.

## REFERENCES


1. Ward R: Familial aggregation and genetic epidemiology of blood pressure: Pathophysiology, Diagnosis, and Management. New York, Raven Press, 1990, 81-100.
2. Pickering G. High blood-pressure evident cause: Essential hypertension. *BMJ* 1965; 5469: 1021-1026.
3. Inagami T. The renin-angiotensin system. *Essays Biochem* 1994; 28: 147-164.
4. Brugts JJ, den Uil CA, Danser AH, Boersma E. The Renin-Angiotensin-Aldosterone System: Approaches to Guide Angiotensin-Converting Enzyme Inhibition in Patients with Coronary Artery Disease. *Cardiology* 2008; 112: 303-312.
5. Agerholm-Larsen B, Nordestgaard BG, Tybjærg-Hansen A. ACE Gene Polymorphism in Cardiovascular Disease : Meta-Analyses of Small and Large Studies in Whites. *Arterioscler Thromb Vasc Biol* 2000; 20: 484-492.
6. Danser AH, Schalekamp MA, Bax WA, van den Brink AM, Saxena PR, Riegger GA, Schunkert H.. Angiotensin-converting enzyme in the human heart. Effect of the deletion/insertion polymorphism. *Circulation* 1995; 92: 1387-1388.
7. Sethi AA, Nordestgaard BG, Tybjaerg-Hansen A. Angiotensinogen gene polymorphism, plasma angiotensinogen, and risk of hypertension and ischemic heart disease: a meta-analysis. *Arterioscler Thromb Vasc Biol* 2003; 23: 1269–1275.
8. Jeunemaitre X, Soubrier F, Kotelevtsev YV, Lifton RP, Williams CS, Charru A, Hunt SC, Hopkins PN, Williams RR, Lalouel JM. Molecular basis of human hypertension: role of angiotensinogen. *Cell* 1992; 71: 169-180.
9. Sayed-Tabatabaei FA, Oostra BA, Isaacs A, van Duijn CM, Witteman JCM. ACE polymorphisms, *Circ Res* 2006; 98: 1123-1133.
10. Niu W, Qi Y, Hou S, Zhai X, Zhou W, Qiu C. Haplotype-based association of the renin-angiotensin-aldosterone system genes polymorphisms with essential hypertension among Han Chinese: the Fangshan study. *J Hypertens* 2009 ;27: 1384-1391.
11. Stanton AV, Dicker P, O'Brien ET. Aliskiren Monotherapy Results in the Greatest and the Least Blood Pressure Lowering in Patients With High- and Low-Baseline PRA Levels, Respectively. *Am J Hypertens* 2009; 22: 954-957.
12. Nussberger J, Gradman AH, Schmieder RE, Lins RL, Chiang Y, Prescott MF. Plasma renin and the antihypertensive effect of the orally active renin inhibitor aliskiren in clinical hypertension. *Int J Clin Pract* 2007; 61: 1461-1468.
13. Harrap S. Tzourio C, Cambien F, Poirier O, Raoux S, Chalmers J, Chapman N, Colman S, Leguennec S, MacMahon S, Neal B, Ohkubo T, Woodward M; PROGRESS Collaborative Group.The ACE gene I/D polymorphism is not associated with the blood pressure and cardiovascular benefits of ACE inhibition. *Hypertension* 2003; 42: 297-303.
14. The EUROPA trial investigators. The European Trial on Reduction of Cardiac Events with Perindopril in Patients with Stable Coronary Artery Disease investigators. Efficacy of perindopril in reduction of cardiovascular events in stable coronary artery disease: randomized, double-blind, placebo controlled, multicentre trial (the EUROPA study). *Lancet* 2003; 362: 782–788.
15. Brugts JJ, de Maat MPM, Boersma E, Witteman JCM, van Duijn C, Uitterlinden AG, Bertrand M, Remme W, Fox K, Ferrrari R, Danser AHJ, Simoons ML. The Rationale and Design of the Perindopril Genetic Association Study: A Pharmacogenetic analysis of ACE-inhibitor therapy in patients with stable coronary artery disease. *Cardiovasc Drugs Ther* 2009; 23: 171-181.
16. Gomma AH, Fox KM, for the EUROPA investigators. The EUROPA trial: design, baseline demography and status of the substudies. C*ardiovasc Drugs Ther* 2001; 15: 169-179.
17. *PROGRESS Collaborative Group* Randomised trial of a perindopril-based blood-pressure-lowering regimen among 6105 individuals with previous stroke or transient ischaemic attack. *Lancet* 2001; 358: 1033–1041.
18. Nederhand RJ, Droog S, Kluft C, Simoons ML, de Maat MP. Logistics and quality control for DNA sampling in large mult-center studies. *J Thromb Haemost* 2003; 1: 987–991.

19. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005; 21: 263-265.
20. de Bakker PIW, Yelensky R, Pe'erI, Gabriel SB, Daly MJ, Altshuler D. Efficiency and power in genetic association studies. *Nature Genetics* 2005; 37: 1217-1223.
21. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, Jones DW, Materson BJ, Oparil S, Wright JT Jr, Roccella EJ; Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. National Heart, Lung, and Blood Institute; National High Blood Pressure Education Program Coordinating Committee. 7th report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood pressure. *Hypertension* 2003; 42: 1206-1252.
22. Churchill GA, Doerge RW. Empirical threshold values for quantitative trait mapping. *Genetics* 1994; 138: 963–971.
23. Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. *Am J Hum Genet* 2002; 70: 425-434.
24. Abdollahi MR, Huang S, Rodriguez S, Guthrie PA, Smith GD, Ebrahim S, Lawlor DA, Day IN, Gaunt TR. Homogeneous assay of rs4343, an ACE I/D proxy, and an analysis in the British Women's Heart and Health Study (BWHHS). *Dis Markers* 2008; 24: 11-17.
25. Danser AHJ, Batenburg WW, van den Meiracker AH, Danilov SM. ACE phenotyping as a first step toward personalized medicine for ACE inhibitors. Why does ACE genotyping not predict the therapeutic efficacy of ACE inhibition? *Pharmacol Ther* 2007; 113: 607-18.
26. Danser AHJ, Derkx FHM, Hense HW, Jeunemaître X, Riegger GA, Schunkert H. Angiotensinogen (M235T) and angiotensin-converting enzyme (I/D) polymorphisms in association with plasma renin and prorenin levels. *J Hypertens* 1998; 16: 1879-1883.
27. Hirose T, Hashimoto M, Totsune K, Metoki H, Asayama K, Kikuya M, Sugimoto K, Katsuya T, Ohkubo T, Hashimoto J, Rakugi H, Takahashi K, Imai Y. Association of (pro)renin receptor gene polymorphism with blood pressure in Japanese men: the Ohasama study. *Am J Hypertens* 2009; 22: 294-299.
28. Batenburg WW, Krop M, Garrelds IM, de Vries R, de Bruin RJA, Burcklé CA, Müller DN, Bader M, Nguyen G, Danser AHJ. Prorenin is the endogenous agonist of the (pro)renin receptor. Binding kinetics of renin and prorenin in rat vascular smooth muscle cells overexpressing the human (pro)renin receptor. *J Hypertens* 2007; 25: 2441-2453.
29. Batenburg WW, Danser AHJ. The (pro)renin receptor: a new addition to the renin-angiotensin system? *Eur J Pharmacol* 2008; 585: 320-324.
30. Batenburg WW, de Bruin RJA, van Gool JM, Müller DN, Bader M, Nguyen G, Danser AHJ. Aliskiren-binding increases the half life of renin and prorenin in rat aortic vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 2008; 28: 1151-1157.
31. Danser AHJ, Deinum J. Renin, prorenin and the putative (pro)renin receptor. *Hypertension* 2005; 46: 1069-1076.
32. Fisher ND, Danser AHJ, Nussberger J, Dole WP, Hollenberg NK. Renal and hormonal responses to direct renin inhibition with aliskiren in healthy humans. *Circulation* 2008; 117: 3199-3205.
33. Feldman DL, Jin L, Xuan H, Contrepas A, Zhou Y, Webb RL, Mueller DN, Feldt S, Cumin F, Maniara W, Persohn E, Schuetz H, Danser AHJ, Nguyen G. Effects of aliskiren on blood pressure, albuminuria, and (pro)renin receptor expression in diabetic TG(mRen-2)27 rats. *Hypertension* 2008; 52: 130-136.
34. Gainer JV, Morrow JD, Loveland A, King DJ, Brown NJ. Effect of bradykinin-receptor blockade on the response to angiotensin-converting-enzyme inhibitor in normotensive and hypertensive subjects. *N Engl J Med* 1998; 339: 1285-1292.
35. Arnett DK, Davis BR, Ford CE, Boerwinkle E, Leiendecker-Foster C, Miller MB, Black H, Eckfeldt JH. Pharmacogenetic Association of the Angiotensin-Converting Enzyme Insertion/Deletion Polymorphism on Blood Pressure and Cardiovascular Risk in Relation to Antihypertensive Treatment The Genetics of Hypertension-Associated Treatment (GenHAT) Study. *Circulation* 2005; 111: 3374-3383.
36. Brugts JJ, Ninomiya T, Boersma E, Remme WJ, Vertrand M, Ferrari R, Fox K, MacMahon S, Chalmers J, Simoons ML. The consistency of the treatment effect of an ACE-inhibitor based treatment regimen in patients with vascular disease or high risk of vascular disease: a combined analysis of individual data of ADVANCE, EUROPA, and PROGRESS trials. *Eur Heart J* 2009; 30, 1385–1394.



# Part VI

## Individualizing ACE-inhibitor therapy in coronary artery disease patients

# Chapter 13

# Tailored-therapy of ACE-inhibitors in coronary artery disease: pharmacogenetic profiling of treatment benefit

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## ABSTRACT

Angiotensin-converting enzyme (ACE) inhibitors are among the most commonly used drugs in stable coronary artery disease (CAD) as these agents have been proven effective in reducing the risk of cardiovascular morbidity and mortality. As with other drugs, individual variation in treatment benefit is likely. Such heterogeneity could be used to target ACE-inhibitor therapy to those patients most likely to benefit of treatment. However, prior attempts to target ACE-inhibitor therapy to those patients who are most likely to benefit of such prophylactic treatment in secondary prevention using clinical characteristics or the level of baseline risk appeared to be not appropriate. A new approach of 'tailored-therapy' could be to integrate more patient-specific characteristics such as the genetic information (DNA) of patients. Pharmacogenetic research of ACE-inhibitors in coronary artery disease patients is in a formative stage and studies are limited. The PERindopril GENEtic association study is a large pharmacogenetic sub-study of the randomized placebo-controlled EUROPA-trial, aimed to assess the feasibility of pharmacogenetic profiling of ACE-inhibitor therapy by perindopril. This review summarizes the recent findings of the PERGENE-study and pharmacogenetic research of the treatment benefit of perindopril in stable coronary artery disease.

## ANGIOTENSIN-CONVERTING ENZYME INHIBITORS

ACE-inhibitors competitively block the conversion of angiotensin-I (AT-1) into angiotensin-II (AT-II). This blockade results in a decrease in circulating and local levels of AT-II, thereby inhibiting the main effects of AT-II: arteriolar vasoconstriction and water and salt retention. ACE-inhibitors do not antagonize the AT-1 receptor and thus do not inhibit the unfavorable effects of AT-II completely. Furthermore, the formation of AT-II is restored, at least partially, due to the reactive rise in rise that occurs when the renin release is blocked by the A-II-induced negative feedback. A second beneficial effect of ACE-inhibitors, and a main difference with angiotensin-receptor antagonists, is the increase in bradykinin levels by a decrease in transformation of bradykinin in inactive peptides [1,2]. The increase in bradykinin levels induced by ACE inhibitors leads to the release of nitric oxide and prostaglandins, with vasodilative effects on vessel walls [2,3].

The efficacy of ACE-inhibitors has been demonstrated by several large clinical trials in patients at high-risk of cardiovascular disease, including those with left ventricular ejection fraction of <40% after myocardial infarction (MI), heart failure or a history of cerebrovascular accidents, and those with a lower risk of cardiovascular events, in particular patients with stable coronary artery disease without overt heart failure [4-10]. Nowadays, the use of ACE inhibitors is recommended in guidelines on the management of hypertension, stable CAD, MI, heart failure, and in the prevention of the progression of renal insufficiency in diabetes mellitus related kidney disease [11-13]. In particular ACE-inhibitors are recommended as secondary prevention for the broad group of patients with known CAD [11]. This review is primarily focused at patients with stable CAD and the ACE-inhibitor perindopril as studied in the EURopean trial On reduction of cardiac events with Perindopril in patients with stable coronary Artery disease (EUROPA) [10,14].

## THE EUROPA TRIAL

The EUROPA trial studied the ACE-inhibitor perindopril in a population with stable coronary artery disease without heart failure [10]. In EUROPA, 12218 patients were randomly assigned perindopril 8 mg once daily (n=6110), or matching placebo (n=6108). The primary endpoint was cardiovascular mortality, myocardial infarction, or cardiac arrest. Mean age of patients was 60 years and 85% were male, 92% were taking platelet inhibitors, 62% beta-blockers, and 58% statins. During a mean follow-up of 4.2 years, perindopril was associated with a 20 % relative reduction in the primary endpoint, from 9.9% to 8.0%, (HR 0.80; 95% CI 0.71-0.91; 8% vs 10%). These benefits were consistent in all clinical subgroups, across several secondary endpoints and independent of baseline blood pressure and use of concomitant medication. Perindopril was safe and well tolerated. To prevent one major cardiovascular event, 50 patients with stable CAD need to be treated for a period of 4.2 years [10]. Several sub-studies of EUROPA have established

that ACE inhibitors have additional effects by improving endothelial function, neurohumoral balance, and reducing of unfavorable remodeling of the coronary arteries [15-18].

## PRIOR ATTEMPTS TO GUIDE ACE-INHIBITOR THERAPY TO THOSE PATIENTS MOST LIKELY TO BENEFIT

Several analyses have been performed to test the consistency of the treatment benefit ACE-inhibitors among patient subgroups based on clinical characteristics [19- 23]. Heterogeneity in the clinical treatment effect of ACE-inhibitors could be used to guide ACE-inhibitor therapy to those patients most likely to benefit of such therapy. Tailored ACE-inhibitor therapy will improve patients' benefit, and reduce unnecessary health care costs and side effects. Using the EUROPA trial data, a risk model based on baseline clinical characteristics was developed [20]. The treatment benefit of perindopril was consistent across different risk categories and therefore not modified by the level baseline risk (figure 1). Renal insufficiency is an important risk factor for developing cardiovascular disease [21]. To study whether patient with normal renal function and impaired real function experienced a different treatment benefit, a subgroup analysis was performed within the EUROPA-trial. This analysis showed that treatment benefit was not modified by renal insufficiency [22]. In a recent meta-analysis of the EUROPA, PROGRESS and ADVANCE trials, investigating the same ACE-inhibitor perindopril, we demonstrated a consistent treatment effect of ACE-inhibitor based regimens independent of clinical characteristics or baseline blood pressure levels [23]. Hence, no heterogeneity of treatment benefit was observed



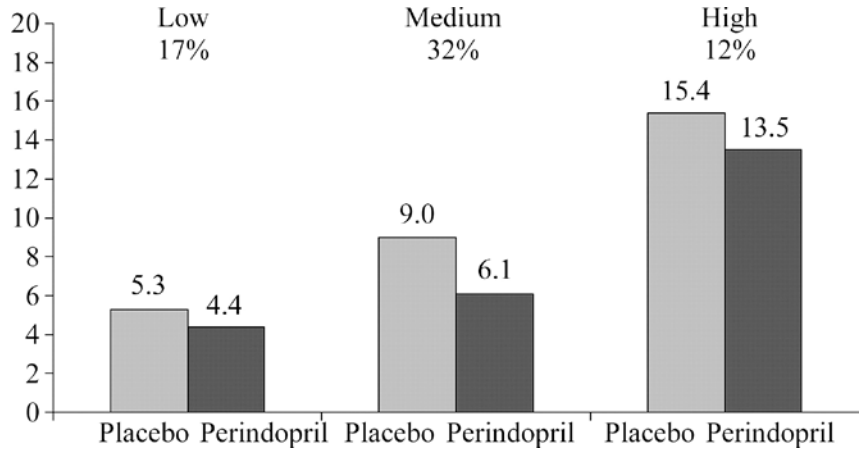


**Figure 1** Consistency of the treatment benefit of perindopril in the EUROPA trial according to a risk models based on clinical characteristics. Adapted from Deckers JW, Goedhart D, Simoons ML, et al. Treatment benefit by perindopril in patients with stable coronary artery disease at different levels of risk. *Eur Heart J*. 2006;27(7):796-801 with permission. P-value for interaction between treatment benefit and risk tertile is non-significant.

according to clinical characteristics. It appeared to be no feasible to guide ACE-inhibitor therapy in patients with stable CAD to specific subgroups that are most likely to benefit of such prolonged prophylactic treatment based on simple clinical characteristics.

## PHARMACOGENETIC APPROACH OF INDIVIDUALIZING ACE-INHIBITOR THERAPY

As simple clinical patient characteristics are inadequate to tailor ACE-inhibition therapy, new approaches that integrate more patient-specific characteristics should be considered, such as pharmacogenetic profiling of the drug response. The new field of cardiovascular pharmacogenetics involves examining the genetic determinants of patients' responses to drugs and is expanding rapidly. Pharmacogenetics is aimed to understand why some drugs work better for some people than others and why some people are more likely than others to experience side-effects. Indeed, pharmacogenetic profiling might be a new way to reach significant advances in individualized cardiovascular medicine. A priori, it is expected for several types of factors that they play a role in determining the response of a patient to therapy. Genetic factors causing differences in drug absorption and metabolic clearance are highly relevant; however; this is yet a relatively unexplored field for ACE-inhibitors. Genetic factors within the direct pharmacodynamic pathway that is affected by the ACE-inhibitors, the renin–angiotensin–aldosteron–system (RAAS) and bradykinin pathways are likely to affect the clinical efficacy of ACE inhibitors. In recent years, several genetic polymorphisms in RAAS genes have been associated with high blood pressure levels or an increased cardiovascular risk [3,24,25]. Nearly all prior studies focused at two polymorphisms, the angiotensin-converting enzyme (ACE) I/D polymorphism and the M235T polymorphism in the angiotensinogen (AGT) gene. Because of limited study sample size and power, results have been inconsistent and these important topics have not yet been answered convincingly. With regard to interaction between genetic variation and ACE-inhibitor treatment response, the results are scarce as clinical data is lacking. No prior research with ACE-inhibitors in stable CAD has been performed at large-scale nor in a randomized trial setting. It has been suggested that the response to drug therapy may be influenced by genetic polymorphisms in different ways. Firstly, pharmacodynamics may be affected by polymorphisms in the genes of all proteins involved in the RAAS and related systems, including receptors and signal transduction molecules. Secondly, variations in drug absorption and metabolic clearance may cause inter-individual variation in pharmacokinetics. Thirdly, variations within genes of the RAAS and related systems may influence atherosclerosis (underlying disease process) and inherent differences in the susceptibility to therapeutic agents such as ACE inhibitors.

The concept of pharmacogenetic research to individualize medicine is emerging rapidly and is clinically highly relevant. Several successes of this approach have recently been demonstrated for different cardiovascular agents, such as the activation of clopidogrel [26,27] and the risk of

rhabdomyolysis associated with statin therapy [28]. Current pharmacogenetic data is often obtained from observational cohort studies or cross sectional data. Large randomized clinical trials with available DNA offer a unique opportunity to study this concept of tailored-therapy and truly test the feasibility of pharmacogenetic profiling of treatment benefit. The objective is to construct a genetic profile which enables the doctor to predict the patient's benefit of treatment in advance. Additionally, pharmacogenetics will teach us more in the individual response mechanism to medications.

## CURRENT LITERATURE

Three studies have performed a pharmacogenetic analysis of ACE-inhibitors or a treatment regimen containing ACE-inhibitors [29-31], two of them only studied the ACE I/D polymorphism and found no associations [29,30], one study examined for relevant genetic targets within the RAAS and found some interesting results [31]:

The Genetics of Hypertension-Associated Treatment (GenHAT) Study first assessed the concept of pharmacogenetics of anti-hypertensive drugs [29]. The investigators used a double-blind, active-controlled randomized trial of antihypertensive treatment that included hypertensives >55 years of age with at least 1 risk factor for cardiovascular disease. The ACE insertion/deletion genotype was determined in 37939 participants randomized to chlorthalidone, amlodipine, lisinopril, or doxazosin treatments and followed up for 4 to 8 years. Primary outcomes included fatal coronary heart disease (CHD) and/or nonfatal myocardial infarction. Fatal and nonfatal CHD occurred in 3096 individuals during follow-up. The hazard rates for fatal and nonfatal CHD were similar across antihypertensive treatments. The ACE I/D genotype group was not associated with fatal and nonfatal CHD (relative risk of DD versus ID and II, 0.99; 95% CI, 0.91 to 1.07). The 6-year hazard rate for fatal and nonfatal CHD in the DD genotype group was not statistically different from the ID and II genotype group by type of treatment. Therefore, the authors concluded that the ACE I/D genotype group was not a predictor of CHD, nor did it modify the response to antihypertensive treatment. The ACE I/D polymorphism is not a useful marker to predict antihypertensive treatment response [29]. Unfortunately, the authors did not study other relevant candidate genes or multiple genetic polymorphisms within the complex RAAS system.

In PROGRESS [30], the insertion/deletion (I/D) polymorphism of the ACE genotype was studied for the effect of a perindopril-based blood pressure-lowering regimen on macro vascular events, dementia, and cognitive decline among hypertensive and nonhypertensive patients with a history of cerebrovascular disease. There were no associations between ACE genotypes and cerebrovascular disease history or cardiovascular risk factors, including baseline blood pressure. The ACE genotype was not associated with the long-term risks of stroke, cardiac events, mortality, dementia, or cognitive decline; neither did the ACE genotype predict the

blood pressure reduction associated with the use of the ACE inhibitor perindopril. Similarly, there was no evidence that the ACE genotype modified the relative benefits of ACE inhibitor-based therapy over placebo. The ACE genotype is not useful for predicting either the risk of disease or the benefits of perindopril-based blood pressure-lowering treatment [30].

Within the Chinese Community-Based Comprehensive Prevention and Control of Hypertension project, investigators studied the genetic contribution to the variation in blood pressure (BP) response to ACE-inhibitors [31]. Fourteen single-nucleotide polymorphisms (SNPs) in the angiotensinogen (AGT), angiotensin receptor 1 (AGTR1), and angiotensin receptor 2 (AGTR2) genes were evaluated for their association with BP response to ACEI in 1447 Chinese patients with hypertension in a 2-stage design from a 3-year benazepril postmarket surveillance. The AGT rs7079 (C/T) SNP (3'-untranslated region) was significantly associated with the response of diastolic BP to benazepril (diastolic BP response: 7.4 mm Hg for subjects with the CC genotype, 8.9 mm Hg for CA, and 10.1 mm Hg for AA; $P<0.001$). Although there was no association of individual SNPs in the AGTR1 gene, there was a graded response between common haplotypes and systolic BP reduction. The total variations in response to ACEI therapy that were explained by the AGT SNP and AGTR1 haplotype groups were 13% for systolic and 9% to 9.6% for diastolic BP, respectively. These findings are useful in future studies, providing genetic markers to predict the hypertensive response to ACE-inhibitor therapy [31]. An important limitation of prior studies is the investigation of one or two polymorphisms within one candidate gene, thereby ignoring the well documented feedback mechanisms within the RAAS but also the fact that there are two angiotensin II receptors (AT1 and AT2), which have counteracting effects. Additionally, the ACE I/D polymorphisms is not a reflection of the entire renin-angiotensin system. We suggest that a more comprehensive coverage of genetic variation in multiple RAAS genes is needed, by using a haplotype approach to study common variation within relevant candidate genes. Combining information from multiple SNPs in the RAAS genes, will result in a more comprehensive in-depth analysis of RAAS and BK system genes in relation to ACE-inhibitor treatment benefit, which is more likely to unravel any existing pharmacogenetic association.

## THE PERGENE STUDY

The PERindopril GENEtic association study (PERGENE) is a pharmacogenetic substudy within the main EUROPA trial [32]. PERGENE aims at assessing the feasibility of pharmacogenetic profiling of treatment benefit of ACE-inhibitors in patients with stable CAD. We hypothesized that genetic polymorphism in the RAAS and kininogen–kallikrein–bradykinin pathways may influence the treatment benefit of ACE-inhibitors in patients with stable CAD. Polymorphisms were selected based on haplotype tagging SNP's using the HapMap genome project to comprehensively cover all genetic variation within genes; additional selection was based on functionality, location within the gene (promoter) or relevant literature. The PERGENE study

is unique in the field of pharmacogenetic studies because of the large sample size, a randomized and placebo-controlled design, and the availability of extensive and accurate phenotypic data. Also, the extensive selection of 52 tagging SNP's in 12 candidate genes in both pathways ensures a new and comprehensive coverage of common genetic variation in the candidate genes. The main outcome measure of PERGENE was the interaction between genetic factors and treatment effect of ACE-inhibitors during follow-up. The size of this pharmacogenetic substudy allows detection with a statistical power of 98% to detect a difference in hazard ratios (treatment effect) of 20% between genotypes with minor allele frequency of 0.20 (two-sided alpha 0.05). More details on the study design, SNP selection procedure and statistical analysis can be found elsewhere [32].

## Clinical treatment effect of ACE-inhibitors

An analysis of heterogeneity in clinical effectiveness of perindopril according to genetic variation in RAAS was further performed in PERGENE [33]. The EUROPA-trial provides a unique opportunity to evaluate this hypothesis, since it is a large randomized double-blind placebo-controlled clinical trial with complete phenotypic data [2,13]. We studied whether genetic polymorphisms in the renin-angiotensin-aldosterone and kallikrein-bradykinin systems modify the treatment benefit of the ACE-inhibitor perindopril. The main EUROPA-trial randomized 12,218 stable CAD patients to perindopril (8 mg/day) or placebo and perindopril was associated with a 20% reduction (HR 0.80; 95% CI 0.71-0.91) in the event rate of the primary endpoint (composite of cardiovascular mortality, non-fatal MI, or resuscitated cardiac arrest) during a mean follow-up of 4.2 years [2]. Event rates were 9.9% with placebo and 8.0% in patients receiving perindopril. In 8907 stable CAD patients from this trial, we analyzed 12 genes within the two pharmacodynamic pathways affected by ACE-inhibitors, using 52 haplotype-tagging SNPs. The primary outcome was the reduction in cardiovascular mortality, non-fatal myocardial infarction and resuscitated cardiac arrest during 4.2 years of follow-up. Cox regression was performed with multiple-testing corrections by permutation analysis.

In unadjusted analysis, 7 SNPs in 4 genes were associated with the treatment effect of perindopril. In multivariate permutation analysis, 3 of these SNPs, located in the angiotensin-II type I receptor and bradykinin type I receptor genes, significantly modified the treatment benefit of perindopril. In the bradykinin type I (BK1) receptor gene; rs12050217 was a strong modifier of the treatment benefit of perindopril. The hazard ratio (95% CI) for the reduction in the event rate of the primary endpoint for AA (62.1%) genotypes was 0.64 (0.55-0.78), for AG (33.2%) genotypes 1.02 (0.79-1.29) and for GG (4.7%) genotypes 1.10 (0.56-2.19). The p-values for interaction were 0.004 (empirical) and 0.012 (permutated) [33]. In the angiotensin-II type I (AT1) receptor gene, rs275651 and rs5182 significantly modified the treatment benefit of perindopril in a similar way, with empirical p-values of 0.008 and 0.011, and permutated p-values of 0.049 and 0.054, respectively (figure 2). No further associations of treatment interaction were observed for the other genes [33].

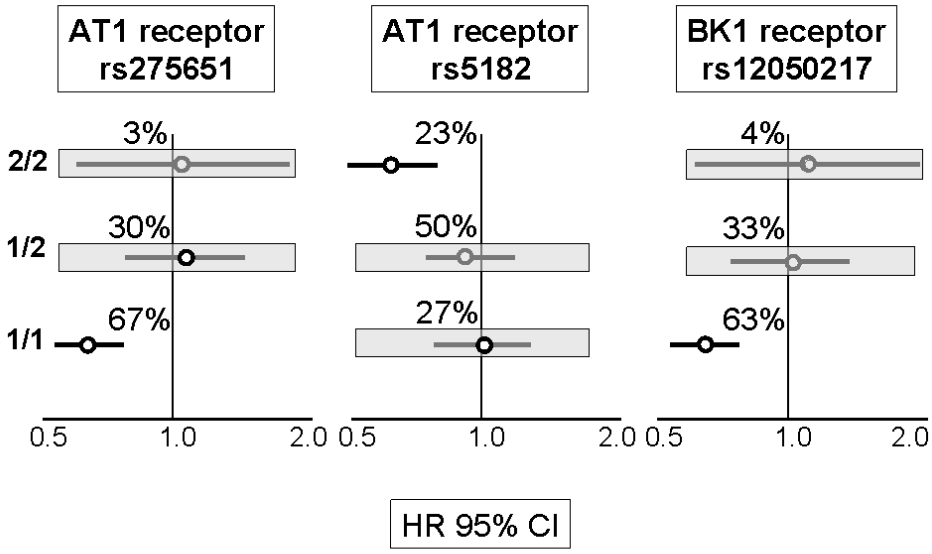


**Figure 2** Treatment effect modifying SNPs in PERGENE 1/1 = homozygous common allele, 1/2 = heterozygous, 2/2 = homozygous common allele. Percentages correspond to the number of patients within each group according to genotype. The x-axis corresponds to the hazard ratio and 95% CI estimates and Y-axis to genotype category.

A pharmacogenetic profile, combining the unfavorable alleles of these 3 SNPs, demonstrated a stepwise decrease in treatment benefit of perindopril with increasing number of unfavorable alleles (interaction p<0.0001). The treatment benefit was concentrated in 73.5% of the patients (responders, HR 0.67; 95% CI 0.56-0.79) and absent in 26.5% of the patients (non-responders, HR 1.26; 95% CI 0.97-1.67) (figure 3) [33]. An interaction effect of similar direction and magnitude was observed in a confirmatory analysis of 1051 patients with cerebrovascular disease from the PROGRESS-trial. This unique pharmacogenetic analysis identified genetic determinants for the treatment benefit of ACE-inhibitor therapy by perindopril in patients with stable CAD for the first time. Haplotype analysis confirmed the association between the identified SNPs and treatment effect modification. In both genes, the haplotype carriers of the unfavorable alleles of the identified SNPs significantly modified the treatment effect of perindopril [33].

The PERGENE study demonstrates that the treatment benefit of ACE-inhibitor therapy by perindopril is modified by genetic variation in the renin-angiotensin-aldosterone and kallikrein-bradykinin systems [33]. Based on the aggregated pharmacogenetic profile, both patients with a higher treatment benefit (responders, 73.5%), and patients with a diminished treatment effect (non-responders, 26.5%), could be identified [34] (figure 4). For the first time, a group with significantly different treatment effect could be identified within the EUROPA trial based upon pharmacogenetic data. Non-responding patients demonstrate a relative resistance or unresponsiveness to ACE-inhibitors.

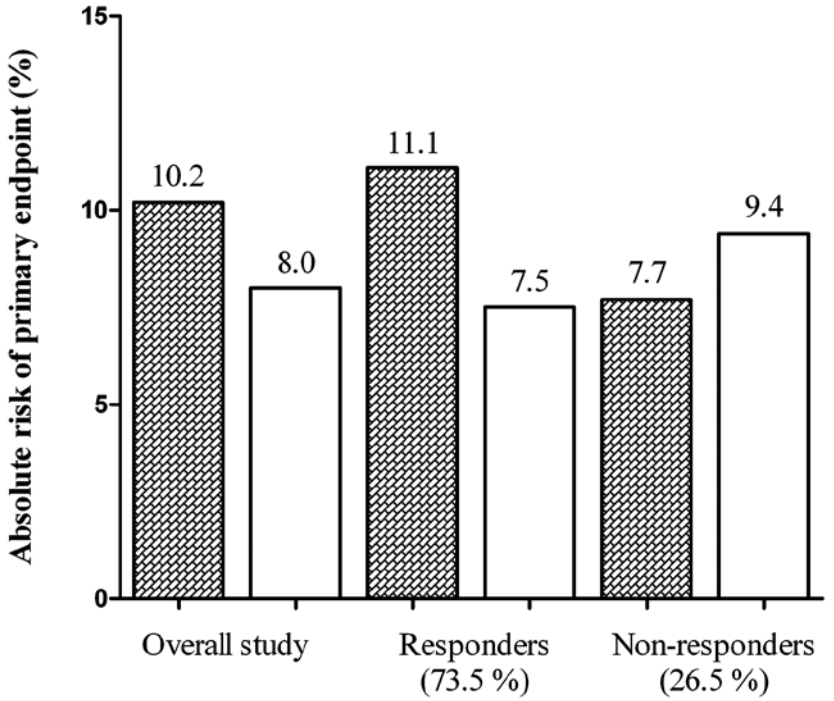


**Figure 3** Treatment effect according to the pharmacogenetic profile categories responders = <3 unfavorable alleles, non-responders ≥ 3 unfavorable alleles.



## Blood pressure and blood pressure reduction by ACE-inhibitor therapy

In the patients which were identified as responders and non-responders with pharmacogenetic profiling, no differences in clinical characteristics were observed between responders and non-responders in PERGENE (table 1) [33]. Responders and non-responders were of similar age (mean age 60 vs 60), gender (% of males, 85 vs 85) and cardiovascular risk factors were evenly distributed between patients (diabetes 12% vs 12%, hypertension 29% vs 28% prior MI 65% vs 66%). Thus, the observed treatment interaction cannot be explained by clinical differences between the genotypes. This is in line with the article by Deckers et al. [20] which also demonstrated that the level of baseline risk, calculated with a risk score of clinical baseline characteristics, did not modify the treatment benefit of perindopril in patients with stable CAD.

We also studied whether the observed differences in treatment benefit could be explained by a difference in the baseline level of blood pressure between responders and non-responders. The level of blood pressure at baseline was identical between responders and non-responders (137/82 mmHg) and did not modify the treatment effect of perindopril. This is in line with the article by Brugts et al. [23] which analyzed thirty-thousand patients with vascular disease treated with ACE-inhibitors and demonstrated that the level of baseline blood pressure did not modify the treatment benefit of ACE-inhibitor therapy. We further studied the level of blood pressure reduction by ACE-inhibitor therapy (perindopril) during the run-in period of four weeks of the

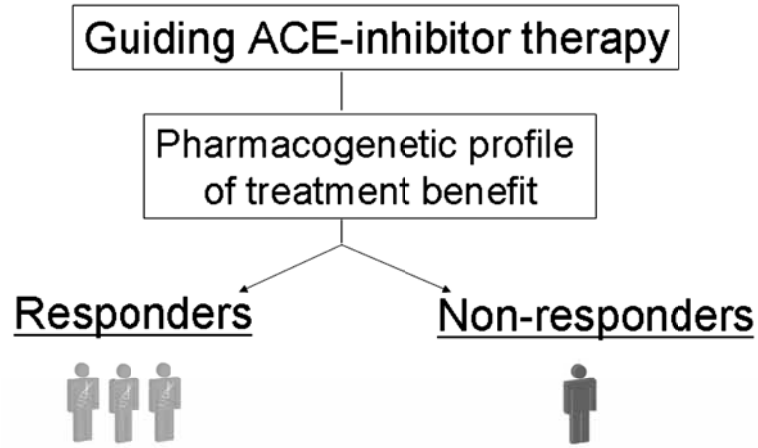


**Figure 4** Heterogeneity in treatment benefit by pharmacogenetic profiling identifies responders and non-responders to ACE-inhibitor therapy.

EUROPA trial. Responders and non-responders demonstrated a similar BP reduction during these four weeks on ACE-inhibitor therapy. A similar results was observed in the analysis by Brugts et al. [23] which demonstrated the independence of the treatment effect of ACE-inhibitors in terms of the level of blood pressure reduction during run-in period. We observed no differences in intermediate phenotypes such as blood pressure and blood pressure reduction which suggests a direct genetic effect which results in a resistance to ACE-inhibitors in patients 3 or more unfavourable alleles of the SNPs located in the AT1 and BK1 receptor. Thus, the observed treatment interaction cannot be explained by clinical differences between the genotypes. To strengthen our results, the BPLTCC consortium studied the blood pressure dependent and independent effects of ACE-inhibitors in 146838 patients and confirmed that blood pressure beneficial effects up and above the blood pressure reduction are present [14]. As perindopril is one of the most competent ACE-I with highest tissue ACE penetrance, blood pressure independent effects are likely as demonstrated in several substudies of the EUROPA trial [23]. Several

**Table 1.** Baseline characteristics according to pharmacogenetic profile category.

| (%) | Responders | Non-responders | p-value |
|---|---|---|---|
| Age, years | 60 | 60 | ns |
| Gender, male | 85 | 85 | ns |
| Diabetes Mellitus | 12 | 12 | ns |
| Hypertension | 29 | 28 | ns |
| Current smoking | 15 | 16 | ns |
| Prior MI | 65 | 66 | ns |
| Prior revascularization | 55 | 54 | ns |
| Blood pressure, mmHg | 137/82 | 137/82 | ns |
| BP-reduction, run-in period | 9/4 | 9/4 | ns |

Legend: responders = <3 unfavorable alleles, non-responders ≥ 3 unfavorable alleles.

sub-studies of EUROPA (PERFECT, PERSPECTIVE and PERTINENT), have studied the additional effects of ACE-inhibitors and have established that ACE inhibitors have additional effects beyond the blood pressure reduction alone such as the improvement of endothelial function, improvement of the neurohumoral balance, and reduction of unfavorable remodeling of the coronary arteries [15-18].

### Absolute risk of events

In our pharmacogenetic analysis of treatment benefit, the combination of ≥3 unfavourable alleles of the 3 SNPs located in AT1 and BK1 receptor genes was associated with a decreased risk of cardiac events in patient receiving placebo, while the risk increased in patients receiving perindopril which is independent of clinical characteristics. It may be suggested that the absolute risk of events in these patients was already low (stable CAD is a relatively low-risk group, in which one can argue whom to treat now), preventing any benefit of the addition of an ACE-inhibitor. However, the absolute risk for cardiovascular death or myocardial infarction in these patients was 7.7 % at four years follow-up. In a previous analysis of the EUROPA trial a consistent treatment benefit was observed in the lower risk tertile (based on assessment of clinical characteristics) with a risk of only 5.3 % in the patients treated with placebo as well as in the higher risk tertiles [7].



## POTENTIAL MECHANISM

Our findings suggest that the genetic variants modifying the clinical treatment effect of perindopril are particularly located in the AT1 and BK1 receptors. The SNPs in the AT1 receptor were located in the promoter (rs275651) and exon (rs5182), the SNP in the BK1 receptor was located in an intron, all three were important tagging SNPs within the candidate gene [33]. Functionality of these SNPs is yet unknown but under extensive investigation. The AT1 receptor is well-known and mediates all the well-known effects of angiotensin II, including vasoconstriction, water and salt retention, aldosterone synthesis and hypertrophy, and thus its appearance in this analysis is not particularly surprising. The role of the BK1 receptor, on the other hand, is less well established but recent reports increase the current interest in this receptor. Bradykinin is a potent vasodilator that also induces anti-atherosclerotic and anti-thrombotic effects, which are mediated by bradykinin type II receptors. Previous studies indicated that the clinical benefit of ACE-inhibitors depends, at least in part, on BK2 receptor activation [34]. In the past year, more data is emerging on the effect of the BK1 receptor, which effects are less well known. BK1 receptors are weakly expressed under physiological conditions, but are strongly induced in response to pathological conditions and/or RAAS blockading agents [35,36]. Recent reports indicate that BK1 receptor deficiency predisposes to atherosclerosis [37] and kinins and the BK1 receptor plays an important deleterious role in this process [38]. Interestingly, it has been suggested that

BK1 receptors are directly activated by ACE-inhibitors (thus resulting in an increase in endothelial NO release, for instance in the cardiac tissue [38,39,40]), by which they do contribute to the cardioprotective beneficial effects of ACE-inhibitors, but this has not been uniformly confirmed by others [41]. Therefore, a more likely possibility is that the up-regulated BK1 receptors (under pathologic conditions) are activated by their endogenous ligand during ACE-inhibition. Such activation results in the hypotensive [42], cardioprotective [34] and cerebroprotective [43] effects of kinins, as observed in animals, and one could speculate that patients with genetic defects in their BK1 receptor display a diminished response or relative resistance to ACE-inhibitors with regard to kinins. Indeed, in our study we observed that especially patients with the minor allele variants of the BK1 receptor were relatively insensitive or resistant to the beneficial effect of the ACE-inhibitor perindopril. In patients with the genetic defect in the BK1 receptor, one could speculate that the kinins can not have their beneficial effects by BK1 receptor activation (up-regulated during ACE-inhibition) and in such way do not benefit from treatment. Likewise, the patients without these genetic defects in the BK1 receptor have a much more pronounced effect as the activation of the BK1 receptor is not negatively affected. Clearly, more work is needed to support this interesting concept for which we have set up an additional basic research project.

The lack of a blood pressure mediated effect of the pharmacogenetic profile by 3 identified SNPs in the treatment effect analysis suggests a different pathway of clinical effect. In the clinical subgroups analyses, the treatment effect was also independent of baseline blood pressure as well as blood pressure reduction which supports our findings. As blood pressure independent effects of ACE-inhibitors is often proposed for the BK pathway [14]. In our analyses, indeed the responding and non-responding patients did not differ in clinical characteristics, baseline blood pressure or BP reduction level by ACE-inhibitor therapy while a clear heterogeneity was observed in the reduction in the event rate of the primary endpoint during follow-up related to the BK1 receptor. The blood pressure lowering effect must be important for the clinical effect, but it might be speculated that the presumed additional effects beyond lowering blood pressure alone which is frequently debated, might me more related to the BK system. Our findings do support that discussion, as it might be speculated that the genetic defects in the BK1 receptor alter the anti-atherosclerotic properties of ACE-inhibitor treatment effect which might be an important cornerstone of the treatment benefit besides blood pressure lowering.

## CLINICAL IMPLICATIONS

The PERGENE study demonstrated that RAAS and BK polymorphisms modified the response to the ACE-inhibitor perindopril in patient with stable CAD. We demonstrated a relative resistance to ACE-inhibitors in patients with unfavorable alleles of the AT1 receptor and BK1 receptor genes. Based on the PERGENE findings, three out of four patients with stable CAD (participating in EUROPA) had an enhanced benefit of ACE-inhibitor therapy (responders, 33% reduction of

cardiovascular death of myocardial infarction, up to 54% in patients without any unfavorable alleles), and one out of four patients experienced a markedly diminished benefit of treatment with perindopril (non-responders).

In the overall study, 50 patients needed to be treated for four years to prevent one cardiovascular event. The relative risk reduction in the primary endpoint was 20% (HR 0.80; 95% CI 0.71-0.91) and absolute risk reduction 2%. In our pharmacogenetic profile categories of patients with respectively <3 and ≥ 3 unfavorable alleles, relative risk reduction were respectively 33% (HR 0.67; 95% CI 0.56-0.79) and +26% (HR 1.26; 95% CI 0.97-1.67). Refraining from treatment with perindopril in this group of patients may considerably reduce healthcare cost and increase overall efficacy of the drug. In the fictive scenario that one would only patient with <3 unfavourable alleles were treated, which compromises 76.5% of the population, the absolute risk would be reduced from 11.1 % in placebo to 7.5 % in perindopril patients. Likewise, the number needed to treat would decrease from 50 to 32 (figure 5). Considering the millions of patients treated with ACE-inhibitors this reduction has huge clinical implications and fictively demonstrates the potential of pharmacogenetic profiling of drug response.



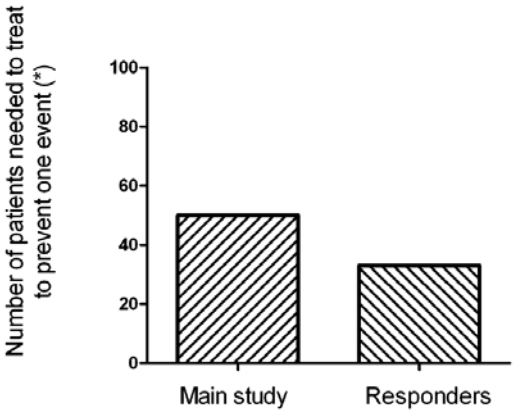


**Figure 5** Numbers needed to treat according to the pharmacogenetic profile

## FEASIBILITY OF PHARMACOGENETIC PROFILING OF ACE-INHIBITORS

The PERGENE study is one of the first pharmacogenetic analyses within a randomized clinical trial (assessing one agent), and one of the first demonstrating results for ACE-inhibitors. The concept of pharmacogenetic should be investigated further and replicated in similar patient populations but also at patients at higher risk of events as stable CAD patients are at relatively low risk of CVD events. When the feasibility of pharmacogenetic profiling of ACE-inhibitor therapy is confirmed in other studies, pharmacogenetic analyses of clinical trials truly open up a perspective to individualize preventive therapy in patients with cardiovascular disease.

Physicians will be able to predict the response to treatment (responders and non-responders) in advance, before starting prescription. Considering the findings of the PERGENE study, further replication must be sought in other cohorts. Additionally, other relevant genetic targets need to be investigated such as genes involved in the metabolism of ACE-inhibitors, fe CYP450 genes (pharmacokinetics). However, until now no specific genetic targets for ACE-inhibitor metabolism have been demonstrated. Ultimately, one would wish to perform a genome wide scan on the PERGENE data to elucidate further relevant pharmacogenetic targets throughout the genome related to the treatment benefit of ACE-inhibitors.

A similar approach could be used for other cardiovascular drugs such as statins to optimize patients' benefits as a strong consistency in the treatment benefit has been demonstrated as well [44]. The combination of these cardiovascular drug trials could be used to develop a pharmacogenetic profile for cardiovascular drugs in general. We advocate that future large scale randomized clinical trials should also integrate a pharmacogenetic analysis in their trial design to prospectively test treatment efficacy in a similar way as usually done with clinical risk factor assessment of trial patients. "Individualized therapy" by pharmacogenetic profiling will avoid unnecessary treatment of non-responding patients and considerably reduce health care costs.

## CONCLUSION

The PERGENE study demonstrated the feasibility of pharmacogenetic profiling of treatment benefit of ACE-inhibitors in patients with stable CAD. Unfavorable alleles of genetic variants in the AT1 and BK1 receptor genes identified patients with a relative resistance to ACE-inhibitors. Patients without the unfavourable alleles experienced a much more pronounced treatment benefit as compared to the overall study results and treatment should not be withheld in these patients.

### Executive summary

- ACE-inhibitors reduce cardiovascular risk in patients with stable CAD
- Assessing the consistency of treatment benefit is crucial for the efficacy and cost-effective prescription of ACE-inhibitors
- The treatment benefit of ACE-inhibitors is not modified by clinical characteristics. Thus guiding ACE-inhibitor therapy appeared not feasible using clinical characteristics.
- The PERGENE study is a pharmacogenetic analysis of treatment benefit of ACE-inhibitors in a large randomized placebo controlled clinical trial of patients with stable CAD
- The PERGENE study demonstrated that genetic variation in the AT1 and BK1 receptor modified the treatment benefit of the ACE-inhibitor perindopril

- The constructed pharmacogenetic profile identified 73.5 % of the patients with treatment benefit – responders- and 26.5 % of the patients with a diminished treatment benefit – non-responders-
- Pharmacogenetic profiling will optimize patients benefit of treatment and reduce unnecessary treatment of patient and reduce health care costs


## Acknowledgements

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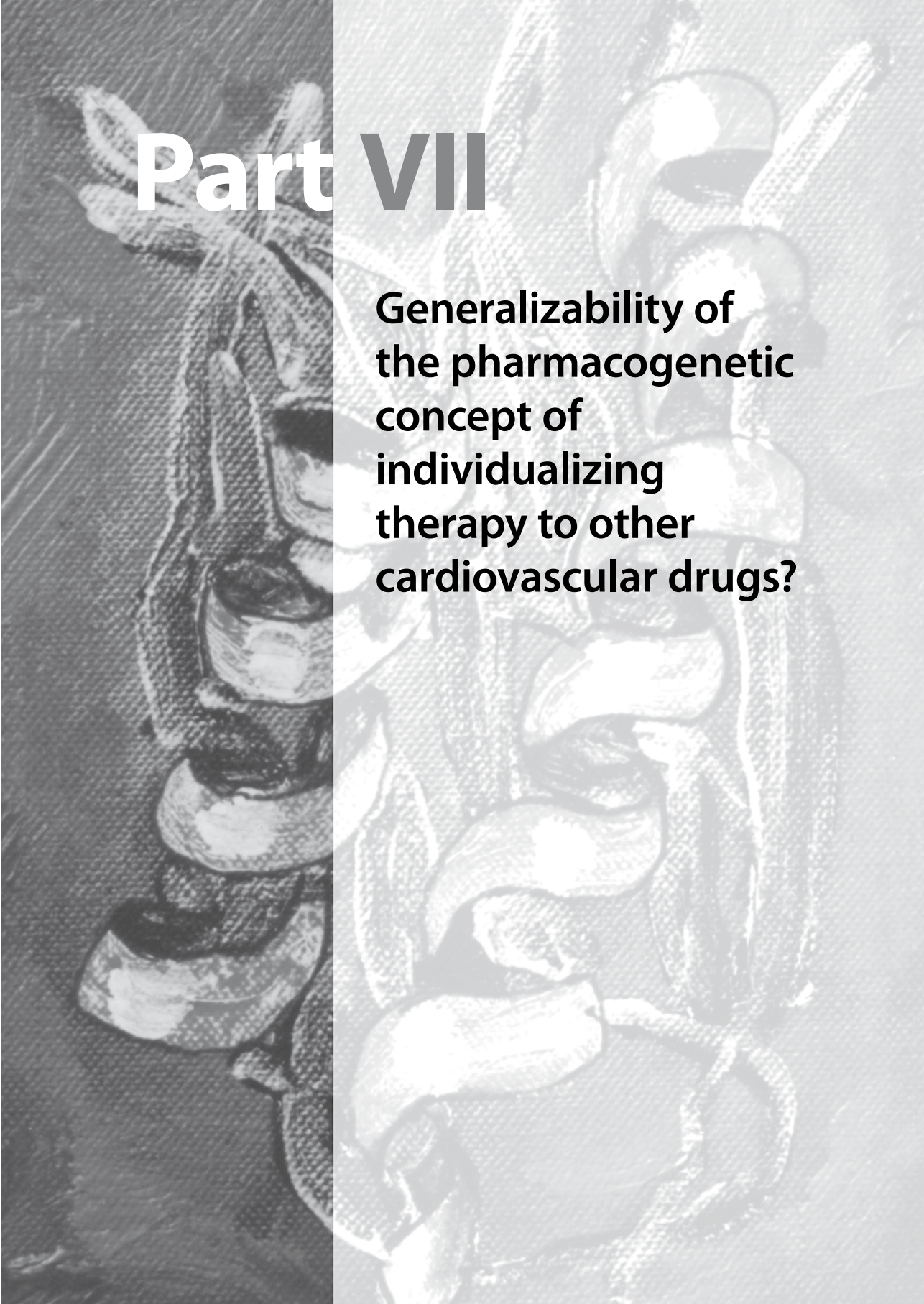
## REFERENCES


1. Pellacani A, Brunner HR, Nussberger J: Plasma kinins increase after angiotensin enzyme inhibition in human subjects. *Clin Sci* (Lond) 1994; 87: 567–574.
2. Ferrari R: Angiotensin-converting enzyme inhibition in cardiovascular disease: evidence with perindopril. *Expert Rev Cardiovasc Ther* 2005; 3: 15–29.
3. Brugts JJ, den Uil CA, Danser AHJ, Boersma E. The Renin-Angiotensin-Aldosterone System: Approaches to Guide Angiotensin-Converting Enzyme Inhibition in Patients with Coronary Artery Disease. *Cardiology 2009*;112:303–312.
4. Pfeffer MA, Braunwald E, Moye LA, SAVE Investigators, et al.Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction and myocardial infarction: results of the survival and ventricular enlargement trial. *N Engl J Med* 1992;327:669–77.
5. The SOLVD investigators. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. *N Engl J Med* 1991;325:293–302.
6. The Acute Infarction Ramipril Efficacy (AIRE) study investigators. Effect of ramipril on mortality and morbidity of survivors of acute myocardial infarction with clinical evidence of heart failure. *Lancet* 1993;342:821–8.
7. Torp-Pedersen C, Kober L, TRACE Study Group. Effect of ACE inhibitor trandolapril on life expectancy of patients with reduced left-ventricular function after acute myocardial infarction. *Lancet* 1999;354:9–12.
8. The Heart Outcomes Prevention Evaluation study investigators. Effects of an angiotensin-converting enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. *N Engl J Med* 2000;342:145–53.
9. The PEACE Trial Investigators. Angiotensin-converting-enzyme inhibition in stable coronary artery disease. *N Engl J Med* 2004;351:2058–68.
10. The European Trial on Reduction of Cardiac Events with Perindopril in Patients with Stable Coronary Artery Disease investigators. Efficacy of perindopril in reduction of cardiovascular events in stable coronary artery disease: randomized, double-blind, placebo controlled, multicentre trial (the EUROPA study). *Lancet* 2003;362:782–8.
11. The Task Force on the Management of Stable Angina Pectoris of the European Society of Cardiology. Guidelines on the management of stable angina pectoris. *Eur Heart J* 2006;27:1341–81.
12. Wood D, DeBacker G, Faergam O, Graham I, Mancia G, Pyörälä K, and the task force. Prevention of coronary disease in clinical practice: recommendation of the second Joint Task Force of European and other societies on Coronary Prevention. *Atherosclerosis* 1998;140:99–270.
13. Gibbons RJ, Abrams J, Chatterjee K, et al. ACC/AHA 2002 guideline update for the management of patients with chronic stable angina—summary article: a report of the American College of Cardiology/American Heart Association Task Force on practice guidelines (Committee on the Management of Patients With Chronic Stable Angina). *J Am Coll Cardiol* 2003;41:159–68.
14. Brugts JJ, Ferrari R, Simoons ML. Angiotesin-converting enzyme inhibition by perindopril in the treatment of cvardiovascular disease. *Exp Rev Cardiovasc* 2009; 7(4): 345-360.
15. Bots ML, Remme WJ, Lüscher TF, et al. ACE inhibition and endothelial function: main findings of PERFECT, a sub-study of the EUROPA trial. *Cardiovasc Drugs Ther* 2007;21:269–79.
16. Rodriguez-Granillo GA, Vos J, De Feyter PJ. Long-term effect of perindopril on coronary atherosclerosis progression (from the PERindopril's Prospective Effect on Coronary aTherosclerosis by Angiography and IntraVascular Ultrasound Evaluation [PERSPECTIVE] Study). *Am J Cardiol* 2007;100:159–63.
17. Ceconi C, Fox KM, Ferrari R, et al. ACE inhibition with perindopril and endothelial function. Results of a substudy of the EUROPA study: PERTINENT. *Cardiovasc Res* 2007;73:237–46.
18. Remme WJ, Deckers JW, Fox KM, Ferrari R, Bertrand M, Simoons ML. for the EUROPA investigators. Secondary prevention of coronary disease with ACE inhibition—does blood pressure reduction with perindopril explain the benefits in EUROPA? *Cardiovasc Drugs Ther* 2009;23

19. Dagenais GR, Pogue J, Fox K, Simoons ML, Yusuf S. Angiotensin converting-enzyme inhibitors in stable vascular disease without left ventricular systolic dysfunction or heart failure: a combined analysis of three trials. *Lancet* 2006;368:581–8.
20. Deckers JW, Goedhart DM, Boersma E, et al. Treatment benefit by perindopril in patients with stable coronary artery disease at different levels of risk. *Eur Heart J* 2006;27:796–801.
21. Brugts JJ, Knetsch AM, Mattace-Raso FU, Hofman A, Witteman JC: Renal function and risk of myocardial infarction in an elderly population: the Rotterdam study. *Arch Intern Med* 2005; 165: 2659–2665.
22. Brugts JJ, Boersma E, Chonchol M, et al. The cardioprotective effects of the angiotensin-converting enzyme inhibitor perindopril in patients with stable coronary artery disease are not modified by mild to moderate renal insufficiency: insights from the EUROPA trial. *J Am Coll Cardiol* 2007;50:2148–55.
23. Brugts JJ, Ninomiya T, Boersma E, Remme WJ, Vertrand M, Ferrari R, Fox K, MacMahon S, Chalmers J, Simoons ML. The consistency of the treatment effect of an ACE-inhibitor based treatment regimen in patients with vascular disease or high risk of vascular disease: a combined analysis of individual data of ADVANCE, EUROPA, and PROGRESS trials. *Eur Heart J* 2009; 30, 1385–1394.
24. Danser AHJ, Schunkert H. Renin–angiotensin system gene polymorphisms: potential mechanisms for their association with cardiovascular diseases. *Eur J Pharmacol* 2000;410:303–16.
25. Brugts JJ, de Maat M, den Uil CA, et al. Pharmacogenetics of ACE inhibition in stable coronary artery disease: steps towards tailored drug therapy. *Curr Opin Cardiol* 2008;23:296–301.
26. Simon T, Verstuyft C, Mary-Kraue M, Quteineh L, Drouet E, Meneveau N, Steg PG, Ferrieres J, Danchin N, Becquemont L, for the FAST-MI Investigators. Genetic Determinants of Response to Clopidogrel and Cardiovascular Events. *New Engl J Med* 2009;360:363-375.
27. The SEARCH collaborative group, SLCO1B1 variants and statin-induced myopathy, a genome wide study, *N Engl J Med* 2008, 359:789-799.
28. International Warfarin Pharmacogenetics Consortium**,** Klein TE**,** Altman RB**,** Eriksson N**,** Gage BF**,** Kimmel SE**,** Lee MT**,** Limdi NA**,** Page D**,** Roden DM**,** Wagner MJ**,** Caldwell MD**,** Johnson JA**.** Estimation of the warfarin dose with clinical and pharmacogenetic data. *N Engl J Med.* 2009 Feb 19;360(8):753-64.
29. Arnett DK, Davis BR, Ford CE, et al. Pharmacogenetic association of the angiotensin-converting enzyme insertion/deletion polymorphism on blood pressure and cardiovascular risk in relation to antihypertensive treatment: the Genetics of Hypertension-Associated Treatment (GenHAT) study. *Circulation* 2005;28;111:3374-83.
30. *PROGRESS Collaborative Group.* The ACE gene I/D polymorphism is not associated with the blood pressure and cardiovascular benefits of ACE inhibition. *Hypertension* 2003;42:297-303.
31. Xiaowen Su, Liming Lee, Xiaohui Li, Jun Lv, Yonghua Hu, Siyan Zhan, Weihua Cao, Ling Mei, Yong-Ming Tang, Dai Wang, Ronald M. Krauss, Kent D. Taylor, Jerome I. Rotter and Huiying Yang**.** Association Between Angiotensinogen, Angiotensin II Receptor Genes, and Blood Pressure Response to an Angiotensin-Converting Enzyme Inhibitor. *Circulation* 2007;115;725-732
32. Brugts JJ, de Maat M, Boersma E, Witteman J, van Duijn C, Uitterlinden A, Bertrand M, Remme W, Fox K, Ferrari R, Danser AHJ, Simoons ML. The Rationale and Design of the Perindopril Genetic Association Study (PERGENE): A Pharmacogenetic Analysis of Angiotensin-Converting Enzyme Inhibitor Therapy in Patients with Stable Coronary Artery Disease. *Cardiovasc Drugs Ther* 2009;23:171–181.
33. Brugts JJ, Isaacs A, Boersma E, van Duijn C, Uitterlinden AG, Remme WJ, Bertrand M, Ninomiya T, Ceconi C, Chalmers J, MacMahon S, Fox K, Ferrari R, Witteman J, Danser AHJ, Simoons ML, de Maat M. Genetic Determinants of Treatment Benefit of ACE-inhibitor Therapy in Patients with Stable Coronary Artery Disease. Eur Heart J 2010 in press.
34. Merino VF, Todiras M, Mori MA, Sales VMT, Fonseca RG, Saul V, Tenner K, Bader M, Pesqueo JB. Predisposition to atherosclerosis and aortic aneurysms in mice deficient in kinin B1 receptor and apolipoprotein E. *J Mol Med* DOI 10.1007/s00109-009-0501-0
35. Luft FC. Aneurysm formation and bradykinin. *J Mol Med* DOI 10.1007/s00109-009-0517-5

36. Gainer JV, Morrow JD, Loveland A, King DJ, Brown NJ. Effect of bradykinin-receptor blockade on the response to angiotensin converting-enzyme inhibitor in normotensive and hypertensive subjects. *N Engl J Med* 1998;339:1285–92.
37. Xu J, Carretero OA, Shesely EG, Rhaleb NE, Yang JJ, Bader M, Yang XP. The Kinin B1 Receptor Contributes to the Cardioprotective Effect of ACE Inhibitors and Angiotensin Receptor Blockers. *Exp Physiol.* 2009 Mar;94(3):322-9.
38. Marin-Castaño ME, Schanstra JP, Neau E, Praddaude F, Pecher C, Ader JL, Girolami JP, Bascands JL. Induction of functional bradykinin b(1)-receptors in normotensive rats and mice under chronic Angiotensin-converting enzyme inhibitor treatment. *Circulation*. 2002;5;627-32.
39. Ignatovic T, Tan F, Brovkovych V, Skidgel RA, Erdos GE. Novel Mode of Action of Angiotensin I Converting Enzyme Inhibitors: Direct activation of the Bradykinin Receptor type I. *The Journal of Biological Chemistry* 2002; 277, 16847–16852.
40. Ignjatovic T, Stanisavljevic S, Brovkovych V, Skidgel RA, Erdos EG. Kinin B1 Receptors Stimulate Nitric Oxide Production in Endothelial Cells: Signaling Pathways Activated by Angiotensin I-Converting Enzyme Inhibitors and Peptide Ligands. *Mol. Pharmacol*. 2004; 66:1310-16.
41. Forten JP, Gobeil F, Adam A, Regoli D, Marceau F. Do angiotensin-converting enzyme inhibitors directly stimulate the kinin B1 receptor? *Am J Physiol Heart Circ Physiol*. 2003;285:H277-82.
42. Su JB, Hoüel R, Héloire F, Barbe F, Beverelli F, Sambin L, Castaigne A, Berdeaux A, Crozatier B, Hittinger L. Stimulation of Bradykinin B1 Receptors Induces Vasodilation in Conductance and Resistance Coronary Vessels in Conscious Dogs. *Circulation* 2000; 101: 1848 - 1853.
43. Austinat M, Braeuninger S, Pesquero JB, Brede M, Bader M, Stoll G, Renné T, Kleinschnitz C. Blockade of bradykinin receptor B1 but not bradykinin receptor B2 provides protection from cerebral infarction and brain edema. *Stroke* 2009;40:285-93.
44. Brugts JJ, Yetgin T, Hoeks S, Gotto A, Knopp R, Shepherd J, Westendorp R, Nakamura H, Ridker P, van Domburg R, Deckers J. The benefit of HMG CoA reductase inhibitors in patients without established cardiovascular disease with cardiovascular risk factors. *BMJ* 2009;30;338:b2376.



# Part VII

## Generalizability of the pharmacogenetic concept of individualizing therapy to other cardiovascular drugs?

# Chapter 14

## The benefit of HMG CoA reductase inhibitors in patients without established cardiovascular disease.

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*BMJ 2009;30(338):2376-2386*

## ABSTRACT

**Objectives:** To investigate whether statins reduce all cause mortality and major coronary and cerebrovascular events in people without established cardiovascular disease but with cardiovascular risk factors, and whether these effects are similar in men and women, in young and older (>65 years) people, and in people with diabetes mellitus.

**Methods:** Meta-analysis of randomised trials. Data sources Cochrane controlled trials register, Embase, and Medline. Two independent investigators identified studies on the clinical effects of statins compared with a placebo or control group and with follow-up of at least one year, at least 80% or more participants without established cardiovascular disease, and outcome data on mortality and major cardiovascular disease events. Heterogeneity was assessed using the Q and $I^2$ statistics. Publication bias was assessed by visual examination of funnel plots and the Egger regression test.

**Results**: Ten trials enrolled a total of 70388 people, of whom 23681 (34%) were women and 16078 (23%) had diabetes mellitus. Mean follow-up was 4.1 years. Treatment with statins significantly reduced the risk of all cause mortality (odds ratio 0.88, 95% confidence interval 0.81 to 0.96), major coronary events (OR 0.70, 95% CI 0.61 to0.81), and major cerebrovascular events (OR 0.81, 95% CI 0.71 to 0.93). No evidence of an increased risk of cancer was observed. There was no significant heterogeneity of the treatment effect in clinical subgroups.

**Conclusion:** In patients without established cardiovascular disease but with cardiovascular risk factors, statin use was associated with significantly improved survival and large reductions in the risk of major cardiovascular events.

## INTRODUCTION

Cardiovascular disease is the leading cause of death and disability in the Western world and contributes substantially to healthcare budgets [1]. Several clinical trials and meta-analyses have shown the beneficial effects of lipid lowering treatment using hydroxylmethyl glutaryl coenzyme A reductase inhibitors (statins) in reducing mortality and cardiovascular morbidity in patients with established cardiovascular disease [2-6]. Statins therefore have a place in the secondary prevention of cardiovascular disease [7-10].

The use of statins in patients without established cardiovascular disease (that is, primary prevention) and at relatively low risk has important public health implications. To date research has provided ambiguous answers. In addition, the reliability of treatment in older people (>65 years), women, and those with diabetes mellitus is uncertain, mainly because of a lack of data or inconsistent findings within these clinically defined groups [11,12]. Most meta-analyses have been carried out on published tabular data and failed to provide consistent answers on treatment effect in these subgroups [13,14]. We carried out a meta-analysis of randomised trials that focused on primary prevention to determine whether statins reduce all cause mortality and the incidence of major coronary and cerebrovascular events in people without established cardiovascular disease but with cardiovascular risk factors. We also assessed whether these effects differed by sex, age, and the presence of diabetes.

## METHODS

We followed the quality of reporting of meta-analysis guidelines [15]. We searched the Cochrane Central Register of Controlled Trials, Medline (1990-November 2008), Embase (1980-November 2008), DARE, and the ACP Journal Club for randomised clinical trials that compared statins with a control group in people without established cardiovascular disease but with cardiovascular risk factors. We identified relevant studies using the MeSH terms "HMG-CoA reductase inhibitor", "atorvastatin", "simvastatin", "pravastatin", "fluvastatin", "rosuvastatin", or "lovastatin", and "cardiovascular disease", "coronary heart disease", "cerebrovascular disease", or "myocardial infarction", and "cholesterol", "LDL" [low density lipoprotein], "HDL" [high density lipoprotein], or "triglycerides", and primary prevention restricted to randomised controlled trials or meta-analyses. In addition we examined the reference lists and related links of retrieved articles in PubMed to detect studies potentially eligible for inclusion.

### Study selection

We included studies if they were randomised trials of statins compared with controls (placebo, active control, or usual care), had a mean follow-up of at least one year, reported on mortality or cardiovascular disease events as primary outcomes, and included at least 80% of people

without established cardiovascular disease or reported data separately on a sole primary prevention group and provided specific numbers for patients and events in that group. Eight studies were excluded that primarily investigated statin related non-clinical and intermediate surrogate end points such as changes in the thickness of the carotid intima media and lipid levels that collectively contributed fewer than 50 clinical events [16-23]. We also excluded one study in patients with renal transplants because of the specific nature of that population,[24] and three studies with design problems, fewer than 20 events overall, and insufficient follow-up [25-27]. Our study therefore focused on people without established cardiovascular disease but with cardiovascular risk factors.

## Validity assessment

Our search identified 1230 studies, of which 10 fulfilled our inclusion criteria.[w1-w10] Figure 1 summarizes the results of the search. We evaluated suitable trials for concealment of treatment allocation, performance of the analysis according to the intention to treat principle, and completeness of follow-up. The Jadad scale was used to score study quality (range 0-5, higher scores indicating better quality) [28]. Study quality was sufficient (≥4) for all included randomised clinical trials.



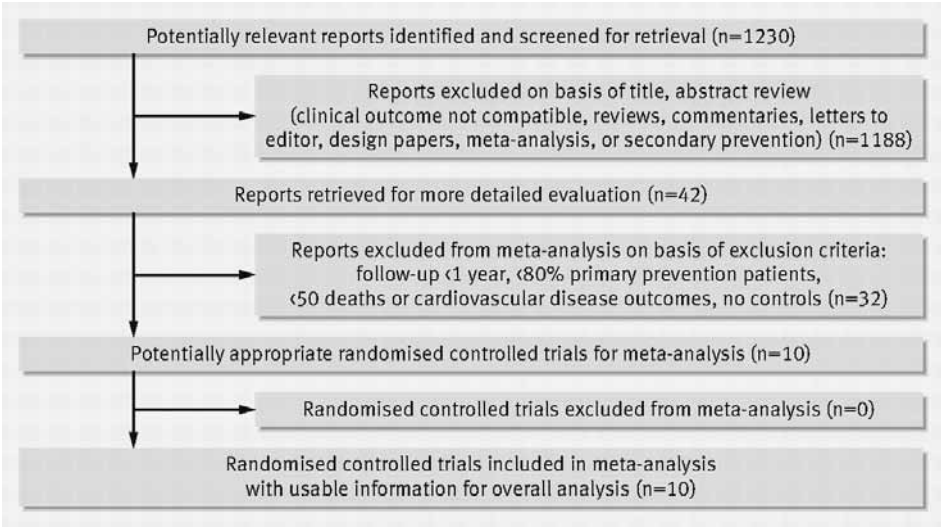


**Figure 1.** Flow diagram of included trials. Odds ratios (95% confidence intervals) for all cause mortality, major coronary events, major cerebrovascular events, and incidence of cancer. Mortality risk based on mean follow-up of 4.1 years, with data from nine trials, and 67476 patients free of cardiovascular disease (no data available from HPS diabetic arm[w5]. Risk of coronary events based on mean follow-up of 4.9 years, with data from eight trials, and 50681 patients free of cardiovascular disease (no data available from ASPEN[w3] and JUPITER[w1]). Risk of cerebrovascular events based on mean follow-up of 4.1 years, with data from nine trials, and 67476 patients free of cardiovascular disease (no data available from HPS diabetic arm). Risk of cancer based on mean follow-up of 3.9 years, with data from six trials, and 52027 patients free of cardiovascular disease (no data available from HPS, ASCOT,[w10] PROSPER,[w6] and ASPEN). See footnote to table 1 for full titles of studies. *Measures of heterogeneity.

## Data abstraction

From each study two investigators separately extracted information on trial characteristics, patient data, outcome measures, and study quality using a standardized protocol and reporting document. Disagreements were resolved by consensus.

## Subgroup analysis

We searched the papers for data on clinically defined subgroups. The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT)[w7] presented data on our prespecified subgroups. The other studies did not publish results stratified by age (<65 or >65 years), sex, or diabetes. To obtain data for these stratified groups we sent an electronic sheet with data fields to the principal investigators of these studies, requesting the number of events and number of patients in the treatment and placebo groups. We obtained data on subgroups for five trials.[w1-w3 w6 w8] Subgroup analyses were therefore done in six studies. [w1-w3 w6 w7] Not all end points were recorded in these studies.

## End points

The primary end point of our meta-analysis was all cause mortality. Secondary end points were the composite of major coronary events defined as death from coronary heart disease and non-fatal myocardial infarction, and the composite of major cerebrovascular events defined as fatal and non-fatal stroke. We also assessed death from coronary heart disease, non-fatal myocardial infarction, revascularisations (percutaneous coronary intervention or coronary artery bypass graft), and cancer (fatal and non-fatal). The clinical outcomes evaluated in the subgroup analysis (data should be reported in two or more studies) were all cause mortality, major coronary events, major cerebrovascular events, and cancer.

## Quantitative data synthesis

For each trial we calculated the summary odds ratios and 95% confidence intervals for the clinical outcomes. We pooled studies using both fixed effect and random effects models [(29)]. A random effects model makes the assumption that individual studies are estimating different treatment effects. Our conclusions were drawn from the results of the random effects model. We were unable to exclude a small proportion of secondary prevention patients from the West of Scotland Coronary Prevention Study (1069/6595; WOSCOPS),[w9] ALLHAT (1470/10355),[w7] and the Anglo-Scandinavian Cardiac Outcomes Trial lipid lowering arm (1906/ 10305; ASCOT-LLA),[w10] and these therefore constitute about 6% of our study population (4445/70388) [(30, w7 w9)]. In a separate analysis we verified whether our results remained consistent after exclusion of these studies. We also investigated whether our results differed when we used the original study results from ASCOT without extended follow-up [(30, w10)].

We assessed the results for heterogeneity in the main analysis and subgroup analysis by examining the forest plots and then calculating a Q statistic, which we compared with a $\chi^2$ distribution, and the $I^2$ index [31]. The Q test indicates the statistical significance of the homogeneity hypothesis and the $I^2$ index measures the extent of the heterogeneity. We considered the results for heterogeneity to be significant at P<0.10 (two sided). Publication bias was assessed for the main end points by visually examining for funnel plot asymmetry and quantified by using the Egger regression test to calculate two tailed P values [32].

## RESULTS

Table 1 shows the characteristics of the 10 included studies [w1-w10]. In total, 70388 participants were randomised, of whom 35138 were allocated to statin therapy and 35250 to control. The number of participants in the trials ranged from 1905 to 17802. The mean age was 63 years (range 55.3 to 75.0), and the mean follow-up was 4.1 years (range 1.9 to 5.3). Thirty four per cent of participants were women and 23% had diabetes. The mean baseline low density lipoprotein cholesterol level was 3.63 mmol/l. The mean reduction in levels of total cholesterol was 17.1%, low density lipoprotein cholesterol was 25.6%, and triglyceride was 9.3%. High density lipoprotein cholesterol increased by a mean 3.3%.



### Mortality, coronary events, and cerebrovascular events

During a mean follow-up of 4.1 years 5.7% (1925/33793) of participants died in the control group compared with 5.1% (1725/33683) in the statin group. Statin therapy was therefore associated with a 12% risk reduction in all cause mortality compared with the control (odds ratio 0.88, 95% confidence interval 0.81 to 0.96; figure 2 and table 2). The annual rate for all cause mortality with placebo in our study was 1.4% (figure 2). Overall, 5.4% (1266/23946) of participants in the control group had a major coronary event compared with 4.1% (966/23 823) in the statin group, a 30% risk reduction (odds ratio 0.70, 95% confidence interval 0.61 to 0.81). The annual rate for major coronary events with placebo in our study was 1.1% (figure 2). Overall, 2.3% (767/33793) of participants in the control group had a major cerebrovascular event compared with 1.9% (627/33683) in the statin group, a 19% risk reduction (OR 0.81, 95% CI 0.71 to 0.93). The annual rate for major cerebrovascular events with placebo in our study was 0.6% (figure 2). The annual rate for coronary heart disease mortality with placebo in our study was 0.3%, for non-fatal myocardial infarction it was 0.6%, for revascularization it was 0.6%, and for incidence of cancer it was 1.2%. The association between statin therapy and risk of cancer was not significant (OR 0.97, 95% CI 0.89 to 1.05; figure 2 and table 2). Table 2 also shows the summary odds ratios for other end points. The outcome of the analyses was not influenced by removal of the three trials that enrolled 4445 patients (6%) with a previous cardiovascular event (all cause mortality OR 0.87, 95% CI 0.78 to 0.97). Also, using only the first reported data from

**Table 1.** Baseline characteristics of study populations.

| Characteristic | WOSCOPS 1995 | AFCAPS 1998 | PROSPER* 2002 | ALLHAT 2002 | ASCOT-LLA 2003 | HPS* 2003 | CARDS 2004 | ASPEN* 2006 | MEGA 2006 | JUPITER 2008 |
|---|---|---|---|---|---|---|---|---|---|---|
| Target population | Men with hypercholesterol-emia (no history of MI) | Patients with average cholesterol levels (without CVD) | Elderly patients with vascular disease risk factors | Patients with hypertension, moderate HCH and at least 1 CHD risk factor | Patients with hypertension, average levels and at least 3 other risk factors | Patients with diabetes | Patients with diabetes and low LDL cholesterol (no CVD) | Patients with diabetes, and low LDL-C levels | Patients with HCH without CVD | Patients without CVD; LDL < 130 mg/dl and Hs-CRP >2.0 mg/l |
| Design | RCT, dbpc | RCT, dbpc | RCT, dbpc | RCT, | RCT, dbpc | RCT, dbpc | RCT, dbpc | RCT, dbpc | RCT, dbpc, | RCT, dbpc |
| Drug | Pravastatin | Lovastatin | Pravastatin | Pravastatin | Atorvastatin | Simvastatin | Atorvastatin | Atorvastatin | Pravastatin | Rosuvastatin |
| Dose, mg/day | 40 | 20-40 | 40 | 20-40 | 10 | 40 | 10 | 10 | 10 20 | 20 |
| Mean follow-up, y. | 4.9 | 5.2 | 3.2 | 4.8 | 5.5† | 4.8 | 3.9† | 4.0† | 5.3 | 1.9† |
| Age range, y. | 45-64 | 45-73 | 70-82 | 51-81 | 40-79 | 40-80 | 40-75 | 40-75 | 40-70 | 60-71 |
| Mean age, y. | 55.3 | 58 | 75 | 66.4 | 63.1 | NA | 61.5 | 60.5 | 58.3 | 66† |
| Women, % | 0 | 15 | 58‡ | 49 | 18.9 | NA | 32 | 38 | 68.4 | 37.9 |
| Diabetes, % | 1 | 3.8 | 12.2‡ | 34.4 | 24.3 | 100 | 100 | 100 | 21 | 0 |
| Smoking, % | 44 | 13 | 33.4‡ | 23.3 | 33.2 | NA | 22 | 12 | 21 | 16 |
| Hypertension, % | 16 | 22 | 71.6‡ | 89.9 | 80.3 | NA | 84 | 52 | 42 | 0 |
| BMI, mean, kg/m$^2$ | 26 | 26.8 | 27‡ | 29.9 | 28.6 | NA | 28.7 | 28.9 | 23.8 | 28.4† |
| Mean SBP, mm Hg | 136 | 138 | 156.6‡ | 145 | 164.2 | NA | 144 | 133 | 132 | 134† |
| Mean DBP, mm Hg | 84 | 78 | 85.2‡ | 84 | 95 | NA | 83 | 77.1 | 78.4 | 80† |
| Lipids (change, %) | | | | | | | | | | |
| Total cholesterol | 7.0 (-20.0) | 5.7 (-19.3) | 5.7 (NA) | 5.9 (-9.6) | 5.5 (-18.2) | NA | 5.4 (-21.8) | 5.0 (-19.8) | 6.3 (-11.0) | 4.8 (NA) |
| LDL-C | 5.0 (-26.0) | 3.9 (-26.5) | 3.8 (NA) | 3.8 (-16.7) | 3.4 (-27.6) | NA | 3.0 (-33.9) | 3.0 (-30.5) | 4.0 (-18.0) | 2.8 (NA) |
| Triglycerides | 1.8 (-12.0) | 1.7 (-12.7) | 1.5 (NA) | 1.7 (0.0) | 1.7 (-12.6) | NA | 2.0 (-15.9) | 1.6 (-4.7) | 1.4 (-7.0) | 1.3 (NA) |

To convert cholesterol to milligrams per deciliter, divide values by 0.0259; to convert triglycerides to milligrams per deciliter, divide values by 0.0113. dbpc=double-blind placebo controlled, RCT=randomized controlled trial, SBP=systolic blood pressure, DBP=diastolic blood pressure, BMI=body mass index, LDL-C=low-density cholesterol, HDL-C=high-density cholesterol, NA=not available. *Primary prevention subgroup data used. †Median; in ASCOT-LLA we used the extended follow-up from the extended observations trial.[37] ‡Data obtained from baseline characteristics publication of PROSPER.[47]

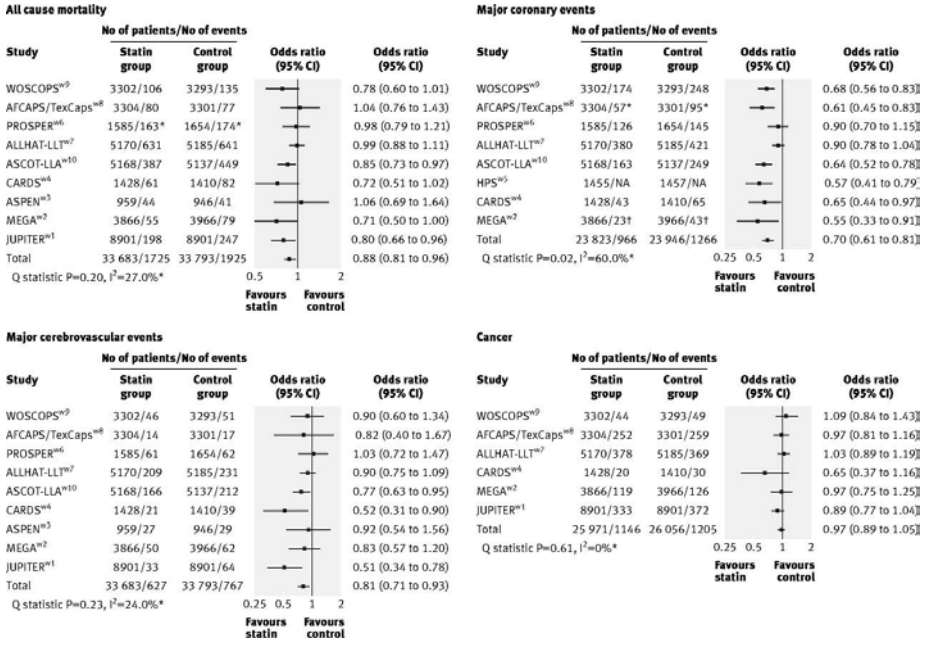


**Figure 2.** Odds ratios (95% CI) for all cause mortality, major coronary events, major cerebrovascular events, and incidence of cancer. NR = not reported. See footnote to table 1 for full titles of studies. *Data from Thavendiranathan et al.[14].Fixed effect and random effect models in meta-analysis gave identical results, making important statistical heterogeneity unlikely. †No data in primary prevention group (n=3239).[w6] ‡Significant heterogeneity; however, a positive trend of statin therapy is observed in all trials, only of different magnitude (no neutral or negative trials).



ASCOT-LLA instead of the extended follow-up data that were published later did not influence the result of the analyses (OR 0.88, 95% CI 0.81 to 0.97) [w10]. When the only study (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin; JUPITER)[w1] that found a significant effect on mortality was removed from the analysis, the reduction in mortality in the other nine trials remained significant (OR 0.89, 95% CI 0.81 to 0.97). No funnel plot asymmetry was visualised for the main end points, and P values using the Egger regression test were greater than 0.10 for all the major end points (all cause mortality: intercept −0.8, 95% CI -3.1 to 1.5; P value 0.42).

## Subgroup analyses

No heterogeneity in treatment effect was observed for end points in men and women and for age (≤65 and >65 years) or diabetic status (figure 3).

**Table 2.** Treatment Effects of Statin Therapy (OR; 95% CI)

| Clinical trial | All-cause mortality | Major Coronary Events | Major Cerebrovascular Events | Fatal or Nonfatal Cancer |
|---|---|---|---|---|
| WOSCOPS | 0.78 (0.60 to 1.01) | 0.68 (0.56 to 0.83) | 0.90 (0.60 to 1.34) | 1.09 (0.84 to 1.43) |
| AFCAPS | 1.04 (0.76 to 1.43) | 0.61 (0.45 to 0.83) | 0.82 (0.40 to 1.67)† | 0.97 (0.81 to 1.16) |
| PROSPER | 0.98 (0.79 to 1.21)† | 0.90 (0.70 to 1.15) | 1.03 (0.72 to 1.47) | NR* |
| ALLHAT-LLT | 0.99 (0.88 to 1.11) | 0.90 (0.78 to 1.04) | 0.90 (0.75 to 1.09) | 1.03 (0.89 to 1.19) |
| ASCOT-LLA | 0.85 (0.73 to 0.97) | 0.64 (0.52 to 0.78) | 0.77 (0.63 to 0.95) | NR |
| HPS | NR | 0.57 (0.41 to 0.79)† | NR | NR |
| CARDS | 0.72 (0.51 to 1.02) | 0.65 (0.44 to 0.97) | 0.52 (0.31 to 0.90) | 0.65 (0.37 to 1.16) |
| ASPEN | 1.06 (0.69 to 1.64) | NR | 0.92 (0.54 to 1.56) | NR |
| MEGA | 0.71 (0.50 to 1.00) | 0.55 (0.33 to 0.91) | 0.83 (0.57 to 1.20) | 0.97 (0.75 to 1.25) |
| JUPITER | 0.80 (0.66 to 0.96) | NR | 0.51 (0.34 to 0.78) | 0.89 (0.77 to 1.04) |
| **Combined:** | | | | |
| - Fixed effects | 0.90 (0.84 to 0.96) | 0.74 (0.68 to 0.81) | 0.82 (0.74 to 0.91) | 0.97 (0.89 to 1.05) |
| - Random effect | 0.88 (0.81 to 0.96) | 0.70 (0.61 to 0.81) | 0.81 (0.71 to 0.93) | 0.97 (0.89 to 1.05) |
| **Heterogeneity:** | | | | |
| - Q-statistic | 0.20 | 0.02‡ | 0.23 | 0.61 |
| - I-square index | Low (27 %) | Moderate (60 %) | Low (24 %) | Low (0 %) |

Data are reported as odds ratios (95% confidence interval). NR=trial did not report data. *No data of PROSPER presented on malignancies in the primary prevention group (N=3239)[33] †Data from Thavendiranathan et al.[14] The fixed effect and random effect models in our study gave almost identical results, which makes it unlikely, that there is important statistical heterogeneity. ‡ significant heterogeneity, however in all trials a positive trend of statin therapy is observed (no neutral or negative trials).

## DISCUSSION

The current meta-analysis totaled 70388 participants without established cardiovascular disease but with cardiovascular risk factors who were randomized to statin therapy or control. Statin therapy was associated with a significant risk reduction in all cause mortality of 12%, in major coronary events of 30%, and in major cerebrovascular events of 19%. Moreover, statin use was not associated with an increased risk of cancer. These results are in line with those previously published on the effects of statins in secondary prevention.

Our meta-analysis differs from earlier analyses in several ways[13,14]. We were able to include several recently published studies targeted at primary prevention that enrolled a large number of women and people with diabetes.[w1-w3] For example, the Management of Elevated Cholesterol in the Primary Prevention Group of Adult Japanese trial (MEGA)[w2] comprised a large number of women (68%, 5356/7832), and we were able to obtain subgroup data. Additionally, the Atorvastatin Study for Prevention of Coronary Heart Disease Endpoints in Non-Insulin-Dependent Diabetes Mellitus study (ASPEN)[w3] was carried out in a large group of people with type 2 diabetes (n=1905) who did not have established cardiovascular disease.

We also included data from the recently published JUPITER trial,[w1] totaling 17802 participants with no apparent vascular disease, low density lipoprotein cholesterol levels less than 3.4

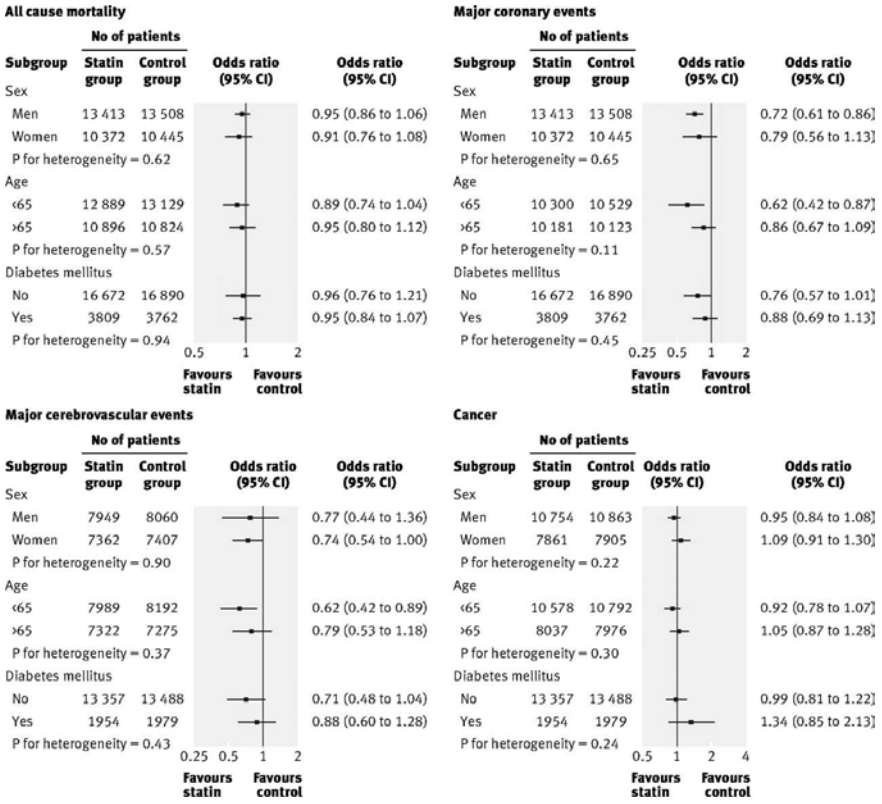


**Figure 3.** Odds ratios (95% CI) for clinically defined subgroups of sex, age, and diabetes for end points of all cause mortality, major coronary events, major cerebrovascular events, and cancer. Odds ratios (95% CI) for clinically defined subgroups of sex, age, and diabetes for end points of all cause mortality, major coronary events, major cerebrovascular events, and cancer. Subgroup data are obtained from AFCAPS,[w8] PROSPER,[w6] ASPEN,[w3] MEGA,[w2] and JUPITER[w1], and for mortality and coronary events from ALLHAT-LLT.[w7] We had complete mortality data from all six trials for sex; for age, no data from PROSPER on age<65; for diabetes, no data from ASPEN and AFCAPS on participants without diabetes, and no data from AFCAPS and JUPITER on participants with diabetes. For cardiovascular events, studies included in subgroup analysis were same as for mortality, except no data from AFCAPS for age groups. For cerebrovascular disease, no data from AFCAPS and ALLHAT for all subgroups; also no data from PROSPER on age <65, from ASPEN for participants without diabetes, and from JUPITER for participants with diabetes. For cancer no subgroup data were obtained from ALLHAT; also no data for age <65 from PROSPER, for participants without diabetes from AFCAPS and ASPEN, and for participants with diabetes from JUPITER and AFCAPS. See footnote to table 1 for full titles of studies.

mmol/l, and increased levels of high sensitivity C reactive protein (>2.0 mg/l).[w1] As our study is based on such large numbers, this meta-analysis, including the subgroups, has significant statistical power. Previously, only the JUPITER trial showed improved survival associated with statin use in high risk participants, but it is clear from the current analysis that a mortality benefit is a shared characteristic of long term statin use in people without previous cardiovascular

disease. The currently observed benefit, a 12% risk reduction in mortality, may even be an underestimation of the true effect because subsequent death after a morbid cardiovascular event was not always considered in individual trials.

The numbers and duration of follow-up of our study allow for relatively strong inferences on risk of cancer with long term statin use. We found no evidence for an increased risk of cancer, fatal or non-fatal. One of the trials (Prospective Study of Pravastatin in the Elderly at Risk; PROSPER)[w6] did report an increased risk of cancer with use of statins among men and women older than 70. Although our results show that statins do not seem to increase the risk of cancer, longer follow-up would be helpful to determine whether new cancer events could occur with time. This is especially critical when statins are used in primary prevention. Follow-up of patients in WOSCOPS for 10 years did not show higher rates of malignancies [34,35]. Concerns might remain about the higher risk of cancer in elderly patients (70-82 years) as in PROSPER[w6] and further follow-up studies in such patients are required. Although this meta-analysis cannot fully remove that uncertainty, it confirms that the risk of cancer is not increased in middle aged patients. Tolerance to statins is also important to tackle in primary prevention. Side effects such as an increase in creatine kinase levels and myopathy have been reported relatively frequently, but rhabdomyolysis and hepatotoxicity are rare[5]. Lastly, by contacting principal investigators of each trial we were able to obtain data on clinically defined subgroups. This allowed us to draw meaningful inferences on treatment effects in large numbers of women, older people, and people with diabetes. Although there is little reason to suspect different treatment effects between such groups from a pathophysiological standpoint, it is reassuring that no significant treatment heterogeneity was found between the sexes, in elderly and young people, and between people with and without diabetes.

## Limitations of the study

Some limitations of our study need to be mentioned. Firstly, we included three trials in the analyses that had recruited a small proportion of patients (about 6%) with clinical cardiovascular disease [30, w7, w9] Exclusion of these trials did not affect the outcome of our analyses. Secondly, the dose and type of statin differed between included trials. Depending on the statin and the dose, some treatment regimens may be more effective in lowering lipid levels. However, according to guidelines from the Adult Treatment Panel III, the statins included in our meta-analysis at their respective doses have similar clinical efficacy [8]. Thirdly, the included trials represented participants with a clinically heterogeneous level of risk (although statistical heterogeneity was low). The benefit observed in the pooled estimate of treatment effect could be of different magnitude depending on the level of risk. However, exclusion of the studies with a small proportion of patients at higher risk did not influence the outcome of the analysis because our subgroup analysis indicated no heterogeneity in clinically defined groups such as elderly participants or those with diabetes mellitus who are at relatively higher risk. Such a risk dependent effect seems unlikely.

## Clinical implications

Our meta-analysis shows that the relative risk reduction from long term statin use in a primary care setting is comparable to that observed in secondary prevention. Our findings confirm the results of JUPITER[w1] regarding the beneficial effect of statins on survival across a broader range of patients (n=70388) at different levels of risk, and show that there is no significant difference in treatment benefit across a range of clinically defined groups (men and women, elderly people, and those with diabetes). Although our study population comprised participants without established cardiovascular disease, the pooled risk was high. The overall annual mortality was in the range of 1.4%, and fatal as well as non-fatal cardiac and cerebrovascular events occurred at an annual rate of about 1.1% and 0.6%, respectively. This is not too different from the event rates reported in trials of patients at relatively low risk in secondary prevention - for example, the European trial on reduction of cardiac events with perindopril in stable coronary artery disease and the Prevention of Events with Angiotensin-Converting Enzyme inhibition trial (PEACE) [(36,37)]. Statin based secondary prevention is considered mandatory in participants in PEACE. Still, the absolute overall treatment benefit observed in the current study population would certainly be less than 1%, and significant numbers of participants would need to be treated to prevent one event. From the currently pooled data it is not possible to exactly define one group of people who would benefit most from long term statin use. From current risk scoring systems, as well from current data, it is obvious that older men (>65 years) with risk factors, or older women with diabetes and risk factors, constitute the highest risk group. In view of the large treatment effects described here, it is likely that a considerable number of such people would benefit from long term statin use at reasonable costs. The correct identification of such people remains a challenge and, in addition to the assessment on the future cardiovascular risk based on standard cardiovascular risk factors, auxiliary diagnostic or prognostic assessments to improve risk prediction could be useful to identify these men and women more accurately. Given the favourable effects of long term statin treatment it would be wrong to deny these benefits to people at increased risk for cardiovascular disease.



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**Competing interests:** AMG is a consultant for Genentech, Kowa, Martek, Merck, and Merck/Schering-Plough, and serves on the board of directors for Aegerion and Arisaph. He is a member of DuPont's health advisory board and serves on the data safety monitoring board for Novartis. JS carried out consultancy work and receives support for research from Bristol-Myers Squibb. RGJW receives support for research from Bristol-Myers Squibb. HN has received travel grants and speaking honorariums from Sankyo. RHK has received research fees and speaking honorariums from Pfizer. PR has received research grant support from the National Heart Lung and Blood Institute, the National Cancer Institute, the Donald W Reynolds Foundation, the Leducq Foundation, Astra-Zeneca, Novartis, Merck, Abbott, Roche, and Sanofi-Aventis; consulting fees and lecture fees from Astra-Zeneca, Novartis, Merck-Schering Plough, Sanofi-Aventis,

ISIS, and Vascular Biogenics; and is listed as a co-inventor on patents held by the Brigham and Women's Hospital that relate to the use of inflammatory biomarkers in cardiovascular disease. These patents have been licensed to Siemens and Astra-Zeneca. Ethical approval: Not required.

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**References list of included studies**

**w1.** Ridker PM, Danielson E, Fonseca FA, Kastelein JJ, Shepherd J, Willerson JT, Glynn RJ; JUPITER Study Group. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med*. 2008 Nov 20;359(21):2195-207.

**w2.** Nakamura H, Arakawa K, Itakura H, Kitabatake A, Goto Y, Toyota T, et al. Primary prevention of cardiovascular disease with pravastatin in Japan (MEGA Study): a prospective randomised controlled trial. *Lancet* 2006;368(9542):1155-63.

**w3.** Knopp RH, d'Emden M, Smilde JG, Pocock SJ. Efficacy and safety of atorvastatin in the prevention of cardiovascular end points in subjects with type 2 diabetes: the Atorvastatin Study for Prevention of Coronary Heart Disease Endpoints in non-insulin-dependent diabetes mellitus (ASPEN). *Diabetes Care* 2006;29(7):1478-85.

**w4.** Colhoun HM, Betteridge DJ, Durrington PN, Hitman GA, Neil HA, Livingstone SJ, et al. Primary prevention of cardiovascular disease with atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): multicentre randomised placebo-controlled trial. *Lancet* 2004;364(9435):685-96.

**w5.** Collins R, Armitage J, Parish S, Sleigh P, Peto R. MRC/BHF Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: a randomized placebocontrolled trial. *Lancet* 2003;361(9374):2005-16.

**w6.** Shepherd J, Blauw GJ, Murphy MB, Bollen EL, Buckley BM, Cobbe SM, et al. Pravastatin in elderly individuals at risk of vascular disease (PROSPER): a randomised controlled trial. *Lancet* 2002;360(9346):1623-30

**w7.** Major outcomes in moderately hypercholesterolemic, hypertensive patients randomized to pravastatin vs usual care: The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT-LLT). *JAMA* 2002;288(23):2998-3007.

**w8.** Downs JR, Clearfield M, Weis S, Whitney E, Shapiro DR, Beere PA, et al. Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS. *JAMA* 1998;279(20):1615-22.

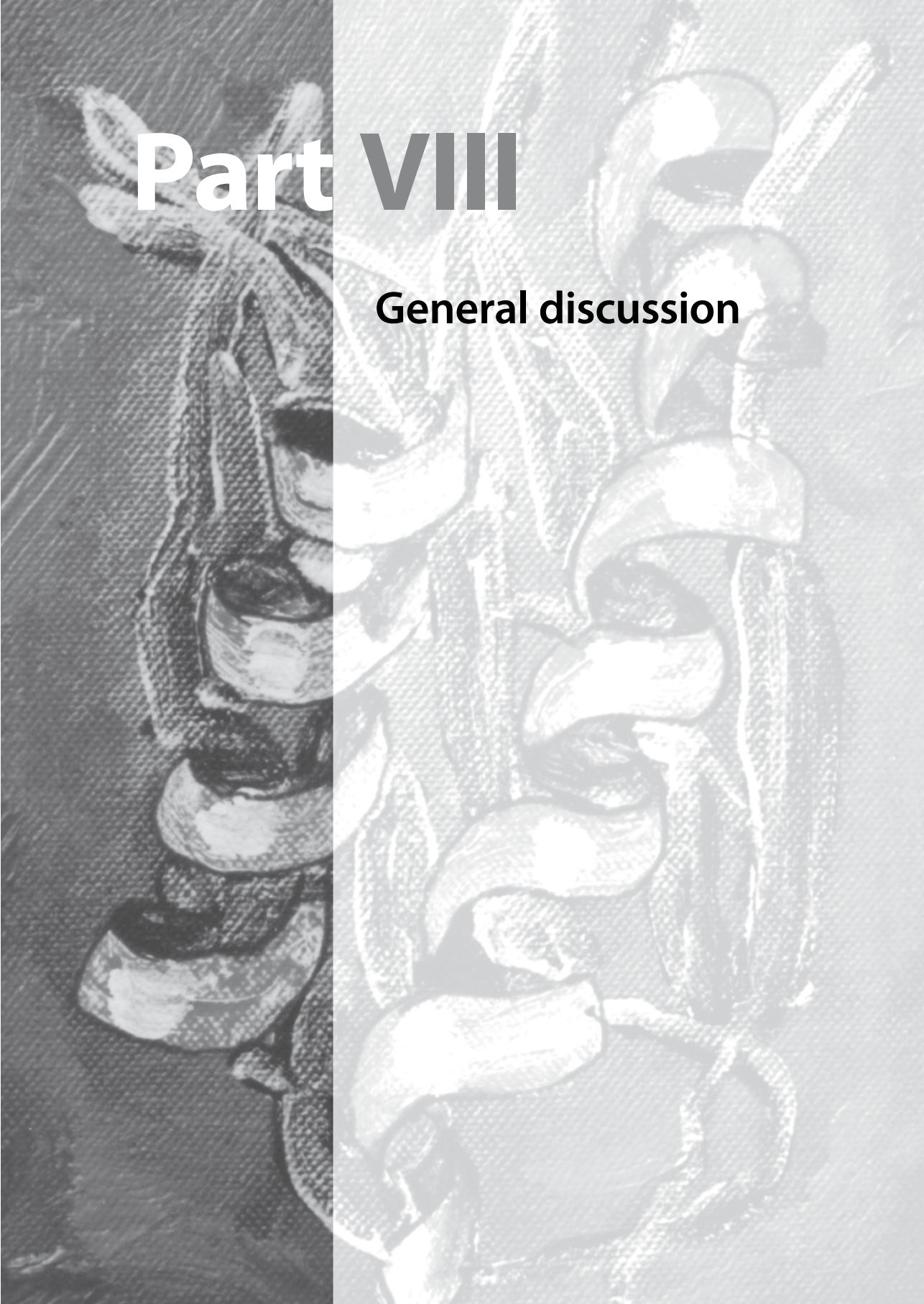
**w9.** Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, MacFarlane PW, et al. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. WSCPS Group. *N Engl J Med* 1995;333(20):1301-7.

**w10.** Sever PS, Poulter NR, Dahlof B, Wedel H, Beevers G, Caulfield M, et al. The Anglo-Scandinavian Cardiac Outcomes Trial lipid lowering arm: extended observations 2 years after trial closure. *Eur Heart J* 2008;29(4):499-508.

## REFERENCES


1. American Heart Association. Heart disease and stroke statistics–2008 update. 2008. www.americanheart.org/downloadable/heart/1200082005246HS_Stats%202008.final.pdf.
2. Strippoli GF, Navaneethan SD, Johnson DW, Perkovic V, Pellegrini F, Nicolucci A, et al. Effects of statins in patients with chronic kidney disease: meta-analysis and meta-regression of randomized controlled trials. *BMJ* 2008;336:645-51.
3. Wei L, Ebrahim S, Bartlett C, Davey PD, Sullivan FM, MacDonald TM. Statin use in the secondary prevention of coronary heart disease in primary care: cohort study and comparison of inclusion and outcome with patients in randomised trials. *BMJ* 2005;330:821.
4. Law MR, Wald NJ, Rudnicka AR. Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease, and stroke: systematic review and meta-analysis. *BMJ* 2003;326:1423.
5. Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet* 2005;366:1267-78.
6. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 1994;344:1383-9.
7. Executive Summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *JAMA* 2001;285:2486-97.
8. Third report of the National Cholesterol Education Program (NCEP) Expert Panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. *Circulation* 2002;106:3143-421.
9. Graham I, Atar D, Borch-Johnsen K, Boysen G, Burell G, Cifkova R, et al. European guidelines on cardiovascular disease prevention in clinical practice: executive summary. *Eur Heart J* 2007;28:2375-414.
10. Grundy SM, Cleeman JI, Merz CN, Brewer HB Jr, Clark LT, Hunninghake DB, et al. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. *Circulation* 2004;110:227-39.
11. Abramson J, Wright JM. Are lipid-lowering guidelines evidence based? *Lancet* 2007;369:168-9.
12. Walsh JM, Pignone M. Drug treatment of hyperlipidemia in women. *JAMA* 2004;291:2243-52.
13. Mills EJ, Rachlis B, Wu P, Devereaux PJ, Arora P, Perri D. Primary prevention of cardiovascular mortality and events with statin treatments: a network meta-analysis involving more than 65,000 patients. *J Am Coll Cardiol* 2008;52:1769-81.
14. Thavendiranathan P, Bagai A, Brookhart MA, Choudhry NK. Primary prevention of cardiovascular diseases with statin therapy: a metaanalysis of randomized controlled trials. *Arch Intern Med* 2006;166:2307-13.
15. Moher D, Cook DJ, Eastwood S,Olkin I, Rennie D, StroupDF. Improving the quality of reports of meta-analyses of randomized controlled trials: the QUOROM statement. Quality of reporting of meta-analyses. *Lancet* 1999;354:1896-900.
16. Anderssen SA,Hjelstuen AK,Hjermann I, Bjerkan K, Holme I. Fluvastatin and lifestyle modification for reduction of carotid intimamedia thickness and left ventricular mass progression in drugtreated hypertensives. *Atherosclerosis* 2005;178:387-97.
17. Furberg CD, Adams HP Jr, Applegate WB, Byington RP, Espeland MA, Hartwell T, et al. Effect of lovastatin on early carotid atherosclerosis and cardiovascular events. Asymptomatic Carotid Artery Progression Study (ACAPS) Research Group. *Circulation* 1994;90:1679-87.
18. Hedblad B, Wikstrand J, Janzon L, Wedel H, Berglund G. Low-dose metoprolol CR/XL and fluvastatin slow progression of carotid intimamedia thickness: main results from the Beta-Blocker Cholesterol-Lowering Asymptomatic Plaque Study (BCAPS). *Circulation* 2001;103:1721-6.

19. MercuriM, BondMG, Sirtori CR, Veglia F, Crepaldi G, Feruglio FS, et al. Pravastatin reduces carotid intima-media thickness progression in an asymptomatic hypercholesterolemic Mediterranean population: the Carotid Atherosclerosis Italian Ultrasound Study. *Am J Med* 1996;101:627-34.
20. Mohler ER 3rd, Hiatt WR, Creager MA. Cholesterol reduction with atorvastatin improves walking distance in patients with peripheral arterial disease. *Circulation* 2003;108:1481-6.
21. Salonen R, Nyyssonen K, Porkkala E, Rummukainen J, Belder R, Park JS, et al. Kuopio Atherosclerosis Prevention Study (KAPS). A population-based primary preventive trial of the effect of LDL lowering on atherosclerotic progression in carotid and femoral arteries. *Circulation* 1995;92:1758-64.
22. Sawayama Y, Shimizu C, Maeda N, Tatsukawa M, Kinukawa N, Koyanagi S, et al. Effects of probucol and pravastatin on common carotid atherosclerosis in patients with asymptomatic hypercholesterolemia. Fukuoka Atherosclerosis Trial (FAST). *J Am Coll Cardiol* 2002;39:610-6.
23. Zanchetti A, Crepaldi G, BondMG, Gallus G, Veglia F, Mancia G, et al. Different effects of antihypertensive regimens based on fosinopril or hydrochlorothiazide with or without lipid lowering by pravastatin on progression of asymptomatic carotid atherosclerosis: principal results of PHYLLIS—a randomized double-blind trial. *Stroke* 2004;35:2807-12.
24. Holdaas H, Fellstrom B, Jardine AG, Holme I, Nyberg G, Fauchald P, et al. Effect of fluvastatin on cardiac outcomes in renal transplant recipients: a multicentre, randomised, placebo-controlled trial. *Lancet* 2003;361:2024-31.
25. Effects of pravastatin in patients with serum total cholesterol levels from 5.2 to 7.8 mmol/liter (200 to 300 mg/dl) plus two additional atherosclerotic risk factors. The Pravastatin Multinational Study Group for Cardiac Risk Patients. *Am J Cardiol* 1993;72:1031-7.
26. Pravastatin use and risk of coronary events and cerebral infarction in Japanese men with moderate hypercholesterolemia: the Kyushu Lipid Intervention Study. *J Atheroscler Thromb* 2000;7:110-21.
27. Asselbergs FW, Diercks GF, Hillege HL, van Boven AJ, Janssen WM, Voors AA, et al. Effects of fosinopril and pravastatin on cardiovascular events in subjects with microalbuminuria. *Circulation* 2004;110:2809-16.
28. Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, Gavaghan DJ, et al. Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin Trials* 1996;17:1-12.
29. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986;7:177-88.
30. Sever PS, Dahlof B, Poulter NR, Wedel H, Beevers G, Caulfield M, et al. Prevention of coronary and stroke events with atorvastatin in hypertensive patients who have average or lower-than-average cholesterol concentrations, in the Anglo-Scandinavian Cardiac Outcomes Trial—Lipid Lowering Arm (ASCOT-LLA): a multicentre randomised controlled trial. *Lancet* 2003;361:1149-58.
31. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003;327:557-60.
32. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997;315:629-34.
33. Ford I,BlauwGJ,MurphyMB, Shepherd J, CobbeSM, BollenEL, et al. A Prospective Study of Pravastatin in the Elderly at Risk (PROSPER): screening experience and baseline characteristics. *Curr Control Trials Cardiovasc Med* 2002;3:1-8.
34. Ford I, Murray H, Packard CJ, Shepherd J, Macfarlane PW, Cobbe SM. Long-term follow-up of the West of Scotland Coronary Prevention Study. *N Engl J Med* 2007;357:1477-86.
35. Strandberg TE, Pyorala K, Cook TJ, Wilhelmsen L, Faergeman O, Thorgeirsson G, et al. Mortality and incidence of cancer during 10- year follow-up of the Scandinavian Simvastatin Survival Study (4S). *Lancet* 2004;364:771-7.
36. Braunwald E, Domanski MJ, Fowler SE, Geller NL, Gersh BJ, Hsia J, et al. Angiotensin-converting-enzyme inhibition in stable coronary artery disease. *N Engl J Med* 2004;351:2058-68.
37. Fox KM, Henderson JR, Bertrand ME, Ferrari R, Remme WJ, Simoons ML. The European trial on reduction of cardiac events with perindopril in stable coronary artery disease (EUROPA). *Eur Heart J* 1998;19(suppl J):J52-5.



# Part VIII

## General discussion

# Chapter 15

## Methodological considerations, clinical implications and directions for future research

The objective of this thesis was to study the feasibility of tailored ACE-inhibitor therapy with the aim to individualize treatment of patients with stable coronary artery disease. Most studies described in this thesis, were conducted within the EUROPA-trial, a randomized double-blind placebo controlled clinical trial studying the effect of the ACE-inhibitor perindopril versus placebo in 12.218 patients with stable CAD during 4 years of follow-up [1]. In the present chapter, methodological considerations with regard to the studies described in this thesis are described and the main findings of this thesis are placed in a broader context. Also, the potential implications for future clinical practice of physicians are discussed. Finally, directions for future research are discussed.

## METHODOLOGICAL CONSIDERATIONS

Methodological considerations pertaining to the separate studies have been described in the specific chapters. Here, general methodological considerations will be discussed with regard to the presented genetic association studies.

### Genetic association studies

In genetic association studies, candidate genes in relevant pathways can be selected, and the association between variation in these genes and disease occurrence or effect of the drug (reduction in disease occurrence) investigated. Genes may be selected on the basis of assumed relation in the pathofysiology of the disease with genetic variants, such as single nucleotide polymorphisms (SNPs). SNPs may be selected because of their functional implications or location in the gene. On the other hand, genetic variants may be selected because they "tag" gene haplotypes. A haplotype is a set of tagging SNPs on a single chromatid that are statistically associated and tend to inherit together. It is thought that the identification of the alleles of a haplotype block can unambiguously identify all other polymorphic sites within its region. As such, the haplotype-approach is important to discover the genetics behind common diseases. In this thesis, we selected the renin-angiotensin and kallikrein-bradykinin system genes as candidate genes as they are the primary targets affected by ACE-inhibitors (pharmacodynamics) [2]. We combined the two described approaches of selecting the SNPs by functional role and haplotype tagging. To ensure a comprehensive coverage of the common genetic variation in these candidate genes, we used a haplotype tagging-SNP approach with multiple tagging SNP's to reach a minimum coverage of at least 90% of the common genetic variation in the candidate gene [2]. In choosing the tagging-SNPs, we preferred functional SNPs or SNPs located in coding or promoter (transcriptional activity) regions of the gene. In addition, we enriched our set of SNPs with SNPs of interest due to prior literature. One of the most frequently studied polymorphisms in the RAAS is the ACE I/D, located in an intronic region of the ACE gene, with only minor effects on ACE plasma levels [3-6]. As it is very laborious SNPs to genotype (286 bp

insertion / deletion) using polymerase chain reactions, we used a direct proxy in the ACE gene: rs4343 which is reported with high LD (D' 1.0; $r^2$ 0.9) with the ACE I/D [3,4]. In total, we studied 12 candidate genes and 52 tagging-SNPs [2]. The current study is one of the most comprehensive analyses of the RAAS and BK pathways ever performed in genetic association studies.

Important to note here is that we only studied the two direct pharmacodynamic pathways of ACE-inhibitors: the renin-angiotensin system and kallikrein-bradykinin system [2,7,8]. ACE-inhibitors inhibit the ACE activity which reduces the activation of AT-2 from AT-1 and increases the degradation of BK. There are other candidate genes to study, most importantly the genes involved in the metabolism of ACE-inhibitors for example genes encoding the CYP450 enzymes. However, the exact targets to study are yet unknown and no relevant metabolic candidate genes could be included. This is a field for ongoing research.

## Plasma-levels

Genetic variants in the RAAS may also influence plasma levels of the hormones involved. For example, it is known that genetic polymorphisms in coding regions of the AGT gene influence the levels of AGT in the human body [7,8]. The M235T polymorphism (rs699) located in exon 5 of the AGT gene influences the plasma levels of AGT [7-9]. In genetic association studies it is very important to have a biologically plausible mechanism for your findings. Investigation of such variants may provide solutions for problems encountered with causal inference. If the blood levels truly increases risk of disease, then carriage of the genetic variant that exposes individuals to an elevation of the blood levels should confer an increased risk of disease proportional to the difference in the marker attributable to the genetic variant. Considering our project, plasma levels of angiotensinogen, angiotensin-converting enzyme, renin, and aldosteron, would have been needed to make remarks on the potential mechanisms of our findings. Unfortunately, as these genetic polymorphisms are rare with about 4-5% homozygous minor allele patients, one would need plasma levels of several hundreds or even thousands of patients to make meaningful inferences. To date, plasma levels of RAAS hormones together with genetic information is only available in limited numbers of fifty to hundred patients which makes it inevitably underpowered. Performing the assays needed for angiotensinogen are expensive and labor intensive. As we found associations in bradykinin receptors, plasma levels of bradykinin would be very interesting, however, no laboratories have these levels in high quantities available. This is a shortcoming of our genetic findings, however, it does open new ways of research and advocates to study the mechanism of action of ACE-inhibitors more intensively, including levels of bradykinin, angiotensinogen, ACE, and AT-II.

## Causality

Genetic association studies in general may have several other shortcomings. An association found between a single variant and a disease outcome may have been caused by linkage

disequilibrium with another genetic variant. A more comprehensive approach is obtained by constructing gene haplotypes that capture the common genetic variation across a gene, as described above. In our study, we found genetic variants in the AT1 and BK1 receptor modifying treatment benefit of perindopril [10]. Both AT1 receptor gene polymorphisms were located in an influential region of the gene, respectively in the promoter and exon. A functional or causal mechanism is plausible. The polymorphism located in the BK1 receptor was located in an intronic region of the gene, and the above mentioned scenario could apply for this SNP [10] but additional research is needed to study the functional role of the BK1 receptor. Several recent studies report a influential role of this receptor in the cascade of the RAAS and cardiovascular risk.

## Sample size

Furthermore, large sample sizes are needed to provide enough power to detect the effect (albeit small) of a genetic variant on disease with a multifactorial origin. Such diseases are thought to result from variation in a large spectrum of genes with small individual effects, the same probably applies for studying the effect of treatments. As demonstrated in chapter 10, the PERGENE study due to the large size and large number of events we had sufficient power for the detection of interaction effects [2]. The size of this pharmacogenetic sub study allows detection with a power of 98% to detect a difference in hazard ratios (treatment effect for the primary endpoint) of 20% between genotypes with minor allele frequency of 0.20, based upon ten-thousand patients (two-sided alpha 0.05). For other genotype distributions, power will be less but for most comparisons still above 80%. For a minor allele frequency of 0.10, statistical power is 88% to detect a difference in treatment effect of 20% with a two-sided alpha 0.05 [2].

## Multiple testing

Multiple testing is an important issue due to the possibility of chance findings in genetic association studies. Correction is therefore necessary. There are several approaches to correct for multiple testing, which are all vividly debated. A clear distinction needs to be made here between genome-wide scans and candidate-genes approaches. In a genome-wide scan, half a million of detectors of SNP's are placed on a chip and tested on the DNA of the patients, rigorous multiple testing correction is necessary due to the large number of tests and SNP are entirely independent of each other. Therefore, p-values of exponent $10^{-15}$ are often needed to reach significance in these studies after Bonferonni correction (0.05 divided by the number of test performed is significance level to be reached). In a candidate gene approach, genes and SNPs are manually selected based upon a strong prior study hypothesis. SNP's are selected by tagging principle, functionality or location in the gene. In our study, we selected candidate genes in the two pharmacodynamic pathways of ACE-inhibitors by study hypothesis and we selected, manually, the 52 tagging SNPs needed in these genes, which are therefore not entirely independent as SNP's are taggers and located within one common pathway [2,10]. To correct for multiple testing, we have chosen for a gene-based permutation analysis. Applying more extensive correction (ie.

Bonferonni or permutations for 52 tests) would be overly conservative due to the strength of the a priori study hypothesis and may fail to notice real existing differences.

### Reproducibility

Finally, reproducibility or replication of the results of genetic association studies is important. Considering the exclusivity of our pharmacogenetic analysis in a randomized clinical trial studying one single drug treatment, no suitable replication cohort of similar size and design does exist. Still, as demonstrated in chapter 11, even with low sample size, the same trend in the direction and magnitude of the interaction effects was observed as compared with the findings in the EUROPA-trial which reassures the validity and consistency of our findings. With replication of your findings, the issue of multiple testing vanishes. Still, as mentioned, finding a suitable replication cohort is one of the major limitations of this project as clearly the data are unique and no other randomized clinical trial with access to DNA in patients with stable CAD was available. For an initial replication of our findings, we had the opportunity to use data of 1051 Caucasian patients of PROGRESS studying the same ACE-inhibitor, perindopril, albeit in lower dose of 4 mg [10,11]. The small sample size results in an underpowered statistical analysis and actual replication of interaction terms would be impossible just to the small number of patients, still a verification of a similar direction of the interaction effect can be made in such small replication studies. PROGRESS enrolled patients with cerebrovascular disease. Because the treatment benefit in PROGRESS was contingent on the combination with indapamide (2,5 mg), we studied patients receiving single therapy with perindopril in the European subjects of PROGRESS, which ensures comparability with the EUROPA-trial subjects [10,11]. Additional replication needs to be sought in different trials with different ACE-inhibitors, to test the generalizability to other patient groups and other ACE-inhibitors as well.



## MAIN FINDINGS

In the current thesis, we demonstrate that guiding ACE-inhibitor therapy was not feasible using clinical characteristics of patients [12-15]. Furthermore, the treatment benefit of perindopril based regimens in a combined analysis of EUROPA, ADVANCE and PROGRESS trials was not modified by baseline risk factors, blood pressure or the blood pressure reduction during run-in period [15]. Still, heterogeneity in treatment effect of ACE-inhibitors is likely as there is a large inter-individual variability in response to ACE-inhibitors. As a new approach, we tested whether genetic variation in the direct pharmacodynamic pathway of ACE-inhibitors, the renin-angiotensin system and kallikrein-bradykinin pathway, modified the treatment benefit of ACE-inhibitors.

The current study demonstrates that the treatment benefit of ACE-inhibitor therapy by perindopril is modified by variation in 2 candidate genes: the AT1 receptor gene, and the BK1

receptor gene [10]. Based on the pharmacogenetic profile, consisting of these variants, both patients with an enhanced treatment benefit (73.5% of the PERGENE population), and patients with a diminished, if not absent, treatment effect (26.5% of the PERGENE population) could be identified. A similar interaction of this profile with the treatment effect was observed in the replication cohort from PROGRESS. The proposed pharmacogenetic profile was directly associated with the treatment benefit of ACE-inhibitors. This association was independent of baseline clinical characteristics and blood pressure, which is in accordance with previous studies in which clinical patient characteristics or subgroup analyses that did not reveal any treatment heterogeneity [10].

In the main analysis of the EUROPA-trial, treatment with perindopril resulted in a relative risk reduction of 20% for the primary endpoint, which was consistent across all clinical subgroups [1]. In contrast, the subgroups based on the proposed genetic profile have a wide range of treatment effects [10], from patients without unfavourable alleles (11.3% of all patients) with a 54% reduction in the primary endpoint during follow-up, via patients with one unfavourable allele (29.8%) who experienced a 39% relative risk reduction and patients with two unfavourable alleles (32.4%) with a 19% relative risk reduction, which is more comparable to the overall study effect. At the other end of the spectrum, patients with ≥3 unfavourable alleles experienced no benefit (26.5%) from perindopril treatment during 4 years of follow-up. Refraining from treatment with perindopril in these patients may considerably reduce healthcare cost and increase the overall efficacy of the drug.

In essence, we have found several genetic determinants related to the treatment benefit of ACE-inhibitors. The aggregated pharmacogenetic profile predicted the response to ACE-inhibitors as it identified responders and non-responders. The current study demonstrates the feasibility of pharmacogenetic profiling of ACE-inhibitor therapy in patients with coronary artery disease.

## PATHOPHYSIOLOGY AND MECHANISM

Our findings suggest that the genetic variants modifying the treatment effect of perindopril are particularly located in the AT1 and BK1 receptor genes. The SNPs in the AT1 receptor were located in the promoter (rs275651) and exon (rs5182), the SNP (rs12050217) in the BK1 receptor was located in an intron. These three SNPs were tagging SNPs and may either be functional themselves or in linkage equilibrium with important functional SNPs. So far, functionality of these three SNPs is unknown. The AT1 receptor does mediate all the well-known effects of angiotensin II, including vasoconstriction, water and salt retention, aldosterone synthesis and hypertrophy [7]. The role of the $B_1$ receptor, on the other hand, is less well established. Bradykinin is a potent vasodilator that also induces anti-atherosclerotic and anti-thrombotic effects, which are mediated by bradykinin type II ($B_2$) receptors [7]. Previous studies indicated

that the clinical benefit of ACE-inhibitors depends, at least in part, on $B_2$ receptor activation [17]. $B_1$ receptors are weakly expressed under physiological conditions, but are strongly induced in response to pathological conditions and/or RAAS blockade [18,19]. Interestingly, it has been suggested that $B_1$ receptors are directly activated by ACE-inhibitors (thus resulting in an increase in endothelial NO release, for instance in the heart, by which they contribute to the cardioprotective effects of ACE-inhibitors [20,21,22], but this has not been uniformly confirmed by others [23]. Therefore, a more likely possibility is that the up-regulated $B_1$ receptors are activated by their endogenous ligand during ACE-inhibition. Given the hypotensive [24], cardioprotective [18] and cerebro-protective [25] effects of such activation, as observed in animals, one might speculate that patients with genetic defects in their $B_1$ receptor display a diminished response to ACE-inhibition. Clearly, more work is needed to support this concept.

## CLINICAL IMPLICATIONS

Our finding show that three out of four patients had an enhanced benefit of ACE-inhibitor therapy (33% reduction of cardiovascular death of myocardial infarction, up to 54% in patients without any unfavourable alleles) and one out of four patients experienced no, or a markedly diminished, benefit of long term perindopril treatment. By developing a pharmacogenetic profile related to treatment response, patients can be selected who are most likely to benefit from such treatment in advance. When the feasibility of pharmacogenetic profiling of ACE-inhibitor therapy is confirmed, physicians will be able to predict the response to treatment (the exciting concept of responders and non-responders) before the start of prescription. Taken together, these pharmacogenetic analyses of clinical trials open up a perspective to individualize preventive therapy in patients with stable CAD, which may avoid unnecessary treatment, and considerably reduce health care costs by the concept of "individualizing therapy" based on genetic data. Moreover, the combination of these trials can be used to identify a genetic profile for cardiovascular drugs at large. As our results demonstrate the feasibility of pharmacogenetic profiling, we advocate that the current pharmacogenetics approach should be integrated in future randomized clinical trials designs as standard procedure to optimize patients' benefit.

## DIRECTIONS OF ONGOING AND FUTURE PHARMACOGENETIC RESEARCH

"Getting the right drug to the right patient"

Through pharmacogenetics, medical science reclaims the art of individual medicine, which serves as the gateway to understanding heterogeneity among individuals in drug response. Pharmacogenetics teaches that different patients respond differently to medications. In the

current clinical trial arenas of therapeutic drug response optimization and individualization strategies, pharmacogenetic testing is used with an ever increasing frequency and is defining a new frontier in diagnostic and therapeutic patient approaches. Correlation between clinical and genetic heterogeneity allows for optimization of therapeutic strategies, while we keep developing new technologies, such as DNA arrays and advanced bioinformatics, in the quest for the Holy Grail, which is "tailoring drug therapies to individual patients". Ultimately, pharmacogenetics will also help in the development of new drugs targeted at critical signalling pathways in disease pathogenesis allowing for both treatment and prevention.

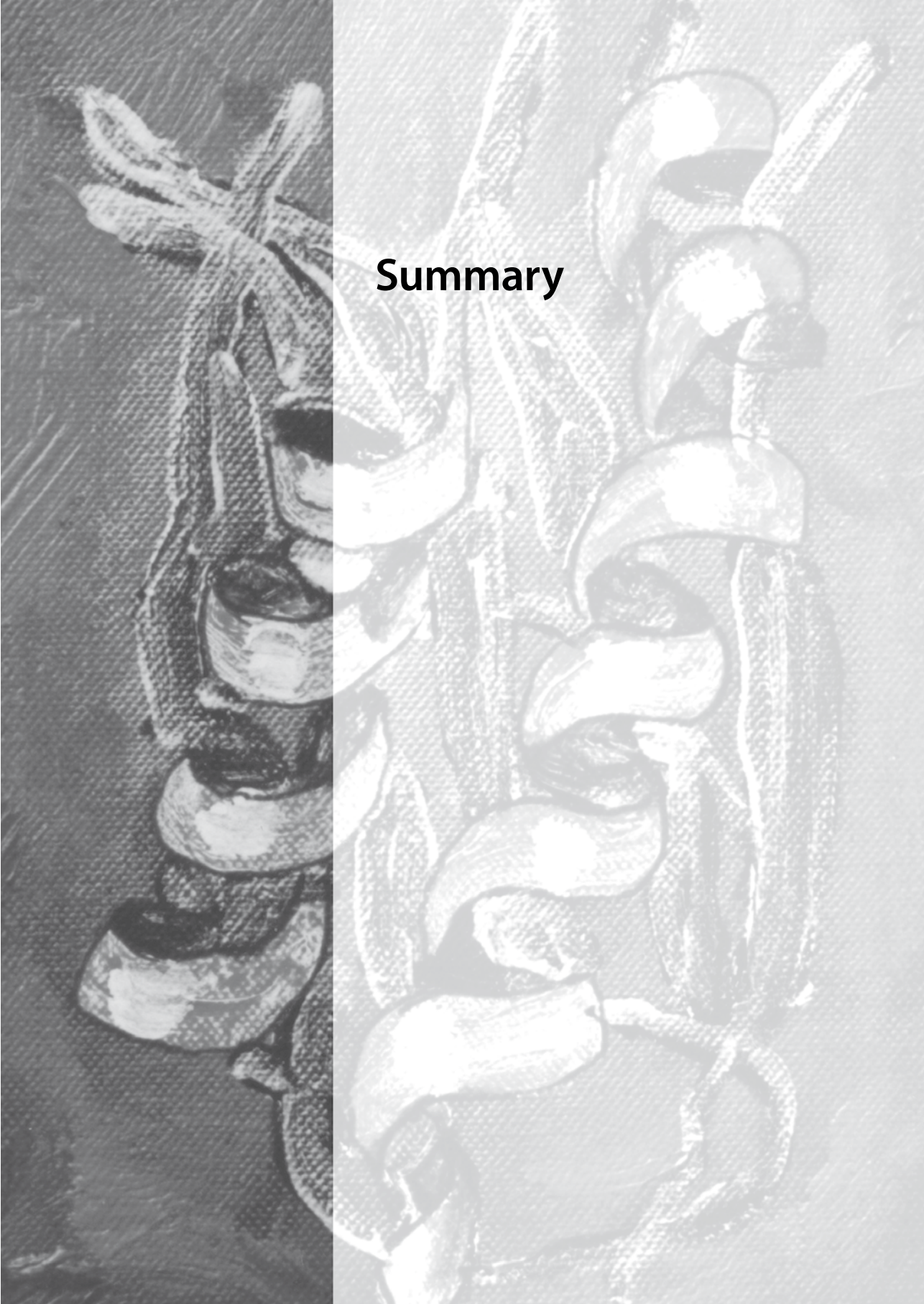
Pharmacogenetic research is emerging rapidly and is expected to revolutionize clinical practice in the next decennia. At this moment, successes are presented for several cardiovascular drugs for example clopidogrel. Pharmacogenetic determinants of the response of patients to clopidogrel contribute to variability in the biologic antiplatelet activity of the drug as well as the clinical efficacy of clopidogrel, especially the genetic variants of genes modulating clopidogrel absorption (ABCB1), metabolic activation (CYP3A5 and CYP2C19), and biologic activity (P2RY12) are under extensive investigation [26,27]. Also pharmacogenetics proves to be important in dosing of coumarin derivates such as warfarin by which the incidence of major hemorrhage can be significantly reduced [28,29]. Clopidogrel and warfarin are just two of many successful (upcoming) examples of pharmacogenetics.

Within the PERGENE study, we will focus on further replication of the main findings in other randomized clinical trials, which studied different patient populations or a different ACE-inhibitor. Another important issue to investigate is the pathophysiological mechanism behind the current findings addressing the functional role of the genetic variants and the role of the bradykinin type I receptor within cardiovascular disease with respect to drug interaction. A new research project has been set up to investigate the functional role of the BK-1 and AT-1 receptor genetic variants and correlate them with plasma levels. Furthermore, genetic research in the metabolic enzyme genes responsible for the absorption and degradation of ACE-inhibitor metabolites is necessary, for example the various CYP450 enzymes. However, no exact target genes have been discovered at the moment. Ultimately, we hope that the current findings will provide opportunities to test the genetic determinants of treatment effect genome-wide. A large-scale genome wide scan of patients across both randomized treatment arms may further reveal relevant genetic targets related to the treatment benefit of ACE-inhibitors.

## REFERENCES:


1. The European Trial on Reduction of Cardiac Events with Perindopril in Patients with Stable Coronary Artery Disease investigators. Efficacy of perindopril in reduction of cardiovascular events in stable coronary artery disease: randomized, double-blind, placebo controlled, multicentre trial (the EUROPA study). *Lancet* 2003;362:782–8.
2. Brugts JJ, de Maat M, Boersma E, Witteman J, van Duijn C, Uitterlinden A, Bertrand M, Remme W, Fox K, Ferrari R, Danser AHJ, Simoons ML. The Rationale and Design of the Perindopril Genetic Association Study (PERGENE): A Pharmacogenetic Analysis of Angiotensin-Converting Enzyme Inhibitor Therapy in Patients with Stable Coronary Artery Disease. *Cardiovasc Drugs Ther* 2009;23:171–181.
3. Brugts JJ, de Maat MPM, Den Uil CA, Danser AHJ. Angiotensinogen haplotypes in patient with hypertension. *Journal of Hypertension* 2008
4. Abdollahi MR, Huang S, Rodriguez S, Guthrie PA, Smith GD, Ebrahim S, Lawlor DA, Day IN, Gaunt TR. Homogeneous assay of rs4343, an ACE I/D proxy, and an analysis in the British Women's Heart and Health Study (BWHHS). *Dis Markers*. 2008;24:11-7.
5. Arnett DK, Davis BR, Ford CE, et al. Pharmacogenetic association of the angiotensin-converting enzyme insertion/deletion polymorphism on blood pressure and cardiovascular risk in relation to antihypertensive treatment: the Genetics of Hypertension-Associated Treatment (GenHAT) study. *Circulation* 2005;28;111:3374-83.
6. *PROGRESS Collaborative Group.* The ACE gene I/D polymorphism is not associated with the blood pressure and cardiovascular benefits of ACE inhibition. *Hypertension* 2003;42:297-303.
7. Brugts JJ, den Uil CA, Danser AHJ, Boersma E. The Renin-Angiotensin-Aldosterone System: Approaches to Guide Angiotensin-Converting Enzyme Inhibition in Patients with Coronary Artery Disease. *Cardiology* 2009;112:303–312
8. Brugts JJ, de Maat M, den Uil CA, et al. Pharmacogenetics of ACE inhibition in stable coronary artery disease: steps towards tailored drug therapy. Curr Opin Cardiol 2008;23:296–301.
9. Danser AHJ, Schunkert H. Renin–angiotensin system gene polymorphisms: potential mechanisms for their association with cardiovascular diseases. *Eur J Pharmacology* 2000.303–316.
10. Brugts JJ, Isaacs A, Boersma E, van Duijn C, Uitterlinden AG, Remme WJ, Bertrand M, Ninomiya T, Ceconi C, Chalmers J, MacMahon S, Fox K, Ferrari R, Witteman J, Danser AHJ, Simoons ML, de Maat M. Genetic Determinants of Treatment Benefit of ACE-inhibitor Therapy in Patients with Stable Coronary Artery Disease. *Eur Heart J 2010 in press.*
11. *PROGRESS Collaborative Group* Randomised trial of a perindopril-based blood-pressure-lowering regimen among 6105 individuals with previous stroke or transient ischaemic attack. *Lancet* 2001; 358: 1033–41.
12. Dagenais GR, Pogue J, Fox K, Simoons ML, Yusuf S. Angiotensin converting-enzyme inhibitors in stable vascular disease without left ventricular systolic dysfunction or heart failure: a combined analysis of three trials. *Lancet* 2006;368:581–8.
13. Deckers JW, Goedhart DM, Boersma E, et al. Treatment benefit by perindopril in patients with stable coronary artery disease at different levels of risk. *Eur Heart J* 2006;27:796–801.
14. Brugts JJ, Knetsch AM, Mattace-Raso FU, Hofman A, Witteman JC: Renal function and risk of myocardial infarction in an elderly population: the Rotterdam study. *Arch Intern Med* 2005; 165: 2659–2665.
15. Brugts JJ, Boersma E, Chonchol M, et al. The cardioprotective effects of the angiotensin-converting enzyme inhibitor perindopril in patients with stable coronary artery disease are not modified by mild to moderate renal insufficiency: insights from the EUROPA trial. *J Am Coll Cardiol* 2007;50:2148–55.
16. Brugts JJ, Ninomiya T, Boersma E, Remme WJ, Vertrand M, Ferrari R, Fox K, MacMahon S, Chalmers J, Simoons ML. The consistency of the treatment effect of an ACE-inhibitor based treatment regimen in patients with vascular disease or high risk of vascular disease: a combined analysis of individual data of ADVANCE, EUROPA, and PROGRESS trials. *Eur Heart J* 2009; 30, 1385–1394.

17. Gainer JV, Morrow JD, Loveland A, King DJ, Brown NJ. Effect of bradykinin-receptor blockade on the response to angiotensin converting-enzyme inhibitor in normotensive and hypertensive subjects. *N Engl J Med* 1998;339:1285–92.
18. Xu J, Carretero OA, Shesely EG, Rhaleb NE, Yang JJ, Bader M, Yang XP. The Kinin B1 Receptor Contributes to the Cardioprotective Effect of ACE Inhibitors and Angiotensin Receptor Blockers. *Exp Physiol.* 2009 Mar;94(3):322-9.
19. Marin-Castaño ME, Schanstra JP, Neau E, Praddaude F, Pecher C, Ader JL, Girolami JP, Bascands JL. Induction of functional bradykinin b(1)-receptors in normotensive rats and mice under chronic Angiotensin-converting enzyme inhibitor treatment. *Circulation.* 2002;5;627-32
20. Ignatovic T, Tan F, Brovkovych V, Skidgel RA, Erdos GE. Novel Mode of Action of Angiotensin I Converting Enzyme Inhibitors: Direct activation of the Bradykinin Receptor type I. *The Journal of Biological Chemistry* 2002; 277, 16847–16852.
21. Ignjatovic T, Stanisavljevic S, Brovkovych V, Skidgel RA, Erdos EG. Kinin B1 Receptors Stimulate Nitric Oxide Production in Endothelial Cells: Signaling Pathways Activated by Angiotensin I-Converting Enzyme Inhibitors and Peptide Ligands. *Mol. Pharmacol*. 2004; 66:1310-16
22. Merino VF, Todiras M, Mori MA, Sales VMT, Fonseca RG, Saul V, Tenner K, Bader M, Pesqueo JB. Predisposition to atherosclerosis and aortic aneurysms in mice deficient in kinin B1 receptor and apolipoprotein E. *J Mol Med* DOI 10.1007/s00109-009-0501-0
23. Forten JP, Gobeil F Jr, Adam A, Regoli D, Marceau F. Do angiotensin-converting enzyme inhibitors directly stimulate the kinin B1 receptor? *Am J Physiol Heart Circ Physiol*. 2003;285:H277-82.
24. Su JB, Hoüel R, Héloire F, Barbe F, Beverelli F, Sambin L, Castaigne A, Berdeaux A, Crozatier B, Hittinger L. Stimulation of Bradykinin $B_1$ Receptors Induces Vasodilation in Conductance and Resistance Coronary Vessels in Conscious Dogs. *Circulation* 2000; 101**:** 1848 - 1853.
25. Austinat M, Braeuninger S, Pesquero JB, Brede M, Bader M, Stoll G, Renné T, Kleinschnitz C. Blockade of bradykinin receptor B1 but not bradykinin receptor B2 provides protection from cerebral infarction and brain edema. *Stroke* 2009;40:285-93.
26. Simon T, Verstuyft C, Mary-Kraue M, Quteineh L, Drouet E, Meneveau N, Steg PG, Ferrieres J, Danchin N, Becquemont L, for the FAST-MI Investigators. Genetic Determinants of Response to Clopidogrel and Cardiovascular Events. *New Engl J Med* 2009;360:363-375
27. Shuldiner AR, O'Connell JR, Bliden KP, Gandhi A, Ryan K, Horenstein RB, Damcott CM, Pakyz R, Tantry US, Gibson Q, Pollin TI, Post W, Parsa A, Mitchell BD, Faraday N, Herzog W, Gurbel PA. Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. *JAMA*. 2009 Aug 26;302(8):849-57.
28. International Warfarin Pharmacogenetics Consortium, Klein TE, Altman RB, Eriksson N, Gage BF, Kimmel SE, Lee MT, Limdi NA, Page D, Roden DM, Wagner MJ, Caldwell MD, Johnson JA. Estimation of the warfarin dose with clinical and pharmacogenetic data. *N Engl J Med.* 2009 Feb 19;360(8):753-64.
29. Anderson JL, Horne BD, Stevens SM, Grove AS, Barton S, Nicholas ZP, Kahn SF, May HT, Samuelson KM, Muhlestein JB, Carlquist JF; Couma-Gen Investigators. Randomized trial of genotype-guided versus standard warfarin dosing in patients initiating oral anticoagulation. *Circulation*. 2007 Nov 27;116(22):2563-70.



# Summary

Worldwide, millions of patients with stable coronary artery disease are treated with ACE-inhibitors according to the recommendations of international treatment guidelines on secondary prevention based on the results of large clinical trials. In the EUROPA-trial, treatment with the ACE-inhibitor perindopril at 8 mg / day was associated with 20% risk reduction in the event rate of cardiovascular mortality, MI or resuscitated cardiac arrest as compared to placebo during four years of follow-up. Also, in other patient categories ACE-inhibitors have proven clinical benefits. These studies were the basis for recommendations on ACE-inhibitors in the treatment guidelines for secondary prevention in patients with cardiovascular disease.

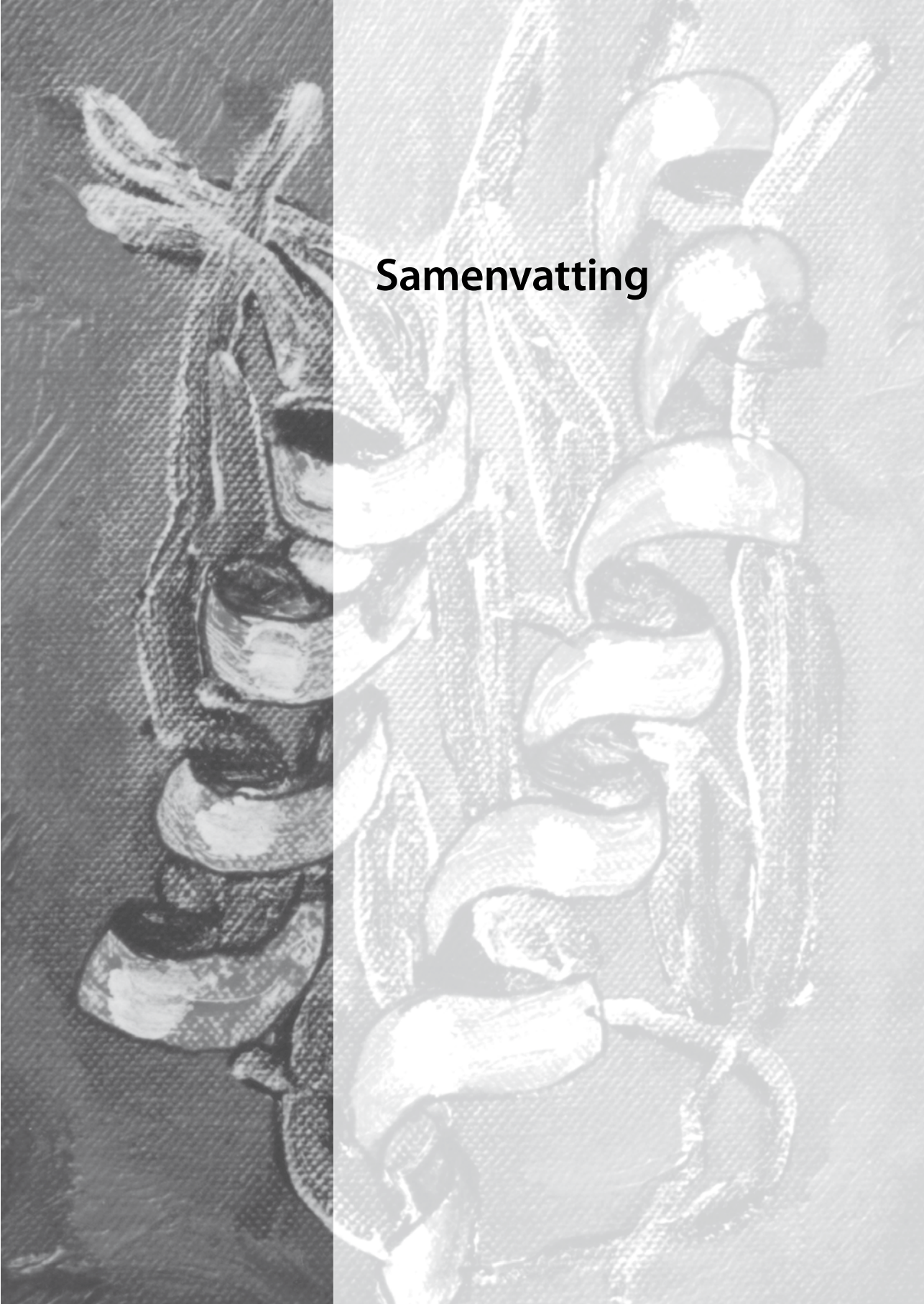
Still, in a relatively low-risk group of stable CAD patients, the absolute risk reduction with perindopril was 2% and 50 patients needed to be treated to prevent one cardiovascular event (200 patient years). To improve the clinical efficacy of ACE-inhibitors, several attempts have been made to target ACE-inhibitors only to those patients most likely to benefit of such prolonged prophylactic treatment. As the results of clinical trials are based on a selected patient group (inclusion and exclusion criteria) and results may not apply to all patients studied. However, treatment guidelines are based on these studies, which in general do not always perfectly reflect the patient in front of your desk as a physician. One should consider that the treatment effect will differ between patients. To optimally treat patients, one would rather know whom to treat rather than to treat everyone. Elucidating an existing heterogeneity in the treatment effect of ACE-inhibitors will be crucial if one would wish to target ACE-inhibitors to those who will benefit (responders versus non-responders). In this thesis, we examined the consistency in treatment benefit of ACE-inhibitor therapy in patients with stable coronary artery disease. Most of the studies have been performed in the EUROPA-trial, a randomized placebo-controlled clinical trial comparing perindopril versus placebo in patients with stable coronary artery disease. We assessed the consistency of the treatment effect of ACE-inhibitor therapy according to clinical characteristics and genetic factors with the aim to detect a possible heterogeneity which could be used to target ACE-inhibitors to those patients most likely to benefit and test the feasibility of pharmacogenetic profiling.

**Part I** focuses on the established role of ACE-inhibitors in the treatment of cardiovascular disease patients. Especially, extensive evidence has been gathered with the ACE-inhibitor, perindopril, which properties will be described in detail (**chapter 2**), as well as an overview of other ACE-inhibitor trials in different patient categories (**chapter 3**). **Part II** focuses on the identification of risk factors which could be involved in a heterogeneity in the treatment benefit of ACE-inhibitors. Renal insufficiency has been identified as an important cardiovascular risk factor of in relatively health subjects (**chapter 4**) as well as in patients with cardiovascular disease (**chapter 5**). Renal insufficiency could therefore be an important modifier of treatment effect of ACE-inhibitors considering pharmacokinetics (renal clearance) as well as patients' increased cardiovascular risk. However, in our analysis in the EUROPA-trial, renal insufficiency did not modify the treatment effect of perindopril (**chapter 6**). In **part III**, we describe a

combined analysis of the EUROPA, ADVANCE and PROGRESS-trials with 30.000 vascular disease patients which could not identify a heterogeneity in the treatment effect according to clinical characteristics including blood pressure (**chapter 7**). From part II and III, we conclude that no heterogeneity in the treatment effect of ACE-inhibitors in stable CAD patients can been elucidated using simple clinical characteristics. More advanced approaches are therefore necessary. In **part IV**, we discuss these new steps needed to guide ACE-inhibitor therapy. A new approach could be to integrate more patient-specific characteristics, such as the genetic information (DNA) of patients (**chapter 8**), especially the pharmacodynamic pathway of ACE-inhibitors – the renin-angiotensin-aldosterone-system – is expected to be involved in the inter-individual variability in the response to ACE-inhibitors (**chapter 9**). In **part V**, we present the design and rationale of the PERindopril GENEtic association study (PERGENE), the largest pharmacogenetic study of ACE-inhibitors and one of the first randomized clinical trials presenting genetic data with regard to treatment effect analyses (**chapter 10**). The aim of the research project was to develop a pharmacogenetic profile related to the treatment effect of ACE-inhibitor therapy predicting patients' response to such prolonged prophylactic treatment. Indeed, several genetic factors were related to the treatment benefit of perindopril (**chapter 11**). These genetic variants were located in the AT1-receptor and BK1-receptor. A pharmacogenetic profile combining the unfavorable alleles of these genetic variants demonstrated a stepwise decrease in the treatment benefit of perindopril. Patients with no or little genetic variants in these receptors experienced a more pronounced treatment benefit of perindopril **(responders, 73.5%)**. In contrast, patients with 3 or more of the unfavorable alleles did not benefit of treatment at all, in other words, no risk reduction of perindopril treatment during follow-up occurred in these patients which were relatively resistant to perindopril **(non-responders, 26.5%)**. These findings were verified in the PROGRESS-trial. This new concept of "tailored-therapy" by pharmacogenetic profiling can reduce unnecessary treatment of non-responding patients and reduce health care costs. The selected candidate genes were also related to blood pressure and blood pressure reduction by perindopril (**chapter 12**). However, the differences in blood pressure reduction were so small that they count not explain the difference in clinical risk reduction, but feed the discussion for BP-independent effects of ACE-inhibitors. The current findings support more extensive research in the mechanism of action of ACE-inhibitors. Finally, in **part VI**, the clinical implications of our findings and "the feasibility of tailored therapy of ACE-inhibitors" will be discussed in **chapter 13**.

In overview, this thesis presents a new concept of individualizing drug prescriptions. The concept of pharmacogenetics can be generalized to other frequently used drugs in cardiology such as beta-blockers, calcium-antagonists, platelet-inhibitors. For one of the most frequently prescribed drugs, statins, the current concept of individualizing therapy is clinically highly relevant (**Part VII, chapter 14**). As chapter 14 demonstrates the strong consistency of the treatment benefit of statins independent of several clinical characteristics, one could again argue that a comparable pharmacogenetic approach would be useful. We advocate that the

pharmacogenetic concept will be integrated in the design of all emerging randomized clinical trials to optimize patients' benefits. In the general discussion (**Part VIII, chapter 15**), methodological considerations with regard to the studies described in this thesis are mentioned and the main findings of this thesis are placed in a broader context. Finally, directions for on-going research and future research are discussed.



# Samenvatting

Miljoenen patiënten met stabiel coronair lijden worden behandeld met ACE-remmers volgens de internationale richtlijnen. In de EUROPA-trial was de behandeling met de ACE-remmer perindopril (8mg/dag) geassocieerd met een daling van 20% in het optreden van cardiovasculaire events (cardiovasculaire sterfte, myocard infarct, of hartstilstand) ten opzichte van placebo tijdens vier jaren van follow-up. Ook in andere patiënten populaties hebben ACE-remmers een bewezen gunstig effect. Dit heeft geleid tot de aanbeveling ACE-remmers voor te schrijven ter secundaire preventie van hart- en vaatziekten in alle internationale richtlijnen. ACE-remmers spelen een belangrijke rol in de dagelijkse behandeling van vele patiënten in de cardiologie.

Aangezien de absolute daling van het risico op events door perindopril 2% was en er 200 patienten behandeld moeten worden gedurende 4 jaren om 1 event te voorkomen, zijn er verscheidene pogingen ondernomen om ACE-remmers te richten op alleen die patiënten die er baat bij hebben. Subgroep analyses, o.a. in de EUROPA trial, hebben de heterogeniteit van het behandeleffect onderzocht maar niet aangetoond. Geen van de klinische kenmerken die onderzocht werden, zoals hoge bloeddruk of het hebben van suikerziekte, bleek van waarde in het gericht voorschrijven van ACE-remmers. Het behandeleffect is consistent in patiënten met stabiel coronair lijden en dus niet afhankelijk van klinische factoren. Om patiënten optimaal te behandelen, is het van belang vooraf te weten wie er meeste baat hebben bij het medicijn. De ontdekking van een mogelijke heterogeniteit in het behandeleffect blijft derhalve cruciaal (wie wel of niet op het medicijn reageren). Dit staat in direct contrast met het medicijn aan iedereen voor te schrijven. Een nieuwe aanpak zou kunnen zijn om meer patiëntspecifieke informatie te gebruiken: de genetica of pharmacogenetica. Pharmacogenetica is een nieuw onderzoeksveld gericht op het ontdekken van genetische factoren gerelateerd aan de response op een medicijn of de bijwerkingen van het medicijn. Wanneer de response op ACE-remmers verschilt op basis van genetische verschillen tussen mensen, kan dat een handvat zijn om een genetisch profiel te ontwikkelen dat de respons op deze behandeling voorspelt. Idealiter voorspelt dit wie wel reageert en wie niet reageert op het medicijn. Een pharmacogenetisch profiel gerelateerd aan behandeleffect kan het onnodig behandelen van patiënten reduceren en de kosten van de gezondheidszorg drukken.

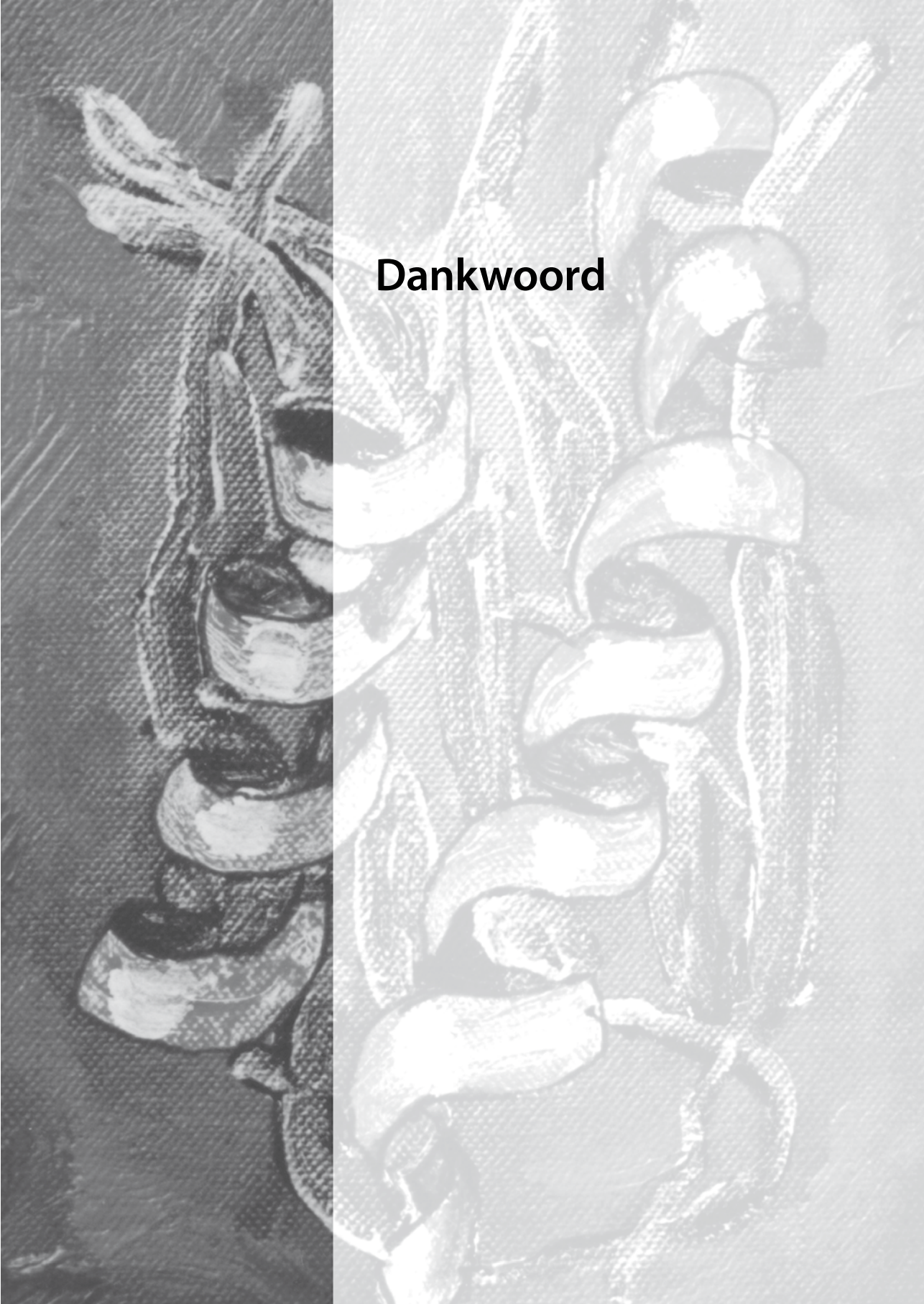
In dit proefschrift hebben we deze aspecten van het gericht voorschrijven van ACE-remmers in verscheidene facetten bestudeerd. De meeste studies zijn verricht in de EUROPA-trial, een gerandomiseerde placebo-gecontroleerde studie naar het behandeleffect van perindopril versus placebo in patiënten met stabiel coronair lijden. Vanuit het bloed van deze deelnemers, werd genetische informatie van deze patiënten gedestileerd en het verband met de response op de ACE-remmer perindopril onderzocht.

**Deel I** is gericht op de rol van ACE-remmers in de cardiologie, vooral de ACE-remmer in kwestie, perindopril, wordt hierin uitvoerig beschreven in verschillende patiënten groepen (**hoofdstuk 2**), evenals een overzicht van de verscheidene andere ACE-remmers trials (**hoofdstuk 3**). **Deel II** is gericht op de zoektocht naar klinische factoren die de response op

ACE-remmers voorspellen. Nierinsufficientie is een belangrijke risicofactor voor het optreden van hartinfarcten of cardiovasculaire sterfte in relatief gezonde mensen (**hoofdstuk 4**) maar zeker ook in hart- en vaatziekten patiënten (**hoofdstuk 5**). Nierinsufficientie, kan derhalve een belangrijke factor zijn om patiënten te identificeren die meer of minder baat hebben bij ACE-remmer behandeling. In onze analyse binnen de EUROPA trial bleek nierinsufficiëntie echter niet het behandeleffect te modificeren richten (**hoofdstuk 6**). Risicofactoren zoals diabetes, hypertensie, of het hebben van eerder myocard infarct bleken in een analyse binnen de EUROPA, ADVANCE en PROGRESS-trial niet gerelateerd aan het behandeleffect van ACE-remmers (**deel III, hoofdstuk 7**). Uit Part II en III kunnen we concluderen dat klinische kenmerken niet gebruikt kunnen worden om te voorspellen wie baat heeft van het medicijn of niet, om de effectiviteit van het medicijn te vergroten zou men dit wel willen. In **deel IV** bestuderen we de nieuwe stappen die nodig zijn om dit te bereiken. Een nieuwe aanpak hierin kan zijn om patiënt-specifiekere gegevens te gebruiken, het DNA van patiënten (**hoofdstuk 8 en 9**). In **deel V** wordt het design en rationale voor het opzetten van een grote genetische studie binnen de EUROPA trial besproken (**hoofdstuk 10**). De hypothese van deze studie was dat er bepaalde genetische factoren, met name in het direct pharmacodynamische pathway van ACE-remmers, een effect hebben op de werking van het medicijn. Inderdaad bleken er genetische factoren in het renine-angiotensine-aldosteron systeem en kallikrein-bradykinin systeem te bestaan die het behandeleffect van perindopril beïnvloeden (**hoofdstuk 11**). Drie afwijkingen in het DNA van patiënten bleken gerelateerd aan het behandel effect. Een gecombineerd risico profiel van deze 3 afwijkingen liet een stapsgewijze afname van het behandeleffect met perindopril zien. Patienten zonder deze genetische afwijkingen lieten een zeer uitgesproken behandeleffect zien **(responders, 73.5%)**. Echter, mensen met 3 of meer van de genetische varianten bleken geen enkel behandeleffect van de behandeling met perindopril **(non-responders, 26.5%)** gedurende de 4 jaren van follow-up te ondervinden. Deze bevindingen werden geverifeerd in de PROGRESS trial. Dit ontwikkelde pharmacogenetisch profiel identificeert responders en non-responders, en kan zo het aantal onnodig behandelde patiënten en de kosten voor de gezondheidszorg reduceren. De genetische factoren in het renine-angiotensine en kallikrein-bradykinin systeem bleken ook gerelateerd aan de bloeddruk van patiënten en de bloeddruk daling op behandeling met ACE-remmers (**hoofdstuk 12**). Echter, de verschillen in deze bloeddruk daling waren zo klein dat dit geen klinische effecten had. Tevens staat het werkingsmechanisme van ACE-remmers, hetzij bloeddruk afhankelijk of bloeddruk onafhankelijk effecten ter discussie, iets wat versterkt wordt door deze genetische bevindingen. Deze resultaten sporen aan tot nader onderzoek in het exacte werkingsmechanisme van ACE-remmers. Tenslotte worden in **deel VI** de klinische implicaties van onze bevindingen besproken: "the feasibility of tailored-therapy of ACE-inhibitors" **hoofdstuk 13**).

Dit proefschrift beschrijft een nieuw concept van tailored-therapy van ACE-remmers door middel van genetische informatie van patiënten. Dit concept valt te generaliseren naar andere medicijnen binnen de cardiologie zoals de beta-blockers, calcium antagonisten en

plaatjesremmers, maar zeker ook de cholesterolverlagers (statines) verdienen een dergelijk aanpak om het juiste medicijn bij de juiste patiënt te krijgen. Statines zijn een van de meest voorgeschreven medicijnen in de cardiologie en tonen net als de ACE-remmers ook een grote consistentie in het behandeleffect (**deel VII, hoofdstuk 14**). Te overwegen valt of een dergelijke pharmacogenetische aanpak standaard procedure dient te worden in de opzet van nieuwe gerandomiseerde klinische trials. In de algemene discussie (**deel VIII, hoofdstuk 15**) worden de methodologische overwegingen met betrekking tot de studies in dit proefschrift beschreven. Ook worden de belangrijkste bevindingen in perspectief geplaatst van de huidige inzichten en worden de klinische implicaties voor de toekomst van de dagelijkse praktijk van een dokter besproken. Tenslotte worden de richtpunten van verder onderzoek besproken.



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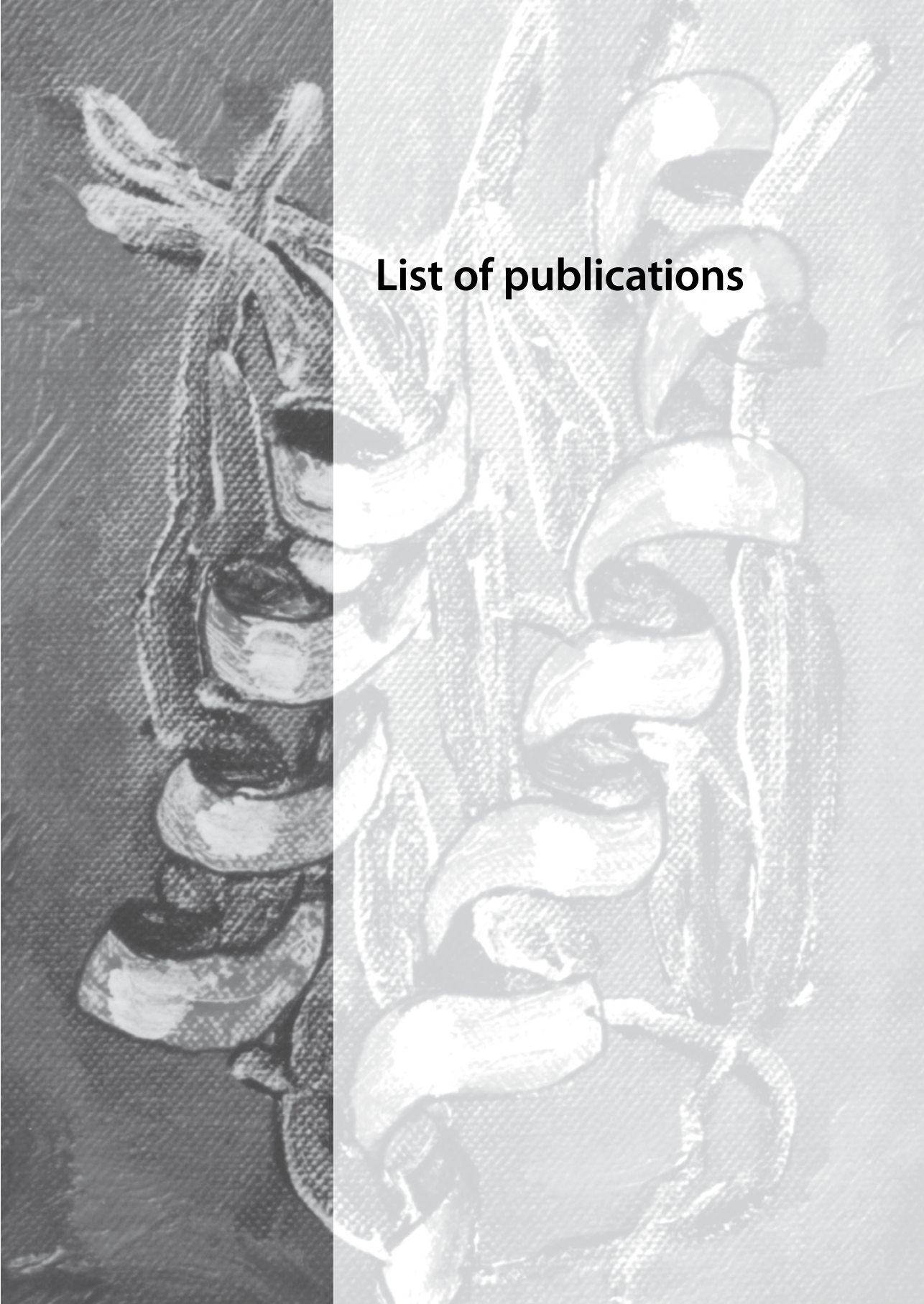


# List of publications

## LIST OF PUBLICATIONS

**Brugts JJ**, Yetgin T, Hoeks S, Gotto AM, Shepherd J, Westendorp R, de Craen T , Knopp R, Nakamura H, Ridker P, van Domburg R, Deckers JW. The benefits of HMG-CoA reductase inhibitors in patients without established cardiovascular disease with traditional cardiovascular risk factors. *BMJ* 2009;30(338):2376-2386.

**Brugts JJ,** Boersma E, Chonchol M, Deckers J, Bertrand M, Remme W, Ferrari R, Fox K, Simoons ML. The cardioprotective effects of the angiotensin-converting enzyme inhibitor perindopril in patients with stable coronary artery disease are not modified by mild to moderate renal insufficiency: insights from the EUROPA trial. *J Am Coll Cardiol.* 2007;27:2148-2155.

**Brugts JJ**, Boersma E, Decker JW, Chalmers J, MacMahon S, Bertrand M, Remme W, Fox K, Ferrari R, Simoons ML. Consistency of the treatment effect of an ACE-inhibitor based treatment regimen in patients with vascular disease or high-risk of vascular disease: A combined analysis of ADVANCE, EUROPA, and PROGRESS. *Eur Heart J.* 2009;30(11):1385-94.

**Brugts JJ**, Knetsch A, Mattace-Raso FUS, Hofman A, Witteman JCM. Renal function and risk of myocardial infarction in an elderly population: the Rotterdam Study. *Arch Intern Med.* 2005; 165:2659-65.

**Brugts JJ,** Mercado N, Ix JH, Shlipak MG, Dixon SR, Gersh B, Teirstein P, Wijns W, Serruys PW, O'Neill W, Boersma E. Periprocedural bleeding complications and long-term outcome after percutaneous coronary intervention (Results from the EXCITE-trial). *Am J Cardiol*. 2008;102(9):1151-5.

**Brugts** JJ, Danser AHJ, de Maat MPM, den Uil CA, Boersma E, Ferrari R, Simoons ML. Pharmacogenetics of ACE inhibition in stable coronary artery disease: steps towards tailored drug therapy. *Curr Opin Cardiol.* 2008;23:296-301.

**Brugts JJ**, Ferrari R, Simoons ML. Drug profile on ACE-inhibitors: pharmacokinetic and pharmacodynacmis aspects of perindopril in the treatment of cardiovascular disease. *Expert Rev Cardiovasc Ther* 2009;7(4):345-60.

**Brugts JJ**, de Maat MPM, Boersma E, Witteman J, van Duijn C, Uitterlinden A, Danser AHJ, Simoons ML. A pharmacogenetic analysis of ACE-inhibition in stable coronary artery disease: Desgin and rationale of the PERindopril GENEtic association study (PERGENE). *Cardiovasc Drugs Ther* 2009;23(2):171-181.

**Brugts JJ,** den Uil CA, Danser AHJ, Boersma E. The Renin-Angiotensin-Aldosterone System: Approaches to guide Angiotensin-Converting Enzyme inhibition in patients with coronary artery disease. *Cardiology* 2008;112(4):303-312.

**Brugts JJ**, de Maat MPM, Den Uil CA, Danser AHJ. Angiotensinogen haplotypes in patient with hypertension. *J Hypertension* 2008;26(12):2451-4.

**Brugts JJ**, Simoons ML. Lessons learned from ACE-inhibitor trials in stable CAD: Meta-analysis of ACE inhibitors in coronary artery disease. In: Coversyl Compendium, Chapter 9, 1st ed. Paris, France. ESC 2008 *Wolters Kluwer-Health, Lippincott, Williams&Wilkins*.

**Brugts JJ**, den Uil CA. Angiotensin-converting enzyme insertion/deletion polymorphism in sepsis and acute respiratory distress syndrome. *Intensive Care Med.* 2008;34(9):1733-1735.

**Brugts JJ**, Boersma E, Simoons ML. Tailored-therapy of angiotensin-converting enzyme inhibitors in coronary artery disease: pharmacogenetic profiling of treatment benefit. *Pharmacogenomics* 2010 *in press*.

**Brugts JJ**, Deckers JW. Statin prescription in those without cardiac disease: What the evidence tells us. *Curr Opin Cardiol 2010* in press.





**Brugts JJ**, Simoons ML. Antihypertensive treatment efficacy of angiotensin-converting enzyme inhibitors, clinical and genetic aspects. *Medicographia* 2010;105 *in press*.

**Brugts JJ**, Danser AHJ, de Maat MPM, Boersma E, Simoons ML. Results and clinical implications of the PERindopril GENEtic association study: pharmacogenetics of ACE-inhibitors. *Neth Heart J* 2010 *in press*.

**Brugts JJ**, Isaacs A, Boersma E, Witteman JCM, van Duijn C, Uitterlinden A, Remme W, Ceconi C, Chalmers J, MacMahon S, Fox K, Bertrand M, Ferrari R, Danser AHJ, Simoons ML, de Maat M. Genetic determinants of the treatment effect of ACE-inhibitor therapy in patients with stable coronary artery disease. Submitted to *European Heart Journal*.

**Brugts JJ,** Isaacs A, Boersma E, Witteman JCM, de Maat MPM, van Duijn C, Uitterlinden A, Remme W, Ceconi C, Akkerhuis K, Ninomiya N, Chalmers J, MacMahon S, Fox K, Bertrand M, Ferrari R, Simoons ML, Danser AHJ. Genetic determinants of blood pressure ands blood pressure response to ACE-inhibitor therapy in patients with stable coronary artery disease. Submitted to *Circulation Genetics*.

**Brugts JJ**, de Maat MPM, van Duijn C, Uitterlinden A, Remme W, Ceconi C, Fox K, Bertrand M, Ferrari R, Danser AHJ, Simoons ML, Boersma E. Genetic variation in the NPPA gene and response to ACE-inhibitors in patients with stable coronary artery disease. *Submitted*.

**Brugts JJ**, de Maat MPM, van Duijn C, Uitterlinden A, Remme W, Ceconi C, Fox K, Bertrand M, Ferrari R, Danser AHJ, Simoons ML. Boersma E. Genetic variation in the adducin-1 gene and response to ACE-inhibitors. *Submitted*.

Mercado N, **Brugts JJ**, Ix JH, Shlipak MG, Dixon SR, Gersh B, Teirstein P, Wijns W, Serruys PW, O'Neill W, Boersma E. Usefulness of Proteinuria as a Prognostic Marker of Mortality and Cardiovascular Events among Patients Undergoing Percutaneous Coronary Intervention (Results from the EXCITE Trial). *Am J Cardiol.* 2008;102(9):1151-5.

Oemrawsingh R, **Brugts JJ**, Akkerhuis KM, de Maat MPM, van Duijn C, Uitterlinden A, Remme W, Ceconi C, Fox K, Bertrand M, Ferrari R, Danser AHJ, Simoons ML Boersma E. Genetic determinants of the risk of recurrent cardiac events in patients with stable coronary artery disease. *Submitted*.

Van Vark L, **Brugts JJ**, Akkerhuis KM, de Maat MPM, van Duijn C, Uitterlinden A, Remme W, Ceconi C, Fox K, Bertrand M, Ferrari R, Danser AHJ, Simoons ML Boersma E. Genetic deteminants of incident hypertension and blood pressure during 4 years of follow-up in patient with stable coronary artery disease. *Submitted*.

Den Uil CA, Lagrand W, **Brugts JJ**, Spronk P. Microcirculation and multi-organ failure in patients with sepsis. *Intensiv Care Med* 2008;34(12):2303-4.

Den Uil CA, Klijn E, **Brugts JJ**, Lagrand WK, Spronk PE. Monitoring of the sublingual microcirculation in cardiac surgery using two-dimensional imaging. *Anesthesiology* 2008; 109(2):353-4

Feringa H, Schouten O, Karagiannis S, **Brugts JJ**, Boersma E, Vidakovic R, van Sambeek M, Noordzij P, Bax JJ, Poldermans D. Intensity of statin therapy in relation to myocardial ischemia, troponin T release, and clinical cardiac outcome in patients undergoing major vascular surgery. *J Am Coll Cardiol.* 2007;50(17):1649-56.

Den Uil CA, Klijn E, Lagrand WK, **Brugts JJ,** Ince C, Spronk PE, Simoons ML. The Microcirculation in Health and Critical Disease. *Progr Cardiovasc Dis* 2008;21:1-10

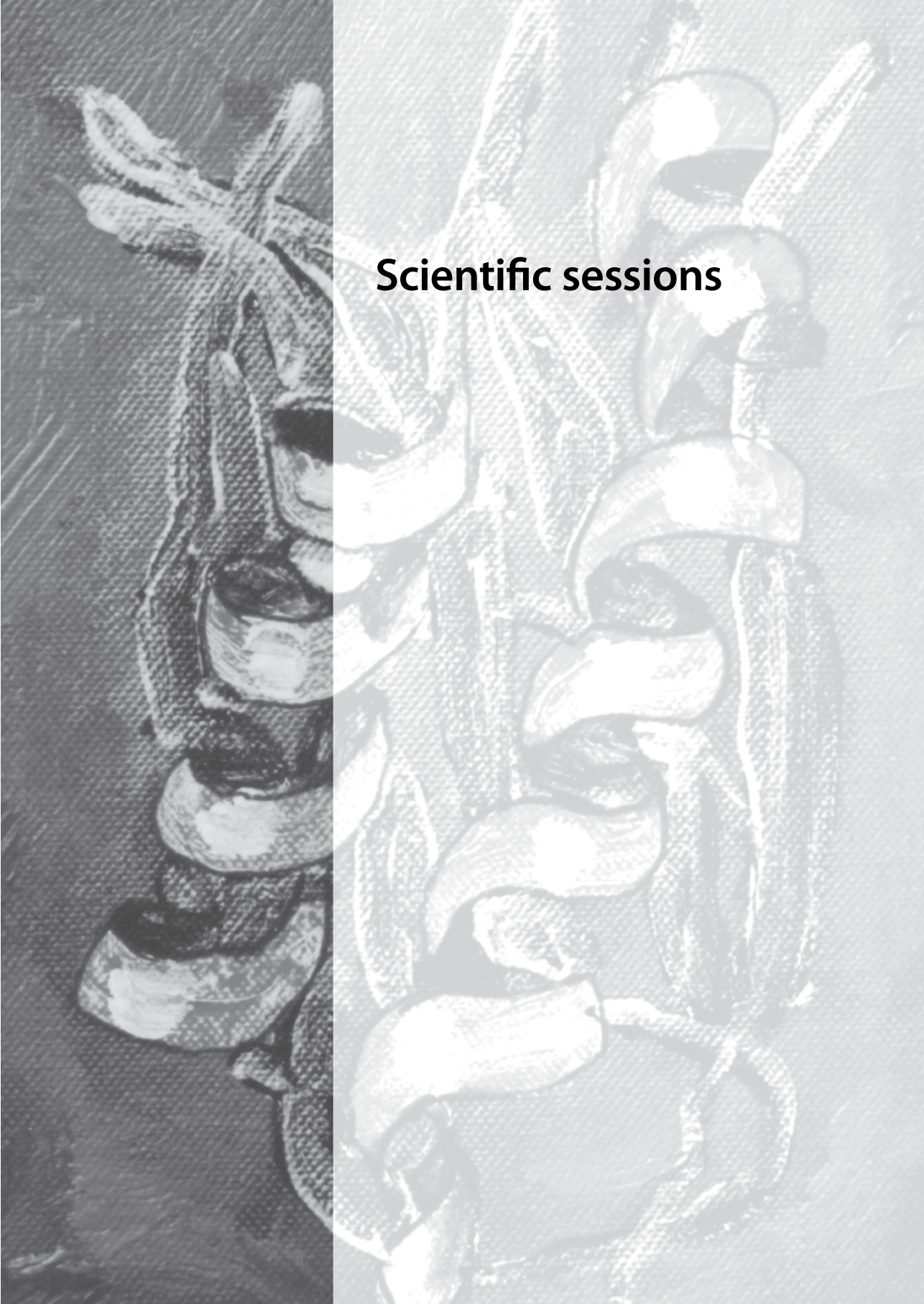
Feringa H, Karagiannis S, Vidakovic R, Noordzij P, **Brugts JJ**, Schouten O, van Sambeek M, Bax JJ, Poldermans D. Comparison of the incidences of cardiac arrhythmias, myocardial ischemia, and

cardiac events in patients treated with endovascular versus open surgical repair of abdominal aortic aneurysms. *Am J Cardiol*. 2007;100(9):1479-84.

Den Uil CA, Lagrand WK, van der Ent M, Jewbali LS, **Brugts JJ**, Spronk PE, Simoons ML. The effects of intra-aotic balloon pump support on macrocirculation and tissue microcirculation in patients with cardiogenic shock. *Cardiology* 2009;114(1):42-46.

Den Uil CA, Lagrand WK, Spronk PE, van der Ent M, Jewbali LS, **Brugts JJ**, Ince C, Simoons ML. Low-dose nitroglycerin improves microcirculation in hospitalized patients with acute heart failure. *Eur J Heart Fail*. 2009;11(4):386-90.

Den Uil CA, Lagrand WK, Spronk PE, van Domburg R, Hofland J, Luthen C, **Brugts JJ**, van der Ent M, Simoons ML. Impaired sublingual microvascular perfusion during surgery with cardiopulmonary bypass: a pilot study. *J Thorac Cardiovasc Surg.* 2008;136(1):129-34.



# Scientific sessions

## ORAL COMMUNICATIONS

### (presenter)

Genetic determinants of treatment effect of ACE-inhibitors in patients with stable coronary artery disease. [*Young Investigator Award*, 58th Annual Scientific Session of the American College of Cardiology 2009, Orlando, Florida, United States of America] *J Am Coll Cardiol* 2009.

Genetic factors related to decreased outcome with ACE-inhibitor therapy in patient with stable CAD. *State of the Art session "Pharmacogenetics, the new frontier of hypertension trials"*, best abstract. European Society of Cardiology congress 2009. Barcelona, Spain. *Eur Heart J* 2009.

Individualized ACE-inhibitor therapy in stable coronary artery disease. Nederlandse Vereniging voor Cardiologie 2009 "*onderzoeksprijs* General Cardiology", Amsterdam, the Netherlands. *Neth Heart J* 2009.

Genetic factors related to blood pressure and blood pressure response to ACE-inhibitor therapy in patient with stable CAD. European Society of Cardiology congress 2009, Barcelona, Spain. *Eur Heart J* 2009.

The Use of Statins in the Primary Prevention of Cardiovascular Disease: A Combined Analysis of Nine Randomized Controlled Trials. Annual Scientific Session of the American Heart Association 2008, New Orleans, United States of America. *Circulation* 2008.

Strong and Independent Association between Angiotensinogen Gene Polymorphisms and Hypertension in 10060 Patients with Stable Coronary Artery Disease. Annual Scientific Session of the American Heart Association 2008, New Orleans, Louisiana, United States of America. *Circulation* 2008.

Tailored-therapy of ACE-inhibitors in cornary artery disease: genetic aspects. American Society of Hypertension 2010, New York, United States of America.

ACE-inhibitors in cardiovascular disease: what about blood pressure and genetics? Cardiology and Vascular Medicine Update and Perspective 2009, European Society of Cardiology, Congress center de Doelen, Rotterdam, the Netherlands.

Renin-angiotensin-aldosterone system genes and treatment effect of ACE-inhibitors. 14th International Conference on Pharmacogenetics and Pharmacogenomics 2008, Seoul, Pusan, Republic of South-Korea.

Pharmacogenetics of ACE-inhibitors in stable coronary artery disease. Cardiovasculair Onderzoeks Instituut Erasmus Universiteit Rotterdam 2008, the Netherlands

Hypertension trials and new aspects of hypertension management. Cardiovasculair Onderzoeks Instituut Erasmus Universiteit Rotterdam 2009, the Netherlands

Impaired outcome of ACE-inhibitors according to genetic variation in the renin-angiotensin system. Cardiovasculair Onderzoeks Instituut Erasmus Universiteit Rotterdam 2009, the Netherlands

Renal function and the risk of myocardial infarction in the elderly. [European Society of Cardiology congress EURO PREVENT; Leuven, Belgium, oral communication] *European Journal of Cardiovascular Prevention & Rehabilitation* 2005;12(3):277-278.

## POSTER SESSIONS

### (first author)

**Brugts JJ**, Boersma E, Chonchol M, Deckers J, Bertrand M, Remme W, Ferrari R, Fox K, Simoons ML. The cardioprotective effects of the ACE-inhibitor perindopril in patients with coronary artery disease are not modified by renal insufficiency (ESC 2007). *Eur. Heart J*. 2007;28:S889-S949.

**Brugts JJ**, Boersma E, Chonchol M, Deckers J, Bertrand M, Remme W, Ferrari R, Fox K, Simoons ML. Treatment benefit of perindopril is consistent independent of renal insufficiency (AHA 2007). *Circulation* 2007;116:783.

**Brugts JJ**, de Maat MPM, Boersma E, Witteman JCM, van Duyn C, Uitterlinden A, Danser AHJ, Fox K, Ferrari R, Simoons ML. Functional polymorphisms in the angiotensinogen gene are associated with hypertension in patients with coronary artery disease (ESC 2008). *Eur Heart J* 2008;29:901-962.

**Brugts JJ**, Mercado N, Wijns W, Serruys PW, O'Neill W, Boersma E. Proteinuria is significantly and independently associated with all-cause mortality among patients undergoing percutaneous coronary intervention (ESC 2008). *Eur Heart J* 2008;29:901-962.

**Brugts JJ**, Boersma E, Deckers JW, Bertrand M, Remme W, Fox K, Ferrari R, Simoons ML. Consistent treatment effect of perindopril in patients with vascular disease or high risk of vascular disease: A combined analysis of three perindopril trials (ESC 2008). *Eur Heart J* 2008;29:901-962.

**Brugts JJ**, de Maat MPM, Boersma E, Witteman JCM, van Duyn C, Uitterlinden A, Danser AHJ, Fox K, Ferrari R, Simoons ML. Promotor polymorphisms of the angiotensinogen gene are associated with hypertension in patients with stable CAD (ESC 2008). *Eur Heart J* 2008;29:901-962.

**Brugts JJ**, Ninomiya T, Boersma E, Remme W, Bertrand M, Chalmers J, MacMahon S, Ferrari R, Fox K, Simoons ML The Treatment Effect of Perindopril in Patients with Vascular Disease or High Risk of Vascular Disease (AHA 2008). *Circulation* 2008;118: S_1138.

**Brugts JJ**, Mercado N, Ix J, Shlipak MG, Gersh BJ Wijns W, O'Neill WW, Serruys PW, Boersma E. The Need for Blood Transfusion Is an Independent Predictor of Incident Cardiovascular Events and All-Cause Mortality among Patients Undergoing Percutaneous Coronary Intervention: Results from the EXCITE trial (AHA 2008). *Circulation* 2008;118: S_1049.

**Brugts JJ**, de Maat M, Boersma E, Witteman JCM, van Duijn C, Uitterlinden A, Remme W, Ceconi C, Fox K, Bertrand M, Ferrari R, Danserr AHJ, Simoons ML. Direct Relation between Angiotensin-Converting Enzyme (ACE) Gene Polymorphisms and Hypertension in Patients with Stable Coronary Artery Disease (AHA 2008). *Circulation* 2008; 118: S_889.

**Brugts JJ**, de Maat M, Boersma E, Witteman JCM, van Duijn C, Uitterlinden A, Remme W, Ceconi C, Fox K, Bertrand M, Ferrari R, Danserr AHJ, Simoons ML. Strong and Independent Association between Angiotensinogen Gene Polymorphisms and Hypertension in 10060 Patients with Stable Coronary Artery Disease (AHA 2008). *Circulation*, 2008;118: S_729.

**Brugts JJ**, Yetgin T, Hoeks SE, Gotto A, Knopp R, Nakamura H, Westendorp R, de Craen A, Shepherd J, Ridker P, van Domburg R, Deckers J. The Use of Statins in the Primary Prevention of Cardiovascular Disease: A Combined Analysis of Nine Randomized Controlled Trials. *Circulation* 2008; 118: S_1150.

**Brugts JJ**, de Maat M, Boersma E, Uitterlinden AG, van Duijn C, Witteman JCM, Danser AHJ, Simoons ML. Pharmacogenetics of ACE-inhibitor therapy: tailored therapy of ACE-inhibitors. Nederlandse Hartstichting wetenschapsdag 2008.

**Brugts JJ**, Isaacs A, Boersma E, Witteman JCM, de Maat MPM, van Duijn C, Uitterlinden A, Remme W, Ceconi C, Fox K, Bertrand M, Ferrari R, Danser AHJ, Simoons ML Genetic variation in renin-angiotensin system genes and the treatment benefit of ACE-inhibitor therapy in patients with stable coronary artery disease: results from the EUROPA trial (ACC 2009). *J Am Coll Cardiol* 2009;53:A331-A366

**Brugts JJ**, Isaacs A, Boersma E, Witteman JCM, de Maat MPM, Danser AHJ, van Duijn C, Uitterlinden A, Remme W, Ceconi C, Fox K, Bertrand M, Ferrari R, Simoons ML. Genetic variation in renin-angiotensin system genes in relation to blood pressure and blood pressure response to ACE-inhibitor therapy in patients with stable coronary artery disease: results from the EUROPA trial (ACC 2009). *J Am Coll Cardiol* 2009;53:A331-A366.

**Brugts JJ**, Ninomiya T, Boersma E, Remme W, Bertrand M, Chalmers J, MacMahon S, Ferrari R, Fox K, Simoons ML. Blood pressure and the treatment effect of perindopril-based regimens in 29463 patients with vascular disease: combined analysis of ADVANCE, EUROPA, PROGRESS trials (ACC 2009). *J Am Coll Cardiol* 2009;53:A367-A402.

**Brugts JJ**, Isaacs A, Boersma E, Witteman JCM, de Maat MPM, van Duijn C, Uitterlinden A, Remme W, Ceconi C, Fox K, Bertrand M, Ferrari R, Simoons ML, Danser AHJ Renin-angiotensin system gene polymorphisms and incident risk for recurrent cardiovascular events in patients with stable coronary artery disease: results from the EUROPA trial (ACC 2009). *J Am Coll Cardiol* 2009;53:A367-A402.





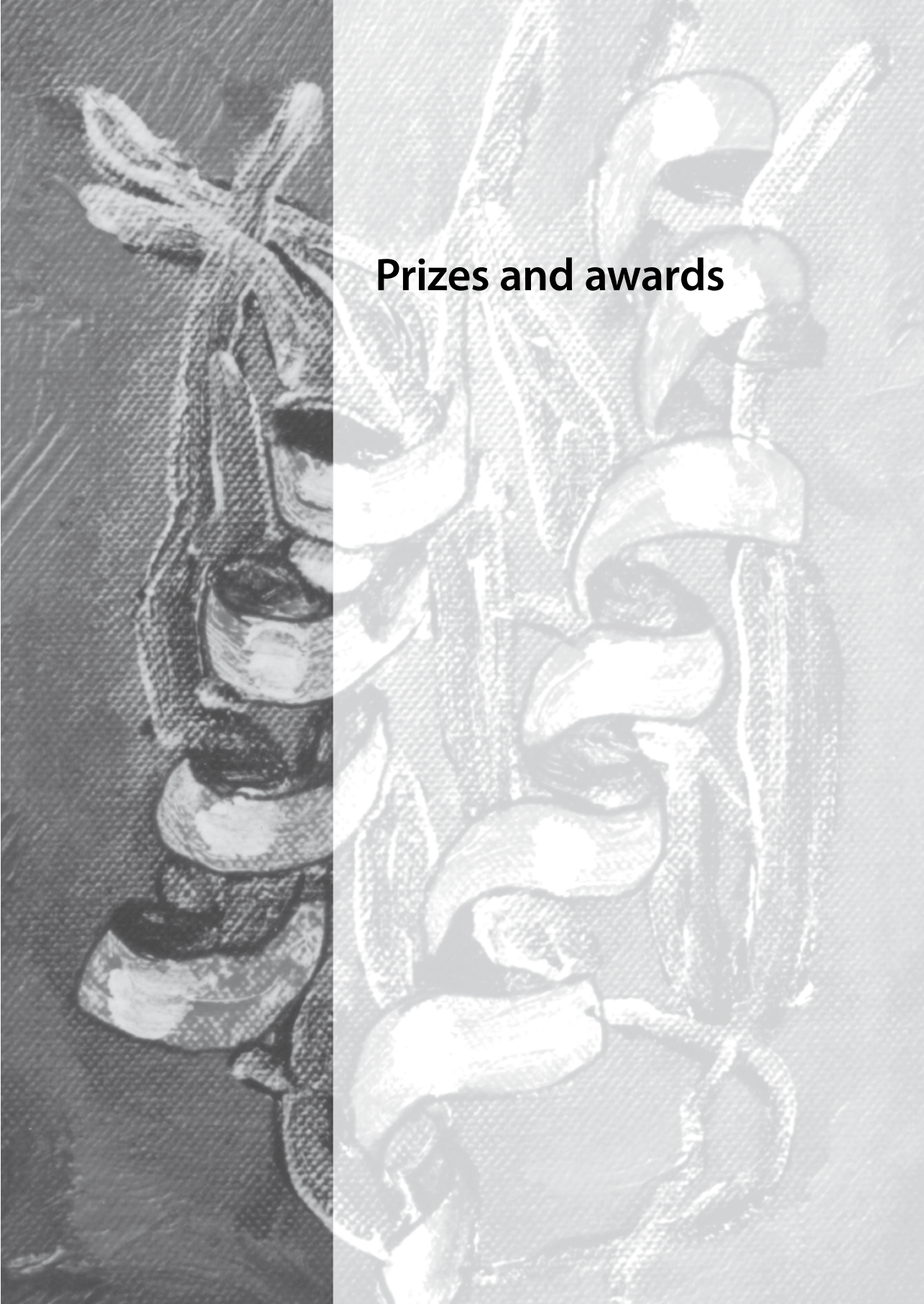
**Brugts JJ**, Isaacs A, Boersma E, Witteman JCM, de Maat MPM, Danser AHJ, van Duijn C, Uitterlinden A, Remme W, Ceconi C, Fox K, Bertrand M, Ferrari R, Simoons ML. Identification of Patients with Impaired Outcome on ACE-inhibitor therapy (ESC 2009). *Eur Heart J* 2009;30:1039-1108.

**Brugts JJ**, Isaacs A, Boersma E, Witteman JCM, de Maat MPM, van Duijn C, Uitterlinden A, Remme W, Ceconi C, Fox K, Bertrand M, Ferrari R, Simoons ML, Danser AHJ. Genetic determinants of blood pressure and blood pressure response to ACE-inhibitor therapy in patients with stable coroanry artery disease (ESC 2009). *Eur Heart J* 2009;30:1039-1108.

**Brugts JJ**, Yetgin T, Hoeks S, Gotto A, Knopp R, Nakamura H, Westendorp R, de Craen A, Shepherd J, Ridker P, van Domburg R, Deckers J.W. The use of statins in primary prevention. (ESC 2009). *Eur Heart J* 2009;30:1039-1108.

**Brugts JJ**, Yetgin T, Hoeks S, Gotto A, Knopp R, Nakamura H, Westendorp R, de Craen A, Shepherd J, Ridker P, van Domburg R, Deckers J.W. The benefits of HMG-CoA reducatse inhibitors in patients without established cardiovascular disease with cardiovascular risk factors: meta-analysis of randomized trials (NVVC congres 2009). *Neth Heart J* 2009.

**Brugts JJ**, de Maat M, Boersma E, Uitterlinden AG, van Duijn C, Witteman JCM, Danser AHJ, Simoons ML. Tailored-therapy of ACE-inhibitors in stable coronary artery disease (NVVC 2009). *Neth Heart J* 2009.



# Prizes and awards

**Young Investigator Award 2009**

58th Annual Scientific Session of the American College of Cardiology, Clinical Cardiology, Orlando, United States of America

**State of the Art lecture**

Pharmacogenetics, the new frontier of hypertension trials.
Best abstract. European Society of Cardiology Congress 2009, Barcelona, Spain

**NVVC onderzoeksprijs "General Cardiology". Best presentation**

Nederlandse Vereniging voor Cardiologie najaarscongres 2009, Amsterdam.

**ZonMW-NWO AGIKO**

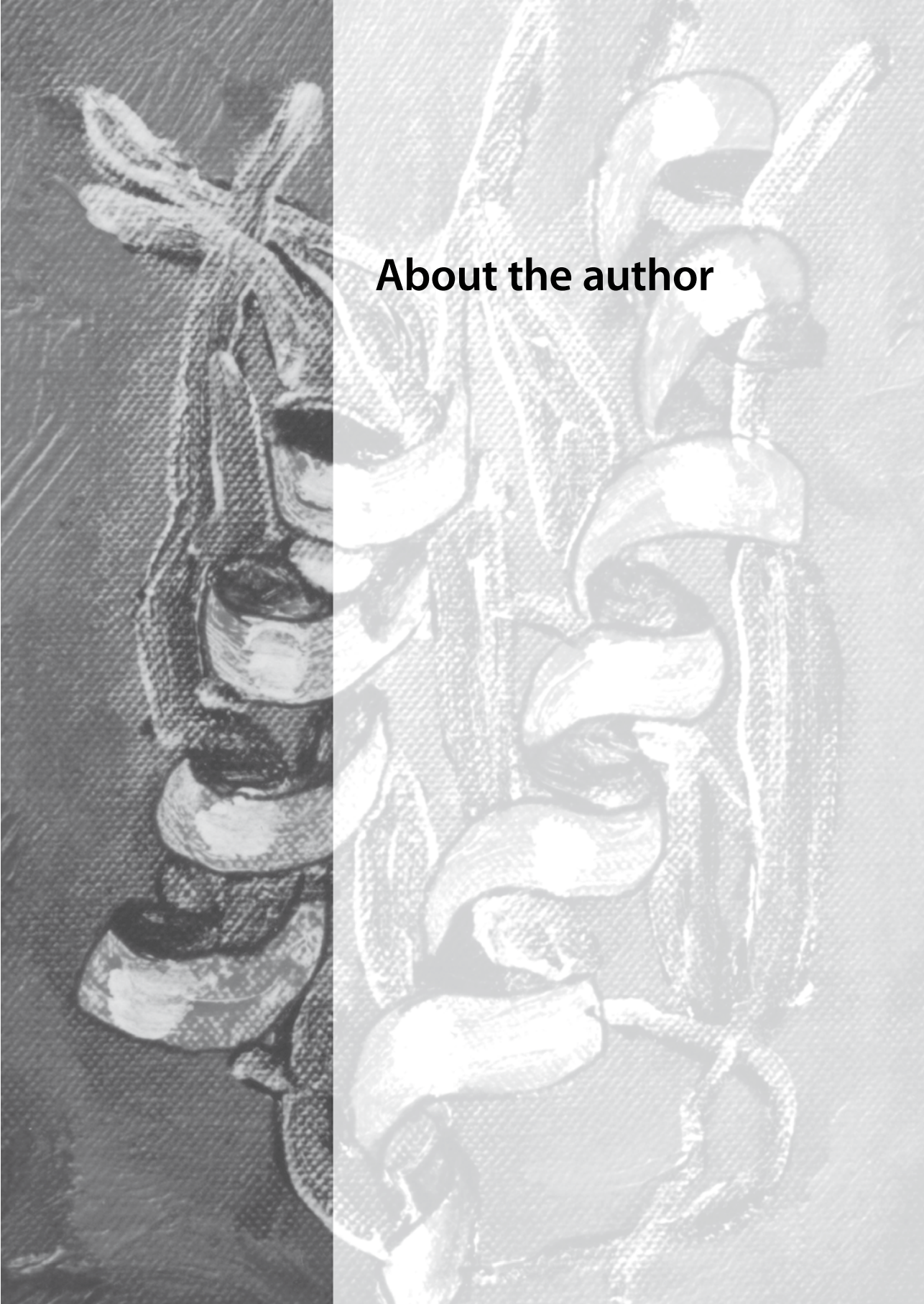
winnaar nationale persoonsgebonden stipendium, 2006

**Nederlandse Hartstichting subsidie onderzoeksproject**

"Pharmacogenetics of ACE-inhibitors in stable CAD: the EUROPA trial"

**Nederlandse Hartstichting**

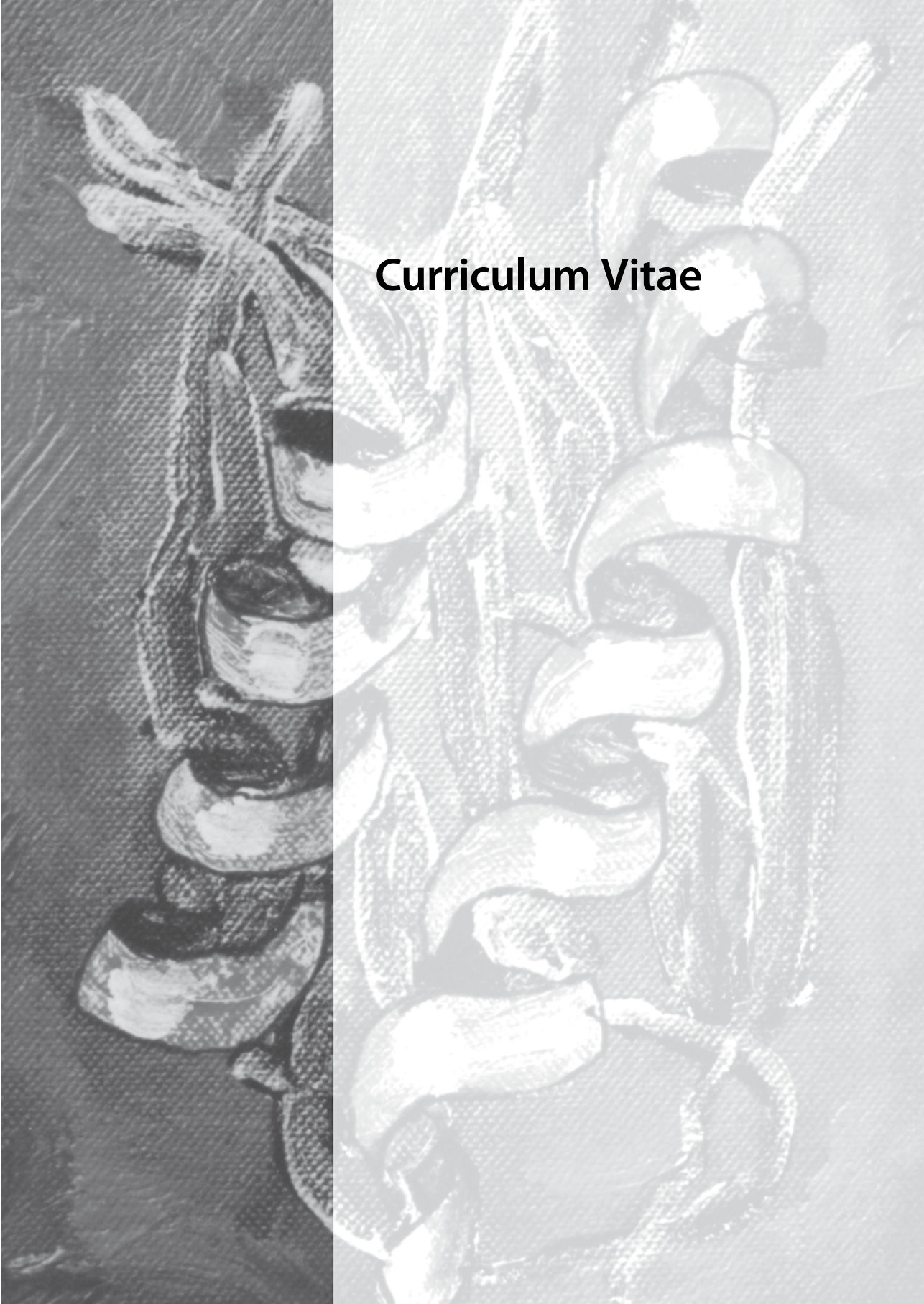
Dr. Stiggelbout onderzoeksubsidie NHS2005B219



# About the author

## ABOUT THE AUTHOR

Jasper Brugts was born on 12 May 1981 in Rotterdam. In 2000, he graduated from the "Erasmiaans Gymnasium" in Rotterdam. Hereafter, he started his medical training at the Erasmus Medical Center in Rotterdam. During medical school, he participated in several electives in anatomy, cardiology and clinical research. In 2002, he was selected to participate in the Master degree programme for excellent medical students at the Erasmus Medical Center to coincide with his medical training. He completed the Master of Science program in Clinical Epidemiology at the Netherlands Institute for Health Sciences in 2004 (head: Prof. Dr. A. Hofman). As part of this program, he studied cardiovascular epidemiology at Cambridge University in Cambridge, United Kingdom and clinical epidemiology and management of health care organizations at Harvard Medical School and Harvard University in Boston, United States of America. In 2004, he participated in research on renal insufficiency in cardiovascular disease with the Cardiovascular Epidemiology group at the department of Epidemiology & Biostatistics (head: Prof Dr J. Witteman). In September 2006, he graduated with honors from medical school at the Erasmus Medical Center. He succeeded in winning the Dutch national selection of the ZonMw AGIKO research stipendium of the year 2006 and started as research fellow with Prof. dr. Simoons at the department of Cardiology on the research project "Pharmacogenetics of ACE-inhibitor therapy in stable CAD, the EUROPA-trial" funded by the Netherlands Heart Foundation. During his PhD, Dr. Brugts succeeded in publishing his work in high-ranked international journals, f.e. BMJ, JACC, Arch Intern Med and Eur Heart J and acted as a reviewer in Circulation, Eur Heart J and Am J Cardiol. In March 2009, he was winner of the Young Investigator Award of the American College of Cardiology in Clinical Cardiology for the work on "individualized ACE-inhibitor therapy: the feasibility of pharmacogenetic profiling" and ranked second during the grand finale of the ACC assembly. Furthermore, he presented the main results of his project at the State of the Art session of the European Society of Cardiology on "pharmacogenetics, the new frontier of hypertension trials" (best abstract) and won the science award in "General Cardiology" of the Dutch Society of Cardiology (2009). In addition, he is a member of the ESC working group on Cardiovascular Drug Therapy, and participates in the Young CardioVascular Clinical Trialist's initiative (global CVCT forum). In April 2009, he started as a resident at the department of Internal Medicine at the Albert Schweitzer hospital in Dordrecht (head: Dr. E.F.H. van Bommel) as part of his Cardiology training (head: Dr. M.J.M. Kofflard). He will continue his training in Cardiology at the Erasmus MC Thoraxcenter (head: Prof.Dr M.L. Simoons).



# Curriculum Vitae

[redacted]

# J.J. BRUGTS M.D. M.SC.

## PERSOONLIJKE GEGEVENS

- Naam: Jan Jasper Brugts (Jasper)
- Adres: Kralinger Esch 266
  3063 NB Rotterdam
- Nationaliteit: Nederlandse
- Geslacht: Mannelijk
- Leeftijd: 28 jaar (12 Mei 1981)
- Geboorteplaats: Rotterdam

## OVERZICHT VAN DIPLOMA'S

| | | |
|---|---|---|
| [1993-2000] | Erasmiaans Gymnasium | Rotterdam |
| [2000-2001] | Propedeuse Geneeskunde Erasmus MC | Rotterdam |
| [2001-2004] | Doctoraal Geneeskunde Erasmus MC | Rotterdam |
| [2002-2004] | Master of Science in Clinical Epidemiology | Utrecht |
| [febr. 2003] | Cardiovascular Disease Epidemiology module | Cambridge |
| [juli 2004] | Harvard Medical School, Management courses | Boston |
| [aug. 2004] | Harvard School of Public Health, Research courses | Boston |
| [sept. 2006] | Artsexamen Geneeskunde Erasmus MC<br>***Cum Laude*** | Rotterdam |

## WERKERVARING

| | | |
|---|---|---|
| [2002-2005] | Thoraxcentrum Erasmus MC<br>Studententeam Cardiologie Medium Care/CCU<br>(patiëntenzorg) | Rotterdam |
| [2004-2006] | Erasmus MC Klinische Fase<br>***Cum Laude*** | Rotterdam |
| [april 2009] | Albert Schweitzer Ziekenhuis<br>Vooropleiding interne geneeskunde<br>(Opleider Dr. van Bommel) | Dordrecht |
| [mei 2009] | Fundamental Critical Care Support | Lunteren |
| [jun. 2009] | Advanced Life Support | Houten |
| [aug. 2009] | Advanced Cardiac Life Support | Houten |

## ONDERZOEKSERVARING

**Master of Science in Clinical Epidemiology**

Netherlands Institute for Health Sciences Research
Postgraduate Research Training (International Degree Programme Erasmus MC).

**Overview MSc.:**

| | |
|---|---|
| [2002 – 2004] | Summer research programme n i h e s / Erasmus MC |
| [2003 – 2004] | Winter research programme n i h e s / Erasmus MC |
| [2002 – 2004] | Spring Courses en Advanced Research Courses<br>Erasmus MC, UMC Utrecht |
| [2003 – 2004] | Methodologische training in statistiek programma's<br>SPSS, Stata, SAS, R. |
| [ 2003 – 2004 ] | Internationale programma's in epidemiologie, en management in gezondheidszorginstellingen<br>*Harvard University, Boston (USA), Cambridge University, Cambridge (UK)* |

## PROMOTIE ONDERZOEK

Promotieonderzoek "Pharmacogenetic aspects of ACE inhibition in stable coronary artery disease, The EUROPA Trial" *(Nederlandse Hartstichting subsidie B219 ).*

## SUBSIDIES EN PRIJZEN

**Young Investigator Award ACC**
58th Annual Scientific Session 2009, clinical cardiology council
American College of Cardiology, Orlando, United States of America

**European Society of Cardiology**
State of the Art lecture "The new fronitier of hypertension trials"
Best abstract, ESC 2009, Barcelona, Spain

**Nederlandse Verening voor Cardiologie**
Onderzoeksprijs "General Cardiology" 2009, Amsterdam.

**Zon MW AGIKO** Cardiologie persoonsgebonden stipendium (voorjaarsronde 2006)

**Dr Stiggelbout** persoonsgebonden subsidie Nederlandse Hartstichting



## NEVENFUNCTIES

| | |
|---|---|
| [2002 – 2005] | KNMG Studenten-Platform, Domus Medica, Utrecht |
| [2002 – 2003] | Faculteitsraad Erasmus Universiteit Faculteit Geneeskunde |
| [2003 – 2004] | Studentenraad Erasmus Universiteit Faculteit Geneeskunde |
| [2003 – 2004] | Commissie Onderwijs&Onderzoek Erasmus MC |
| [2000 – 2004] | Onderwijsraad MORE Erasmus MC |
| [2004 – 2006] | Jaarvertegenwoordiging collegejaar 1-4 |
| [2004 – 2006] | Co-raad Erasmus MC (Commissie ECG & Echo. cursussen) |
| [2002 – 2006] | Hart- en Vaatziekten groep Afd. Epidemiologie Erasmus MC) |

## ACTIVITEITEN

- European Society of Cardiology Working Group on Cardiovascular Drug Therapy.
- Young CardioVascular Clinical Trialists' Initiative (global CVCT forum).
- Editorial Board World Journal of Cardiology.

## REFERENTIES

- Prof. Dr. A. Hofman, Wetenschappelijk Directeur NIHES.
- Prof. Dr. M.L. Simoons, afdelingshoofd Cardiologie, thoraxcentrum.
- Prof. Dr. E. Boersma, hoofd Cardiovasculaire Epidemiologie Thoraxcentrum.

## BIJLAGE

### Followed Courses

### Summer and Winter Programmes Erasmus MC

| | |
|---|---|
| Principles of Research in Medicine and Epidemiology | 2002 |
| Introduction to Data-Analysis | 2003 |
| Clinical Decision Analysis | 2002 |
| Regression Analysis | 2003 |
| Methods of Clinical Research | 2002 |
| Topics in Meta-Analysis | 2004 |
| Pharmaco-epidemiology | 2002 |
| Survival Analysis | 2003 |
| Decision Making in Medicine I | 2002 |
| Topics in evidence-based medicine | 2002 |
| Epidemiology and Public Health | 2002 |
| Study design for Scientific Medicine | 2004 |

### Core curriculum MSc. Clinical Epidemiology

| | |
|---|---|
| Study design | 2002 |
| Data-Analysis | 2003 |
| Discussion Meetings Research Proposals | 2003 |

### Skills courses

| | |
|---|---|
| Medical Writing | 2004 |
| Computer Facilities | 2002 |
| Working with SPSS for windows | 2004 |
| How to publish in medicine | 2004 |

### Advanced courses, Erasmus MC

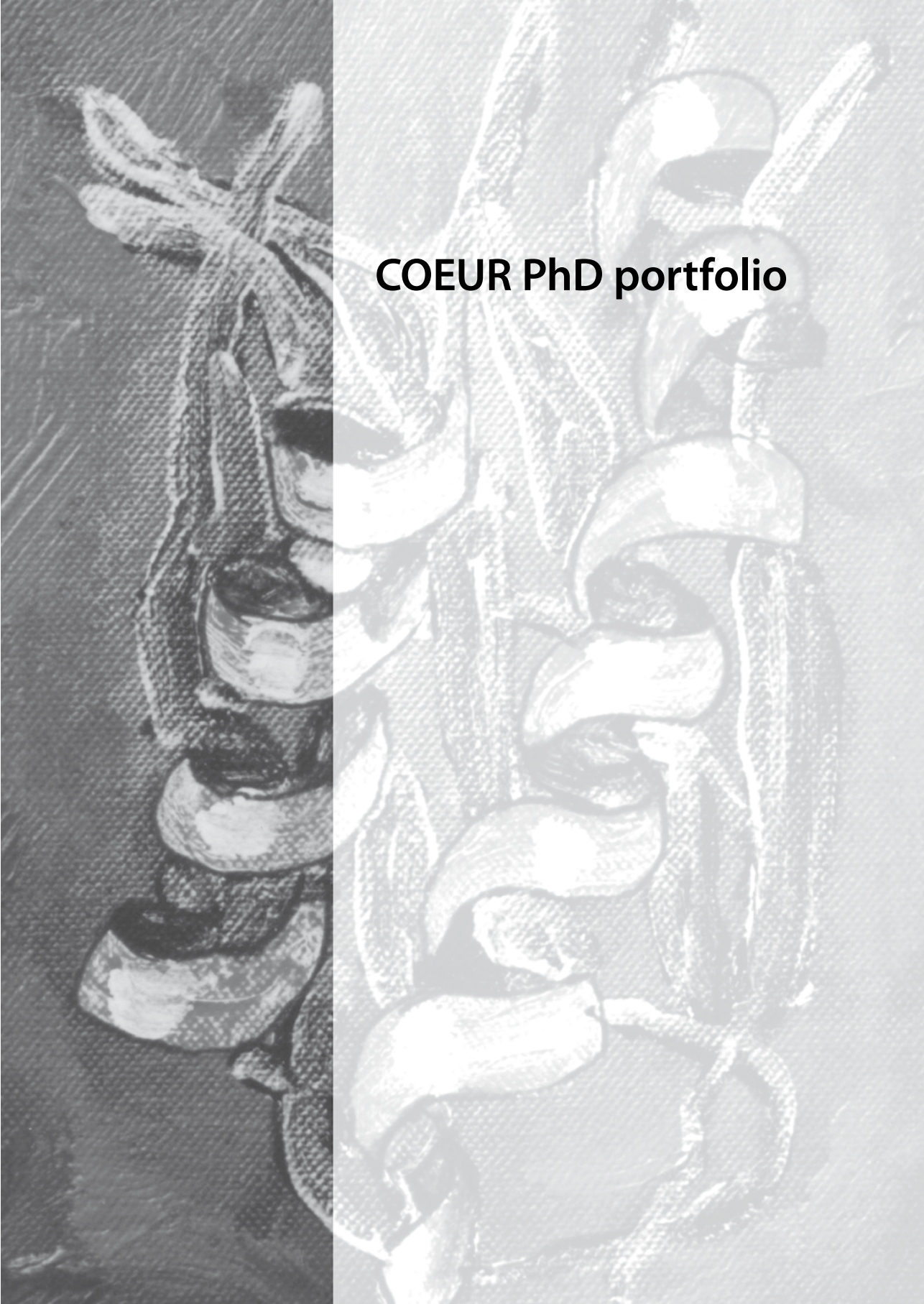
| | |
|---|---|
| Analysis of time-varying Exposures | 2004 |
| Introduction to Clinical Research | 2004 |
| Decision Making in Medicine II | 2004 |

### Advanced Courses, University of Utrecht

| | |
|---|---|
| Clinic trials and Drug Risk Assessment | 2004 |

### International courses and programmes

**Cambridge University**, Cambridge (United Kingdom) 2003
*"Cardiovascular Disease Epidemiology"* module (Prof. K. Khaw)
**Harvard Medical School and School of Public Heath** 2004
Boston, Massachusetts (United States of America)
*"Management in Health Care Organizations"* (grade: A)
*"Clinical Epidemiology"* (grade: A)



# COEUR PhD portfolio

Erasmus MC
Universitair Medisch Centrum Rotterdam

## PHD PORTFOLIO SUMMARY

**Summary of PhD training and teaching activities**

Name PhD student: J.J.Brugts PhD period: 2006-2009
Erasmus MC Department: Cardiology Promotor(s): Prof. dr. Maarten Simoons
Research School: C.O.E.U.R. Prof. dr. Eric Boersma

### 1. PhD training

| | Year | Workload (Hours/ECTS) |
|---|---|---|
| **General academic skills** | | |
| - Biomedical English Writing and Communication | 2004 | 1.5 |
| - Research Integrity, Statistical software (SPSS,SAS) | 2004 | 1.5 |
| **Research skills** | | |
| - Master of Science in Clinical Epidemiology (NIHES) | 2002-2006 | 30 |
| - Statistics (multiple statistics and advanced statistics courses) | 2002-2006 | 10 |
| - Methodology (study design, randomized trials) | 2002-2006 | 10 |
| **In-depth courses (e.g. Research school, Medical Training)** | | |
| - COEUR courses on heart failure, clinical epidemiology, cardiovascular imaging (CT, MRI, echocardiography), interventional cardiology, cardiovascular pharmacology, hypertension research. | 2006-2009 | 7.5 |
| - Cardiovascular epidemiology module, Cambridge University, Cambridge, United Kingdom. | 2004-2005 | 1.5 |
| - Management courses in Health Care Organization, Harvard Medical School, Boston, United States of America. | 2004-2005 | 4.5 |
| **Oral presentations** | | |
| - 6x Oral Communication ESC, AHA or ACC scientific sessions | 2006-2009 | 4.8 |
| - 3x COEUR research seminar presenter (hypertension research and pharmacogenetics) | 2006-2009 | 1.6 |
| - 4x Stafpresentaties cardiologie en interne geneeskunde | 2006-2009 | 6.0 |

| | | |
|---|---|---|
| - 3x presenter Vascular Medicine & Cardiology Update (ESC), Dutch Heart Foundation congress (NHS), Dutch Society of Cardiology congress (NVCC) | 2008-2009 | 2.4 |

**International conferences**

| | | |
|---|---|---|
| - European Society of Cardiology, American College of Cardiology, American Heart Association scientific sessions | 2006-2009 | 12 |
| - Human Genome Variation, Human Genome, Pharmacogenomics congresses | 2006-2009 | 4.5 |

**Seminars and workshops**

| | | |
|---|---|---|
| - COEUR research seminars, courses Molecular Medicine research school | 2006-2009 | 2.5 |

## 2. Teaching activities

**Lecturing:**

| | | |
|---|---|---|
| - Keuzeonderwijs begeleiding van 8 tweedejaars-studenten (3 weken) | 2007-2008 | 2.4 |
| - Keuzeonderzoek begeleiding van 1 zesdejaars-student (6 maanden) | 2008-2009 | 3.0 |