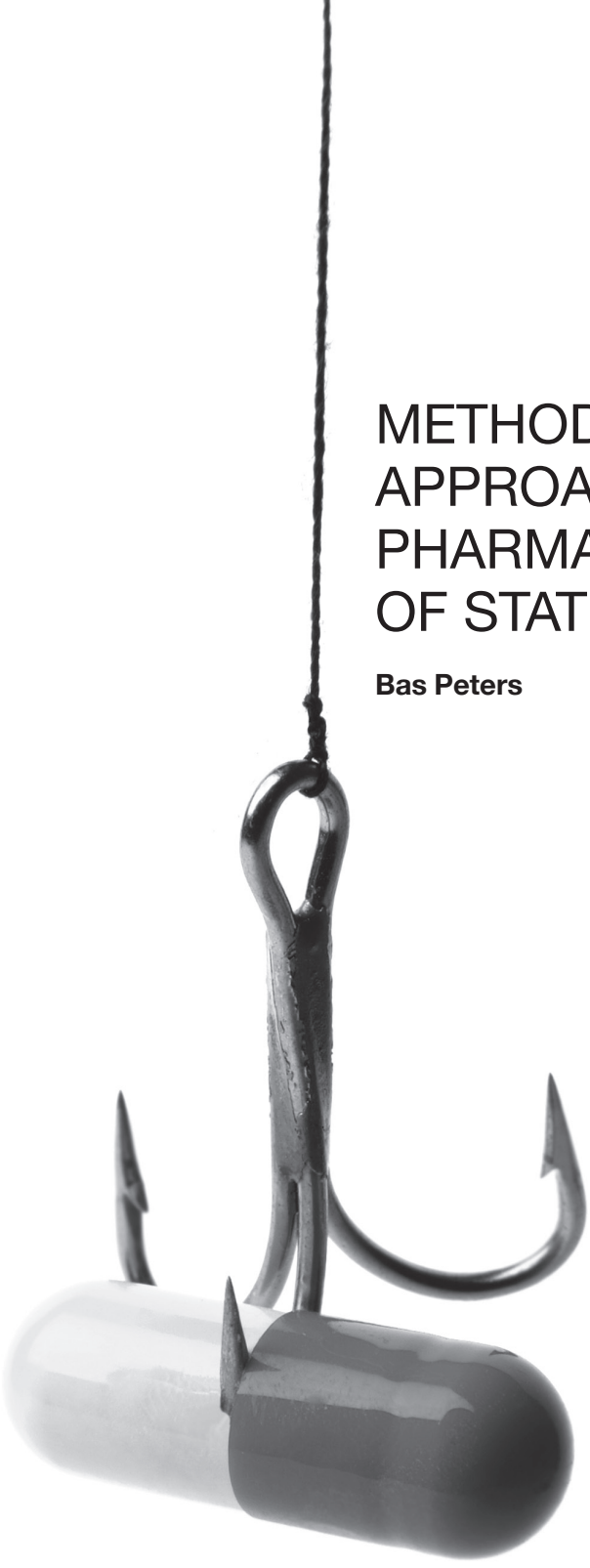

# METHODOLOGICAL APPROACHES TO THE PHARMACOGENOMICS OF STATINS

**Bas Peters**

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# METHODOLOGICAL APPROACHES TO THE PHARMACOGENOMICS OF STATINS

METHODOLOGISCHE BENADERINGEN TER BESTUDERING VAN DE FARMACOGENETICA VAN STATINES

(met een samenvatting in het Nederlands)

Proefschrift
ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. J.C. Stoof, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op maandag 21 december 2010 des middags te 4.15 uur

door
Bas Johannes Maria Peters

Geboren op 28 september 1982 te Veghel

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| Promotor | Prof.dr. A. de Boer |
| Co-promotoren | Dr. A.H. Maitland-van der Zee |
| | Dr. O.H. Klungel |

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# 1 GENERAL INTRODUCTION

## GENERAL INTRODUCTION

Around 1900, the relationship between genes and proteins, and the phenomena of biochemical individuality of humans was first proposed by the British physician Garrod. Long after, it was postulated that genetic variations could affect response to drugs, a phenomenon called pharmacogenetics.[1] Throughout the second half of the 20th century, an increasing number of pharmacogenetic discoveries have been reported over time. Starting point was the discovery of N-acetyltransferase 2 (encoded by *NAT2*) variability and the capacity to N-acetylate isoniazid in the early 1950s. Soon after, glucose-6-phosphat dehydrogenase (encoded by *G6PD*) deficiency, pseudocholinesterase deficiency, cytochrome P450 2D6 (encoded by *CYP2D6*) and thiopurine methyltransferase (encoded by *TPMT*) were linked to interindividual variability in response to a variety of drugs. The invention of the polymerase chain reaction (PCR) in 1983 by Nobel Prize winner Kary Mullis heralded a new era in (pharmaco)genetics. The PCR made it possible to identify disease loci and to investigate responders and non-responders to drugs based on their genotype rather than their phenotype. A fair amount of articles in the field of pharmacogenetics of cardiovascular medicine have been published in the 90's. These studies included mostly a one Single Nucleotide Polymorphism (SNP) in a candidate gene that was hypothesized to affect the response to the drug of interest. With the introduction of relatively cheap high throughput genotyping techniques and the launching of the human genome project[2] and International HapMap Project,[3] the start of the 21st century was characterized by a tremendous surge in the field of (pharmaco)genetic research.[4]

## METHODOLOGICAL CHALLENGES IN PHARMACOGENETIC RESEARCH

Discovery of the promising *CETP* taq1B and *ADD1* $^{Gly}460^{Trp}$ treatment interactions, with the cholesterol lowering drug pravastatin[5] and the antihypertensive agent hydrochlorothiazide,[6] respectively, initially led to speculations about genotype guided pharmacotherapy. However, numerous subsequent studies that have been published to date have still not been able to pass judgment on the exact role of these polymorphisms in the efficacy of these widely prescribed cardiovascular drugs. Unfortunately, non replication and inconclusiveness about the exact mechanism and implication of many pharmacogenetic interactions seems to be more the rule than the exception. On a brighter note, there are also successful examples of pharmacogenetic research such as the aforementioned (*TPMT*, *CYP2D6*, and *G6PD*) examples. Another successful example of explaining interindividual variability in response to the anticoagulants coumarins with genetics, is the implication of the metabolizing enzyme encoded by *CYP2C9* that has consistently been associated with drug safety.[7] In fact, genotype guided coumarin dosing has already been tested in a small trial.[8] Current pharmacogenetic research greatly benefits from the advances in technology. Nonetheless, the problem of non replication and inconclusiveness about many pharmacogenetic interaction remains present. This common pattern may be attributed to a range of characteristics of the pharmacogenetic research that has been conducted to date, among which power problems, small magnitude of effects, (single) SNP approach, poor understanding of biological mechanisms, and study design.

## PHARMACOGENETICS OF STATINS

Worldwide, statins are among the most prescribed drugs. Statins are highly effective in reducing the risk of cardiovascular events,[9] primarily by lowering low density lipoprotein (LDL) cholesterol. In addition, there is overwhelming evidence that statins do not solely lower cholesterol, but also exert beneficial non-lipid or pleiotropic effects,[10] with anti-inflammatory actions as the principal component. The most convincing example for statin pharmacogenetics involves the *APOE* gene: Carriers of the *APOE2* allele may have a better LDL response to statin treatment compared to *APOE3* and/or *APOE4* carriers.[11] However, the field of the pharmacogenetics of statins is no exception with respect to the aforementioned challenges that pharmacogenetic research is facing.[11]
It is recognized that future research will have to find a way to combine different methods and different disciplines to make real progress. Certainly, much progress has been made, but the scientific community realizes full well that genetics comes with great complexity. Therefore new initiatives are needed that combine genomics with disciplines such as epigenetics, proteomics, bioinformatics, etcetera to aim for a system's biology approach.

## OBJECTIVES OF THIS THESIS

The subject of this thesis is "methodological approaches to the pharmacogenetics of statins". The first main objective is to study common genetic variability in genes involved in the pharmacokinetics, cholesterol lowering pathway of statins, the non-lipid pathway of statins, and cardiovascular candidate genes in more general for modification of the response to statins. The response of statin includes both the protective effect of statins against myocardial infarction (MI) and the risk of discontinuation (as a proxy for adverse drug reactions/ineffectiveness). The second main objective is to test and improve new methods that can be used in multi-locus approaches in (pharmaco-) genetic research.

## OUTLINE OF THIS THESIS

This thesis starts off with a review of the current knowledge of the pharmacogenetics of the four mostly prescribed cardiovascular drugs (coumarins, statins, antihypertensives, and platelet aggregation inhibitors). In addition, the same chapter describes a review of the methodological and statistical challenges pharmacogenetics research is facing.
Chapter 3 focuses on Single Nucleotide Polymorphisms (SNPs) that are candidates for modification of statin effectiveness. In Chapter 3.1, we conducted an observational study to investigate the effect of the well known functional C825T polymorphism in the G protein beta polypeptide 3 (*GNB3*) gene. In Chapter 3.2, nine SNPs in coagulation genes were tested for their effect on statin effectiveness in a post-hoc analysis of the Genetics of Hypertension Associated Treatment (GenHAT) trial. Chapter 4 describes studies that consider all common variability in candidate genes within the pharmacokinetic, cholesterol lowering, and non-lipid pathway of statins, for the pharmacogenetics of statins, instead of a single SNP approach as described in Chapter 3. The pathways that are included in Chapter 4.1, 4.2, and 4.3 are a pharmacokinetic, anti-inflammatory, and cholesterol lowering pathway. Subsequently, Chapter 5 includes a study that utilizes a gene-centric

cardiovascular 50K SNP array to study those genetic variants for modification of statin treatment, and to introduce and test a new method to address epistasis in pharmacogenetic research. Although statins are generally well tolerated, muscular side effects are often experienced by patients. Chapter 6 focuses on statin safety. The first part reviews the contribution of genetics to the susceptibility and management of statin-induced myopathy. In this chapter, we also investigate the effect of a pharmacokinetic transporter gene (*SLCO1B1*) on the risk of discontinuing statin treatment.
Finally, the Chapter 7 provides a general discussion of the findings in a broader perspective, including the implications for clinical practice and recommendations for future research.

## REFERENCES

1. Lennard MS, Tucker GT, Woods HF. Inborn 'errors' of drug metabolism. Pharmacokinetic and clinical implications. Clin Pharmacokinet 1990;19(4):257-63.
2. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, et al. Initial sequencing and analysis of the human genome. Nature 2001;409(6822):860-921.
3. Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA, et al. A second generation human haplotype map of over 3.1 million SNPs. Nature 2007;449(7164):851-61.
4. Meyer UA. Pharmacogenetics - five decades of therapeutic lessons from genetic diversity. Nat Rev Genet 2004;5(9):669-76.
5. Kuivenhoven JA, Jukema JW, Zwinderman AH, de Knijff P, McPherson R, Bruschke AV, et al. The role of a common variant of the cholesteryl ester transfer protein gene in the progression of coronary atherosclerosis. The Regression Growth Evaluation Statin Study Group. N Engl J Med 1998;338(2):86-93.
6. Sciarrone MT, Stella P, Barlassina C, Manunta P, Lanzani C, Bianchi G, et al. ACE and alpha-adducin polymorphism as markers of individual response to diuretic therapy. Hypertension 2003;41(3):398-403.
7. Wadelius M, Pirmohamed M. Pharmacogenetics of warfarin: current status and future challenges. Pharmacogenomics J 2007;7(2):99-111.
8. Anderson JL, Horne BD, Stevens SM, Grove AS, Barton S, Nicholas ZP, et al. Randomized trial of genotype-guided versus standard warfarin dosing in patients initiating oral anticoagulation. Circulation 2007;116(22):2563-70.
9. Cheung BM, Lauder IJ, Lau CP, Kumana CR. Meta-analysis of large randomized controlled trials to evaluate the impact of statins on cardiovascular outcomes. Br J Clin Pharmacol 2004;57(5):640-51.
10. Jain MK, Ridker PM. Anti-inflammatory effects of statins: clinical evidence and basic mechanisms. Nat Rev Drug Discov 2005;4(12):977-87.
11. Maitland-van der Zee AH, Klungel OH, Stricker BH, Monique Verschuren WM, Kastelein JJ, Leufkens HG, et al. Genetic polymorphisms: importance for response to HMG-CoA reductase inhibitors. Atherosclerosis 2002;163(2):213-22.

# 2 BACKGROUND PAPERS

# CHAPTER 2.1

# PHARMACOGENETICS OF CARDIOVASCULAR DRUG THERAPY

Bas J.M. Peters
Anthonius de Boer
Tom Schalekamp
Olaf H. Klungel
Anke-Hilse Maitland-van der Zee

Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, Utrecht, The Netherlands

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# INTRODUCTION

Cardiovascular disease (CVD) covers a wide range of conditions and diseases among which hypertension, hypercholesterolemia, congestive heart failure, cerebrovascular disease and coronary heart disease (CHD). Especially in developed countries, CVD is one of the leading causes of death because of its high prevalence and high impact on morbidity and mortality.[1] To reduce the risk of CVD, lifestyle adjustments are generally recommended and have been shown to be very effective in reducing CVD risk. These recommendations include physical activity, smoking cessation, low sodium intake, low fat diet etcetera. However, lifestyle interventions do not always provide satisfactory CVD risk reductions. Moreover, for a variety of reasons, adherence to a healthy lifestyle is a difficult task for many people. Therefore, cardiovascular drugs are abundantly prescribed all over the world for the treatment of conditions such as hypertension and hyperlipidemia. Although a huge variety of drugs are available for the treatment of CVD, major essential pharmacological groups are considered platelet aggregation inhibitors (PAI), statins, antihypertensive drugs and anticoagulants.

There is notable interindividual variation in response to these drugs, which is partially explained by factors such as gender, age, diet, concomitant drug use and environmental factors. Nevertheless, large part of this variability remains unknown. Genetic variability may contribute to the explanation of variability in response to these cardiovascular drugs.[2] In the past decade, field of pharmacogenetics has enjoyed a tremendous surge in activity due to advances in technology and many association studies have been published. This chapter gives an overview of the state of affairs of the pharmacogenetics of the most commonly prescribed drugs in the management of cardiovascular disease.

# CARDIOVASCULAR DISEASE

The World Health Organization (WHO) estimated that cardiovascular disease accounted for 17.5 million global deaths in 2005. Deaths due to myocardial infarctions (MI) and cerebrovascular accidents (CVA) represented the majority of these deaths.[3 4] Estimates demonstrate that in 2005, over 80 million people (about 1 in 3 adults) in the USA were suffering from at least one cardiovascular condition or form of CVD.[5] In more detail, these patients accounted for approximately 73.6 million hypertension, 16.8 million CHD (angina pectoris and myocardial infarction), 6.5 million stroke, 5.7 million heart failure diagnoses.[5] Future prospects are that CVD will continue to cause great medical and economic burden.[3 4]

## PATHOPHYSIOLOGY

The majority of deaths due to cardiovascular disease are caused by cardiovascular conditions like hypertension and hypercholesterolemia. The aetiology of most cases of hypertension and hypercholesterolemia is poorly understood and are therefore classified as primary hypertension and primary hypercholesterolemia. Both conditions contribute significantly to the development of atherosclerosis, which is characterized by hardening of the arteries, itself enhancing hypertension severity. Atherosclerotic plaques are the result of accumulation of cholesterol - mediated by low-density-lipoproteins (LDL) - in the arterial

walls, which in turn leads to a macrophage-induced inflammatory process. Rupture of an atherosclerotic plaque exposes the lipid core, smooth-muscle cells, macrophages and collagen to the bloodstream resulting in adhesion of blood platelets and the activation of the coagulation cascade. Ultimately, a thrombus is formed that can either completely or incompletely occlude an artery, resulting in clinical events such as MI and CVA.[6] Myocardial necrosis may be a consequence of cardiac ischemia. Loss of cardiac function may initiate other CVD such as congestive heart failure, characterized by a poor prognosis.

### CLINICAL MANIFESTATION

Symptoms of a patient suffering from CVD can vary from none to sudden (cardiac) death. Conditions like hypercholesterolemia, hypertension and atherosclerosis may elapse unnoticed, because high cholesterol levels, high blood pressure and atherosclerosis are often non symptomatic. A MI and CVA generally do not elapse unnoticed. Preceding a MI, some patients experience chest pains known as angina pectoris. Specific symptoms of a patient suffering an MI are pain in the chest, arms, left shoulder, jaw and/or back. A CVA is often characterized by confusion, impaired understanding of speech, speaking and/or seeing, unilateral weakness or numbness of the face, arm and/or leg.

## CARDIOVASCULAR THERAPY

Cardiovascular conditions such as hypertension and hypercholesterolemia do not necessarily require acute medical treatment. Essential lifestyle changes to reduce the risk of CVD include non pharmacological cornerstone interventions like smoking cessation, healthy diet, low sodium intake, weight loss and physical activity.[7] If these interventions do not achieve the desired level of risk reduction, many pharmacological interventions are available.

Besides smoking, the most prevalent CVD risk factors are hypertension and hypercholesterolemia. Heavily depending on the total cardiovascular risk (including factors such as diabetes status, age, gender, smoking status, BMI, cholesterol level), treatment with an antihypertensive drug may be indicated when the blood pressure BP is within the high normal range (130–139/85–89 mmHg) or higher. All subjects with a total plasma cholesterol of >5 mmol/L (190 mg/dL) and LDL-cholesterol of >3 mmol/L (115 mg/dL) should be considered for pharmacological intervention with HMG CoA reductase inhibitors (statins), also depending on the total cardiovascular risk. Similar to the treatment of hypertension, lower total cholesterol and LDL cholesterol target levels reduces the risk of CVD even more in patients with other risk factors such as diabetes.[8]

PAI is another drug class to reduce the risk of cardiovascular events. By inhibiting platelet aggregation, drugs such as clopidogrel and aspirin prevent formation of a thrombus that could lead to a MI or CVA. CVD such as atrial fibrillation give rise to high risk of thromboembolisms and require anticoagulation with warfarin, phenprocoumon or acenocoumarol.

# GENETIC DETERMINANS OF RESPONSE TO CARDIOVASCULAR DRUGS

## PLATELET AGGREGATION INHIBITORS

Pharmacotherapy with PAI such as aspirin and clopidogrel is an important intervention in cardiovascular risk management. In high risk patients, the efficacy in the primary and particularly secondary prevention of cardiovascular death, myocardial infarction and stroke is well established.[9] However, part of the patients suffer a (recurrent) thromboembolic vascular event despite PAI therapy.[10] Results from meta-analyses have shown that patients resistant for aspirin experience a four times greater risk for cardiovascular events,[11] whereas patients resistant for clopidogrel have an eight times greater risk.[12] A variety of tests and clinical outcomes can be used to measure sensitivity to PAI. However, there is little consistency which measure to use to define non response to PAI. Tests to measure platelet function include thromboxane $A_2$ ($TXA_2$) production (measured as urinary or serum 11-dehydro-thromboxane $B_2$ ($dTxB_2$)), optical aggregometry using agonist such as arachidonic acid (AA), adenosine diphosphate (ADP) and collagen, the Platelet Function Analyzer 100 and others.[13] Numerous studies have been conducted to investigate possible underlying genetic mechanisms leading to treatment failure. Most of these studies used one of the platelet function tests as the outcome measure and fewer studies included clinically relevant outcomes such as MI. Aspirin irreversibly inhibits the cycloxygenase-1 (COX-1) enzyme, ultimately resulting in a decreased amount of $TXA_2$. $TXA_2$ is responsible for activation of platelet aggregation. Therefore, polymorphisms in the *COX1* gene may affect response to aspirin therapy. In 2005, Maree et al. reported an association between a polymorphism in the *COX1* gene and platelet function in response to aspirin.[14] Five common SNPs were genotyped in 144 patients with cardiovascular disease who were treated with aspirin for at least 2 weeks. Aspirin response, determined by serum $dTxB_2$ levels and AA induced platelet aggregation, was associated with the A842G polymorphism. Patients carrying the -842G polymorphism were less sensitive to aspirin treatment.[14] Lepantalo et al. included 101 patients undergoing elective percutaneous coronary intervention and reported similar results: 60% of the non-responders carried the -842G allele compared to 17% of the responders.[15] Gonzalez-Conejero et al. investigated the C50T polymorphism which was in complete linkage disequilibrium with the A842G polymorphism. Only the results of the TXB2 assay were similar to those reported in literature, whereas no drug gene interaction was shown using the AA induced platelet aggregation.[16] Other studies report contradictory results suggesting -842G allele carriers to be more sensitive to aspirin.[17 18] The exact mechanism of the possible interaction between aspirin and the A842G and C50T polymorphisms has not been elucidated yet.

Another gene that has been investigated many times with regard to the pharmacogenetics of both aspirin and clopidogrel is the gene coding for the platelet glycoprotein IIIa subunit, *ITGB3*. It is part of the glycoprotein IIb/IIIa receptor which is present on the activated platelet surface and responsible for platelet aggregation by binding of fibrinogen. Most research focused on the *ITGB3* PlA1/A2 polymorphism, in which the PlA1 is the wild-type variant.[19] Undas et al. were first to report on the effect of this polymorphism on platelet functioning after patients were exposed to aspirin, showing that subjects carrying the PlA1/PlA2 genotype were less sensitive to aspirin than homozygous PlA1 carriers.[20]

These findings have been replicated in small studies,[21 22] whereas other larger studies could not find such an association[23-26] or even showed opposite findings,[27] corroborating results from earlier in vitro studies.[28 29] Similar for clopidogrel response, some articles report (contradicting) associations[30 31] whereas others report no difference between different genotype groups.[24 32 33] The PlA1/PlA2 polymorphism does not seem to explain a large part of the interindividual variability in response to PAI when considering current evidence. In addition to the *COX1* and *ITGB3* gene, the genetic variability in *ITGA2*, *COX2*, *P2Y1*, *P2Y12* and several other genes have been investigated with respect to modified response to aspirin, but were studied in a very small number of patients and larger studies did not replicate these results.[34 35]

Goodman et al. conducted a meta-analysis on the pharmacogenetics of aspirin to increase statistical power and to take in account the different biochemical and functional methodologies.[34] Combination of studies could not show a pharmacogenetic interaction between aspirin and the polymorphisms that were included (*ITGA2* C807T, *COX1* A842G/C50T, *P2Y12* H1/H2, *P2Y1* A1622G and *ITGB3* PlA1/PlA2). However, after stratification by study population or measurement technique, the PlA2 allele of the *ITGB3* gene was associated with aspirin resistance: small but significant effects were found in the healthy population (4 studies) and in the studies using bleeding time as the outcome measure (only 2 studies).

Clopidogrel is a prodrug and has to be activated by hepatic cytochrome P450 (CYP) isoenzymes in order to inhibit platelet aggregation. After activation, clopidogrel irreversibly blocks the ADP receptor on platelet cell membranes and thereby activation of the glycoprotein IIb/IIIa pathway. *CYP2C19*2* is a common genetic variant of the *CYP2C19* gene that encodes a deficient version of the enzyme. Hulot et al. showed in 28 healthy subjects that heterozygous carriers of the *CYP2C19*2* allele had decreased platelet responsiveness to clopidogrel.[36] This finding was confirmed in other studies both pharmacokinetically[37 38] as well as pharmacodynamically.[37-40] In addition studies on clinical outcomes showed that carriers of loss-of-function alleles of the *CYP2C19* gene have a higher risk of major adverse cardiovascular events when treated with clopidogrel after suffering acute coronary syndromes.[38 41 42] In conclusion, the *CYP2C19*2* polymorphism explains part of the interindividual response to clopidogrel. It is not yet clear what this compelling evidence means for daily practice. Should *CYP2C19*2* carriers receive higher dosages that may overcome the loss of effect or should those patients be treated with an alternative PAI that does not require metabolism by the CYP2C19 enzyme? In the first case, research should provide the evidence that a higher dose for patients carrying the *CYP2C19*2* allele are as effective as the normal dose in wild-type carriers. If so, patients have to be genotyped before starting clopidogrel. However, genotype information is unlikely to be available when starting clopidogrel treatment in the early high risk phase of an MI. A P2Y12-mediated platelet function test could be a more rapid approach.[43] Moreover, treating patients carrying the *CYP2C19*2* genotype with an alternative PAI that does not require activation by the CYP2C19 enzyme may be an appropriate solution. Currently, there is no data available that can shed light on these important questions. A prospective study should demonstrate how patients carrying the *CYP2C19*2* allele should be treated.

Also other cytochrome P450 system enzymes have been investigated with regard to the pharmacogenetics of clopidogrel. However, current status of evidence shows neither

convincing nor conclusive evidence for interactions between clopidogrel and genetic variances in *CYP3A4*, *CYP3A5*, *CYP2C9*, *CYP2B6* or *CYP1A2*.[44]

The active thiol metabolite of clopidgrel binds irreversibly to the P2Y12 receptor. Genetic variability within the genes coding for the P2Y12 receptor and another ADP dependent P2Y1 receptor present on the platelet surface have been investigated. Unfortunately, research on polymorphisms within the *P2Y12*[24 45-49] and *P2Y1* gene[24 50] have not been able to explain any of the variability in clopidogrel response.

More comprehensive reviews that cover the pharmacogenetics of aspirin[34 35 44 51] and clopidogrel[44 51] can be found elsewhere.

## ANTICOAGULANTS

Oral anticoagulants of the coumarin type are highly efficacious for the treatment and prevention of thromboembolic diseases.[52] Warfarin is the most common coumarin, but acenocoumarol and phenprocoumon are frequently being used in several European countries.[53 54] Dosing of coumarins is difficult as a consequence of a narrow therapeutic range and large interindividual and intra-individual differences in dose response.[55] Consequently, an overdose will increase the risk of haemorrhage and an insufficient dose may lead to failure of prevention of thromboembolisms. Large intraindividual variability in response to coumarins[56] and strong overanticoagulation[57] are important risk factors for haemorrhages. Patients' characteristics such as weight, diet, disease state and concomitant use of other medications have been shown to affect the response to coumarins.[58] In addition, genetic variation contributes to the interindividual variation. To date, studies on the pharmacogenetics of coumarins have mainly focused on polymorphisms within the *CYP2C9* and *VKORC1* gene and their modifying effect on mean daily dosage, overanticoagulation, time to stable anticoagulation and the risk of bleeding.

The *CYP2C9* gene encodes the CYP2C9 enzyme which is principally responsible for the metabolism of the pharmacologically more effective S-enantiomer of coumarins. The most extensively investigated genetic variants are *CYP2C9**2 (C430T) and *CYP2C9**3 (A1075C), which both encode enzymes with a decreased activity compared to the wild type, *CYP2C9**1. Sanderson et al. reported in a systematic review and meta-analysis that lower maintenance doses of warfarin are required in subjects carrying either variant allele. They showed that *2 and *3 carriers have an increased risk of bleeding as a result of higher plasma levels of warfarin due to slow metabolism of warfarin by the CYP2C9 enzyme.[55 59] In another meta-analysis of 39 studies with a total of 7907 warfarin users Lindh et al. found that being carrier of more than one polymorphism resulted in a progressive reduction in warfarin dose requirements, warfarin dose in patients with the *CYP2C9**3/*3 genotype being 78% less than in patients with the *CYP2C9**1/*1 genotype.[60] For acenocoumarol, the *CYP2C9**2 and *CYP2C9**3 variant have also been associated with low dose requirements.[61-64] Moreover, the *CYP2C9**3 genotype has been associated with a decreased chance to achieve stable anticoagulation within 6 months, an increased risk for over anticoagulation[65] and an increased risk of major bleeding events.[66] Although research on phenprocoumon is sparse and although the contribution of CYP2C9 to the metabolism of phenprocoumon is smaller than for warfarin and acenocoumarol,[67] the *CYP2C9**2 and *CYP2C9**3 allele have also been associated with lower dosage requirements, a decreased chance to achieve stability, an increased risk of over anticoagulation[68] and bleeding.[69]

These results could not be replicated in the Rotterdam study,[64 66] possibly due to power problems.

Coumarins exert their anticoagulant effect by inhibiting the vitamin K epoxide reductase complex (VKOR), preventing VKOR from converting vitamin K epoxide to reduced vitamin K,[70] which is essential for functioning of several clotting factors such as factor II, VII, IX and X. The target of coumarins is VKORC1, encoded by the homonymous gene *VKORC1*. Being carrier of the noncoding SNP C1173T has been associated with reduced dose needs of warfarin,[55] acenocoumarol,[71-73] and phenprocoumon[71 72] compared with non carriers of this SNP. The single C1173T SNP proved to be as informative as five haplotype constructs of 10 SNPs in the *VKORC1* gene which have been allocated to low and high dose warfarin haplotype groups and which account for almost all the total haplotypes in Caucasian and Asian American populations.[74] Furthermore, it has been reported that the *VKORC1* and *CYP2C9* genotypes modify each others effects on overanticoagulation in users of acenocoumarol,[75] risk being highest in patients with both a *CYP2C9* and a *VKORC1* polymorphism. However, a recent study demonstrated that the bleeding risk in users of warfarin was associated with the *CYP2C9*, but not with the *VKORC1* genotype.[76]

Inconclusive results for polymorphisms in the *F2,*[77-79] *F7*,[77 79] and *F10*[77 79] genes - encoding the vitamin K dependent clotting factors II, VII and X - suggest a minor influence of these coagulation genes on warfarin dosing. Genetic variability in other downstream genes of coumarin action such as *GGCX*, *EPHX1*, *PROC*, and *APOE* have shown limited effects, if any, on coumarin dosing.[80] The *GGCX* gene encodes γ-glutamylcarboxylase, the enzyme catalyzing the carboxylation of vitamin K dependent clotting factors, the *PROC* gene encodes the clotting factor Va and VIIIa inactivating protein C and the *EPHX1* gene encodes microsomal epoxide hydrolase, a subunit of the VKOR complex harbouring a vitamin K epoxide binding site. The *APOE* gene has been considered a candidate gene because it encodes the vitamin K liver-uptake facilitating ligand Apolipoprotein E.

Moreover, a large study which included 183 polymorphisms in 29 candidate genes could not reveal major importance for genes other than *CYP2C19* and *VKORC1*.[80] Similar conclusions were drawn from the results from the only genome-wide association study to date, suggesting that it is unlikely that common SNPs with large effects on warfarin dose are to be discovered outside of the *CYP2C9* and *VKORC1* gene.[81]

The above mentioned polymorphisms of *CYP2C9* and *VKORC1* are common in Caucasian populations, allele frequencies ranging from 8-19% for *CYP2C9**2, from 4-16% for *CYP2C9**3[82 83] and from 37-41% for the *VKORC1* 1173C>T allele.[80 84]

Large studies have been able to explain around 50% of the variability in warfarin dosing with *CYP2C9* and *VKORC1* genotype,[84 85] although estimates vary.[58] The next step would be to use this pharmacogenetic knowledge to develop coumarin dosing algorithms that include *CYP2C9* and *VKORC1* genotype information. However, research should first demonstrate that genotype guided dosing improves safety and efficacy of coumarin therapy in daily practice. Several small randomized studies assessing genotype-guided warfarin have already been conducted. The one study that included both the *CYP2C9* as well as the *VKORC1* genotype in a randomized controlled trial showed genotype-guided warfarin dosing to be moderately favorable.[86] Generally, other studies of genetic testing for warfarin therapy initiation have not been able to demonstrate improvement of safety or efficacy of warfarin therapy.[87] Large randomized clinical trials to evaluate genotype-

guided dosing of coumarins are underway and will decide about the future implication for treatment with coumarins. More comprehensive overviews of the pharmacogenetics of coumarins can be found elsewhere.[55 58]

## ANTIHYPERTENSIVE DRUGS IN HYPERTENSION

The most prevalent indication in cardiovascular drug therapy is hypertension. Its high prevalence and the strong association with cardiovascular morbidity have given rise to the question of whom to treat with which drug. In the past decades, the search for markers that can predict response to therapy has experienced a tremendous surge. The major drug classes available for the treatment of hypertension are diuretics, beta-blockers, ACE inhibitors, angiotensin II type 1 receptor antagonists and calcium channel blockers (CCB). In this chapter, the most important genetic variants within the pharmacogenetics of hypertension are highlighted.

### DIURETICS

Diuretics are considered the first line pharmacological intervention for most patients with hypertension.[88] The long term beneficial effects of thiazides are thought to result mainly from a reduction of the peripheral vascular resistance.

To date, many pharmacogenetic studies have been published on the pharmacogenetics of diuretics. The best-studied polymorphism is the $^{Gly}460^{Trp}$ variant of the alpha adducin (*ADD1*) gene. In addition, multiple studies have been conducted on the angiotensin converting enzyme (*ACE*) I/D, angiotensin (*AGT*) -6A, angiotensin receptor (*AGTR1*) A1166C and G-protein beta-3 subunit (*GNB3*) C825T polymorphism and the response to diuretics.

The *ADD1* gene encodes the alpha subunit of the adducin protein, which is involved in the activation of the renal $Na^+/K^+$ ATPase. Carriers of the *ADD1* $460^{Trp}$ allele have a higher activity of the $Na^+/K^+$ pump in the nephron. The *ADD1* $^{Gly}460^{Trp}$ polymorphism has been shown to affect renal proximal tubule sodium reabsorption in hypertension with increased reabsorption in patients carrying the $460^{Trp}$ allele.[89] Hypothetically, $460^{Trp}$ allele carriers could benefit more from therapy as diuretics could trigger less counter-regulatory mechanisms. Cusi et al. were first to report on the *ADD1* $^{Gly}460^{Trp}$ polymorphism and the association with altered response to hydrochlorothiazide in hypertensive patients.[90] They found that heterozygous hypertensive patients experienced a greater fall in mean arterial pressure in response to two months' treatment with hydrochlorothiazide than wild-type homozygous hypertensive patients.[90] Following this study, these results were replicated in two other studies also showing that carriers of the *ADD1* $460^{Trp}$ allele respond better to hydrochlorothiazide.[91 92] Retrospective large scale studies with up to 36,000 patients[93] could not replicate these findings[93-96] and thereby suggest a minor role for the *ADD1* $^{Gly}460^{Trp}$ polymorphism in predicting blood pressure response to diuretics.

Furthermore, the *ADD1* $460^{Trp}$ allele has been associated with a better response to diuretics with regard to clinical outcomes like stroke or MI,[97] a result which was not found in other studies on clinical outcomes.[93 98-100] It would be a myopic judgement to say the *ADD1* $^{Gly}460^{Trp}$ does not affect the response to diuretics. First, there is a good rationale for the pharmacogenetic interaction between *ADD1* and use of diuretics. Second, the positive studies all point in the same direction. Third, a closer look at the study designs that were used shows that the three positive studies on blood pressure conducted excellent

studies including patients with a newly discovered diagnosis of hypertension and were never treated before. On the other hand, large observational studies consistently fail to show such association. The association of the *ADD1* $^{Gly}460^{Trp}$ polymorphism with altered response to diuretics seems genuine, but so far of no clinical importance.

Besides the *ADD1* gene, researchers have taken interest in polymorphisms in genes involved in the renin angiotensin system (RAS), namely the *ACE* gene encoding the angiotensin converting enzyme, *AGT* encoding angiotensinogen and *AGTR1* encoding the angiotensin II receptor, type 1. The rationale for the selection of these candidate genes for the pharmacogenetics of diuretics as well as other antihypertensive drugs is obvious as the RAS system is activated by diuretics and regulates the water and electrolyte balance and thereby the blood volume and blood pressure.

The D allele of the *ACE* I/D polymorphism is associated with increased plasma and tissue levels of ACE.[101] Because low plasma renin activity is a predictor of greater BP response to thiazide diuretics,[102] it can be hypothesized that I allele carriers would respond better to thiazides. Sciarroni et al. indeed reported I allele carriers to responds better to hydrochlorothiazide.[92] Schwartz et al. showed similar results for women and opposite results in men.[103] Although, the study of Sciarroni et al. confirms the initial hypothesis, the myriad of studies that could not confirm such association[95 104-106] suggest a minor role for the *ACE* I/D polymorphism. Other than the *ACE* I/D polymorphism, Frazier et al. included polymorphisms within the other RAS genes *AGT* and *AGTR1* and showed an association with both genes in African American women only.[105] These results were not corroborated by the Doetinchem Cohort Study[95] and were contradicted by Jiang et al.[107] More studies are needed to elucidate the possible role of these polymorphisms in the response to diuretics.

Furthermore, an association with the *GNB3* C825T polymorphism has been reported and replicated, both studies showing that subjects carrying the TT genotype would benefit more from diuretics in lowering blood pressure.[95 108] The mechanistic pathway of this possible gene-treatment interaction is that the T allele of the *GNB3* C825T polymorphism has been associated with low plasma renin and an elevated aldosterone-renin ratio[109] which could influence the response to diuretics. A study by Schelleman et al. could not corroborate previous findings concerning the association between the *GNB3* C825T polymorphism and clinical events.[98]

## BETA-BLOCKERS

The main target of beta-blockers is the beta1-adrenoreceptor encoded by the *ADRB1* gene, in which the $^{Arg}389^{Gly}$ and the $^{Ser}49^{Gly}$ polymorphism are most extensively investigated. The $^{Arg}389$ and $^{Ser}49$ allele may enhance response to beta blockers as receptors containing $^{Arg}389$ possess a three- to four-fold higher adenylyl cyclase activity[110] and $49^{Gly}$ polymorphic receptors are more prone to agonist-promoted receptor downregulation.[111] Initially, O'Shaughnessy did not report an association of the *ADRB1* polymorphism and altered response to atenolol or bisoprolol.[112] In the years after, four articles were published reporting an association of the $^{Arg}389$ allele (in combination with $^{Ser}49$ allele)[113 114] with better response to beta-blockers, either SBP,[114-116] DBP,[113 114] or resting MBP.[114 115] This observation could not be confirmed by other studies.[117-121] Nonetheless, the hypothesis should not be rejected as there is a consistency in findings for studies with the beta-blocker metoprolol. Moreover, given the similarities in the pharmacology of beta blockers, differences in study

design may well explain the inconsistency of results. The positive associations point in the same direction, as expected with regard to the initial hypothesis, and suggest a true pharmacogenetic interaction. Major clinical importance of the *ADRB1* $^{Arg}389^{Gly}$ and $^{Ser}49^{Gly}$ polymorphism for the pharmacogenetics of beta blockers seems unlikely. Beta adrenergic receptors are G-protein coupled receptors. Therefore the *GNB3* C825T polymorphism possibly modifies the blood pressure response to beta blockade. In females only, the carriers of the CC genotype has been associated with better blood pressure response to beta blockers,[118] a finding that was not confirmed in the Doetinchem study.[95]
Finally, the RAS gene *AGT* (A(-6)G polymorphism) has been associated with the pharmacogenetics of beta blockers, but the association has only been reported once[122] whereas other studies failed to confirm this association.[123 124]

ACE INHIBITORS

The aforementioned (Section 4.3.1) *ACE* gene - coding for the drug target of ACE inhibitors - has been studied over 20 times. Studies that evaluated blood pressure response to ACE inhibitors according to *ACE* I/D genotype include treatment duration ranging from single doses to several years, small scale studies as well as larger studies and studies in both healthy subjects and patients. Carriers of the D allele -accompanied by higher ACE levels[101]- could benefit less from ACE inhibitors because of underdosing or could benefit more due to a poorer baseline condition. At first, smaller studies reported a more beneficial effect of ACE inhibitors on blood pressure in subjects carrying II. Large studies that have been published more recently[104 125] could not confirm these results. Uncertainty about the expected biological mechanism based direction of the association together with many studies showing opposite or no association directions of the association suggest that the *ACE* I/D polymorphism is not likely to be a strong modifier of blood pressure response to treatment with ACE inhibitors. Also stratification of results by study design, study population or type of ACE inhibitor cannot explain the inconsistency.
Another gene involved in the RAS that has been associated with modified response to ACE inhibitors is the *AGT* gene. Initially, a prospective study in 125 subjects showed that the T allele of the M235T polymorphism was associated with better blood pressure lowering response to ACE inhibitors compared to homozygous M allele carriers.[126] Bis et al. showed similar results for the outcome stroke.[127] Several other studies, both retrospective as well as prospective, found no such association,[95 96 128-131] concluding that there is very little evidence for an interaction between the *AGT* M235T polymorphism and the response to ACE inhibitors.

OTHER ANTIHYPERTENSIVE DRUGS

Another category of antihypertensives that act on the RAS, is the angiotensin II type 1 receptor antagonists. Studies in the SILVHIA trial which included almost 50 subjects taking irbesartan reported that the *ACE* I/D[123] and *CYP11B2* C-344T polymorphism were associated with modified blood pressure response.[123 132] *CYP11B2* - encoding aldosterone synthase – is required for the final steps of aldosterone biosynthesis. Subjects carrying the *ACE* II polymorphism123 and subjects carrying the *CYP11B2* TT variant[132] had a more pronounced blood pressure response to irbesartan. The finding of the *CYP11B2* C-344T polymorphism association could not be replicated (results point in opposite directions),[133]

nor could a prospective study including 206 subjects treated with telmisartan find an association with any of the RAS genes (*AGT*, *ACE*, and *AGTR1*) polymorphisms.[134] Currently, the role of polymorphisms in genes of the RAS system and the response to angiotensin II antagonists is unknown.
CCB and central alpha-adrenergic agonists have insufficiently been investigated and future research should provide more information about the genetic contribution to variability in response.

### ANTIHYPERTENSIVE DRUGS AND SIDE EFFECTS

In addition to the impact of genetic variability on the effectiveness of antihypertensive drugs, several studies have investigated the genetic influence on the incidence of certain side effects. A frequent reason for discontinuation of ACE inhibitor therapy is a persistent, dry cough[135] As a result from ACE inhibition, metabolism of bradykinin by ACE is impaired. Local accumulation of bradykinin in the airways is thought to cause the persistent, dry cough. Variability in RAS genes as well as the gene coding for the bradykinin B2 receptor (*BDKRB2*) has been proposed to influence the susceptibility to develop a cough due to ACE inhibition. The T allele of the T-58C promoter polymorphism in *BDKRB2* results in a higher transcription rate[136] and has been associated with elicitation of the adverse effect coughing.[136 137] All but one[138] study have not been able to find a relation between the *ACE* I/D polymorphism and cough.[136 137 139] In addition, beneficial effects of ACE inhibition regarding incidence of diabetes was only seen in hypertensive subjects homozygous for *AGTR1* 1166A and/or carriers of the *ACE* I allele.[140]
A complete review of the large amount of literature of the pharmacogenetics of antihypertensives can be found elsewhere.[141-144]

### CHOLESTEROL LOWERING DRUGS (STATINS)

Statins primarily reduce the risk on coronary artery disease (CAD) by lowering blood cholesterol through inhibition of the HMG-CoA reductase enzyme. Although large clinical trials found a 27% average relative risk reduction of major coronary events,[145] there is large variability in benefits from statin therapy. Many genes involved in the pharmacodynamic pathway of statins have been part of pharmacogenetic research in patients with hypercholesterolemia, with an emphasis on genes involved in the cholesterol pathway, although genes involved with possible pleiotropic effects of statins gain more and more interest. The enormous amount of candidate genes that have been part of research in the pharmacogenetics of statins, proves the potentially high impact of future findings on treatment with statins. Moreover, it highlights the complexity of the mechanism by which statins exert their beneficial effect.
HMG-CoA reductase, encoded by the *HMGCR* gene, is responsible for the conversion of HMG-CoA to mevalonic acid, an intermediate in the cholesterol synthesis. The minor alleles of two SNPs, SNP12 and SNP29 (rs17238540), jointly define haplotype 7 of the *HMGCR* gene.[146] The minor alleles for both these SNPs were associated with less pronounced total cholesterol (TC) and LDL cholesterol reduction in response to pravastatin treatment (PRINCE-study).[146] A pharmacogenetic study in the ACCESS trial, could not replicate these findings.[147] This may be due to a statistical power problem as both a large observational study in diabetics[148] as well as a prospective study in hypercholesterolemic patients

(treatment with simvastatin)[149] reported comparable results for these SNPs. In addition, no association with lipid response was found in the elderly population of the PROSPER trial[150] and no association was found considering clinical outcomes such as MI and CVA.[150 151] Both SNP 12 and SNP 29 are located in a non-coding region and further research should determine whether there is a molecular explanation for the results that were found in several studies. Possibly SNP 12 and SNP 29 explain a small part of the variability in response to statins due to genetics. Recently, Medina et al. discovered a HMGCR isoform, encoded by an alternatively spliced transcript which expression is influenced by SNP rs3846662. The isoform was shown to affect response to statins.[152]

Cholesterol is transported throughout the body by apolipoproteins. The apolipoprotein E is a major component of very low-density lipoproteins (VLDL) and ligand for the LDL receptor. Moreover, apolipoprotein E is involved in intestinal cholesterol absorption and reverse cholesterol transport (RCT). Apolipoprotein E is a genetically polymorphic protein defined by three alleles, *APOE**2, *3, and *4, encoding proteins with increasing affinity for the LDL receptor. Consequently, lipoproteins carrying the *4 isoform are cleared most efficiently from the circulation and cholesterol synthesis and thereby HMG-CoA reductase levels are lower. As a result, *4 carriers could benefit less from statin therapy. Ordovas et al. reported that carriers of the *2 genotype experience greatest LDL reduction in response to statin therapy in comparison to *3 and *4 carriers.[153] Many similar studies were conducted trying to clarify the role of the *APOE* polymorphism in the pharmacogenetics of statins. There is a reasonable body of evidence supporting the findings from Ordovas et al.,[147 154-160] among which are some large scale studies.[147 155 156 160] Nonetheless, studies that could not show similar results have also been reported.[146 161-166] *APOE**2 carriers seem to benefit most from statin therapy regarding lipid profile improvement, however a sub study of the Scandinavian Simvastatin Survival Study (S4) and the GISSI-Prevenzione study (both multi-centre, double-blind, randomized trials) reported subjects carrying the *APOE**4 genotype to have the largest risk reduction of mortality.[167 168] These results were not replicated in an observational study.[169]

Besides *APOE*, genetic variations in the genes coding for other apolipoproteins have been part of pharmacogenetic research with inconclusive results.[170 171]

Lowering the hepatic cholesterol biosynthesis increases the amount of LDL receptors. Some functional mutations in the *LDLR* gene, encoding the low density lipoprotein receptor, cause familial hypercholesterolemia (FH). Generally, small studies have associated these mutations with variable response to statins.[172] More common genetic variations in the *LDLR* have been suggested to modify the cholesterol lowering response to statins. However, inconsistent results of *LDLR* polymorphisms in the 3' UTR region (*LDLR* C44857T and A44964G)[146 150 151] and other intronic SNPs (AvaII, PvuII and HincII)[147 173 174] have been reported. The genetic contribution of genetic variability of the *LDLR* gene should be investigated in detail for the important reason that the LDL receptor plays a pivotal role in the cholesterol housekeeping and working mechanism of statins. In addition, genetic variation within the LDLR expression/degradation regulating *SREBF1*, *SCAP* and *PCSK9* genes have been subject of research without conclusive or consistent results.[170 171]

The cholesteryl ester transfer protein (CETP) enzyme plays a central role in transport of cholesterol from peripheral tissues back to the liver, RCT. Taq1B is a common polymorphism in the *CETP* gene and is associated with variations in lipid transfer activity and high-density

lipoprotein (HDL) levels[175] and may therefore alter response to statins. CETP concentrations are believed to be highest in homozygous B1 carriers and lowest in *CETP* homozygous B2 carriers. The effect of Taq1B on statin therapy was first investigated by Kuivenhoven et al., showing that pravastatin therapy slowed the progression of coronary atherosclerosis in B1B1 carriers, whereas B2B2 carriers did not benefit from pravastatin therapy although higher HDL levels were observed.[176] Very recently, the same research group performed a 10 year follow-up analysis in the same REGRESS cohort, showing similar results: more benefit from statin therapy for B1B1 carriers on cardiovascular clinical outcomes and all cause mortality despite higher HDL levels observed in B2 carriers.[177] Several other studies investigated the *CETP* polymorphism as well but could not find an association of the Taq1B polymorphism with altered efficacy of statins in preventing cardiovascular diseases[178-181] or found the opposite.[182] A large meta-analysis including over 13,000 patients found no gene treatment interaction between statins and the Taq1B polymorphism of the *CETP* gene.[183] A common pattern of contradictory results is seen for genes encoding lipoprotein lipase (*LPL*) and hepatic lipase (*LIPC*), enzymes that transfer lipids between lipoproteins and mediate lipolysis.[147 184 185]

The foremost cause of CAD is atherosclerosis, which is characterized by inflammation.[186] In addition to lowering cholesterol, statins have been shown to exert lipid-independent pleiotropic effects, among which beneficial effects on the process of inflammation.[187] Therefore not only candidate genes in the lipid lowering pathway, but also genes involved in the atherosclerosis inflammatory pathway have been designated as candidate genes.

The kinesin-like protein 6 is encoded by *KIF6* and is expressed in many tissues and cell types among which vascular cells. The $^{Trp}719^{Arg}$ polymorphism in the *KIF6* gene has been associated with CVD[188-191] and altered response to statins in three studies.[188 192] These three pharmacogenetic studies included more than 6,200 patients exposed to pravastatin 40 mg daily (versus placebo)[188] or 80 mg atorvastatin (versus 40 mg pravastatin) daily.[192] The results from the PROVE IT-TIMI 22 trial[192] are consistent with those observed in two other clinical trial population (CARE and WOSCOPS).[188] Carriers of the *KIF6* $719^{Arg}$ allele receive significantly greater benefit from (intensive) statin therapy than do non-carriers. Proteins of the kinesin family are involved in intracellular transport but the exact role of *KIF6*, the $^{Trp}719^{Arg}$ polymorphism and response to statins are not understood. The differential benefit from statins has been suggested to be distinct from lipid or CRP lowering.[192]

The toll like receptor mediates innate and adaptive immunity. The $^{Asp}299^{Gly}$ polymorphism in the *TLR4* gene has been studied in REGRESS and SAS, both including approximately 650 patients. Both studies showed that Gly allele carriers treated with statins had a lower risk of cardiovascular events than non carriers treated with statins,[193 194] suggesting that statins interact with inflammatory factors like the toll like receptor through an unknown biological mechanism. Pharmacogenetic research furthermore focused on polymorphisms in other examples of genes that are putatively involved in pleiotropic statin response are *eNOS*, *IL6*, *ITGB3* and *PAI-1*.[170 171]

Statins undergo different metabolizing pathways. Lovastatin, atorvastatin, simvastatin are mainly metabolized by CYP3A4, fluvastatin by CYP2C9 and pravastatin, pitavastatin and rosuvastatin are practically not metabolized by CYP enzymes.[195] There is little evidence for polymorphism within genes encoding CYP enzymes to be of much importance to the

pharmacogenetics of statins.[170 171] Genes coding for transporters involved in the hepatic uptake and hepatic elimination of statins may also be of great importance for variability in response. Polymorphisms in pharmacokinetic genes encoding the solute carrier organic transporter (*SCLO1B1*) involved in the hepatic uptake of statins and the adenosine triphosphate-binding cassette (ABC) B1 transporter involved in the hepatobiliary excretion of statins may influence the pharmacokinetics of statins and thereby its lipid lowering response. The variant allele of the *SCLO1B1* T521C polymorphism - a functional SNP causing a Val>Ala amino acid change - results in impaired hepatic statin uptake and thereby an attenuated pharmacodynamic effect. This has been shown for both the pharmacokinetics as well as pharmacodynamics of statins, in particular pravastatin.[196] The TT genotype of the *SCLO1B1* T521C polymorphism has been associated with greater response to statins in lowering total cholesterol,[197 198] although controversy exists.[147 199 200] Variance in the *SCLO1B1* gene is likely to affect the response to statins to some extent.
Many genes encoding members of the ABC (ATP-binding cassette) transporter family are involved in the pharmacodynamics (*ABCA1*, *ABCG5* and *ABCG8*) or pharmacokinetics (*ABCB1*, *ABCC2*, *ABCG2* and *ABCB11*) of statins.[171] Most of these genes have been subject of pharmacogenetic research related to statins, but the most extensively investigated gene encoding a ABC transporter is the *ABCB1* gene. Research has not been able to produce definitive results to show the role – if any - of the contribution of this gene to statin response.[171 201 202]

### STATINS AND SIDE EFFECTS

Adverse effects to statin treatment, in particular statin induced myopathy, have been associated with genetic variability in certain candidate genes.[203] There is convincing evidence about the association between the *SLCO1B1* T521C polymorphism and statin induced myopathy. The C allele is known to cause high plasma levels of statins[196 204 205] and a genome wide scan by the SEARCH collaboration indeed demonstrated a strong association with statin induced myopathy. In fact, they reported an increased risk for myopathy by 4.5 times for each copy of a C allele and an increased risk of 16.9 times for CC versus TT,[206] supporting the hypothesis that statin induced myopathy is related to plasma levels of statin.
An excellent review published by Mangravite et al. provides a comprehensive overview of most studies conducted in the pharmacogenetics of statins.[170 171 207]

## CLINICAL IMPLICATIONS

By addressing the most extensively investigated pharmacogenetic associated genes in cardiovascular drug therapy, it is clear that there is little clinical implication. Although many initial associations of a polymorphism with modified drug response seemed very promising, the reality is that most associations cannot be replicated. Furthermore, most effects that were found are very small. Therefore implementation of these interactions in clinical practice is still far away. Only for coumarins, there is a real opportunity for pharmacogenetics by genotyping the *VKORC1* and *CYP2C9* gene to optimize anticoagulant therapy. Even though the effect of the existing variations in these genes is quite clear, clinical trials should provide evidence for the effectiveness of genotyping regarding prevention of adverse drug

events and cost-effectiveness, before genotype guided dosing will be a part of every day anticoagulant therapy. These studies are currently underway and will possibly advocate for a global implementation of *VKORC1* and *CYP2C9* genotyping into the anticoagulant therapy guidelines. Nevertheless, in August 2007 the Food and Drug Administration updated the warfarin prescribing information and highlighted the opportunity to use genetic tests to improve their initial estimate of warfarin dose.

For the other cardiovascular drugs discussed in this chapter, future research may well elucidate the exact role of genetics in the response to these drugs, but current knowledge is somewhat disappointing after high expectations of personalized medicine a decade ago. Nevertheless, also for cardiovascular drugs, other than coumarins, some real progress has been made. There is a reasonable body of evidence that clopidogrel response partly depends on *CYP2C19* genotype, response to beta blockers is affected by the *ADRB1* genotype status and statin efficacy is highly likely to depend on variation in the *HMGCR* gene whereas myopathy is related to a polymorphism in the *SLCO1B1*.

## FUTURE DIRECTIONS

Current approaches in pharmacogenetic research do not seem to lead to results that meet our expectations of individualized medicine. Therefore, new approaches are needed addressing issues and challenges such as the number of SNPs studied, study power, study design and application of new statistical methods in (pharmaco-)genetic analysis.

Most studies have only examined one polymorphism in a candidate gene associated with modified response to a certain drug. However, drug response is likely to result from complex interactions among various biologic pathways. Hence, future studies should consider a set of candidate genes and/or a genome wide scan (GWS) rather than addressing a single or small number of SNPs. In recent years, the costs for a GWS have considerably decreased and will be increasingly available.

Examining multiple SNPs will require sufficient sample size, that many studies to date lack. Moreover, analysis of large numbers of SNPs to identify a combination of SNPs that influence drug efficacy, will be a huge challenge due to statistical problems. Not only the issue of multiple testing of many SNP should be addressed with new tools in statistical analysis, also possible important effects of gene-gene interactions should be considered.[208] Although a definitive statistical method for characterising statistical patterns of epistasis in not known yet, conventional statistical methods only will not be the appropriate tool to decipher the complexity of pharmacogenetics.

Finally, to elucidate mechanisms that lie behind the genetic associations, other fields of research including proteomics and transcriptomics should be integrated in the field of pharmacogenomics.[209]

## CONCLUSION

In conclusion, although pharmacogenetic testing is already part of everyday clinical practice in some areas (chemotherapy, psychiatry), for cardiovascular drugs currently only oral anticoagulant therapy seems to have a real opportunity to benefit from pharmacogenetic testing. In spite of the tremendous amount of publications in this field, there is no reason to advocate for genetic testing for any other cardiovascular drugs yet. Although future research will certainly benefit from emerging genetic technology as high throughput genome wide scans will be readily available, finding the genetic profile that will predict response to cardiovascular drugs will be a major challenge.

## REFERENCES

1. McGovern PG, Jacobs DR, Jr., Shahar E, Arnett DK, Folsom AR, Blackburn H, et al. Trends in acute coronary heart disease mortality, morbidity, and medical care from 1985 through 1997: the Minnesota heart survey. Circulation 2001;104(1):19-24.
2. Weinshilboum R. Inheritance and drug response. N Engl J Med 2003;348(6):529-37.
3. World Health Organization.
4. Mackay J, Mensah G. The Atlas of Heart Disease and Stroke: World Health Organization, 2004.
5. Lloyd-Jones D, Adams R, Carnethon M, De Simone G, Ferguson TB, Flegal K, et al. Heart Disease and Stroke Statistics--2009 Update. A Report From the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 2008.
6. Yeghiazarians Y, Braunstein JB, Askari A, Stone PH. Unstable angina pectoris. N Engl J Med 2000;342(2):101-14.
7. Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franch HA, et al. Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. Circulation 2006;114(1):82-96.
8. 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. Atherosclerosis 2007;194(1):1-45.
9. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. BMJ 2002;324(7329):71-86.
10. Grotemeyer KH, Scharafinski HW, Husstedt IW. Two-year follow-up of aspirin responder and aspirin non responder. A pilot-study including 180 post-stroke patients. Thromb Res 1993;71(5):397-403.
11. Krasopoulos G, Brister SJ, Beattie WS, Buchanan MR. Aspirin "resistance" and risk of cardiovascular morbidity: systematic review and meta-analysis. Bmj 2008;336(7637):195-8.
12. Snoep JD, Hovens MM, Eikenboom JC, van der Bom JG, Jukema JW, Huisman MV. Clopidogrel nonresponsiveness in patients undergoing percutaneous coronary intervention with stenting: a systematic review and meta-analysis. Am Heart J 2007;154(2):221-31.
13. Michelson AD, Frelinger AL, 3rd, Furman MI. Current options in platelet function testing. Am J Cardiol 2006;98(10A):4N-10N.
14. Maree AO, Curtin RJ, Chubb A, Dolan C, Cox D, O'Brien J, et al. Cyclooxygenase-1 haplotype modulates platelet response to aspirin. J Thromb Haemost 2005;3(10):2340-5.
15. Lepantalo A, Mikkelsson J, Resendiz JC, Viiri L, Backman JT, Kankuri E, et al. Polymorphisms of COX-1 and GPVI associate with the antiplatelet effect of aspirin in coronary artery disease patients. Thromb Haemost 2006;95(2):253-9.

16. Gonzalez-Conejero R, Rivera J, Corral J, Acuna C, Guerrero JA, Vicente V. Biological assessment of aspirin efficacy on healthy individuals: heterogeneous response or aspirin failure? Stroke 2005;36(2):276-80.
17. Halushka MK, Walker LP, Halushka PV. Genetic variation in cyclooxygenase 1: effects on response to aspirin. Clin Pharmacol Ther 2003;73(1):122-30.
18. Lim E, Carballo S, Cornelissen J, Ali ZA, Grignani R, Bellm S, et al. Dose-related efficacy of aspirin after coronary surgery in patients With PI(A2) polymorphism (NCT00262275). Ann Thorac Surg 2007;83(1):134-8.
19. Nurden AT. Platelet glycoprotein IIIa polymorphism and coronary thrombosis. Lancet 1997;350(9086):1189-91.
20. Undas A, Sanak M, Musial J, Szczeklik A. Platelet glycoprotein IIIa polymorphism, aspirin, and thrombin generation. Lancet 1999;353(9157):982-3.
21. Szczeklik A, Undas A, Sanak M, Frolow M, Wegrzyn W. Relationship between bleeding time, aspirin and the PlA1/A2 polymorphism of platelet glycoprotein IIIa. Br J Haematol 2000;110(4):965-7.
22. Undas A, Brummel K, Musial J, Mann KG, Szczeklik A. PI(A2) polymorphism of beta(3) integrins is associated with enhanced thrombin generation and impaired antithrombotic action of aspirin at the site of microvascular injury. Circulation 2001;104(22):2666-72.
23. Pamukcu B, Oflaz H, Nisanci Y. The role of platelet glycoprotein IIIa polymorphism in the high prevalence of in vitro aspirin resistance in patients with intracoronary stent restenosis. Am Heart J 2005;149(4):675-80.
24. Lev EI, Patel RT, Guthikonda S, Lopez D, Bray PF, Kleiman NS. Genetic polymorphisms of the platelet receptors P2Y(12), P2Y(1) and GP IIIa and response to aspirin and clopidogrel. Thromb Res 2007;119(3):355-60.
25. Jefferson BK, Foster JH, McCarthy JJ, Ginsburg G, Parker A, Kottke-Marchant K, et al. Aspirin resistance and a single gene. Am J Cardiol 2005;95(6):805-8.
26. Papp E, Havasi V, Bene J, Komlosi K, Czopf L, Magyar E, et al. Glycoprotein IIIA gene (PlA) polymorphism and aspirin resistance: is there any correlation? Ann Pharmacother 2005;39(6):1013-8.
27. Macchi L, Christiaens L, Brabant S, Sorel N, Ragot S, Allal J, et al. Resistance in vitro to low-dose aspirin is associated with platelet PlA1 (GP IIIa) polymorphism but not with C807T(GP Ia/IIa) and C-5T Kozak (GP Ibalpha) polymorphisms. J Am Coll Cardiol 2003;42(6):1115-9.
28. Cooke GE, Bray PF, Hamlington JD, Pham DM, Goldschmidt-Clermont PJ. PlA2 polymorphism and efficacy of aspirin. Lancet 1998;351(9111):1253.
29. Michelson AD, Furman MI, Goldschmidt-Clermont P, Mascelli MA, Hendrix C, Coleman L, et al. Platelet GP IIIa PI(A) polymorphisms display different sensitivities to agonists. Circulation 2000;101(9):1013-8.
30. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Alfonso F, Sabate M, Fernandez C, et al. PlA polymorphism and platelet reactivity following clopidogrel loading dose in patients undergoing coronary stent implantation. Blood Coagul Fibrinolysis 2004;15(1):89-93.
31. Dropinski J, Musial J, Jakiela B, Wegrzyn W, Sanak M, Szczeklik A. Anti-thrombotic action of clopidogrel and P1(A1/A2) polymorphism of beta3 integrin in patients with coronary artery disease not being treated with aspirin. Thromb Haemost 2005;94(6):1300-5.
32. Cooke GE, Liu-Stratton Y, Ferketich AK, Moeschberger ML, Frid DJ, Magorien RD, et al. Effect of platelet antigen polymorphism on platelet inhibition by aspirin, clopidogrel, or their combination. J Am Coll Cardiol 2006;47(3):541-6.
33. Papp E, Havasi V, Bene J, Komlosi K, Talian G, Feher G, et al. Does glycoprotein IIIa gene (PI(A)) polymorphism influence clopidogrel resistance? : a study in older patients. Drugs Aging 2007;24(4):345-50.
34. Goodman T, Ferro A, Sharma P. Pharmacogenetics of aspirin resistance: a comprehensive systematic review. Br J Clin Pharmacol 2008;66(2):222-32.
35. Goodman T, Sharma P, Ferro A. The genetics of aspirin resistance. Int J Clin Pract 2007;61(5):826-34.
36. Hulot JS, Bura A, Villard E, Azizi M, Remones V, Goyenvalle C, et al. Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. Blood 2006;108(7):2244-7.

37. Brandt JT, Close SL, Iturria SJ, Payne CD, Farid NA, Ernest CS, 2nd, et al. Common polymorphisms of CYP2C19 and CYP2C9 affect the pharmacokinetic and pharmacodynamic response to clopidogrel but not prasugrel. J Thromb Haemost 2007;5(12):2429-36.
38. Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, et al. Cytochrome P-450 Polymorphisms and Response to Clopidogrel. N Engl J Med 2008.
39. Frere C, Cuisset T, Morange PE, Quilici J, Camoin-Jau L, Saut N, et al. Effect of cytochrome p450 polymorphisms on platelet reactivity after treatment with clopidogrel in acute coronary syndrome. Am J Cardiol 2008;101(8):1088-93.
40. Fontana P, Hulot JS, De Moerloose P, Gaussem P. Influence of CYP2C19 and CYP3A4 gene polymorphisms on clopidogrel responsiveness in healthy subjects. J Thromb Haemost 2007;5(10):2153-5.
41. Simon T, Verstuyft C, Mary-Krause M, Quteineh L, Drouet E, Meneveau N, et al. Genetic Determinants of Response to Clopidogrel and Cardiovascular Events. N Engl J Med 2008.
42. Collet JP, Hulot JS, Pena A, Villard E, Esteve JB, Silvain J, et al. Cytochrome P450 2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: a cohort study. Lancet 2009;373(9660):309-17.
43. Storey RF. Clopidogrel in acute coronary syndrome: to genotype or not? Lancet 2009;373(9660):276-8.
44. Feher G, Feher A, Pusch G, Lupkovics G, Szapary L, Papp E. The genetics of antiplatelet drug resistance. Clin Genet 2008.
45. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramirez C, Cavallari U, Trabetti E, et al. Lack of association between the P2Y12 receptor gene polymorphism and platelet response to clopidogrel in patients with coronary artery disease. Thromb Res 2005;116(6):491-7.
46. Bura A, Bachelot-Loza C, Ali FD, Aiach M, Gaussem P. Role of the P2Y12 gene polymorphism in platelet responsiveness to clopidogrel in healthy subjects. J Thromb Haemost 2006;4(9):2096-7.
47. von Beckerath N, von Beckerath O, Koch W, Eichinger M, Schomig A, Kastrati A. P2Y12 gene H2 haplotype is not associated with increased adenosine diphosphate-induced platelet aggregation after initiation of clopidogrel therapy with a high loading dose. Blood Coagul Fibrinolysis 2005;16(3):199-204.
48. Cuisset T, Frere C, Quilici J, Morange PE, Saut N, Lambert M, et al. Role of the T744C polymorphism of the P2Y12 gene on platelet response to a 600-mg loading dose of clopidogrel in 597 patients with non-ST-segment elevation acute coronary syndrome. Thromb Res 2007;120(6):893-9.
49. Giusti B, Gori AM, Marcucci R, Saracini C, Sestini I, Paniccia R, et al. Cytochrome P450 2C19 loss-of-function polymorphism, but not CYP3A4 IVS10 + 12G/A and P2Y12 T744C polymorphisms, is associated with response variability to dual antiplatelet treatment in high-risk vascular patients. Pharmacogenet Genomics 2007;17(12):1057-64.
50. Sibbing D, von Beckerath O, Schomig A, Kastrati A, von Beckerath N. P2Y1 gene A1622G dimorphism is not associated with adenosine diphosphate-induced platelet activation and aggregation after administration of a single high dose of clopidogrel. J Thromb Haemost 2006;4(4):912-4.
51. Maree AO, Fitzgerald DJ. Variable platelet response to aspirin and clopidogrel in atherothrombotic disease. Circulation 2007;115(16):2196-207.
52. Hirsh J, Guyatt G, Albers GW, Harrington R, Schunemann HJ, American College of Chest P. Antithrombotic and thrombolytic therapy: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). Chest 2008;133(6 Suppl):110S-112S.
53. Ansell J, Hollowell J, Pengo V, Martinez-Brotons F, Caro J, Drouet L. Descriptive analysis of the process and quality of oral anticoagulation management in real-life practice in patients with chronic non-valvular atrial fibrillation: the international study of anticoagulation management (ISAM). J Thromb Thrombolysis 2007;23(2):83-91.
54. Pengo V, Pegoraro C, Cucchini U, Iliceto S. Worldwide management of oral anticoagulant therapy: the ISAM study. J Thromb Thrombolysis 2006;21(1):73-7.

55. Wadelius M, Pirmohamed M. Pharmacogenetics of warfarin: current status and future challenges. Pharmacogenomics J 2007;7(2):99-111.
56. Fihn SD, McDonell M, Martin D, Henikoff J, Vermes D, Kent D, et al. Risk factors for complications of chronic anticoagulation. A multicenter study. Warfarin Optimized Outpatient Follow-up Study Group. Ann Intern Med 1993;118(7):511-20.
57. Hylek EM, Singer DE. Risk factors for intracranial hemorrhage in outpatients taking warfarin. Ann Intern Med 1994;120(11):897-902.
58. Yin T, Miyata T. Warfarin dose and the pharmacogenomics of CYP2C9 and VKORC1 - rationale and perspectives. Thromb Res 2007;120(1):1-10.
59. Sanderson S, Emery J, Higgins J. CYP2C9 gene variants, drug dose, and bleeding risk in warfarin-treated patients: a HuGEnet systematic review and meta-analysis. Genet Med 2005;7(2):97-104.
60. Lindh JD, Holm L, Andersson ML, Rane A. Influence of CYP2C9 genotype on warfarin dose requirements-a systematic review and meta-analysis. Eur J Clin Pharmacol 2008.
61. Thijssen HH, Verkooijen IW, Frank HL. The possession of the CYP2C9*3 allele is associated with low dose requirement of acenocoumarol. Pharmacogenetics 2000;10(8):757-60.
62. Tassies D, Freire C, Pijoan J, Maragall S, Monteagudo J, Ordinas A, et al. Pharmacogenetics of acenocoumarol: cytochrome P450 CYP2C9 polymorphisms influence dose requirements and stability of anticoagulation. Haematologica 2002;87(11):1185-91.
63. Hermida J, Zarza J, Alberca I, Montes R, Lopez ML, Molina E, et al. Differential effects of 2C9*3 and 2C9*2 variants of cytochrome P-450 CYP2C9 on sensitivity to acenocoumarol. Blood 2002;99(11):4237-9.
64. Visser LE, van Vliet M, van Schaik RH, Kasbergen AA, De Smet PA, Vulto AG, et al. The risk of overanticoagulation in patients with cytochrome P450 CYP2C9*2 or CYP2C9*3 alleles on acenocoumarol or phenprocoumon. Pharmacogenetics 2004;14(1):27-33.
65. Schalekamp T, van Geest-Daalderop JH, de Vries-Goldschmeding H, Conemans J, Bernsen Mj M, de Boer A. Acenocoumarol stabilization is delayed in CYP2C93 carriers. Clin Pharmacol Ther 2004;75(5):394-402.
66. Visser LE, van Schaik RH, van Vliet M, Trienekens PH, De Smet PA, Vulto AG, et al. The risk of bleeding complications in patients with cytochrome P450 CYP2C9*2 or CYP2C9*3 alleles on acenocoumarol or phenprocoumon. Thromb Haemost 2004;92(1):61-6.
67. Ufer M, Kammerer B, Kahlich R, Kirchheiner J, Yasar U, Brockmoller J, et al. Genetic polymorphisms of cytochrome P450 2C9 causing reduced phenprocoumon (S)-7-hydroxylation in vitro and in vivo. Xenobiotica 2004;34(9):847-59.
68. Schalekamp T, Oosterhof M, van Meegen E, van Der Meer FJ, Conemans J, Hermans M, et al. Effects of cytochrome P450 2C9 polymorphisms on phenprocoumon anticoagulation status. Clin Pharmacol Ther 2004;76(5):409-17.
69. Hummers-Pradier E, Hess S, Adham IM, Papke T, Pieske B, Kochen MM. Determination of bleeding risk using genetic markers in patients taking phenprocoumon. Eur J Clin Pharmacol 2003;59(3):213-9.
70. Suttie JW. The biochemical basis of warfarin therapy. Adv Exp Med Biol 1987;214:3-16.
71. Bodin L, Verstuyft C, Tregouet DA, Robert A, Dubert L, Funck-Brentano C, et al. Cytochrome P450 2C9 (CYP2C9) and vitamin K epoxide reductase (VKORC1) genotypes as determinants of acenocoumarol sensitivity. Blood 2005;106(1):135-40.
72. Reitsma PH, van der Heijden JF, Groot AP, Rosendaal FR, Buller HR. A C1173T dimorphism in the VKORC1 gene determines coumarin sensitivity and bleeding risk. PLoS Med 2005;2(10):e312.
73. Schalekamp T, Brasse BP, Roijers JF, Chahid Y, van Geest-Daalderop JH, de Vries-Goldschmeding H, et al. VKORC1 and CYP2C9 genotypes and acenocoumarol anticoagulation status: interaction between both genotypes affects overanticoagulation. Clin Pharmacol Ther 2006;80(1):13-22.
74. Rieder MJ, Reiner AP, Gage BF, Nickerson DA, Eby CS, McLeod HL, et al. Effect of VKORC1 haplotypes on transcriptional regulation and warfarin dose. N Engl J Med 2005;352(22):2285-93.

75. Schalekamp T, Brasse BP, Roijers JF, van Meegen E, van der Meer FJ, van Wijk EM, et al. VKORC1 and CYP2C9 genotypes and phenprocoumon anticoagulation status: interaction between both genotypes affects dose requirement. Clin Pharmacol Ther 2007;81(2):185-93.
76. Limdi NA, McGwin G, Goldstein JA, Beasley TM, Arnett DK, Adler BK, et al. Influence of CYP2C9 and VKORC1 1173C/T genotype on the risk of hemorrhagic complications in African-American and European-American patients on warfarin. Clin Pharmacol Ther 2008;83(2):312-21.
77. Aquilante CL, Langaee TY, Lopez LM, Yarandi HN, Tromberg JS, Mohuczy D, et al. Influence of coagulation factor, vitamin K epoxide reductase complex subunit 1, and cytochrome P450 2C9 gene polymorphisms on warfarin dose requirements. Clin Pharmacol Ther 2006;79(4):291-302.
78. D'Ambrosio RL, D'Andrea G, Cappucci F, Chetta M, Di Perna P, Brancaccio V, et al. Polymorphisms in factor II and factor VII genes modulate oral anticoagulation with warfarin. Haematologica 2004;89(12):1510-6.
79. Shikata E, Ieiri I, Ishiguro S, Aono H, Inoue K, Koide T, et al. Association of pharmacokinetic (CYP2C9) and pharmacodynamic (factors II, VII, IX, and X; proteins S and C; and gamma-glutamyl carboxylase) gene variants with warfarin sensitivity. Blood 2004;103(7):2630-5.
80. Wadelius M, Chen LY, Lindh JD, Eriksson N, Ghori MJ, Bumpstead S, et al. The largest prospective warfarin-treated cohort supports genetic forecasting. Blood 2009;113(4):784-92.
81. Cooper GM, Johnson JA, Langaee TY, Feng H, Stanaway IB, Schwarz UI, et al. A genome-wide scan for common genetic variants with a large influence on warfarin maintenance dose. Blood 2008;112(4):1022-7.
82. Mizutani T. PM frequencies of major CYPs in Asians and Caucasians. Drug Metab Rev 2003;35(2-3):99-106.
83. Xie HG, Prasad HC, Kim RB, Stein CM. CYP2C9 allelic variants: ethnic distribution and functional significance. Adv Drug Deliv Rev 2002;54(10):1257-70.
84. Gage BF, Eby C, Johnson JA, Deych E, Rieder MJ, Ridker PM, et al. Use of pharmacogenetic and clinical factors to predict the therapeutic dose of warfarin. Clin Pharmacol Ther 2008;84(3):326-31.
85. Wadelius M, Chen LY, Lindh JD, Eriksson N, Ghori MJ, Bumpstead S, et al. The largest prospective warfarin-treated cohort supports genetic forecasting. Blood 2008.
86. Anderson JL, Horne BD, Stevens SM, Grove AS, Barton S, Nicholas ZP, et al. Randomized trial of genotype-guided versus standard warfarin dosing in patients initiating oral anticoagulation. Circulation 2007;116(22):2563-70.
87. Hynicka LM, Cahoon WD, Jr., Bukaveckas BL. Genetic testing for warfarin therapy initiation. Ann Pharmacother 2008;42(9):1298-303.
88. Psaty BM, Lumley T, Furberg CD, Schellenbaum G, Pahor M, Alderman MH, et al. Health outcomes associated with various antihypertensive therapies used as first-line agents: a network meta-analysis. JAMA 2003;289(19):2534-44.
89. Manunta P, Burnier M, D'Amico M, Buzzi L, Maillard M, Barlassina C, et al. Adducin polymorphism affects renal proximal tubule reabsorption in hypertension. Hypertension 1999;33(2):694-7.
90. Cusi D, Barlassina C, Azzani T, Casari G, Citterio L, Devoto M, et al. Polymorphisms of alpha-adducin and salt sensitivity in patients with essential hypertension. Lancet 1997;349(9062):1353-7.
91. Glorioso N, Manunta P, Filigheddu F, Troffa C, Stella P, Barlassina C, et al. The role of alpha-adducin polymorphism in blood pressure and sodium handling regulation may not be excluded by a negative association study. Hypertension 1999;34(4 Pt 1):649-54.
92. Sciarrone MT, Stella P, Barlassina C, Manunta P, Lanzani C, Bianchi G, et al. ACE and alpha-adducin polymorphism as markers of individual response to diuretic therapy. Hypertension 2003;41(3):398-403.
93. Davis BR, Arnett DK, Boerwinkle E, Ford CE, Leiendecker-Foster C, Miller MB, et al. Antihypertensive therapy, the alpha-adducin polymorphism, and cardiovascular disease in high-risk hypertensive persons: the Genetics of Hypertension-Associated Treatment Study. Pharmacogenomics J 2007;7(2):112-22.

94. Turner ST, Chapman AB, Schwartz GL, Boerwinkle E. Effects of endothelial nitric oxide synthase, alpha-adducin, and other candidate gene polymorphisms on blood pressure response to hydrochlorothiazide. Am J Hypertens 2003;16(10):834-9.
95. Schelleman H, Stricker BH, Verschuren WM, de Boer A, Kroon AA, de Leeuw PW, et al. Interactions between five candidate genes and antihypertensive drug therapy on blood pressure. Pharmacogenomics J 2006;6(1):22-6.
96. Schelleman H, Klungel OH, Witteman JC, Hofman A, van Duijn CM, de Boer A, et al. The influence of the alpha-adducin G460W polymorphism and angiotensinogen M235T polymorphism on antihypertensive medication and blood pressure. Eur J Hum Genet 2006;14(7):860-6.
97. Psaty BM, Smith NL, Heckbert SR, Vos HL, Lemaitre RN, Reiner AP, et al. Diuretic therapy, the alpha-adducin gene variant, and the risk of myocardial infarction or stroke in persons with treated hypertension. JAMA 2002;287(13):1680-9.
98. Schelleman H, Klungel OH, Witteman JC, Breteler MM, Hofman A, van Duijn CM, et al. Diuretic-gene interaction and the risk of myocardial infarction and stroke. Pharmacogenomics J 2007;7(5):346-52.
99. Gerhard T, Gong Y, Beitelshees AL, Mao X, Lobmeyer MT, Cooper-DeHoff RM, et al. Alpha-adducin polymorphism associated with increased risk of adverse cardiovascular outcomes: results from GENEtic Substudy of the INternational VErapamil SR-trandolapril STudy (INVEST-GENES). Am Heart J 2008;156(2):397-404.
100. van Wieren-de Wijer DB, Maitland-van der Zee AH, de Boer A, Kroon AA, de Leeuw PW, Schiffers P, et al. Interaction between the Gly460Trp alpha-adducin gene variant and diuretics on the risk of myocardial infarction. J Hypertens 2009;27(1):61-8.
101. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 1990;86(4):1343-6.
102. Preston RA, Materson BJ, Reda DJ, Williams DW, Hamburger RJ, Cushman WC, et al. Age-race subgroup compared with renin profile as predictors of blood pressure response to antihypertensive therapy. Department of Veterans Affairs Cooperative Study Group on Antihypertensive Agents. JAMA 1998;280(13):1168-72.
103. Schwartz GL, Turner ST, Chapman AB, Boerwinkle E. Interacting effects of gender and genotype on blood pressure response to hydrochlorothiazide. Kidney Int 2002;62(5):1718-23.
104. Arnett DK, Davis BR, Ford CE, Boerwinkle E, Leiendecker-Foster C, Miller MB, 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(25):3374-83.
105. Frazier L, Turner ST, Schwartz GL, Chapman AB, Boerwinkle E. Multilocus effects of the renin-angiotensin-aldosterone system genes on blood pressure response to a thiazide diuretic. Pharmacogenomics J 2004;4(1):17-23.
106. Schelleman H, Klungel OH, van Duijn CM, Witteman JC, Hofman A, de Boer A, et al. Drug-gene interaction between the insertion/deletion polymorphism of the angiotensin-converting enzyme gene and antihypertensive therapy. Ann Pharmacother 2006;40(2):212-8.
107. Jiang X, Sheng HH, Lin G, Li J, Lu XZ, Cheng YL, et al. Effect of renin-angiotensin-aldosterone system gene polymorphisms on blood pressure response to antihypertensive treatment. Chin Med J (Engl) 2007;120(9):782-6.
108. Turner ST, Schwartz GL, Chapman AB, Boerwinkle E. C825T polymorphism of the G protein beta(3)-subunit and antihypertensive response to a thiazide diuretic. Hypertension 2001;37(2 Part 2):739-43.
109. Schunkert H, Hense HW, Doring A, Riegger GA, Siffert W. Association between a polymorphism in the G protein beta3 subunit gene and lower renin and elevated diastolic blood pressure levels. Hypertension 1998;32(3):510-3.
110. Mason DA, Moore JD, Green SA, Liggett SB. A gain-of-function polymorphism in a G-protein coupling domain of the human beta1-adrenergic receptor. J Biol Chem 1999;274(18):12670-4.

111. Rathz DA, Brown KM, Kramer LA, Liggett SB. Amino acid 49 polymorphisms of the human beta1-adrenergic receptor affect agonist-promoted trafficking. J Cardiovasc Pharmacol 2002;39(2):155-60.
112. O'Shaughnessy KM, Fu B, Dickerson C, Thurston D, Brown MJ. The gain-of-function G389R variant of the beta1-adrenoceptor does not influence blood pressure or heart rate response to beta-blockade in hypertensive subjects. Clin Sci (Lond) 2000;99(3):233-8.
113. Johnson JA, Zineh I, Puckett BJ, McGorray SP, Yarandi HN, Pauly DF. Beta 1-adrenergic receptor polymorphisms and antihypertensive response to metoprolol. Clin Pharmacol Ther 2003;74(1):44-52.
114. Liu J, Liu ZQ, Yu BN, Xu FH, Mo W, Zhou G, et al. beta1-Adrenergic receptor polymorphisms influence the response to metoprolol monotherapy in patients with essential hypertension. Clin Pharmacol Ther 2006;80(1):23-32.
115. Liu J, Liu ZQ, Tan ZR, Chen XP, Wang LS, Zhou G, et al. Gly389Arg polymorphism of beta1-adrenergic receptor is associated with the cardiovascular response to metoprolol. Clin Pharmacol Ther 2003;74(4):372-9.
116. Sofowora GG, Dishy V, Muszkat M, Xie HG, Kim RB, Harris PA, et al. A common beta1-adrenergic receptor polymorphism (Arg389Gly) affects blood pressure response to beta-blockade. Clin Pharmacol Ther 2003;73(4):366-71.
117. Zateyshchikov DA, Minushkina LO, Brovkin AN, Savel'eva EG, Zateyshchikova AA, Manchaeva BB, et al. Association of CYP2D6 and ADRB1 genes with hypotensive and antichronotropic action of betaxolol in patients with arterial hypertension. Fundam Clin Pharmacol 2007;21(4):437-43.
118. Filigheddu F, Reid JE, Troffa C, PinnaParpaglia P, Argiolas G, Testa A, et al. Genetic polymorphisms of the beta-adrenergic system: association with essential hypertension and response to beta-blockade. Pharmacogenomics J 2004;4(3):154-60.
119. Karlsson J, Lind L, Hallberg P, Michaelsson K, Kurland L, Kahan T, et al. Beta1-adrenergic receptor gene polymorphisms and response to beta1-adrenergic receptor blockade in patients with essential hypertension. Clin Cardiol 2004;27(6):347-50.
120. Lemaitre RN, Heckbert SR, Sotoodehnia N, Bis JC, Smith NL, Marciante KD, et al. beta1- and beta2-adrenergic receptor gene variation, beta-blocker use and risk of myocardial infarction and stroke. Am J Hypertens 2008;21(3):290-6.
121. Pacanowski MA, Gong Y, Cooper-Dehoff RM, Schork NJ, Shriver MD, Langaee TY, et al. beta-adrenergic receptor gene polymorphisms and beta-blocker treatment outcomes in hypertension. Clin Pharmacol Ther 2008;84(6):715-21.
122. Kurland L, Liljedahl U, Karlsson J, Kahan T, Malmqvist K, Melhus H, et al. Angiotensinogen gene polymorphisms: relationship to blood pressure response to antihypertensive treatment. Results from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation vs Atenolol (SILVHIA) trial. Am J Hypertens 2004;17(1):8-13.
123. Kurland L, Melhus H, Karlsson J, Kahan T, Malmqvist K, Ohman KP, et al. Angiotensin converting enzyme gene polymorphism predicts blood pressure response to angiotensin II receptor type 1 antagonist treatment in hypertensive patients. J Hypertens 2001;19(10):1783-7.
124. Dudley C, Keavney B, Casadei B, Conway J, Bird R, Ratcliffe P. Prediction of patient responses to antihypertensive drugs using genetic polymorphisms: investigation of renin-angiotensin system genes. J Hypertens 1996;14(2):259-62.
125. Harrap SB, Tzourio C, Cambien F, Poirier O, Raoux S, Chalmers J, et al. The ACE gene I/D polymorphism is not associated with the blood pressure and cardiovascular benefits of ACE inhibition. Hypertension 2003;42(3):297-303.
126. Hingorani AD, Jia H, Stevens PA, Hopper R, Dickerson JE, Brown MJ. Renin-angiotensin system gene polymorphisms influence blood pressure and the response to angiotensin converting enzyme inhibition. J Hypertens 1995;13(12 Pt 2):1602-9.

127. Bis JC, Smith NL, Psaty BM, Heckbert SR, Edwards KL, Lemaitre RN, 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(12):1011-7.
128. Mondorf UF, Russ A, Wiesemann A, Herrero M, Oremek G, Lenz T. Contribution of angiotensin I converting enzyme gene polymorphism and angiotensinogen gene polymorphism to blood pressure regulation in essential hypertension. Am J Hypertens 1998;11(2):174-83.
129. Schelleman H, Klungel OH, Witteman JC, Breteler MM, Yazdanpanah M, Danser AH, 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(4):478-84.
130. Su X, Lee L, Li X, Lv J, Hu Y, Zhan S, et al. Association between angiotensinogen, angiotensin II receptor genes, and blood pressure response to an angiotensin-converting enzyme inhibitor. Circulation 2007;115(6):725-32.
131. Yu H, Lin S, Liu G, Zhang Y, Deng C, Ma W. T1198C polymorphism of the angiotensinogen gene and antihypertensive response to angiotensin-converting enzyme inhibitors. Hypertens Res 2005;28(12):981-6.
132. Kurland L, Melhus H, Karlsson J, Kahan T, Malmqvist K, Ohman P, et al. Aldosterone synthase (CYP11B2) -344 C/T polymorphism is related to antihypertensive response: result from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation versus Atenolol (SILVHIA) trial. Am J Hypertens 2002;15(5):389-93.
133. Ortlepp JR, Hanrath P, Mevissen V, Kiel G, Borggrefe M, Hoffmann R. Variants of the CYP11B2 gene predict response to therapy with candesartan. Eur J Pharmacol 2002;445(1-2):151-2.
134. Redon J, Luque-Otero M, Martell N, Chaves FJ. Renin-angiotensin system gene polymorphisms: relationship with blood pressure and microalbuminuria in telmisartan-treated hypertensive patients. Pharmacogenomics J 2005;5(1):14-20.
135. Fuller RW, Choudry NB. Increased cough reflex associated with angiotensin converting enzyme inhibitor cough. Br Med J (Clin Res Ed) 1987;295(6605):1025-6.
136. Mukae S, Aoki S, Itoh S, Iwata T, Ueda H, Katagiri T. Bradykinin B(2) receptor gene polymorphism is associated with angiotensin-converting enzyme inhibitor-related cough. Hypertension 2000;36(1):127-31.
137. Mukae S, Itoh S, Aoki S, Iwata T, Nishio K, Sato R, et al. Association of polymorphisms of the renin-angiotensin system and bradykinin B2 receptor with ACE-inhibitor-related cough. J Hum Hypertens 2002;16(12):857-63.
138. Lee YJ, Tsai JC. Angiotensin-converting enzyme gene insertion/deletion, not bradykinin B2 receptor -58T/C gene polymorphism, associated with angiotensin-converting enzyme inhibitor-related cough in Chinese female patients with non-insulin-dependent diabetes mellitus. Metabolism 2001;50(11):1346-50.
139. Zee RY, Rao VS, Paster RZ, Sweet CS, Lindpaintner K. Three candidate genes and angiotensin-converting enzyme inhibitor-related cough: a pharmacogenetic analysis. Hypertension 1998;31(4):925-8.
140. Bozkurt O, Verschuren WM, van Wieren-de Wijer BM, Knol MJ, de Boer A, Grobbee DE, et al. Genetic variation in the renin-angiotensin system modifies the beneficial effects of ACE inhibitors on the risk of diabetes mellitus among hypertensives. J Hum Hypertens 2008;22(11):774-80.
141. Arnett DK, Claas SA, Glasser SP. Pharmacogenetics of antihypertensive treatment. Vascul Pharmacol 2006;44(2):107-18.
142. Shin J, Johnson JA. Pharmacogenetics of beta-blockers. Pharmacotherapy 2007;27(6):874-87.
143. Mellen PB, Herrington DM. Pharmacogenomics of blood pressure response to antihypertensive treatment. J Hypertens 2005;23(7):1311-25.
144. Schelleman H, Stricker BH, De Boer A, Kroon AA, Verschuren MW, Van Duijn CM, et al. Drug-gene interactions between genetic polymorphisms and antihypertensive therapy. Drugs 2004;64(16):1801-16.
145. Cheung BM, Lauder IJ, Lau CP, Kumana CR. Meta-analysis of large randomized controlled trials to evaluate the impact of statins on cardiovascular outcomes. Br J Clin Pharmacol 2004;57(5):640-51.

146. Chasman DI, Posada D, Subrahmanyan L, Cook NR, Stanton VP, Jr., Ridker PM. Pharmacogenetic study of statin therapy and cholesterol reduction. JAMA 2004;291(23):2821-7.
147. Thompson JF, Man M, Johnson KJ, Wood LS, Lira ME, Lloyd DB, et al. An association study of 43 SNPs in 16 candidate genes with atorvastatin response. Pharmacogenomics J 2005;5(6):352-8.
148. Donnelly LA, Doney AS, Dannfald J, Whitley AL, Lang CC, Morris AD, et al. A paucimorphic variant in the HMG-CoA reductase gene is associated with lipid-lowering response to statin treatment in diabetes: a GoDARTS study. Pharmacogenet Genomics 2008;18(12):1021-6.
149. Krauss RM, Mangravite LM, Smith JD, Medina MW, Wang D, Guo X, et al. Variation in the 3-hydroxyl-3-methylglutaryl coenzyme a reductase gene is associated with racial differences in low-density lipoprotein cholesterol response to simvastatin treatment. Circulation 2008;117(12):1537-44.
150. Polisecki E, Muallem H, Maeda N, Peter I, Robertson M, McMahon AD, et al. Genetic variation at the LDL receptor and HMG-CoA reductase gene loci, lipid levels, statin response, and cardiovascular disease incidence in PROSPER. Atherosclerosis 2008;200(1):109-14.
151. Hindorff LA, Lemaitre RN, Smith NL, Bis JC, Marciante KD, Rice KM, et al. Common genetic variation in six lipid-related and statin-related genes, statin use and risk of incident nonfatal myocardial infarction and stroke. Pharmacogenet Genomics 2008;18(8):677-82.
152. Medina MW, Gao F, Ruan W, Rotter JI, Krauss RM. Alternative splicing of 3-hydroxy-3-methylglutaryl coenzyme A reductase is associated with plasma low-density lipoprotein cholesterol response to simvastatin. Circulation 2008;118(4):355-62.
153. Ordovas JM, Lopez-Miranda J, Perez-Jimenez F, Rodriguez C, Park JS, Cole T, et al. Effect of apolipoprotein E and A-IV phenotypes on the low density lipoprotein response to HMG CoA reductase inhibitor therapy. Atherosclerosis 1995;113(2):157-66.
154. Ye P, Shang Y, Ding X. The influence of apolipoprotein B and E gene polymorphisms on the response to simvastatin therapy in patients with hyperlipidemia. Chin Med Sci J 2003;18(1):9-13.
155. Pedro-Botet J, Schaefer EJ, Bakker-Arkema RG, Black DM, Stein EM, Corella D, et al. Apolipoprotein E genotype affects plasma lipid response to atorvastatin in a gender specific manner. Atherosclerosis 2001;158(1):183-93.
156. Ballantyne CM, Herd JA, Stein EA, Ferlic LL, Dunn JK, Gotto AM, Jr., et al. Apolipoprotein E genotypes and response of plasma lipids and progression-regression of coronary atherosclerosis to lipid-lowering drug therapy. J Am Coll Cardiol 2000;36(5):1572-8.
157. Nestel P, Simons L, Barter P, Clifton P, Colquhoun D, Hamilton-Craig I, et al. A comparative study of the efficacy of simvastatin and gemfibrozil in combined hyperlipoproteinemia: prediction of response by baseline lipids, apo E genotype, lipoprotein(a) and insulin. Atherosclerosis 1997;129(2):231-9.
158. Takane H, Miyata M, Burioka N, Shigemasa C, Shimizu E, Otsubo K, et al. Pharmacogenetic determinants of variability in lipid-lowering response to pravastatin therapy. J Hum Genet 2006;51(9):822-6.
159. Ojala JP, Helve E, Ehnholm C, Aalto-Setala K, Kontula KK, Tikkanen MJ. Effect of apolipoprotein E polymorphism and XbaI polymorphism of apolipoprotein B on response to lovastatin treatment in familial and non-familial hypercholesterolaemia. J Intern Med 1991;230(5):397-405.
160. Donnelly LA, Palmer CN, Whitley AL, Lang CC, Doney AS, Morris AD, et al. Apolipoprotein E genotypes are associated with lipid-lowering responses to statin treatment in diabetes: a Go-DARTS study. Pharmacogenet Genomics 2008;18(4):279-87.
161. Pena R, Lahoz C, Mostaza JM, Jimenez J, Subirats E, Pinto X, et al. Effect of apoE genotype on the hypolipidaemic response to pravastatin in an outpatient setting. J Intern Med 2002;251(6):518-25.
162. Sanllehy C, Casals E, Rodriguez-Villar C, Zambon D, Ojuel J, Ballesta AM, et al. Lack of interaction of apolipoprotein E phenotype with the lipoprotein response to lovastatin or gemfibrozil in patients with primary hypercholesterolemia. Metabolism 1998;47(5):560-5.

163. Tavintharan S, Lim SC, Chan YH, Sum CF. Apolipoprotein E genotype affects the response to lipid-lowering therapy in Chinese patients with type 2 diabetes mellitus. Diabetes Obes Metab 2007;9(1):81-6.
164. Zuccaro P, Mombelli G, Calabresi L, Baldassarre D, Palmi I, Sirtori CR. Tolerability of statins is not linked to CYP450 polymorphisms, but reduced CYP2D6 metabolism improves cholesteraemic response to simvastatin and fluvastatin. Pharmacol Res 2007;55(4):310-7.
165. Maitland-van der Zee AH, Jukema JW, Zwinderman AH, Hallman DM, De Boer A, Kastelein JJ, et al. Apolipoprotein-E polymorphism and response to pravastatin in men with coronary artery disease (REGRESS). Acta Cardiol 2006;61(3):327-31.
166. Fiegenbaum M, da Silveira FR, Van der Sand CR, Van der Sand LC, Ferreira ME, Pires RC, et al. Pharmacogenetic study of apolipoprotein E, cholesteryl ester transfer protein and hepatic lipase genes and simvastatin therapy in Brazilian subjects. Clin Chim Acta 2005;362(1-2):182-8.
167. Gerdes LU, Gerdes C, Kervinen K, Savolainen M, Klausen IC, Hansen PS, et al. The apolipoprotein epsilon4 allele determines prognosis and the effect on prognosis of simvastatin in survivors of myocardial infarction : a substudy of the Scandinavian simvastatin survival study. Circulation 2000;101(12):1366-71.
168. Chiodini BD, Franzosi MG, Barlera S, Signorini S, Lewis CM, D'Orazio A, et al. Apolipoprotein E polymorphisms influence effect of pravastatin on survival after myocardial infarction in a Mediterranean population: the GISSI-Prevenzione study. Eur Heart J 2007;28(16):1977-83.
169. Maitland-van der Zee AH, Stricker BH, Klungel OH, Kastelein JJ, Hofman A, Witteman JC, et al. The effectiveness of hydroxy-methylglutaryl coenzyme A reductase inhibitors (statins) in the elderly is not influenced by apolipoprotein E genotype. Pharmacogenetics 2002;12(8):647-53.
170. Mangravite LM, Krauss RM. Pharmacogenomics of statin response. Curr Opin Lipidol 2007;18(4):409-14.
171. Mangravite LM, Thorn CF, Krauss RM. Clinical implications of pharmacogenomics of statin treatment. Pharmacogenomics J 2006;6(6):360-74.
172. Choumerianou DM, Dedoussis GV. Familial hypercholesterolemia and response to statin therapy according to LDLR genetic background. Clin Chem Lab Med 2005;43(8):793-801.
173. Lahoz C, Pena R, Mostaza JM, Laguna F, Garcia-Iglesias MF, Taboada M, et al. Baseline levels of low-density lipoprotein cholesterol and lipoprotein (a) and the AvaII polymorphism of the low-density lipoprotein receptor gene influence the response of low-density lipoprotein cholesterol to pravastatin treatment. Metabolism 2005;54(6):741-7.
174. Salazar LA, Hirata MH, Quintao EC, Hirata RD. Lipid-lowering response of the HMG-CoA reductase inhibitor fluvastatin is influenced by polymorphisms in the low-density lipoprotein receptor gene in Brazilian patients with primary hypercholesterolemia. J Clin Lab Anal 2000;14(3):125-31.
175. Hannuksela ML, Liinamaa MJ, Kesaniemi YA, Savolainen MJ. Relation of polymorphisms in the cholesteryl ester transfer protein gene to transfer protein activity and plasma lipoprotein levels in alcohol drinkers. Atherosclerosis 1994;110(1):35-44.
176. Kuivenhoven JA, Jukema JW, Zwinderman AH, de Knijff P, McPherson R, Bruschke AV, et al. The role of a common variant of the cholesteryl ester transfer protein gene in the progression of coronary atherosclerosis. The Regression Growth Evaluation Statin Study Group. N Engl J Med 1998;338(2):86-93.
177. Regieli JJ, Jukema JW, Grobbee DE, Kastelein JJ, Kuivenhoven JA, Zwinderman AH, et al. CETP genotype predicts increased mortality in statin-treated men with proven cardiovascular disease: an adverse pharmacogenetic interaction. Eur Heart J 2008;29(22):2792-9.

178. de Grooth GJ, Zerba KE, Huang SP, Tsuchihashi Z, Kirchgessner T, Belder R, et al. The cholesteryl ester transfer protein (CETP) TaqIB polymorphism in the cholesterol and recurrent events study: no interaction with the response to pravastatin therapy and no effects on cardiovascular outcome: a prospective analysis of the CETP TaqIB polymorphism on cardiovascular outcome and interaction with cholesterol-lowering therapy. J Am Coll Cardiol 2004;43(5):854-7.
179. Freeman DJ, Samani NJ, Wilson V, McMahon AD, Braund PS, Cheng S, et al. A polymorphism of the cholesteryl ester transfer protein gene predicts cardiovascular events in non-smokers in the West of Scotland Coronary Prevention Study. Eur Heart J 2003;24(20):1833-42.
180. Klerkx AH, de Grooth GJ, Zwinderman AH, Jukema JW, Kuivenhoven JA, Kastelein JJ. Cholesteryl ester transfer protein concentration is associated with progression of atherosclerosis and response to pravastatin in men with coronary artery disease (REGRESS). Eur J Clin Invest 2004;34(1):21-8.
181. Marschang P, Sandhofer A, Ritsch A, Fiser I, Kvas E, Patsch JR. Plasma cholesteryl ester transfer protein concentrations predict cardiovascular events in patients with coronary artery disease treated with pravastatin. J Intern Med 2006;260(2):151-9.
182. Carlquist JF, Muhlestein JB, Horne BD, Hart NI, Bair TL, Molhuizen HO, et al. The cholesteryl ester transfer protein Taq1B gene polymorphism predicts clinical benefit of statin therapy in patients with significant coronary artery disease. Am Heart J 2003;146(6):1007-14.
183. Boekholdt SM, Sacks FM, Jukema JW, Shepherd J, Freeman DJ, McMahon AD, et al. Cholesteryl ester transfer protein TaqIB variant, high-density lipoprotein cholesterol levels, cardiovascular risk, and efficacy of pravastatin treatment: individual patient meta-analysis of 13,677 subjects. Circulation 2005;111(3):278-87.
184. Sing K, Ballantyne CM, Ferlic L, Brugada R, Cushman I, Dunn JK, et al. Lipoprotein lipase gene mutations, plasma lipid levels, progression/regression of coronary atherosclerosis, response to therapy, and future clinical events. Lipoproteins and Coronary Atherosclerosis Study. Atherosclerosis 1999;144(2):435-42.
185. Lahoz C, Pena R, Mostaza JM, Laguna F, Garcia-Iglesias MF, Taboada M, et al. The -514C/T polymorphism of the hepatic lipase gene significantly modulates the HDL-cholesterol response to statin treatment. Atherosclerosis 2005;182(1):129-34.
186. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 2005;352(16):1685-95.
187. Jain MK, Ridker PM. Anti-inflammatory effects of statins: clinical evidence and basic mechanisms. Nat Rev Drug Discov 2005;4(12):977-87.
188. Iakoubova OA, Tong CH, Rowland CM, Kirchgessner TG, Young BA, Arellano AR, et al. Association of the Trp719Arg polymorphism in kinesin-like protein 6 with myocardial infarction and coronary heart disease in 2 prospective trials: the CARE and WOSCOPS trials. J Am Coll Cardiol 2008;51(4):435-43.
189. Bare LA, Morrison AC, Rowland CM, Shiffman D, Luke MM, Iakoubova OA, et al. Five common gene variants identify elevated genetic risk for coronary heart disease. Genet Med 2007;9(10):682-9.
190. Shiffman D, Chasman DI, Zee RY, Iakoubova OA, Louie JZ, Devlin JJ, et al. A kinesin family member 6 variant is associated with coronary heart disease in the Women's Health Study. J Am Coll Cardiol 2008;51(4):444-8.
191. Shiffman D, O'Meara ES, Bare LA, Rowland CM, Louie JZ, Arellano AR, et al. Association of gene variants with incident myocardial infarction in the Cardiovascular Health Study. Arterioscler Thromb Vasc Biol 2008;28(1):173-9.
192. Iakoubova OA, Sabatine MS, Rowland CM, Tong CH, Catanese JJ, Ranade K, et al. Polymorphism in KIF6 gene and benefit from statins after acute coronary syndromes: results from the PROVE IT-TIMI 22 study. J Am Coll Cardiol 2008;51(4):449-55.
193. Boekholdt SM, Agema WR, Peters RJ, Zwinderman AH, van der Wall EE, Reitsma PH, et al. Variants of toll-like receptor 4 modify the efficacy of statin therapy and the risk of cardiovascular events. Circulation 2003;107(19):2416-21.

194. Holloway JW, Yang IA, Ye S. Variation in the toll-like receptor 4 gene and susceptibility to myocardial infarction. Pharmacogenet Genomics 2005;15(1):15-21.
195. Shitara Y, Sugiyama Y. Pharmacokinetic and pharmacodynamic alterations of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors: drug-drug interactions and interindividual differences in transporter and metabolic enzyme functions. Pharmacol Ther 2006;112(1):71-105.
196. Konig J, Seithel A, Gradhand U, Fromm MF. Pharmacogenomics of human OATP transporters. Naunyn Schmiedebergs Arch Pharmacol 2006;372(6):432-43.
197. Tachibana-Iimori R, Tabara Y, Kusuhara H, Kohara K, Kawamoto R, Nakura J, et al. Effect of genetic polymorphism of OATP-C (SLCO1B1) on lipid-lowering response to HMG-CoA reductase inhibitors. Drug Metab Pharmacokinet 2004;19(5):375-80.
198. Zhang W, Chen BL, Ozdemir V, He YJ, Zhou G, Peng DD, et al. SLCO1B1 521T-->C functional genetic polymorphism and lipid-lowering efficacy of multiple-dose pravastatin in Chinese coronary heart disease patients. Br J Clin Pharmacol 2007;64(3):346-52.
199. Igel M, Arnold KA, Niemi M, Hofmann U, Schwab M, Lutjohann D, et al. Impact of the SLCO1B1 polymorphism on the pharmacokinetics and lipid-lowering efficacy of multiple-dose pravastatin. Clin Pharmacol Ther 2006;79(5):419-26.
200. Niemi M, Neuvonen PJ, Hofmann U, Backman JT, Schwab M, Lutjohann D, et al. Acute effects of pravastatin on cholesterol synthesis are associated with SLCO1B1 (encoding OATP1B1) haplotype *17. Pharmacogenet Genomics 2005;15(5):303-9.
201. Kajinami K, Brousseau ME, Ordovas JM, Schaefer EJ. Polymorphisms in the multidrug resistance-1 (MDR1) gene influence the response to atorvastatin treatment in a gender-specific manner. Am J Cardiol 2004;93(8):1046-50.
202. Fiegenbaum M, da Silveira FR, Van der Sand CR, Van der Sand LC, Ferreira ME, Pires RC, et al. The role of common variants of ABCB1, CYP3A4, and CYP3A5 genes in lipid-lowering efficacy and safety of simvastatin treatment. Clin Pharmacol Ther 2005;78(5):551-8.
203. Vladutiu GD. Genetic predisposition to statin myopathy. Curr Opin Rheumatol 2008;20(6):648-55.
204. Niemi M, Schaeffeler E, Lang T, Fromm MF, Neuvonen M, Kyrklund C, et al. High plasma pravastatin concentrations are associated with single nucleotide polymorphisms and haplotypes of organic anion transporting polypeptide-C (OATP-C, SLCO1B1). Pharmacogenetics 2004;14(7):429-40.
205. Pasanen MK, Neuvonen M, Neuvonen PJ, Niemi M. SLCO1B1 polymorphism markedly affects the pharmacokinetics of simvastatin acid. Pharmacogenet Genomics 2006;16(12):873-9.
206. Link E, Parish S, Armitage J, Bowman L, Heath S, Matsuda F, et al. SLCO1B1 variants and statin-induced myopathy--a genomewide study. N Engl J Med 2008;359(8):789-99.
207. Mangravite LM, Wilke RA, Zhang J, Krauss RM. Pharmacogenomics of statin response. Curr Opin Mol Ther 2008;10(6):555-61.
208. McKinney BA, Reif DM, Ritchie MD, Moore JH. Machine learning for detecting gene-gene interactions: a review. Appl Bioinformatics 2006;5(2):77-88.
209. Evans WE, Relling MV. Moving towards individualized medicine with pharmacogenomics. Nature 2004;429(6990):464-8.

# CHAPTER 2.2

# METHODOLOGICAL AND STATISTICAL ISSUES IN PHARMACOGENOMICS

Bas J.M. Peters[a]
Andrei S. Rodin[b]
Anthonius de Boer[a]
Anke-Hilse Maitland-van der Zee[a]

a Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, Utrecht, The Netherlands
b Human Genetics Center, School of Public Health, University of Texas Health Science Center, Houston, TX, USA

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## ABSTRACT

Pharmacogenomics strives to explain the interindividual variability in response to drugs due to genetic variation. Although technological advances have provided us with relatively easy and cheap methods for genotyping, promises about personalized medicine have not yet met our high expectations. Successful results that have been achieved within the field of pharmacogenomics so far are, to name a few, *HLA-B**5701 screening to avoid hypersensitivity to the antiretroviral abacavir, thiopurine S-methyltransferase genotyping to avoid thiopurine toxicity, and *CYP2C9* and *VKORC1* genotyping for better dosing of the anticoagulant warfarin. However, few pharmacogenetic examples have made it into clinical practice in the treatment of complex diseases. Unfortunately, lack of reproducibility of results from observational studies involving many genes and diseases seems to be a common pattern in pharmacogenomic studies.

In this article we address some of the methodological and statistical issues within study design, gene and single nucleotide polymorphism (SNP) selection and data analysis that should be considered in future pharmacogenomic research. First, we discuss some of the issues related to the design of epidemiological studies, specific to pharmacogenomic research. Second, we describe some of the pros and cons of a candidate gene approach (including gene and SNP selection) and a genome-wide scan approach. Finally, conventional as well as several innovative approaches to the analysis of large pharmacogenomic datasets are proposed that deal with the issues of multiple testing and systems biology in different ways.

## INTRODUCTION

For many decades we have known that patients respond differently to drugs. The contribution of genetic variation to the interindividual response to isoniazid was described by Hughes et al. as early as 1954.[1] However, although technological advances have provided us with relatively easy and cheap methods for genotyping, promises about personalized medicine have not yet been met.

A recently published trial investigated the clinical value of screening *HLA-B**5701 for hypersensitivity to the antiretroviral abacavir and showed that genetic screening resulted in a significant reduction in the risk of hypersensitivity to abacavir.[2] However, few pharmacogenetic examples have made it into clinical practice in the treatment of complex diseases, although observational studies have described many pharmacogenetic interactions involving many genes and diseases. For instance, in 1998 Kuivenhoven et al. reported a pharmacogenetic interaction between response to pravastatin and the cholesteryl ester transfer protein (*CETP*) TaqIb polymorphism.[3] Homozygous carriers of the B1 allele experienced greatest benefit from pravastatin compared with placebo in terms of progression of coronary atherosclerosis. Ten years and many publications later, this interaction was not replicated in any other study.[4] Unfortunately, the lack of reproducibility seems to be a common pattern in (pharmaco-)genetic studies.[5 6] although there have been success stories such as the aforementioned abacavir–gene interaction. An insightful review article by Evans and Relling provides a thoughtful elaboration on why pharmacogenomics has not reached clinical practice to any significant extent.[7] Briefly, the obstacles include:

(i) education of the medical community; (ii) difficulties encountered in conducting definitive clinical pharmacogenomics studies (lack of funding, study design issues), and (iii) technical challenges of genetic testing comparable to other molecular diagnostics.
In this article we address some of the methodological and statistical issues within study design, selection of genes and single nucleotide polymorphisms (SNPs) and data analysis that should be considered in future pharmacogenomic research.

## METHODOLOGICAL ISSUES

Randomized clinical trials are considered the highest level of evidence and are essential in convincing practicing clinicians of the value of genotyping. An example is the forthcoming European Pharmacogenomics Approach to Coumarin Therapy (EU-PACT) trial, which will evaluate the benefit of genotyping to coumarin dosing and the risk of clinical events.[8] However, most commonly used in pharmacogenomics is the case-control design, largely because of its high efficiency: relatively few patients have to be genotyped (researchers can select a certain patient group based on disease status beforehand), the relative ease of patient recruitment, and late-onset diseases can be used as outcome measures without follow-up problems. An issue that may arise in pharmacogenomic studies is the inclusion of subjects. Subjects are asked to give their informed consent to the researcher to collect a sample and analyze the DNA. Privacy is always of high importance and patients' anonymity is therefore guaranteed in publications. However, it is important to realize that as few as 75 independent SNPs could unequivocally lead to an individual person,[9] although in practice we have not experienced this as a major problem in the process of data collection.[10]
In recent years, traditional issues (pros and cons) relating to the different designs of epidemiological studies have been discussed in countless comprehensive reviews.[11 12] For that reason, we focus on study design specific to pharmacogenomics: candidate gene approach (CGA) versus genome-wide scan, and confounding (population structure).

***Candidate gene approach and genome-wide association studies***
A pharmacogenomic study with a CGA typically involves a couple to tens of SNPs within each candidate gene that is possibly involved in the response to a particular drug. In contrast, a genome-wide association study (GWAS) seeks to identify variants that modify the response to a certain drug throughout the whole genome. CGA and GWAS both have their pros and cons, and differ in several significant ways. To begin with, the number of SNPs for a CGA can range from dozens to thousands, whereas in a GWAS between 100,000 and more than 1,000,000 SNPs are genotyped. Importantly, in contrast to a GWAS that comes with a fixed standard array, custom arrays for CGA studies allow SNPs to be selected by the researcher. A CGA on a genome-wide scale is also possible, although the SNPs in the selected candidate genes will only be those available on the standard arrays.
A GWAS is largely data driven (hypothesis-free) whereas a CGA is hypothesis driven because the selection of genes and SNPs is based on prior (expert) knowledge. The result is that GWAS can detect SNPs in genes that were not considered candidate genes before, or SNPs located outside of genes. It is very unlikely that these SNPs would have been found using a CGA. On the other hand, a CGA may detect associations that would not have been identified in a GWAS because of power issues (discussed below). Lastly,

although the costs of a GWAS have plummeted, budget constraints may still only allow a CGA. For the CGA, the first step is the selection of genes related to the research question. Candidate genes can be genes that have previously been reported to be associated in the research field of interest. In addition, genes involved in the pharmacokinetics (absorption, distribution, metabolism and elimination) and pharmacodynamics (drug targets) of a drug should be considered as candidate genes. Finally, genes related to the underlying disease or intermediate phenotype may be important for the pharmacogenomics of a certain drug. In addition to a straightforward manual literature search, more advanced methods are now available, one example being a method by Hansen et al.,[13] who describe a candidate gene-selection method for pharmacogenomic studies that specifically ranks 12,460 genes in the human genome according to the potential relevance to a drug and its indication. Interestingly, it uses gene–drug, gene–gene, and data from drug-drug similarities to construct a network for gene ranking. For several drugs, they were able to identify new candidate genes.[13]

SNPs come in different forms: synonymous, in which the mutation does not change the polypeptide sequence, and non-synonymous, in which the polymorphism results in a different polypeptide sequence. SNPs in non-coding regions may also be important because they can affect processes such as expression and gene splicing. Different types of SNPs should be considered for a CGA: SNPs that were previously associated, SNPs with functional annotation (coding SNPs) and tag SNPs. Coding SNPs can be found in the dbSNP database of NCBI (www.ncbi.nlm.nih.gov/SNP). The main source for many SNPs that are available is the HapMap project (www.hapmap.org), in which 270 individuals with different ancestry have been genotyped for over 3.5 million SNPs. Within the HapMap, so called tag SNPs have been identified in four populations. Tag SNPs are in strong linkage disequilibrium with other SNPs so they can serve as a proxy for the other SNPs, thus tremendously reducing the number of SNPs needed to contain the genetic variance of a gene.[14] Different methodologies for SNP selection are available which not only take advantage of tag SNPs but also give the option of including coding SNPs and defining the size of the flanking region. Two such web-based services based primarily on the international HapMap project are QuickSNP[15] and Tagger.[16] It is important to consider the $r^2$, which is a measure of the required linkage disequilibrium strength (usually set at 0.8), and the allele frequency of a SNP in the research population, as a low allele frequency may ultimately lead to low power. The first pharmacogenomic GWAS are starting to emerge.[17 18] When designing a GWAS, one should be aware of the computational burden, as up to more than one million variables are available in the epidemiological dataset. Furthermore, the recommendations made by the Wellcome Trust Case Control Consortium (WTCCC) should be considered.[19] First, the WTCCC stresses the importance of careful quality control, as small systematic differences can easily produce effects that may obscure true associations. Second, the potential for hidden population structure is a phenomenon that should be recognized (discussed below). Third, even with many cases and controls (2,000 and 3,000, respectively), the study power is limited to the detection of common variants with large effects only. Therefore, meta-analysis of existing GWAS is encouraged if possible. Furthermore, the WTCCC underlines the importance of replication studies to confirm true associations, and functional studies to gain more insight into, and mechanistic understanding of, the underlying biological molecular mechanisms.[19] Because tag SNPs that show an association are likely to be in

linkage disequilibrium with the causal variants, re-sequencing of this region is of major importance for the identification of causal variants.

***Population structure***

Dealing with confounding in epidemiology is a huge challenge and has been the subject of many discussions.[20] Besides the conventional confounding in epidemiology, pharmacogenomic research is faced with other potential confounding such as 'hidden population structure' (or population stratification). This phenomenon is present when genetically incompletely mixed distinct subpopulations exist within the research population.[21] Associations may then reflect confounding due to the different prevalence of a variant allele and prevalence or magnitude of the outcome of interest. Moreover, the exposure to a drug can be unevenly distributed among genetically different subgroups. Therefore, the gene–drug interactions may be biased by the population structure.[21]

Minimizing irrelevant allelic differences in groups can be achieved by sampling cases and controls from the same population and/or by matching cases and controls on the basis of genetic background using surrogate markers such as geographic proximity, physical characteristics and self-reported ethnicity.[22]

Most widely used methods to detect and adjust for this problem are genomic control, structured association methods and the EIGENSTRAT method.[23] The genomic control method, developed by Devlin and Roeder, corrects for variance inflation caused by population structure, using SNPs that are unrelated to the outcome (case or control).[24] The variance inflation factor, denoted by λ, is based on the assumption that λ is the same across the genome for all null SNPs, and can be calculated by dividing the median of the Armitage test statistic for the 'null' SNPs by 0.456 (the median of a chi squared with one degree of freedom [$\chi^2$ distribution, df = 1]). λ is expected to be larger than 1 but in the absence of population stratification may also be smaller than 1 (if this is the case, it is suggested to be set to 1).[25] Subsequently, the Armitage test statistic for the candidate SNPs are divided by λ. This method has also been extended for continuous outcome measures.[26]

In addition, Pritchard and colleagues developed a two-phase structured association method that can test for association in the presence of population structure.[27] The first phase uses the 'null' SNPs to identify the presence of population structure - assuming any of the associations to be the result of population structure - to subsequently assign the individuals to putative subpopulations. In the second phase, associations are tested conditionally on the subpopulation allocation.[27] Of note, the result of this computationally demanding method is highly sensitive to the assumed and unknown number of subpopulations. Finally, a popular tool to detect and correct for population structure is the EIGENSTRAT method,[28] based on principal component analysis. First, principal component analysis is applied to genotype data ('null' SNPs) to infer continuous axes of genetic variation. Second, using the residuals of linear regression, the observed genotypes and phenotypes are continuously adjusted by the amounts attributable to ancestry along each axis. Finally, use of the ancestry-adjusted genotypes and phenotypes used to calculate association statistics takes into account the population structure.

## DATA ANALYSIS

The relationship between variation in DNA sequence and clinical endpoints is likely to involve gene–gene (epistatic) interactions. The term epistasis is not unequivocal,[29 30] as it is used in different contexts. Generally, epistasis can be defined as either biological or statistical. Biologically, epistasis is the physical interactions among proteins or other biomolecules that affect the phenotype. Statistically, epistasis is generally defined in terms of deviation from a model of additive effects.[29] Gene–gene interactions may actually be a plausible explanation for non-replication of positive associations, since these interactions may vary between populations.

Traditional statistics is not well suited to dealing with gene–gene and gene–environment interactions on a large scale. In pharmacogenomics we are faced with an additional challenge – the primary goal of our analyses is not the genetic association with the phenotype, but rather the effect of genetics on the association between a certain drug and the phenotype.

***Multiple comparisons in regression models***

As the number of SNPs increases, data analysis becomes a statistical challenge because of the multiple testing (comparisons) problem. Generally, the P-value threshold that is considered significant in biomedical research is set at 0.05. This P value is not appropriate when testing many variables, as the frequency of type I errors will increase. Testing 20 random variables will give a 64% chance of finding one significantly associated SNP at random (P (≥ 1 significant result) = 1 – P (no significant results) = 1 – $(1 – 0.05)^{20}$ = approx 0.64). There are different ways of dealing with this issue. The Bonferroni correction can be applied by setting the significance cut-off at the P value for one test (i.e. 0.05) divided by the number of tests.[31] When testing 100 SNPs, the null hypothesis will then only be rejected when the P value is below 0.05/100 = 0.0005. In (pharmaco)genomics, the Bonferroni correction can be considered too stringent, as it may wipe out many small effects that one may actually expect (increased rate of type II errors). One of the reasons Bonferroni correction is too conservative is that many SNPs are not independent.

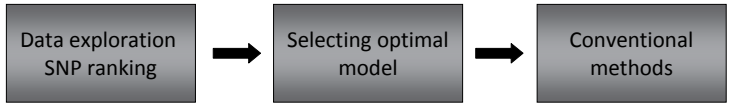
Another way of dealing with multiple testing is the increasingly popular false discovery rate (FDR) approach.[32] The FDR estimates the expected proportion of false positives among the tests declared significant, expressed as a q value. In the case where the FDR gives a q value of 0.2 for 50 significantly associated SNPs, the proportion of false positives would be 20% (10 SNPs). This approach is very different from using a threshold P value. Only a few of the 50 SNPs that were associated using the FDR would have been associated when applying a Bonferroni correction. There is no threshold q value that is considered standard. Depending on the study (number of patients, number of SNPs, biological plausibility), different q values might be chosen.[33]

Performing numerous tests that are necessary to analyze the large number of SNPs, epistasis, the exposure to a drug, and drug–/gene–environment interactions may have a detrimental effect on the ability to detect small effects because of the avalanche of multiple testing issues. It may therefore be necessary to deviate from conventional methods to other methods. Data analysis within the Bayesian framework, for example, may be of great value as there is no penalty for multiple analyses of the data. After all, the prior probability

of an association should not be affected by the tests that the investigator chooses to carry out.[23]

An alternative multi-stage analysis strategy proposed for pharmacogenomic data exploration is shown in Figure 1. The first step entails variable (SNP) selection and ranking, where the number of potentially predictive SNPs is significantly reduced. Second, a set of SNPs with high predictive potential is refined, and a descriptive/predictive model is fitted, in order to reverse-engineer the biological relationships underlying the system in question. Finally, traditional statistical methods are used to calculate odds ratios or relative risks for the specific associations between SNPs, phenotypes, exposures and other epidemiological factors.

***Figure 1.*** A strategy for analyzing large (pharmaco)genomic datasets

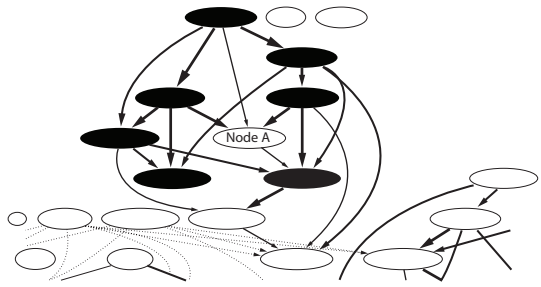



For step 1, conventional univariate methods (such as logistic regression or simple contingency tables) can be used to rank SNPs and to reduce them to a smaller subset (several hundred SNPs) based on the association strength. The choice of the P-value cut-off point is somewhat arbitrary (e.g. 'top 100'), but should ideally be a function of the data itself (i.e. how many SNPs actually carry the signal, as opposed to noise) and thus should probably depend on the number of SNPs that were tested. This univariate approach (known as 'filtering' in computer science vernacular[34]) does not take into account the interactions that may play a role in predicting the outcome. An alternative variable ranking/selection method is a random forests (RF) classifier.[35] RF is capable of accounting for some epistatic interactions, because it aggregates many (thousands, usually) single classification and regression trees (CARTs).[36] A single decision tree is generated by recursively partitioning the data set into subsets. In the whole data set the best possible predictor of the case status is selected to split the root node into two 'child' nodes (e.g. smoking, non-smoking). In the next steps, recursively, these child nodes are split again, using the best remaining predictors. This process continues until either all cases and controls are separated, or the terminal nodes are too small to split. To build an RF, two randomization mechanisms are added. First is bootstrapping, where a number of randomized samples are generated from the original dataset by using resampling with replacement.[37] The second randomization mechanism is the selection of a random (and small) subset of predictors (SNPs) to build each single tree. Once a 'forest' (consisting of thousands of randomized single decision trees) is built to classify a new observation, each tree in the forest classifies it separately; the class that gets the most votes predicts the class of the new observation. RF is capable of accounting for variable interactions because many possible variable combinations are encountered repeatedly within the forest. Another aspect of RF that makes it particularly attractive for large-scale studies is that it is more computationally efficient than comparable classifiers when the number of variables is high. Numerous RF implementations are available.[38-40]

In step 2, a set of SNPs with high predictive potential will be selected, and the relationships between these SNPs and other factors will be ascertained. Methods like Bayesian or belief network (BN) modeling, multifactor-dimensionality reduction (MDR), boosted classifiers and also RF are suitable for this step.

A BN provides a systems biology analytic approach for identifying interactions between genetic, physiological and environmental variables, including the outcome of interest.[41 42] A biological network modeling genotype-to-phenotype relationship is represented visually as a graph consisting of nodes (indicating discrete and continuous variables, such as SNPs, environmental factors, metabolite concentration, phenotypes, etc.) and directed edges (or arrows) that link mutually dependent nodes. Absence of an edge between two nodes indicates their conditional independence. The edge directionality is somewhat arbitrary and is not intended to imply causation; rather, it is used for mathematical convenience to distinguish between the 'parent' and the 'child' nodes. The edge strength indicates the relative magnitude of the dependency between the two variables, given the other interrelationship, and is measured as the marginal likelihood ratio test of the BN with the edge versus the otherwise identical BN without the edge. An edge between two SNPs is indicative of strong linkage disequilibrium; therefore, BN can simultaneously take into account linkage disequilibrium while doing genotype–phenotype association analyses. Since we are interested in predicting the efficacy of a drug on the outcome of interest, the BN can be reduced to a sub-network of the outcome of interest and a limited number of immediately predictive variables. A formal conceptualization of such a sub-network is the Markov 'blanket'.[43] By definition, the Markov blanket of node A consists of the parents of A, the offspring of A and the nodes that share an offspring with A (Figure 2). Given its Markov blanket, the outcome variable is independent of all the other variables in the network. Dependencies within the Markov blanket may be checked for statistical robustness using bootstrapping or subsequent standard statistical tests. Because the BN data analysis can be carried out simultaneously with other analysis methods, the level of overfitting (sensitivity/specificity balance) can be adjusted so that the number of predictive variables (SNPs) generated is roughly the same across the whole palette of analysis methods, and the predictive variable rankings generated by the different analysis methods can be compared directly. Alternatively, simulation studies can be performed to ascertain the optimal balance explicitly.

***Figure 2.*** Bayesian network with Markov Blanket. In a Bayesian network, the Markov blanket of node A includes the black nodes (parents, children and the other parents of all of its children).



Recently, BNs were used to study the pharmacogenomics of short-acting bronchodilator medication.[44] Himes et al. reported 15 of 426 SNPs in 15 of 254 genes to be predictive of bronchodilator response using a BN model with fair accuracy. They compared the BN (multivariate) model with a single-gene approach, and demonstrated that the BN model was much better at predicting bronchodilator response, suggesting that some of the relationships among SNPs and bronchodilator response were potentially true biological relationships. Interestingly, they found two relationships between two SNPs and bronchodilator response, a relationship that cannot be captured using traditional statistics.[44]

Another efficient descriptive/predictive modeling method is boosted classifiers.[45] While RF is a robust and scalable classifier, the complexity of the generated model (thousands of single decision trees) means that it is hardly interpretable by a human expert. On the other hand, single decision trees such as CART are not particularly robust. An attractive compromise is the boosted classifier in which there is more than one tree but their construction is adaptive rather than random (each new tree is aimed primarily at the observations misclassified by the preceding tree), and the number of trees is low (no more than a dozen, typically). The model then can be expressed as a set of 'if/then' rules that also cluster the sample into groups of similar individuals, for example, 'out of 200 individuals, 24 have SNP 1 = AA, SNP 2 = AG and SNP 3 = AC; out of these 24 individuals, 22 are cases, and 2 are controls'. Therefore, by representing the resulting decision tree models as sets of rules ('rule sets'), we perform both classification and sample clustering, thus accounting (to some extent) for genetic heterogeneity within the sample. Various implementations of boosted classifiers are available.[40 46 47]

MDR is also a computer-science based method, developed by Ritchie et al.[48] for the explicit identification and characterization of high-order gene–gene and gene–environment interactions in relatively small-scale studies. MDR is capable of doing so by reducing genotype predictors from multiple dimensions to one by pooling multilocus genotypes into high- and low-risk groups. In other words, a one-dimensional multilocus-genotype variable is computed for each model (combination of predicting variables).

A good example of how MDR has been applied in pharmacogenomic research is that of Motsinger et al., who investigated the effect of variants in a set of selected genes on the pharmacokinetics and treatment response to efavirenz. They showed that combinations of variants in *CYP2B6* and *ABCB1* were the most predictive for the 24 hours area under the plasma–concentration time curve for efavirenz and for virologic failure and toxicity failure.[49] Unfortunately, the number of variables that can be included in the model is limited; this is the price one pays for addressing the non-additive interaction issue explicitly. MDR software is publicly available at www.epistasis.org/software.html.

It should be mentioned that many other novel (mostly computer science-derived) methods can be used for variable selection and descriptive and predictive modeling. A useful internet resource and a convenient starting point for further exploration of data mining software can be found at www.kdnuggets.com/software/index.html.

Step 3 completes the analyses by using conventional statistical methods to calculate odds ratios or hazard ratios, allowing a direct epidemiological interpretation. This is beyond the scope of this article.

## FUTURE PERSPECTIVES

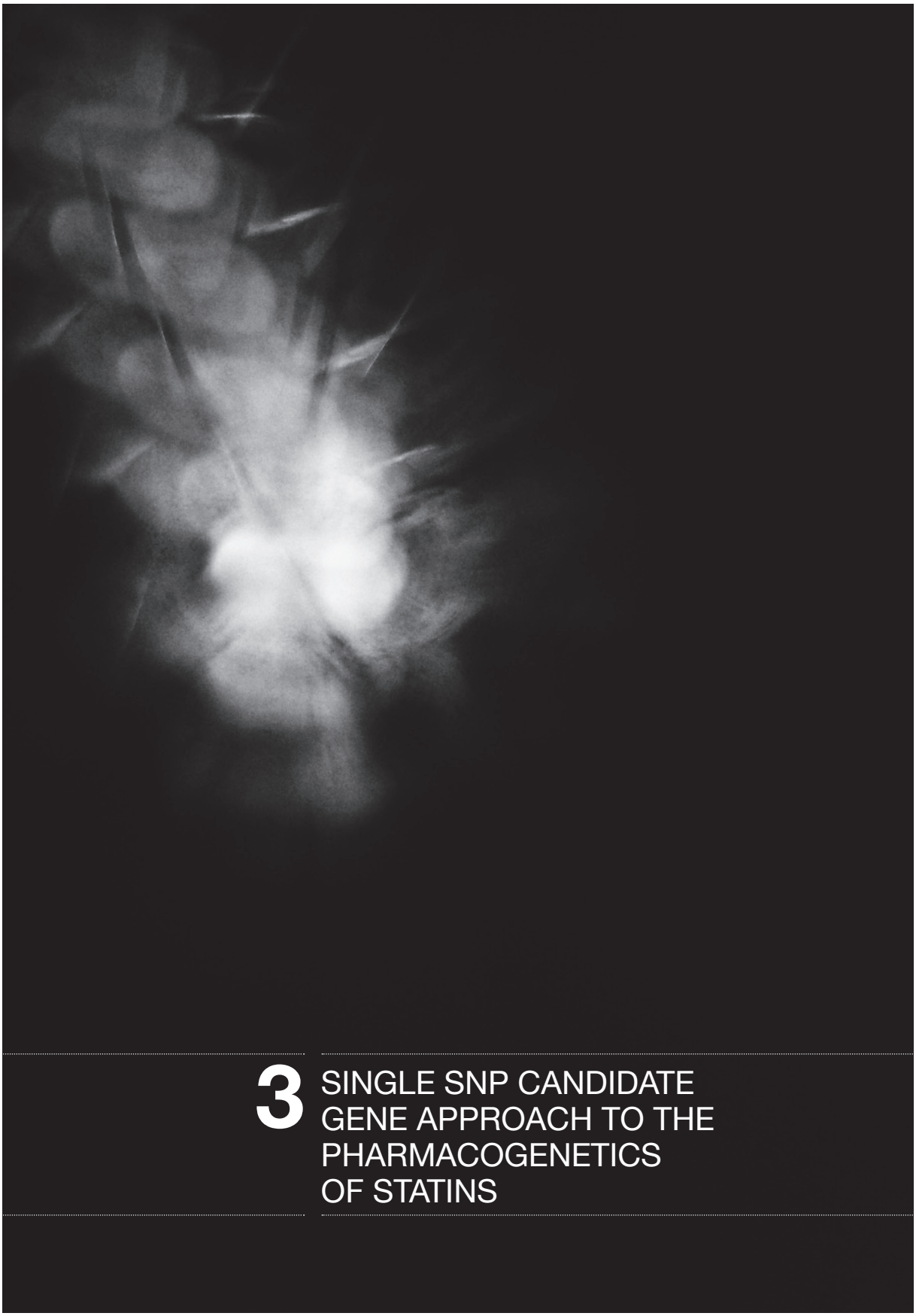
Although there are several well-established examples,[2 50-52] pharmacogenomics is still relatively uncommon in clinical practice. Concomitant to the issues we have discussed in this paper, future research should benefit from the technical advantages that modern technology has to offer. Pharmacogenomics is a staggeringly complex research field that requires a multi-disciplinary approach. Therefore, genome-wide methods at the level of expression, genotype scans and proteomics should be combined with what is already known about a drug. In addition, bioinformatics and ontology-based approaches should play important roles in sorting through the large amounts of data currently available.

## REFERENCES

1. Hughes HB, Biehl JP, Jones AP, Schmidt LH. Metabolism of isoniazid in man as related to the occurrence of peripheral neuritis. Am Rev Tuberc 1954;70(2):266-73.
2. Mallal S, Phillips E, Carosi G, Molina JM, Workman C, Tomazic J, et al. HLA-B*5701 screening for hypersensitivity to abacavir. N Engl J Med 2008;358(6):568-79.
3. Kuivenhoven JA, Jukema JW, Zwinderman AH, de Knijff P, McPherson R, Bruschke AV, et al. The role of a common variant of the cholesteryl ester transfer protein gene in the progression of coronary atherosclerosis. The Regression Growth Evaluation Statin Study Group. N Engl J Med 1998;338(2):86-93.
4. Boekholdt SM, Sacks FM, Jukema JW, Shepherd J, Freeman DJ, McMahon AD, et al. Cholesteryl ester transfer protein TaqIB variant, high-density lipoprotein cholesterol levels, cardiovascular risk, and efficacy of pravastatin treatment: individual patient meta-analysis of 13,677 subjects. Circulation 2005;111(3):278-87.
5. Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K. A comprehensive review of genetic association studies. Genet Med 2002;4(2):45-61.
6. Peters BJM, Klungel OH, de Boer A, Stricker BHC, Maitland-van der Zee AH. Pharmacogenetics of Cardiovascular Drug Therapy. Clin Cases in Miner Bone Metab 2009 January-April;6(1):55-65.
7. Evans WE, Relling MV. Moving towards individualized medicine with pharmacogenomics. Nature 2004;429(6990):464-8.
8. van Schie RM, Wadelius MI, Kamali F, Daly AK, Manolopoulos VG, de Boer A, et al. Genotype-guided dosing of coumarin derivatives: the European pharmacogenetics of anticoagulant therapy (EU-PACT) trial design. Pharmacogenomics 2009;10(10):1687-95.
9. Lin Z, Owen AB, Altman RB. Genetics. Genomic research and human subject privacy. Science 2004;305(5681):183.
10. van Wieren-de Wijer DB, Maitland-van der Zee AH, de Boer A, Stricker BH, Kroon AA, de Leeuw PW, et al. Recruitment of participants through community pharmacies for a pharmacogenetic study of antihypertensive drug treatment. Pharm World Sci 2009;31(2):158-64.
11. Cardon LR, Bell JI. Association study designs for complex diseases. Nat Rev Genet 2001;2(2):91-9.
12. Dempfle A, Scherag A, Hein R, Beckmann L, Chang-Claude J, Schafer H. Gene-environment interactions for complex traits: definitions, methodological requirements and challenges. Eur J Hum Genet 2008;16(10):1164-72.
13. Hansen NT, Brunak S, Altman RB. Generating genome-scale candidate gene lists for pharmacogenomics. Clin Pharmacol Ther 2009;86(2):183-9.
14. Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA, et al. A second generation human haplotype map of over 3.1 million SNPs. Nature 2007;449(7164):851-61.
15. Grover D, Woodfield AS, Verma R, Zandi PP, Levinson DF, Potash JB. QuickSNP: an automated web server for selection of tagSNPs. Nucleic Acids Res 2007;35(Web Server issue):W115-20.

16. de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D. Efficiency and power in genetic association studies. Nat Genet 2005;37(11):1217-23.
17. Cooper GM, Johnson JA, Langaee TY, Feng H, Stanaway IB, Schwarz UI, et al. A genome-wide scan for common genetic variants with a large influence on warfarin maintenance dose. Blood 2008;112(4):1022-7.
18. Link E, Parish S, Armitage J, Bowman L, Heath S, Matsuda F, et al. SLCO1B1 variants and statin-induced myopathy--a genomewide study. N Engl J Med 2008;359(8):789-99.
19. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447(7145):661-78.
20. Rothman KJ, Greenland S. Modern Epidemiology. 2nd ed. Greenland: Lippincott-Raven, 1998.
21. Umbach DM. Invited commentary: on studying the joint effects of candidate genes and exposures. Am J Epidemiol 2000;152(8):701-3.
22. Cardon LR, Palmer LJ. Population stratification and spurious allelic association. Lancet 2003;361(9357):598-604.
23. Balding DJ. A tutorial on statistical methods for population association studies. Nat Rev Genet 2006;7(10):781-91.
24. Devlin B, Roeder K. Genomic control for association studies. Biometrics 1999;55(4):997-1004.
25. Bacanu SA, Devlin B, Roeder K. The power of genomic control. Am J Hum Genet 2000;66(6):1933-44.
26. Bacanu SA, Devlin B, Roeder K. Association studies for quantitative traits in structured populations. Genet Epidemiol 2002;22(1):78-93.
27. Pritchard JK, Stephens M, Rosenberg NA, Donnelly P. Association mapping in structured populations. Am J Hum Genet 2000;67(1):170-81.
28. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet 2006;38(8):904-9.
29. Moore JH, Williams SM. Traversing the conceptual divide between biological and statistical epistasis: systems biology and a more modern synthesis. Bioessays 2005;27(6):637-46.
30. Phillips PC. Epistasis--the essential role of gene interactions in the structure and evolution of genetic systems. Nat Rev Genet 2008;9(11):855-67.
31. Shaffer JP. Multiple Hypothesis Testing. Annual Review of Psychology 1995;46:561-584.
32. Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proceedings of the National Academy of Sciences of the United States of America 2003;100(16):9440-9445.
33. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society, Series B (Methodological) 1995;57(1):289–300.
34. Guyon I, Elisseeff A. An Introduction to Variable and Feature Selection. Journal of Machine Learning Research 2003;3:1157-1182.
35. Breiman L. Random Forests. Machine Learning 2001;45(1):5-32.
36. Breiman L, Friedman JH, Olshen RA, Stone CJ. Classification and Regression Trees. Wadsworth International Group, Belmont, California. 1984.
37. Efron B. Bootstrap methods: another look at the jackknife. The Annals of Statistics 1979;7(1):1–26.
38. Breiman L, Cutler A. Random Forests. www.stat.berkeley.edu/users/breiman/RandomForests/ [accessed 11 November 2008].
39. Breiman L, Cutler A. Salford Systems Software: Random Forests. www.salfordsystems.com/randomforests.php [accessed 11 November 2008].
40. Witten IH, Frank E. Data Mining: Practical machine learning tools and techniques. 2nd ed. San Francisco: Morgan Kaufmann, 2005.
41. Pearl J. Probabilistic reasoning in intelligent systems. San Fransisco, CA: Morgan Kaufman, 1988.
42. Rodin AS, Boerwinkle E. Mining genetic epidemiology data with Bayesian networks I: Bayesian networks and example application (plasma apoE levels). Bioinformatics 2005;21(15):3273-8.

43. Judea P. Probabilistic reasoning in intelligent systems: networks of plausible inference: Morgan Kaufmann Publishers Inc., 1988.
44. Himes BE, Wu AC, Duan QL, Klanderman B, Litonjua AA, Tantisira K, et al. Predicting response to short-acting bronchodilator medication using Bayesian networks. Pharmacogenomics 2009;10(9):1393-412.
45. Freund Y, Schapire RE. A short introduction to boosting. J. Japan. Soc. for Artif. Intel. 1999;14(5):771-780.
46. The R Project for Statistical Computing. www.r-project.org [accessed 11 November, 2008].
47. Cohen W. The SLIPPER Rule Learning System. www.cs.cmu.edu/~wcohen/slipper/ [accessed 11 November 2008].
48. Ritchie MD, Hahn LW, Roodi N, Bailey LR, Dupont WD, Parl FF, et al. Multifactor-dimensionality reduction reveals high-order interactions among estrogen-metabolism genes in sporadic breast cancer. Am J Hum Genet 2001;69(1):138-47.
49. Motsinger AA, Ritchie MD, Shafer RW, Robbins GK, Morse GD, Labbe L, et al. Multilocus genetic interactions and response to efavirenz-containing regimens: an adult AIDS clinical trials group study. Pharmacogenet Genomics 2006;16(11):837-45.
50. Lennard L, Van Loon JA, Weinshilboum RM. Pharmacogenetics of acute azathioprine toxicity: relationship to thiopurine methyltransferase genetic polymorphism. Clin Pharmacol Ther 1989;46(2):149-54.
51. Muss HB, Thor AD, Berry DA, Kute T, Liu ET, Koerner F, et al. c-erbB-2 expression and response to adjuvant therapy in women with node-positive early breast cancer. N Engl J Med 1994;330(18):1260-6.
52. Weinshilboum R. Inheritance and drug response. N Engl J Med 2003;348(6):529-37.



# 3 SINGLE SNP CANDIDATE GENE APPROACH TO THE PHARMACOGENETICS OF STATINS

# CHAPTER 3.1

# EFFECTIVENESS OF STATINS IN THE REDUCTION OF THE RISK OF MYOCARDIAL INFARCTION IS MODIFIED BY THE *GNB3* C825T VARIANT

Bas J.M. Peters[a]
Anke-Hilse Maitland-van der Zee[a]
Bruno H.Ch. Stricker[b]
Diane B.M.A. van Wieren-de Wijer[a]
Anthonius de Boer[a]
Abraham A. Kroon[c]
Peter W de Leeuw[c]
Paul Schiffers[d]
Rob G.J.H. Janssen[e,f]
Cornelia M. van Duijn, C.M.[b]
Olaf H. Klungel[a]

a Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, Utrecht, The Netherlands
b Erasmus Medical Center Rotterdam, Department of Epidemiology & Biostatistics
c University of Maastricht, Department of Internal Medicine, Cardiovascular Research Institute Maastricht
d University of Maastricht, Department of Pharmacology and Toxicology, Cardiovascular Research Institute Maastricht
e University of Maastricht, Department of Population Genetics, Genomics & Bioinformatics, Genome Center Maastricht
f Current affiliation: Westburg BV, Leusden, The Netherlands

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## ABSTRACT

Introduction: The *GNB3* C825T polymorphism has been shown to affect lipid parameters, atherosclerosis progression, and incidence of myocardial infarction (MI). Therefore, we assessed whether the effectiveness of statins in reducing the risk of MI was modified by the *GNB3* C825T polymorphism.

Methods: In a population-based registry of pharmacy records linked to hospital discharge records (PHARMO), we used a nested case-control design. We selected patients hospitalized for MI as cases if they used antihypertensive drugs and had a diagnosis of hypercholesterolemia before their first MI. Controls met the same eligibility criteria, but were not hospitalized for MI. Logistic regression analysis was used to calculate odds ratios (OR) and synergy index (SI) with corresponding 95% confidence intervals (CI), and to adjust for potential confounding factors.

Results: We included 459 cases and 1805 controls. The risk of MI was significantly lower among participants exposed to statins compared with participants not exposed to statins (adjusted OR: 0.37, 95% CI: 0.29-0.47). The *GNB3* T allele was associated with a reduced risk of MI (adjusted OR: 0.74, 95% CI: 0.60-0.92). Among homozygous wild-type (CC) individuals (n = 1119), exposure to statins was associated with a lower risk of MI (OR: 0.48, 95% CI: 0.34-0.67). However, T allele carriers (CT and TT) who used statins had an even stronger reduced risk of MI (OR 0.27; 95% CI: 0.19-0.39). Overall, the interaction between exposure to statins and the *GNB3* C825T polymorphism was significantly increased on the multiplicative scale (SI: 1.67, 95% CI: 1.06-2.65).

Conclusion: Our findings show that T allele carriers of the *GNB3* C825T polymorphism have less risk of MI and are more likely to benefit from statin therapy in a hypercholesterolemic population of antihypertensive drug users.

## INTRODUCTION

Cardiovascular diseases (CVDs) are among the leading causes of death, especially in developed countries.[1] Statins are abundantly prescribed to reduce the risk of CVD. Large randomized controlled clinical trials have demonstrated an average risk reduction of 27% for major coronary events.[2] Nevertheless, there is notable interindividual variation in the response to statins, of which the origins are poorly understood. Genetic factors may be partly responsible for the variation in response.[3 4] G proteins are ubiquitously expressed heterotrimers, composed of α, β, and γ subunits that are key components for intracellular signal transduction, mainly activated by G protein-coupled receptors. The β3 subunit is encoded by the G protein β3 subunit (*GNB3*) gene, consisting of 11 exons and located on chromosome 12p13. The most extensively studied polymorphism of the *GNB3* gene is the C825T variant, in which the T allele results in enhanced G protein signaling through the pertussis toxin (PTX)-sensitive G protein pathway.[5] Studies in Caucasian populations have shown an association between the T allele and an increased risk for hypertension.[5-11] After an initial focus on the *GNB3* C825T variant and hypertension, multiple studies have been conducted showing associations with several features of the metabolic syndrome,[12 13] including obesity,[8] impaired glucose tolerance/insulin resistance,[8 14] dyslipidemia,[15] and hypercholesterolemia.[16-18] In addition,

genetic variation in the *GNB3* gene has been associated with development of coronary artery disease,[19] and the 825T allele is thought to influence the pathophysiological process of atherosclerosis. Indeed, this allele has been associated with more advanced carotid atherosclerosis,[14] increased arterial stiffness,[20] radial artery hypertrophy,[21] and more frequent unstable angina.[22] Moreover, clinical endpoints such as stroke and myocardial infarction (MI) are more frequently observed in patients carrying the 825T allele,[23-25] although this has not always been confirmed in other studies.[9 26]

At present, cardiovascular pharmacogenetic research with regard to the *GNB3* gene has been limited to the effect of the C825T variant on the efficacy of antihypertensive drugs.[27-29] However, the *GNB3* polymorphism has been shown to affect lipid parameters, progression of atherosclerosis, and incidence of MI. In addition, the PTX-sensitive G protein pathway has been shown to be involved in the regulation of low-density lipoprotein (LDL) receptor gene expression in vascular smooth muscle cells.[30] Therefore, the aim of our study was to investigate the association between the *GNB3* C825T polymorphism and the risk of MI, and to assess whether the effectiveness of statins in the reduction of the risk of MI is modified by this polymorphism.

## MATERIALS AND METHODS

### *Design and setting*

A nested case-control design was used to assess whether the *GNB3* C825T polymorphism modifies the effect of statins on the risk of MI. Participants were enrolled from the population-based Pharmaco-Morbidity Record Linkage System (PHARMO). PHARMO links drug dispensing histories from a representative sample of Dutch community pharmacies to the national registrations of hospital discharges (LMR) from 1985 and ever since. Currently, the base population of PHARMO covers about 2,000,000 community-dwelling inhabitants of several population-defined areas in The Netherlands, a sample comparable to the general Dutch population. Approval for this study was obtained from the Medical Ethics Committee of the University Medical Center Utrecht, The Netherlands.

### *Case and control definition*

In the PHARMO database, patients who were using an antihypertensive drug were selected. From this cohort, patients hospitalized for MI (International statistical Classification of Diseases-9 code 410) were included as cases if they had at least one prescription for antihypertensive drugs in the 3 months (last prescription less than 100 days before index date; 90 days plus 10 days to account for irregularity of refills) before their first hospitalization for MI and were registered in PHARMO for at least 1 year. The index date was defined as the date of hospitalization for the first MI. Subsequently, patients were excluded if they were younger than 18 years, if they had had a previous MI, or if the *GNB3* genotype was not available. Controls met the same eligibility criteria as the cases, but had not sustained a MI. Controls were matched with the cases in age (± 1 year), sex and region. They were assigned the same index date as the cases to whom they had been matched. Initially, we randomly selected six control participants for each case from the subset of antihypertensive drug users, assuming a 50% participation rate. However, the number of controls that we selected for each case was increased to 12 because this

rate was lower than expected and the effort to include a sufficient number of controls per case was substantial. For this study, we only included patients with a diagnosis of hypercholesterolemia (self-reported diagnosis or ever use of cholesterol lowering drugs according to pharmacy records).

***Patient recruitment***

Patients were recruited through community pharmacies, which participate in PHARMO. These pharmacists asked the patients whether they were willing to participate in the study after the purpose of the study had been explained. They were asked to return an informed consent form together with a questionnaire. Subsequently, the participant was sent a package with three tubes and three cotton swabs for a buccal swab procedure. All participants were explicitly asked for permission to collect, store and genotype the buccal swab material.

***Ascertainment of exposure to statins***

Coded pharmacy records were used to ascertain exposure to statins. In PHARMO, complete pharmacy records are available as of 1991. Pharmacy records provided details on dispense date, daily dose, and duration of therapy. On the basis of these records, participants' cumulative duration of statin use was calculated. To define exposure, sensitivity analyses were performed assessing the effect of both current/past user status and cumulative duration of statin use on the odds ratio (OR) for the association between use of statins and risk of MI. A patient was considered current user when the last prescription was less than 100 days before index date; 90 days plus 10 days to account for irregularity of refills. Past users had at least one prescription as of 1991 and discontinued their medication at least 100 days before the index date. In sensitivity analyses, we assessed the influence of varying definitions of exposure to statins (cumulative use of 90, 180, 360 and 720 days either combined with current user status or not). The different definitions were analyzed as a variable with three categories (unexposed, less than the number of days, and greater than or equal to the number of days) and as a variable with two categories (unexposed + less than the number of days and greater than or equal to the number of days). Preliminary data showed that statins were not effective in reducing the risk of MI in patients exposed less than 180 days. The efficacy of statins in patients exposed more than 180 days did not differ from a cumulative exposure more than 360 or 720 days. Participants were considered exposed when the cumulative duration of statin use was 180 days or more, whereas a cumulative duration of use of less than 180 days was considered nonexposed.

***Assessment of potential confounding factors and effect-modifiers***

We collected information on smoking, hypertension, hypercholesterolemia, diabetes mellitus, use of alcohol, diet, history of CVDs, family history of CVDs, weight and height through self-administered questionnaires, linkage with automated general practice and laboratory registrations. For all participants, we collected information about risk factor data available only before the index date.

***Buccal cell collection and DNA extraction***

Individuals who agreed to participate in the study were asked to supply a sample of buccal cells, collected by self-performed buccal swabs. They received three cotton swabs, three 15 ml tubes containing 2 ml buffer (1880 µl sodium chloride-Tris-EDTA (100 mmol/l NaCl, 10 mmol/l EDTA, 10 mmol/l Tris), 100 µl 10% SDS and 20 µl of 10 mg/ml Proteinase K (Qiagen, Hilden, Germany), and instructions how to collect a sample of buccal cells. On arrival at the laboratory, the Proteinase K concentration was increased to 0.2 mg/ml and the sample was lysed by incubation at 65°C for 2 h. The cotton tips were placed in a syringe cover inside a 50 ml tube and centrifuged at 1000 rpm for 60 s. The remaining buffer from the original 15 ml tube was poured into the 50 ml tube. DNA was then purified by adding 0.2 volumes of potassium acetate and putting the sample on ice for 15 min. The aqueous phase was extracted with 1 volume chloroform/isoamyl alcohol (24:1) and mixed for 30 min. After 15 min of centrifuging at 3000 rpm, the aqueous phase was transferred to a clean 50 ml tube. DNA was precipitated by adding 2 volumes ethanol absolute and pelleted by centrifugation (3000 rpm for 10 min). After washing with 70% ethanol twice, the pellet was dried and resuspended in Tris-EDTA (200 µl). DNA samples were stored at -30°C. As genotyping failed for some samples, DNA had to be purified using a Minikit (Qiagen). For the remainder of the samples, DNA was extracted using a maxikit (Qiagen).

***Genotyping***

Genotypes were assessed using a multiplex Single Base Extension method. Multiplex Single Base Extension was performed with SNaPshotTM as described by the manufacturer (Applied Biosystems, Foster City, California, USA). This method was described earlier, but adapted to this new set of polymorphisms. Laboratory personnel were blinded both to case-control status and antihypertensive as well as statin drug-therapy status. All participants were classified either as homozygous carriers of the *GNB3* wild-type genotype or as heterozygous or homozygous carriers of the *GNB3T* allele.

***Statistical analysis***

Logistic regression was used to study the association between statin use and the risk of MI and to adjust for potential confounders. Matching variables age, sex, region, and index date were included in our statistical model. To adjust for potential confounders, variables strongly associated with the risk of MI were included in the analysis (smoking, BMI and diabetes status). The inclusion of other potential confounders in the logistic regression model was based on assessment of the influence of each potential confounder on the OR for the association between use of statins and risk of MI. Among other variables such as use of different cardiovascular drugs (antihypertensives, platelet aggregation inhibitors, anticoagulants, and other cholesterol-lowering drugs), use of alcohol, physical activity, family history of CVDs, only the covariate ischemic heart disease showed a >10% change of this OR. For the *GNB3* C825T genotype, the CT and TT genotype were analyzed together as one group because the associated cellular phenotype has been shown to be similar.5 Also, the TT group was too small to be analyzed as a separate category. ORs were calculated separately in the two strata defined by genotype. We estimated the synergy index (SI), which is the ratio of the OR in those with the variant to the OR in those without the variant. An SI of one means that the OR in the two strata are the same and that there

is no interaction on the multiplicative scale. An SI of greater than one means that the joint effect of gene and drug is larger than expected from the product of their individual effects.[31] The Hardy-Weinberg equilibrium was tested using a $\chi^2$ goodness-of-fit test. Analysis was performed using SPSS statistical software (version 12.0.1 for Windows, SPSS, Chicago, Illinois, USA).

## RESULTS

A total of 2,264 patients were included (459 cases and 1,805 controls). In all patients, the *GNB3* genotype was assessed. Table 1 shows an overview of the baseline characteristics, according to the case-control status. BMI, diabetes mellitus, physical activity, and use of alcohol, other cholesterol-lowering drugs, or β blockers were not significantly associated with case-control status. The prevalence of current smoking and ischemic heart disease was higher in cases compared to controls. Furthermore, use of antihypertensive drugs and use of aspirin were associated with case-control status. Of the *GNB3* gene, T allele carriers were more frequently seen in the control group. In the control group, the *GNB3* genotype was in the Hardy-Weinberg equilibrium.
The risk of MI was significantly lower among participants exposed to statins compared to participants not exposed to statins (adjusted OR: 0.37, 95% CI: 0.29-0.47, Table 2). The sensitivity analyses performed to define exposure to statins showed no significant changes from our results.

***Table 2.*** The association between statin exposure/*GNB3* C825T genotype and the risk of myocardial infarction

| | Cases | Controls | OR (95% CI)[a] | OR (95% CI)[b] |
|---|---|---|---|---|
| No exposure | 321 | 850 | (Ref) | (Ref) |
| Statin exposed | 138 | 955 | 0.44 (0.35-0.55) | 0.37 (0.29-0.47) |
| CC | 249 | 819 | (Ref) | (Ref) |
| CT/TT | 210 | 986 | 0.73 (0.59-0.90) | 0.74 (0.60-0.92) |

CI, confidence interval; OR, odds ratio
[a] Adjusted for sex, age, region and index date.
[b] Adjusted for sex, age, region, index date, smoking, BMI, diabetes, ischemic heart disease.

***Table 1.*** Baseline Characteristics by case-control status

| | | Cases n=459 | % | Controls n=1805 | % | P value |
|---|---|---|---|---|---|---|
| Sex | Female | 130 | 28.3 | 525 | 29.1 | 0.75 |
| Age | Years (SD) | 63 | 9.8 | 64 | 9.2 | 0.59 |
| GNB3 genotype | CC | 249 | 54.2 | 819 | 45.4 | 0.001 |
| | CT | 180 | 39.2 | 804 | 44.5 | |
| | TTx | 30 | 6.5 | 182 | 10.1 | |
| Smoking | Never | 124 | 29.5 | 569 | 33.5 | 0.001 |
| | Current | 97 | 23.1 | 260 | 15.3 | |
| | Past | 199 | 47.4 | 872 | 51.3 | |
| BMI | >30 kg/m² | 98 | 23.6 | 312 | 19.5 | 0.07 |
| Diabetes | No | 356 | 78.6 | 1386 | 77.4 | 0.53 |
| | Diet | 43 | 9.5 | 157 | 8.8 | |
| | Drug | 54 | 11.9 | 248 | 13.8 | |
| Use of Alcohol | no use | 84 | 19.3 | 351 | 19.8 | 0.16 |
| | <2 units/day | 123 | 28.2 | 536 | 30.2 | |
| | >2 units/day | 14 | 3.2 | 119 | 6.7 | |
| | Unknown quantity | 215 | 49.3 | 771 | 43.4 | |
| Physical Activity | >=4 hrs/week | 321 | 72.1 | 1358 | 75.4 | 0.16 |
| Ischemic Heart Disease | | 216 | 47.1 | 675 | 37.4 | 0.000 |
| Statin exposure | Non-exposed | 321 | 70.0 | 850 | 47.1 | 0.000 |
| | Exposed | 138 | 30.0 | 955 | 52.9 | |
| Current use of other cholesterol-lowering drugs | | 8 | 1.7 | 52 | 2.9 | 0.18 |
| Current use of aspirin | | 143 | 31.2 | 685 | 38.0 | 0.007 |
| Current use of thiazides | | 77 | 16.8 | 393 | 21.8 | 0.02 |
| Current use of betablockers | | 233 | 50.8 | 928 | 51.4 | 0.80 |
| Current use of calcium-antagonists | | 129 | 28.1 | 424 | 23.5 | 0.04 |
| Current use of ace inhibitor | | 103 | 22.4 | 590 | 32.7 | 0.000 |
| Current use of at2-antagonist | | 42 | 9.2 | 226 | 12.5 | 0.05 |

In the present study, carriers of the T allele were less likely to have been hospitalized for an MI (adjusted OR: 0.74, 95% CI: 0.60-0.92, Table 2), independent of their medication. Among homozygous wild-type (CC) individuals (n = 1,068), participants who were exposed to statins had a statistically significantly lower risk of MI compared with those who were not exposed to statins (OR: 0.48, 95% CI: 0.34-0.67, Table 3). However, T allele carriers (n = 1,196) who used statins had an even stronger reduced risk of MI compared with homozygous wild-type carriers (OR 0.27; 95% CI: 0.19-0.39, Table 3). Overall the interaction between current use of statins and the *GNB3* C825T polymorphism was significantly increased on the multiplicative scale (SI: 1.67, 95% CI: 1.09-2.70).

***Table 3.*** The association between statin exposure and the risk of myocardial infarction, stratified by *GNB3* genotype

| | | Cases | Controls | OR (95% CI)[a] | OR (95% CI)[b] | SI (95% CI)[a] | SI (95% CI)[b] |
|---|---|---|---|---|---|---|---|
| CC | No exposure | 164 | 395 | (Ref) | (Ref) | | |
| | Exposure | 85 | 424 | 0.57 (0.42-0.77) | 0.48 (0.34-0.67) | (Ref) | (Ref) |
| CT/TT | No exposure | 157 | 455 | (Ref) | (Ref) | | |
| | Exposure | 53 | 531 | 0.32 (0.22-0.45) | 0.27 (0.19-0.39) | 1.72 (1.09-2.70) | 1.67 (1.06-2.65) |

CI, confidence interval; OR, odds ratio; SI, synergy index
[a] Adjusted for sex, age, region and index date.
[b] Adjusted for sex, age, region, index date, smoking, BMI, diabetes, ischemic heart disease.

## DISCUSSION

In the present study, the *GNB3* T allele was associated with a protective effect on MI. This is contrary to other studies,[24 25] where an increased risk of MI in T allele carriers was shown. The use of statins was associated with a large risk reduction of MI. Compared with homozygous wild-type individuals, carriers of the T allele were more likely to benefit from statin therapy in this hypercholesterolemic population of antihypertensive drug users.

Naber et al.[24] were the first to report the association between the *GNB3* T allele and an increased risk of MI. In Japanese men, Yamada et al.[25] reported the same association. However, a large cross-sectional study of Hengstenberg et al.[9] and a cohort study by Renner et al.[26] could not confirm these results. Our findings were different from the results from all these studies. An explanation could be that our population is different from populations that were studied before. Ethnic variability and geographical latitude have been shown to influence genetic susceptibility.[32] Furthermore, different study designs were used to investigate the effect of the *GNB3* C825T polymorphism on the risk of MI.

In addition, there are several possible mechanisms that may explain the interaction between the *GNB3* C825T polymorphism and modified response to statins. One study in healthy young men showed T allele carriers to have increased total cholesterol levels.[17] Similar results were reported in older Japanese populations.[16 18] Besides studies finding no association with cholesterol levels,[8 13] others found that the risk of hyperlipidemia was

lower in individuals carrying the CT allele compared with CC allele carriers.[33] Although findings are conflicting and further studies are needed to elucidate the role of *GNB3* in the lipid metabolism, literature shows that *GNB3* is likely to be involved in the cholesterol housekeeping. This hypothesis is also supported by other research showing that the PTX-sensitive G protein pathway is involved in the regulation of LDL receptor gene expression in vascular smooth muscle cells.[30] Therefore, responsiveness to statins may be altered by the *GNB3* C825T polymorphism.

In addition, the signaling pathway through G protein-coupled receptors via PTX-sensitive, Gi-type G proteins has been suggested to be involved in lipid-related inflammatory processes, such as uptake of LDL in macrophages.[34] Virchow et al. demonstrated enhanced chemotaxis of human neutrophils in T allele carriers of the *GNB3* C825T polymorphism in response to IL-8. [35] Moreover, the PTX-sensitive G protein pathway has been shown to be involved in the regulation of endothelial basic fibroblast growth factor expression in response to oxidized LDL, mediating angiogenesis in atherosclerotic plaque neovascularization.[36] Finally, it has been shown that *GNB3* C825T polymorphism affects platelet activation, which could play a role in plaque disruption.[37] Besides lowering cholesterol, statins exert a variety of other beneficial effects that contribute to the risk reduction of MI, known as pleiotropic effects.[38 39] These include effects on vascular inflammation, endothelial functioning, smooth muscle cell proliferation, LDL oxidation and platelet activity[38 39] that may be modified by the *GNB3* C825T polymorphism, as this gene is an important component in the PTX-sensitive G protein pathway.

To our knowledge, this is the first study to report an interaction between the *GNB3* gene and the use of statins. Judging from the current knowledge about the *GNB3* gene, the intended effects of statins might be altered by the polymorphisms within this gene. Nonetheless, our study cannot give more insight into the mechanism(s) of the *GNB3* gene-statin interaction. Therefore, a combination of pharmacogenetics and studies in the field of proteomics may give a better understanding of the exact mechanism of this interaction.

The OR in this study seems to be relatively low compared with the relative risk reduction of 27% from a meta-analysis of randomized controlled trials.[2] A possible explanation is the high number of events (cases) in our dataset (20%), resulting in an OR that is overestimated when interpreted as a relative risk.[40] However, our main interest was not in estimating the actual risk reduction achieved by statins, but in assessing whether the risk reduction was modified by the *GNB3* polymorphism. Although confounding may exist in our analysis, it does not change the interaction between the *GNB3* C825T polymorphism and the use of statins, assuming confounding to be equally distributed among genotypes.

One of the limitations of our study is that we focused on only one SNP, whereas other SNPs in the *GNB3* gene may attribute to the gene-treatment interaction. In addition, possible gene-gene-treatment interactions were not taken into account. Furthermore, the assessment of potential confounding factors was partly based on self-reported data. However, the confirmation of established risk factors for MI such as smoking suggests that these data are valid. Finally, a replication study is needed to confirm our findings.

The strengths of this study are its large sample size, availability of hospital records, and complete pharmacy records. Statin exposure was defined on the basis of pharmacy records. Exposure to statins was thoroughly investigated by performing sensitivity analyses.

In conclusion, our findings show that compared to homozygous C allele carriers, T allele

carriers of the *GNB3* C825T polymorphism have less risk of MI and are more likely to benefit from statin therapy. The pharmacogenetics of statins is a polygenetic matter, probably involving many small genetic effects that contribute to interindividual differences in response. Therefore, more complete gene assessment of candidate genes involved in both the cholesterol-lowering and pleiotropic pathway should be implemented in pharmacogenetic research. The *GNB3* gene should be considered as a candidate gene in future studies of the pharmacogenetics of statins.

## REFERENCES

1. McGovern PG, Jacobs DR, Jr., Shahar E, Arnett DK, Folsom AR, Blackburn H, et al. Trends in acute coronary heart disease mortality, morbidity, and medical care from 1985 through 1997: the Minnesota heart survey. Circulation 2001;104(1):19-24.
2. Cheung BM, Lauder IJ, Lau CP, Kumana CR. Meta-analysis of large randomized controlled trials to evaluate the impact of statins on cardiovascular outcomes. Br J Clin Pharmacol 2004;57(5):640-51.
3. Maitland-van der Zee AH, Boerwinkle E. Pharmacogenetics of response to statins: where do we stand? Curr Atheroscler Rep 2005;7(3):204-8.
4. Mangravite LM, Krauss RM. Pharmacogenomics of statin response. Curr Opin Lipidol 2007;18(4):409-14.
5. Siffert W, Rosskopf D, Siffert G, Busch S, Moritz A, Erbel R, et al. Association of a human G-protein beta3 subunit variant with hypertension. Nat Genet 1998;18(1):45-8.
6. Beige J, Hohenbleicher H, Distler A, Sharma AM. G-Protein beta3 subunit C825T variant and ambulatory blood pressure in essential hypertension. Hypertension 1999;33(4):1049-51.
7. Benjafield AV, Jeyasingam CL, Nyholt DR, Griffiths LR, Morris BJ. G-protein beta3 subunit gene (GNB3) variant in causation of essential hypertension. Hypertension 1998;32(6):1094-7.
8. Brand E, Wang JG, Herrmann SM, Staessen JA. An epidemiological study of blood pressure and metabolic phenotypes in relation to the Gbeta3 C825T polymorphism. J Hypertens 2003;21(4):729-37.
9. Hengstenberg C, Schunkert H, Mayer B, Doring A, Lowel H, Hense HW, et al. Association between a polymorphism in the G protein beta3 subunit gene (GNB3) with arterial hypertension but not with myocardial infarction. Cardiovasc Res 2001;49(4):820-7.
10. Schunkert H, Hense HW, Doring A, Riegger GA, Siffert W. Association between a polymorphism in the G protein beta3 subunit gene and lower renin and elevated diastolic blood pressure levels. Hypertension 1998;32(3):510-3.
11. Bagos PG, Elefsinioti AL, Nikolopoulos GK, Hamodrakas SJ. The GNB3 C825T polymorphism and essential hypertension: a meta-analysis of 34 studies including 14,094 cases and 17,760 controls. J Hypertens 2007;25(3):487-500.
12. Pollex RL, Hanley AJ, Zinman B, Harris SB, Khan HM, Hegele RA. Metabolic syndrome in aboriginal Canadians: prevalence and genetic associations. Atherosclerosis 2006;184(1):121-9.
13. Yamamoto M, Abe M, Jin JJ, Wu Z, Tabara Y, Mogi M, et al. Association of GNB3 gene with pulse pressure and clustering of risk factors for cardiovascular disease in Japanese. Biochem Biophys Res Commun 2004;316(3):744-8.
14. Wascher TC, Paulweber B, Malaimare L, Stadlmayr A, Iglseder B, Schmoelzer I, et al. Associations of a human G protein beta3 subunit dimorphism with insulin resistance and carotid atherosclerosis. Stroke 2003;34(3):605-9.
15. Pollex RL, Hanley AJ, Zinman B, Harris SB, Hegele RA. Clinical and genetic associations with hypertriglyceridemic waist in a Canadian aboriginal population. Int J Obes (Lond) 2006;30(3):484-91.
16. Ishikawa K, Imai Y, Katsuya T, Ohkubo T, Tsuji I, Nagai K, et al. Human G-protein beta3 subunit variant is associated with serum potassium and total cholesterol levels but not with blood pressure. Am J Hypertens 2000;13(2):140-5.

17. Saller B, Nemesszeghy P, Mann K, Siffert W, Rosskopf D. Glucose and lipid metabolism in young lean normotensive males with the G protein beta3 825T-allele. Eur J Med Res 2003;8(3):91-7.
18. Suwazono Y, Kobayashi E, Uetani M, Miura K, Morikawa Y, Ishizaki M, et al. G-protein beta 3 subunit polymorphism C1429T and low-density lipoprotein receptor-related protein 5 polymorphism A1330V are risk factors for hypercholesterolemia in Japanese males--a prospective study over 5 years. Metabolism 2006;55(6):751-7.
19. von Beckerath N, Schusterschitz Y, Koch W, Griesser K, Mehilli J, Gorchakova O, et al. G protein beta 3 subunit 825T allele carriage and risk of coronary artery disease. Atherosclerosis 2003;167(1):135-9.
20. Nurnberger J, Opazo Saez A, Mitchell A, Buhrmann S, Wenzel RR, Siffert W, et al. The T-allele of the C825T polymorphism is associated with higher arterial stiffness in young healthy males. J Hum Hypertens 2004;18(4):267-71.
21. Hanon O, Luong V, Mourad JJ, Bortolotto LA, Safar M, Girerd X. Association between the G protein beta3 subunit 825t allele and radial artery hypertrophy. J Vasc Res 2002;39(6):497-503.
22. von Beckerath N, Kastrati A, Koch W, Bottiger C, Mehilli J, Seyfarth M, et al. G protein beta3 subunit polymorphism and risk of thrombosis and restenosis following coronary stent placement. Atherosclerosis 2000;149(1):151-5.
23. Morrison AC, Doris PA, Folsom AR, Nieto FJ, Boerwinkle E. G-protein beta3 subunit and alpha-adducin polymorphisms and risk of subclinical and clinical stroke. Stroke 2001;32(4):822-9.
24. Naber CK, Husing J, Wolfhard U, Erbel R, Siffert W. Interaction of the ACE D allele and the GNB3 825T allele in myocardial infarction. Hypertension 2000;36(6):986-9.
25. Yamada Y, Izawa H, Ichihara S, Takatsu F, Ishihara H, Hirayama H, et al. Prediction of the risk of myocardial infarction from polymorphisms in candidate genes. N Engl J Med 2002;347(24):1916-23.
26. Renner W, Hoffmann MM, Grunbacher G, Winkelmann BR, Boehm BO, Marz W. G-protein beta3 subunit (GNB3) gene polymorphisms and cardiovascular disease: the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. Atherosclerosis 2007;192(1):108-12.
27. Nurnberger J, Dammer S, Mitchell A, Siffert W, Wenzel RR, Gossl M, et al. Effect of the C825T polymorphism of the G protein beta 3 subunit on the systolic blood pressure-lowering effect of clonidine in young, healthy male subjects. Clin Pharmacol Ther 2003;74(1):53-60.
28. Filigheddu F, Reid JE, Troffa C, PinnaParpaglia P, Argiolas G, Testa A, et al. Genetic polymorphisms of the beta-adrenergic system: association with essential hypertension and response to beta-blockade. Pharmacogenomics J 2004;4(3):154-60.
29. Turner ST, Schwartz GL, Chapman AB, Boerwinkle E. C825T polymorphism of the G protein beta(3)-subunit and antihypertensive response to a thiazide diuretic. Hypertension 2001;37(2 Part 2):739-43.
30. Nicholson AC, Hajjar DP. Transforming growth factor-beta up-regulates low density lipoprotein receptor-mediated cholesterol metabolism in vascular smooth muscle cells. J Biol Chem 1992;267(36):25982-7.
31. Khoury MJ, Flanders WD. Nontraditional epidemiologic approaches in the analysis of gene-environment interaction: case-control studies with no controls! Am J Epidemiol 1996;144(3):207-13.
32. Young JH, Chang YP, Kim JD, Chretien JP, Klag MJ, Levine MA, et al. Differential susceptibility to hypertension is due to selection during the out-of-Africa expansion. PLoS Genet 2005;1(6):e82.
33. Hayakawa T, Takamura T, Abe T, Kaneko S. Association of the C825T polymorphism of the G-protein beta3 subunit gene with hypertension, obesity, hyperlipidemia, insulin resistance, diabetes, diabetic complications, and diabetic therapies among Japanese. Metabolism 2007;56(1):44-8.
34. Vijayagopal P, Srinivasan SR, Radhakrishnamurthy B, Berenson GS. Studies on the mechanism of uptake of low density lipoprotein-proteoglycan complex in macrophages. Biochim Biophys Acta 1991;1092(3):291-7.
35. Virchow S, Ansorge N, Rosskopf D, Rubben H, Siffert W. The G protein beta3 subunit splice variant Gbeta3-s causes enhanced chemotaxis of human neutrophils in response to interleukin-8. Naunyn Schmiedebergs Arch Pharmacol 1999;360(1):27-32.

36. Chang PY, Luo S, Jiang T, Lee YT, Lu SC, Henry PD, et al. Oxidized low-density lipoprotein downregulates endothelial basic fibroblast growth factor through a pertussis toxin-sensitive G-protein pathway: mediator role of platelet-activating factor-like phospholipids. Circulation 2001;104(5):588-93.
37. Naber C, Hermann BL, Vietzke D, Altmann C, Haude M, Mann K, et al. Enhanced epinephrine-induced platelet aggregation in individuals carrying the G protein beta3 subunit 825T allele. FEBS Lett 2000;484(3):199-201.
38. Davignon J. Beneficial cardiovascular pleiotropic effects of statins. Circulation 2004;109(23 Suppl 1):III39-43.
39. Takemoto M, Liao JK. Pleiotropic effects of 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitors. Arterioscler Thromb Vasc Biol 2001;21(11):1712-9.
40. Zhang J, Yu KF. What's the relative risk? A method of correcting the odds ratio in cohort studies of common outcomes. JAMA 1998;280(19):1690-1.

# CHAPTER 3.2

# THE EFFECT OF NINE COMMON POLYMORPHISMS IN COAGULATION FACTOR GENES *(F2, F5, F7, F12* AND *F13)* ON THE EFFECTIVENESS OF STATINS: THE GENHAT STUDY

Anke-Hilse Maitland-van der Zee[a]
Bas J.M. Peters[a]
Amy I. Lynch[b]
Eric Boerwinkle[c]
Donna K. Arnett[d]
Suzanne Cheng[e]
Barry R. Davis[c]
Catherine Leiendecker-Foster[b]
Charles E. Ford[c]
John H. Eckfeldt[b]

a Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, Utrecht, The Netherlands
b University of Minnesota, Department of Laboratory Medicine and Pathology, 420 Delaware Street SE MMC 609, Minneapolis, MN 55455
c University of Texas Health Science Center at Houston, School of Public Health, Houston, Tx USA
d University of Alabama at Birmingham, 1665 University Avenue, Birmingham, AL, 35294
e Department of Human Genetics, Roche Molecular Systems, Inc., Alameda, CA.

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## ABSTRACT

Background: Pharmacogenetic research has shown that genetic variation may influence statin responsiveness. Statins exert a variety of beneficial effects beyond lipid lowering, including antithrombotic effects, which contribute to the risk reduction of cardiovascular disease. Statins have been shown to influence the expression of coagulation factors II, V, VII, XII and XIII.

Aim: Data from a large randomized clinical trial of pravastatin, designed to show efficacy relative to usual care, were used to investigate whether a pharmacogenetic effect of polymorphisms in genes coding for coagulation factors II, V, VII, XII and XIII is associated with reduced fatal coronary heart disease (CHD) and nonfatal myocardial infarction, combined CHD and all-cause mortality.

Methods: The Genetics of Hypertension Associated Treatment is an ancillary study of the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial. The genotyped population in the lipid-lowering trial of Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial included 9,624 participants randomly assigned to pravastatin or to usual care. The efficacy of pravastatin in reducing risk of all-cause mortality, CHD and nonfatal myocardial infarction and combined CHD, was compared among genotype strata by examining an interaction term in a proportional hazards model.

Results: None of the polymorphisms were associated with the clinical outcomes. For the *F7* (–323) ins/del polymorphism there was no interaction with pravastatin for either outcome. For both the *F5* $^{Arg}506^{Gln}$ G>A (rs6025) polymorphism and *F7* $^{Arg}353^{Gln}$ G>A (rs6046) polymorphism there were no interactions with pravastatin in relation to all-cause mortality, but there were significant interactions with combined CHD [interaction hazard ratio = 1.33, 95% confidence interval (1.01–1.76) and interaction hazard ratio = 1.92, 95% confidence interval (1.00–3.65), respectively]. There were no interactions between the polymorphisms in the other coagulation genes and pravastatin in relation to any outcome.

Conclusion: Polymorphisms in anticoagulation genes (*F5* and *F7*) seem to modify the efficacy of pravastatin in reducing risk of cardiovascular events.

## INTRODUCTION

The coagulation system plays a pivotal role in the development of arterial thrombosis. Rupture of an atherosclerotic plaque exposes the lipid core, smooth-muscle cells, macrophages and collagen to the bloodstream resulting in the activation of the coagulation cascade. Ultimately, a thrombus is formed that can either completely or incompletely occlude an artery, resulting in clinical events such as myocardial infarction and stroke.[1] Statins are mainstay drugs in cardiovascular risk management. The efficacy of statin therapy has been well established. Average risk reductions of 27% and 15% have been demonstrated for major coronary events and all-cause mortality, respectively.[2] Importantly, these risk reductions are averages where there is individual variation in the response to statins. Pharmacogenetic research has shown that genetic variation may influence statin responsiveness.[3] Statins exert a variety of other beneficial effects beyond lipid lowering which may contribute to the risk reduction of cardiovascular disease, known as pleiotropic effects.[4] These include antithrombotic effects. Among other coagulation factors, statins

have been shown to influence the expression of coagulation factors II, V, VII, XII and XIII.[5 6] Genetic variability within these coagulation genes has been shown to alter activity or expression of the coagulation factors and therefore the coagulation state. In addition, a range of polymorphisms in the aforementioned genes have previously been associated with coronary artery disease.[7 8] The effects of statins on coagulation haemostasis to reduce clot formation or reduce stability of fibrin clots may be affected by polymorphisms in these genes. In this study, data from a large randomized clinical trial of pravastatin designed to show efficacy against cardiovascular disease was used to investigate whether the *F13* $^{Pro}564^{Leu}$ C>T (rs5982), *F13* $^{Val}35^{Leu}$ C>T (rs5985), *F12* -46 C>T, *F7* -401 G>T (rs7981123), *F7* -402 G>A (rs762637), *F7* (-323) ins/del (rs5742910), *F7* $^{Arg}353^{Gln}$ G>A (rs6046), *F5* $^{Arg}506^{Gln}$ G>A (rs6025) and *F2* 20210 G>A (rs1799963) polymorphisms are associated with the efficacy of statins with respect to clinical outcomes.

## METHODS

### *Study Population and Design*

The Genetics of Hypertension Associated Treatment (GenHAT) study is an ancillary study of the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). The Lipid-Lowering Treatment (LLT) component of ALLHAT was designed to evaluate the impact of large sustained cholesterol reductions on all-cause mortality in a hypertensive cohort with at least one other CHD risk factor and to assess CHD reduction and other benefits in populations that had been excluded or underrepresented in previous trials, particularly older persons, women, racial and ethnic minority groups, and persons with diabetes. GenHAT is a post-hoc analysis of ALLHAT-LLT. The genotyped population in the lipid lowering component of ALLHAT (ALLHAT-LLT) included 9,624 participants. The primary outcome was all-cause mortality. Furthermore two a priori secondary outcomes were analyzed: (i) a combination of CHD death (fatal CHD, coronary revascularization-related mortality, previous angina or MI and no known potentially lethal noncoronary disease process) and nonfatal MI, and (ii) combined CHD (CHD death, coronary revascularization, hospitalized angina). The design of ALLHAT, including the LLT, and its participant and clinical site recruitment and selection have been reported elsewhere.[9-12] Briefly, ALLHAT-LLT was a randomized, open-label, large simple trial conducted from February 1994 through March 2002 at 513 clinical centres in the United States, Puerto Rico, US Virgin Islands, and Canada. The intervention was open-label pravastatin (40 mg/day) versus usual care. Participants were drawn exclusively from the ALLHAT antihypertensive trial. The protocol of ALLHAT was approved by each participating centre's institutional review board. The GenHAT study was approved by the institutional review boards of the University of Alabama at Birmingham, the University of Minnesota and the University of Texas Health Science Centre at Houston.

### *Genotyping*

DNA was isolated on FTA paper from blood samples. Genotyping was performed using the amplified DNA products of a multiplex PCR and detected using a colorimetric reaction of allele-specific oligonucleotide probes hybridized to a nylon membrane (i.e. Roche strip) as described previously.[13]

***Statistical methods***

STATA© version 9.2 (STATA Corporation, College Station, Texas) was used for all analyses. Tests for differences between treatment groups for baseline characteristics were done using ANOVA for continuous variables and chi-square tests for categorical variables. Hardy-Weinberg equilibrium was assessed using chi-square tests. Due to the small number of minor allele homozygotes, genotypes were modeled dominantly by collapsing the minor homozygotes with the heterozygotes, resulting in two categories for each variant. For each outcome, Cox proportional-hazards regression was used for testing the main effect of pravastatin within genotype-specific groups, and the genotype-by-treatment interactions, resulting in hazard ratios (HR) and ratio of hazard ratios (Interaction Hazard Ratio, IHR) point estimates, respectively. Adjustment variables included sex, ethnicity (black/non-black), smoking status (current smoker/non-smoker), type 2 diabetes status (yes/no), age, BMI, history of CHD, years of education, as well as baseline values of SBP, HDL cholesterol and LDL cholesterol. The Kaplan-Meier risk estimates were evaluated at 6 years follow-up, the Cox regression models used the full follow-up time available. A value of $P<0.05$ was used for statistical significance. However, given the many genotype, multivariate and interaction analyses performed, statistical significance at this level should be interpreted with caution To account for multiple statistical testing, q values were calculated.[14] The q-value of a test gives the proportion of false positives incurred (false discovery rate) when that particular test is considered significant.[15] Calculations were carried out using the R package implemented QValue software available from http://genomics.princeton.edu/storeylab/qvalue/.[14] To adjust for multiple testing, p-values of 27 pharmacogenetic interactions (9 gene treatment interactions with 3 outcomes) were used to calculate the q values.

## RESULTS

***Hardy-Weinberg equilibrium***

The *F7* (-323) del/ins, *F5* $^{Arg}506^{Gln}$ G>A, *F7* -401 G>T, *F7* -402 G>A and *F13* $^{Val}35^{Leu}$ C>T variants were in Hardy-Weinberg equilibrium (HWE) when tested in an ethnicity-specific manner, whereas the *F2* 20210 G>A variant was not. The *F7* $^{Arg}353^{Gln}$ G>A was not in HWE for blacks or non-blacks ($p=0.01$ and $p=0.003$, respectively). However, when further stratified by self-identified Hispanic status, both Hispanic black and Hispanic non-black participants were in HWE ($p=0.52$ and $p=0.72$, respectively), while non-Hispanic black and non-Hispanic non-black participants were not ($p=0.03$ and $p<0.001$, respectively). Furthermore, the HWE was violated by the non-black non-Hispanic group for the *F12* -46 C>T polymorphism ($p<0.001$) and by the non-Hispanic black participants for the *F13* Pro564Leu C>T polymorphism ($p=0.01$).

***Effect of genotype on outcomes***

There were no statistically significant effects from the genotypes on the outcomes. (table 2)

***Gene-treatment interactions***

There were no statistically significant interactions detected between the *F13* $^{Pro}564^{Leu}$ C>T, *F13* $^{Val}35^{Leu}$ C>T, *F12* -46 C>T, *F7* -401 G>T, *F7* -402 G>A, *F7* (-323) ins/del and *F2* 20210 G>A polymorphisms and pravastatin on coronary heart disease or all cause mortality (table 3a)

***Table 1.*** Baseline characteristics for participants by treatment group

| Characteristic | Pravastatin | Usual care | p-value* |
|---|---|---|---|
| **Sample size, n (%) by treatment** | 4808 (50.0) | 4818 (50.1) | |
| Age (y), mean (sd) | 66.4 (7.7) | 66.3 (7.5) | 0.35 |
| **Race, n (col %)** | | | |
| White | 2719 (56.6) | 2787 (57.9) | |
| Black | 1804 (37.5) | 1723 (35.8) | |
| Other | 285 (5.9) | 308 (6.4) | 0.17 |
| **Sex (male), n (%)** | 2469 (51.4) | 2450 (50.9) | 0.62 |
| Systolic Blood Pressure (mm Hg), mean (sd) | 144.9 (13.9) | 145.0 (14.0) | 0.72 |
| Diastolic Blood Pressure (mm Hg), mean (sd) | 84.1 (9.8) | 83.9 (9.8) | 0.37 |
| Body Mass Index, mean (sd) | 29.8 (5.9) | 29.9 (6.2) | 0.55 |
| Smoker, n (%) | 1128 (23.5) | 1133 (23.5) | 0.95 |
| History of type 2 diabetes, n (%) | 1697 (35.3) | 1639 (34.0) | 0.19 |
| History of MI or stroke, n (%) | 818 (17.0) | 842 (17.5) | 0.55 |
| History of CHD, n (%) | 654 (13.6) | 733 (15.2) | 0.02 |
| Education (y), mean (sd) | 10.7 (4.0) | 10.8 (4.1) | 0.80 |
| Total cholesterol (mg/dL), mean (sd) | 223.5 (26.9) | 223.6 (26.5) | 0.92 |
| HDL cholesterol (mg/dL), mean (sd) | 47.5 (13.4) | 47.3 (13.5) | 0.41 |
| LDL cholesterol (mg/dL), mean (sd) | 145.4 (21.4) | 145.3 (21.3) | 0.84 |
| **Antihypertensive treatment group, n (col %)** | | | |
| Doxazosin | 1008 (21.0) | 1029 (21.4) | |
| Chlorthalidone | 1743 (36.3) | 1741 (36.1) | |
| Amlodipine | 1039 (21.6) | 1037 (21.5) | |
| Lisinopril | 1018 (21.2) | 1011 (21.0) | 0.97 |
| *F7* **(-323) del/ins, n (col%)** | | | |
| DD | 3158 (65.7) | 3172 (65.9) | |
| DI | 1432 (29.8) | 1418 (29.5) | |
| II | 215 (4.5) | 223 (4.6) | 0.89 |
| *F7* **arg353gln G>A, n (col%)** | | | |
| GG | 3705 (77.4) | 3728 (77.8) | |
| GA | 994 (20.8) | 969 (20.2) | |
| AA | 87 (1.8) | 94 (2.0) | 0.72 |
| *F5* **arg506gln G>A, n (col%)** | | | |
| GG | 4675 (97.4) | 4673 (97.1) | |
| GA | 122 (2.5) | 138 (2.9) | 0.02 |
| AA | 5 (0.1) | 0 (0.0) | |
| *F2* **20210 G>A, n (col%)** | | | |
| GG | 4633 (97.3) | 4673 (97.6) | |
| GA | 126 (2.6) | 106 (2.2) | |
| AA | 5 (0.1) | 7 (0.2) | 0.34 |

*tests for differences between groups: ANOVA for continuous variables, chi-square for categorical variables.

***Table 2.*** Main effect (Hazard Rates) of genotypes on outcomes

| | Fatal CHD + Non-fatal MI | Combined CHD | All-cause mortality |
|---|---|---|---|
| ***F7* (-323) del/ins, n (col%)** | | | |
| DD (n=6330) | 1.00 | 1.00 | 1.00 |
| DI + II (n=3288) | 0.89 (0.75-1.05) | 0.93 (0.82-1.06) | 0.92 (0.80-1.04) |
| p-value | 0.16 | 0.27 | 0.19 |
| ***F7* arg353gln G>A, n (col%)** | | | |
| GG (n=7433) | 1.00 | 1.00 | 1.00 |
| GA + AA (n=2144) | 0.97 (0.80-1.16) | 0.90 (0.78-1.04) | 0.89 (0.77-1.03) |
| p-value | 0.71 | 0.14 | 0.12 |
| ***F5* arg506gln G>A, n (col%)** | | | |
| GG (n=9348) | 1.00 | 1.00 | 1.00 |
| GA + AA (n=265) | 1.00 (0.65-1.53) | 1.02 (0.74-1.39) | 0.87 (0.59-1.27) |
| p-value | 0.99 | 0.92 | 0.46 |
| ***F2* 20210 G>A, n (col%)** | | | |
| GG (n=9306) | 1.00 | 1.00 | 1.00 |
| GA + AA (n=244) | 1.07 (0.67-1.71) | 0.80 (0.54-1.18) | 0.95 (0.63-1.44) |
| p-value | 0.78 | 0.26 | 0.82 |
| ***F12* -46 C>T, n (col%)** | | | |
| CC (n=4537) | 1.00 | 1.00 | 1.00 |
| CT + TT (n=4988) | 1.00 (0.86-1.17) | 1.05 (0.93-1.17) | 0.99 (0.87-1.11) |
| p-value | 0.98 | 0.44 | 0.82 |
| ***F13* Val35Leu C>T, n (col%)** | | | |
| CC (n=5811) | 1.00 | 1.00 | 1.00 |
| CT + TT (n=3716) | 0.97 (0.83-1.13) | 1.00 (0.89-1.12) | 0.95 (0.84-1.07) |
| p-value | 0.69 | 0.98 | 0.38 |
| ***F13* Pro564Leu C>T, n (col%)** | | | |
| CC (n=6405) | 1.00 | 1.00 | 1.00 |
| CT + TT (n=3124) | 1.07 (0.92-1.25) | 1.11 (0.99-1.25) | 1.03 (0.91-1.17) |
| p-value | 0.39 | 0.08 | 0.65 |
| ***F7* -402 G>A, n (col%)** | | | |
| GG (n=6105) | 1.00 | 1.00 | 1.00 |
| GA + AA (n=3442) | 0.92 (0.79-1.08) | 0.99 (0.88-1.11) | 0.97 (0.85-1.10) |
| p-value | 0.33 | 0.87 | 0.58 |
| ***F7* -401 G>T, n (col%)** | | | |
| GG (n=6315) | 1.00 | 1.00 | 1.00 |
| GT + TT (n=3232) | 0.87 (0.73-1.03) | 0.93 (0.82-1.06) | 0.90 (0.79-1.03) |
| p-value | 0.10 | 0.29 | 0.12 |

*adjusted for sex, ethnicity (black/non-black), smoking status (current smoker/non-smoker), type 2 diabetes status (yes/no), age, BMI, history of CHD, years of education, as well as baseline values of SBP, HDL cholesterol and LDL cholesterol

***Table 3a.*** Interaction adjusted ratio of HRs (95% CI) pravastatin vs. UC on outcomes*

| | Fatal CHD + Non-fatal MI | Combined CHD | All-cause mortality |
|---|---|---|---|
| *F2* **20210 G>A** | | | |
| GG (n=9306) | 1 | 1 | 1 |
| GA + AA (n=244) | 1.21 (0.47-3.11) | 0.72 (0.33-1.56) | 0.62 (0.27-1.43) |
| *F5* **arg506gln G>A** | | | |
| GG (n=9348) | 1 | 1 | 1 |
| GA + AA (n=265) | 2.12 (0.88-5.12) | **1.92 (1.00-3.65)** | 1.15 (0.54-2.48) |
| *F7* **arg353gln G>A** | | | |
| GG (n=7433) | 1 | 1 | 1 |
| GA + AA (n=2144) | **1.53 (1.06-2.20)** | **1.33 (1.01-1.76)** | 1.19 (0.89-1.60) |
| *F7* **(-323) del/ins** | | | |
| DD (n=6330) | 1 | 1 | 1 |
| DI + II (n=3288) | 1.08 (0.78-1.50) | 1.04 (0.81-1.32) | 1.05 (0.81-1.35) |
| *F7* **-402 G>A** | | | |
| GG (n=6105) | 1 | 1 | 1 |
| GA + AA (n=3442) | 1.00 (0.73-1.36) | 1.09 (0.87-1.38) | 1.00 (0.78-1.28) |
| *F7* **-401 G>T** | | | |
| GG (n=6315) | 1 | 1 | 1 |
| GT + TT (n=3232) | 1.04 (0.75-1.44) | 1.02 (0.80-1.30) | 1.06 (0.83-1.37) |
| *F12* **-46 C>T** | | | |
| CC (n=4537) | 1 | 1 | 1 |
| CT + TT (n=4988) | 1.11 (0.83-1.50) | 1.03 (0.82-1.29) | 1.19 (0.94-1.51) |
| *F13* **Val35Leu C>T** | | | |
| CC (n=5811) | 1 | 1 | 1 |
| CT + TT (n=3716) | 0.88 (0.65-1.20) | 0.91 (0.72-1.14) | 0.92 (0.72-1.17) |
| *F13* **Pro564Leu C>T** | | | |
| CC (n=6405) | 1 | 1 | 1 |
| CT + TT (n=3124 | 0.99 (0.72-1.36) | 0.95 (0.75-1.20) | 1.12 (0.87-1.44) |

*adjusted for sex, ethnicity (black/non-black), smoking status (current smoker/non-smoker), type 2 diabetes status (yes/no), age, BMI, history of CHD, years of education, as well as baseline values of SBP, HDL cholesterol and LDL cholesterol. Boldfaced interaction ratios = p-value<0.05

There was a significant interaction for *F5* $^{Arg}506^{Gln}$ G>A on combined CHD (HR 1.92, 95%CI 1.00-3.65). For the *F7* $^{Arg}353^{Gln}$ G>A polymorphism there was no interaction with pravastatin in preventing all cause mortality, but there was a significant interaction with fatal CHD + nonfatal MI, and with combined CHD.

When randomized to pravastatin, subjects with the *F7* $^{Arg}353^{Gln}$ GA or AA genotype had a higher risk of fatal CHD + non-fatal MI (HR 1.23, 95%CI 0.89-1.70) than those randomized to usual care, while the more common GG genotype group had a decreased risk of this outcome (HR 0.80, 95%CI 0.67-0.95). This difference between the genotype groups (the pharmacogenetic effect) was statistically significant (IHR 1.53, 95%CI 1.06-2.20) with a q-value of 0.45. There was a similar pharmacogenetic effect on combined CHD: GA or AA participants randomized to pravastatin had increased risk of combined CHD (HR 1.23, 95%CI 0.95-1.57) compared to the usual care group, while the GG group on pravastatin had a decreased risk (HR 0.91, 95%CI 0.81-1.04) compared to the usual care group, and again the pharmacogenetic effect was significant (IHR 1.33, 95%CI 1.01-1.76) with a q value of 0.45 (table 3b).

***Table 3b.*** *F7* $^{Arg}353^{Gln}$ G>A gene-treatment interaction: outcome frequency, risk, HRs and RHRs

| Genotype:<br>Treatment:<br>sample size | GG<br>Pravastatin<br>3705 | <br>U.C.<br>3728 | GA + AA<br>Pravastatin<br>1081 | <br>U.C.<br>1063 |
|---|---|---|---|---|
| **Fatal CHD + Nonfatal MI** | | | | |
| total number of events | 255 | 324 | 90 | 70 |
| adjusted HR (95% CI) for prav vs. UC by genotype | 0.80 (0.67-0.95), p=0.01 | | 1.23 (0.89-1.70), p=0.22 | |
| interaction adjusted ratio of HRs (95% CI) prav vs. UC | 1.00 | | **1.53 (1.06-2.20), p=0.02** | |
| **Combined CHD** | | | | |
| total number of events | 490 | 548 | 150 | 124 |
| adjusted HR (95% CI) for prav vs. UC by genotype | 0.91 (0.81-1.04), p=0.16 | | 1.23 (0.95-1.57), p=0.11 | |
| interaction adjusted ratio of HRs (95% CI) prav vs. UC | 1.00 | | **1.33 (1.01-1.76), p<0.05** | |
| **All cause mortality** | | | | |
| total number of events | 457 | 465 | 134 | 113 |
| adjusted HR (95% CI) for prav vs. UC by genotype | 0.94 (0.82-1.08), p=0.38 | | 1.10 (0.85-1.44), p=0.46 | |
| interaction adjusted ratio of HRs (95% CI) prav vs. UC | 1.00 | | 1.19 (0.89-1.60), p=0.25 | |

Boldfaced interaction ratios = p-value<0.05

For the *F5* $^{Arg}506^{Gln}$ G>A polymorphism there was no interaction with pravastatin in preventing all cause mortality. There was a trend towards an interaction with pravastatin on fatal CHD + nonfatal MI: the minor allele carriers (GA and AA genotypes) had an increased risk when randomized to pravastatin versus usual care (HR 2.24, 95%CI 0.91-5.55), while the more common GG genotype group had decreased risk when randomized to pravastatin versus usual care (HR 0.85, 95%CI 0.73-0.99), though this difference between genotype groups was not statistically significant (IHR 2.12, 95%CI 0.88-5.12). There was a significant pharmacogenetic effect of pravastatin in preventing combined CHD: when randomized to pravastatin, the GA and AA group had increased risk of combined CHD outcomes compared to the usual care group (HR 1.87, 95%CI 0.97-3.59), while the GG genotype group had slightly reduced risk (HR 0.95, 95%CI 0.85-1.06), and this pharmacogenetic effect was statistically significant (IHR 1.92, 95%CI 1.00-3.65) with a q value of 0.45 (table 3c).

***Table 3c.*** *F5* $^{Arg}506^{Gln}$ G>A gene-treatment interaction: outcome frequency, risk, HRs and RHRs

| Genotype: | GG | | GA + AA | |
|---|---|---|---|---|
| Treatment: | Pravastatin | U.C. | Pravastatin | U.C. |
| sample size | 4675 | 4673 | 127 | 138 |
| **Fatal CHD + Nonfatal MI** | | | | |
| total number of events | 332 | 390 | 16 | 8 |
| adjusted HR (95% CI) for prav vs. UC by genotype | **0.85 (0.73-0.99), p=0.04** | | 2.24 (0.91-5.55), p=0.08 | |
| interaction adjusted ratio of HRs (95% CI) prav vs. UC | 1.00 | | 2.12 (0.88-5.12), p=0.10 | |
| **Combined CHD** | | | | |
| total number of events | 615 | 662 | 29 | 16 |
| adjusted HR (95% CI) for prav vs. UC by genotype | 0.95 (0.85-1.06), p=0.35 | | 1.87 (0.97-3.59), p=0.06 | |
| interaction adjusted ratio of HRs (95% CI) prav vs. UC | 1.00 | | **1.92 (1.00-3.65), p=0.05** | |
| **All cause mortality** | | | | |
| total number of events | 576 | 568 | 15 | 14 |
| adjusted HR (95% CI) for prav vs. UC by genotype | 0.96 (0.85-1.09), p=0.56 | | 1.06 (0.48-2.32), p=0.89 | |
| interaction adjusted ratio of HRs (95% CI) prav vs. UC | 1.00 | | 1.15 (0.54-2.48), p=0.71 | |

*smoking status taken out of model – unstable due to small numbers
Boldfaced interaction ratio = p-value<0.05

## DISCUSSION

The GenHAT-LLT study is a large pharmacogenetic trial of pravastatin versus usual care. Based on genotype data collected from almost 10,000 individuals who were followed for cardiovascular events using standard, well-defined definitions for cardiovascular outcomes, we found that polymorphisms in two genes that are involved in anticoagulation (the *F7* $^{Arg}353^{Gln}$ G>A polymorphism and the *F5* $^{Arg}506^{Gln}$ G>A polymorphism (also known as Factor V Leiden)) changed the efficacy of pravastatin in preventing coronary heart disease. For the *F7* $^{Arg}353^{Gln}$ G>A variant, that is located in exon 8, there was a trend towards risk reduction in subjects with the GG genotype (wild type), while subjects with the GA or AA genotype had an increased risk of CHD events when randomized to pravastatin versus usual care. The variant allele has been associated with low plasma levels of Factor VIIa and also with a decreased risk of myocardial infarction.[16] In our study there was no statistically significant relation between the polymorphisms in *F7* and the outcomes, but as expected the variant allele seemed to be protective compared to the wild type. Statin treatment might decrease Factor VII production and activation.[5] The carriers of the wild type allele have a higher Factor VIIa baseline level and this explains our result that wild type carrier have a larger risk reduction with statin therapy.

The other polymorphisms that were studied in the same gene (*F7* ins/del(-323), *F7* -401 G>T, *F7* -402 G>A polymorphism) are located in the promoter region of the gene. There were no associations with outcomes, and no interactions with pravastatin found.

The *F5* $^{Arg}506^{Gln}$ G>A polymorphism changes the cleavage site for activated protein C. The mutation prevents efficient inactivation of Factor V. When Factor V remains active, it facilitates overproduction of thrombin leading to excess fibrin generation and excess clotting. Therefore carriers of factor V Leiden have a well established increased risk of venous thrombosis. Two large meta-analyses have reported an odds ratio of approximately 1.3 on myocardial infarction in carriers of the factor V Leiden mutation.[17 18] In another meta-analysis no association with myocardial infarction was found.[19] In our study there was also no association found. Statins possibly decrease Factor V activation and increase inactivation of FVa.[5] In our study carriers of the mutation do not seem to benefit from statin therapy, and in fact have increased risk of CHD events when randomized to pravastatin versus usual care, though the association does not quite reach statistical significance (HR 2.24, p 0.08). The fact that the interaction term is not statistically significant for fatal CHD and nonfatal MI is probably due to the small numbers in the variant group. This seems especially true because the value of the interaction term (IHR 2.12) is similar to the statistically significant interaction term for combined CHD (IHR 1.92). We hypothesize that statins are not able to inactivate Factor V Leiden in these patients because of the mutation.

The *F2* gene encodes for prothrombin. The *F2* 20210 G>A polymorphism is located in the 3'-untranslated region. The polymorphism is associated with a 25% increase in plasma thrombin activity and has been associated with a higher risk of MI.[20] The variant allele of the -46 C>T polymorphism in the promoter region of the *F12* gene has been strongly associated with lower plasma levels activated factor XII, but no association with coronary artery disease has been shown so far.[21] The two polymorphisms in the *F13* gene (*F13* $^{Pro}564^{Leu}$ C>T and *F13* $^{Val}35^{Leu}$ C>T) have been shown to affect the activity of factor XIII,[22 23] but only for the variant allele of *F13* $^{Val}35^{Leu}$ C>T has been reported to be protective against

myocardial infarction,[7] In our study there were no differences in the risk on coronary heart disease, and even though statins are more or less likely to decrease these generation of these coagulation factors,[5] there was also no interaction with statins on preventing coronary heart disease. There were no differences for the outcome total mortality for all genotypes. Total mortality includes non-cardiac causes of death, and therefore the effect in this endpoint might be diluted.

***Study limitations***

Small numbers in the mutation groups forced us to combine homozygous and heterozygous carriers. Therefore we were not able to distinguish between these groups. Furthermore this might have led to non-significant results in those groups. Because of the large number of tests performed our significant results might be due to chance. Therefore we accounted for multiple testing by calculating q values. All of the significant interactions had a q value of 0.45. This means that we have to conclude that there is a fair chance there our findings were chance findings. Therefore the pharmacogenetic interactions that were found should be considered hypothesis generating and replication in other populations is of major importance. Currently, there are no clinical implications of this study.

The GenHAT study selected individuals based on pre-existing coronary risk factors and hypertension. Therefore there is some uncertainty about the applicability of the findings of this study to the association in subjects without these risk factors. In the ALLHAT study no overall beneficial effect of pravastatin was demonstrated on the primary outcome. After 6 years of follow-up, 26% of subjects in the usual care arm used statins, and 16% in the pravastatin arm did not use a lipid lowering drug anymore. This might have also led to a dilution of the interaction effect that was found.

Furthermore, for some population strata the polymorphisms were not in HWE. This might be due to non-random sampling since the subjects from this study were all drawn from a hypertensive cohort and not from the general population. Deviations from HWE can also be due to inbreeding, population stratification or selection. They can also signify a disease association. Population stratification and/or genetic admixture is present in the GenHAT trial due to the inclusion of black and white subjects, both groups including self identified Hispanics. This could affect the estimate of the main effect of a gene on an outcome, however, it is unlikely that it influences the pharmacogenetic effects, because it is assumed that whatever the population (and level of admixture present), it is randomly distributed among the treatment groups. All of these causes are all unlikely in our trial.

Apparent deviations from HWE can arise in the presence of a common deletion polymorphism, because of a mutant PCR-primer site or because of a tendency to miscall heterozygotes as homozygotes. Quality control in a subset for two SNPs showed for *F2* 20210 G>A, that only 2 (0.3%) out of 722 results disagreed. For *F7* $^{Arg}353^{Gln}$, 6 (0.8%) out of 729 comparisons were not in agreement. These quality control results suggest that genotyping errors are an unlikely explanation for why these SNPs are out of HWE. However the results of these two polymorphisms should be interpreted with caution.[24]

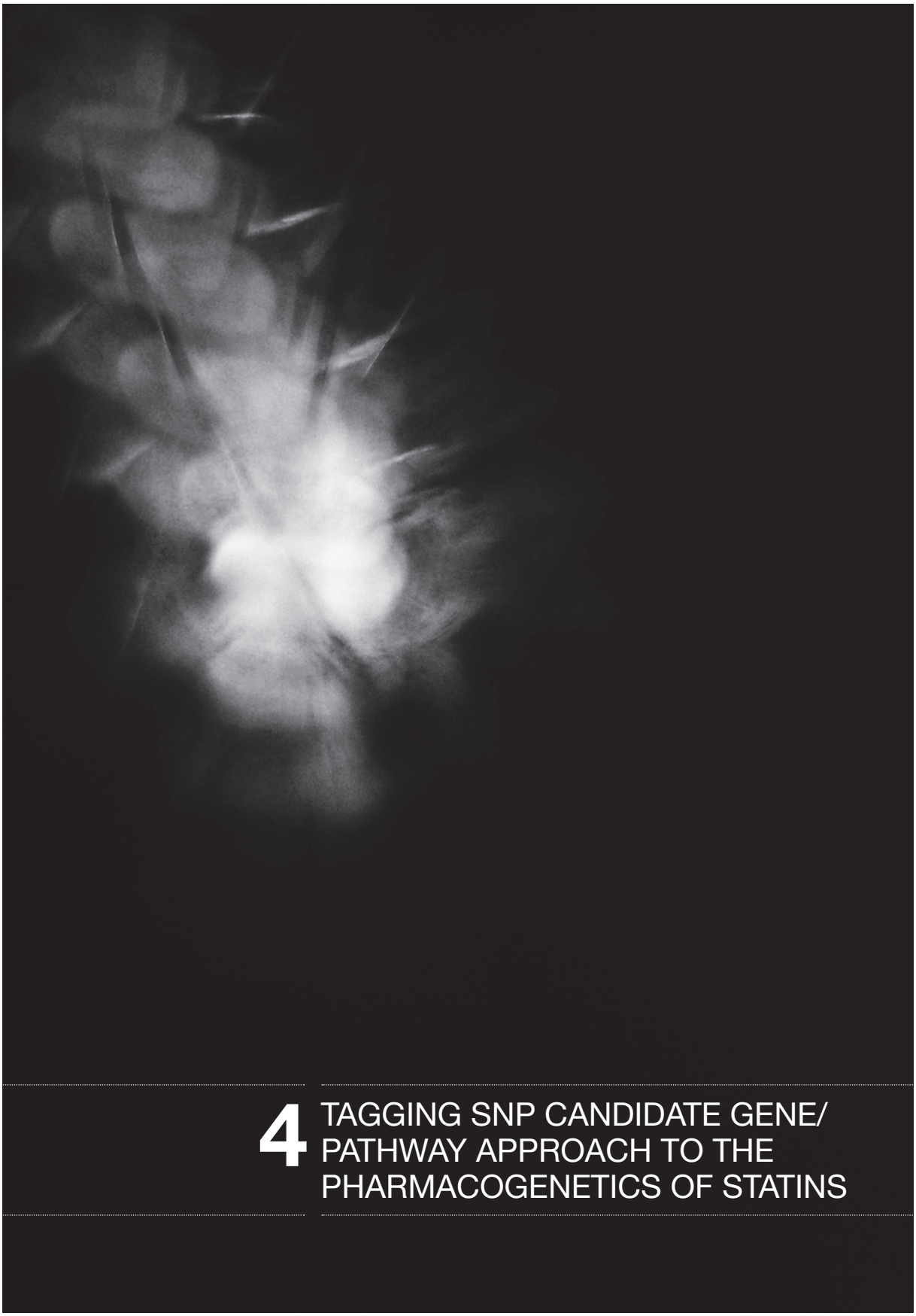
***Future research***

This is a first report to show that genes involved in anticoagulation might be important for the pharmacogenetics of pravastatin. Replication studies with genes involved in the blood coagulation pathway, and with more SNPs in these genes are necessary to clarify if differences in those genes might be clinically relevant.

## REFERENCES

1. Yeghiazarians Y, Braunstein J, Askari A, Stone P. Unstable angina pectoris. N Engl J Med 2000;342(2):101-14.
2. Cheung BM, Lauder IJ, Lau CP, Kumana CR. Meta-analysis of large randomized controlled trials to evaluate the impact of statins on cardiovascular outcomes. Br J Clin Pharmacol 2004;57(5):640-51.
3. Mangravite LM, Thorn CF, Krauss RM. Clinical implications of pharmacogenomics of statin treatment. Pharmacogenomics J 2006;6(6):360-74.
4. Liao JK, Laufs U. Pleiotropic effects of statins. Annu Rev Pharmacol Toxicol 2005;45:89-118.
5. Undas A, Brummel-Ziedins KE, Mann KG. Statins and blood coagulation. Arterioscler Thromb Vasc Biol 2005;25(2):287-94.
6. Dick J, McClaren M, Ford R. The effect of atorvastatin on activated factor XII, fibrinogen and lipid profile. Haemostasis 2000;30:42.
7. Endler G, Mannhalter C. Polymorphisms in coagulation factor genes and their impact on arterial and venous thrombosis. Clin Chim Acta 2003;330(1-2):31-55.
8. Ye Z, Liu EH, Higgins JP, Keavney BD, Lowe GD, Collins R, et al. Seven haemostatic gene polymorphisms in coronary disease: meta-analysis of 66,155 cases and 91,307 controls. Lancet 2006;367(9511):651-8.
9. 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.
10. Davis BR, Cutler JA, Gordon DJ, Furberg CD, Wright JT, Jr., Cushman WC, et al. Rationale and design for the Antihypertensive and Lipid Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). ALLHAT Research Group. Am J Hypertens 1996;9(4 Pt 1):342-60.
11. Pressel S, Davis BR, Louis GT, Whelton P, Adrogue H, Egan D, et al. Participant recruitment in the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). Control Clin Trials 2001;22(6):674-86.
12. Wright JT, Jr., Cushman WC, Davis BR, Barzilay J, Colon P, Egan D, et al. The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT): clinical center recruitment experience. Control Clin Trials 2001;22(6):659-73.
13. Cheng S, Grow MA, Pallaud C, Klitz W, Erlich HA, Visvikis S, et al. A multilocus genotyping assay for candidate markers of cardiovascular disease risk. Genome Res 1999;9(10):936-49.
14. Storey JD. A direct approach to false discovery rates. J R Statist Soc B 2002;64:479-498.
15. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Statist Soc B 1995;57:289-300.
16. Girelli D, Russo C, Ferraresi P, Olivieri O, Pinotti M, Friso S, et al. Polymorphisms in the factor VII gene and the risk of myocardial infarction in patients with coronary artery disease. N Engl J Med 2000;343(11):774-80.
17. Juul K, Tybjaerg-Hansen A, Steffensen R, Kofoed S, Jensen G, Nordestgaard BG. Factor V Leiden: The Copenhagen City Heart Study and 2 meta-analyses. Blood 2002;100(1):3-10.
18. Boekholdt SM, Bijsterveld NR, Moons AH, Levi M, Buller HR, Peters RJ. Genetic variation in coagulation and fibrinolytic proteins and their relation with acute myocardial infarction: a systematic review. Circulation 2001;104(25):3063-8.

19. Wu AH, Tsongalis GJ. Correlation of polymorphisms to coagulation and biochemical risk factors for cardiovascular diseases. Am J Cardiol 2001;87(12):1361-6.
20. Franco RF, Reitsma PH. Gene polymorphisms of the haemostatic system and the risk of arterial thrombotic disease. Br J Haematol 2001;115(3):491-506.
21. Kohler HP, Futers TS, Grant PJ. FXII (46C-->T) polymorphism and in vivo generation of FXII activity--gene frequencies and relationship in patients with coronary artery disease. Thromb Haemost 1999;81(5):745-7.
22. Ariens RA, Kohler HP, Mansfield MW, Grant PJ. Subunit antigen and activity levels of blood coagulation factor XIII in healthy individuals. Relation to sex, age, smoking, and hypertension. Arterioscler Thromb Vasc Biol 1999;19(8):2012-6.
23. Kohler HP, Futers TS, Grant PJ. Prevalence of three common polymorphisms in the A-subunit gene of factor XIII in patients with coronary artery disease. Thromb Haemost 1999;81(4):511-5.
24. Balding DJ. A tutorial on statistical methods for population association studies. Nat Rev Genet 2006;7(10):781-91.



# 4 TAGGING SNP CANDIDATE GENE/ PATHWAY APPROACH TO THE PHARMACOGENETICS OF STATINS

# CHAPTER 4.1

# PHARMACOGENETIC INTERACTIONS BETWEEN *ABCB1* AND *SLCO1B1* TAGGING SNPS AND THE EFFECTIVENESS OF STATINS IN THE PREVENTION OF MYOCARDIAL INFARCTION

Bas J.M. Peters[a]
Andrei S. Rodin[b]
Olaf H. Klungel[a]
Cornelia M. van Duijn[c]
Bruno H.Ch. Stricker[c]
Ruben van 't Slot[d]
Anthonius de Boer[a]
Anke-Hilse Maitland-van der Zee[a]

a Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, Utrecht, The Netherlands
b Human Genetics Center, School of Public Health, University of Texas Health Science Center, Houston, TX 77030, USA
c Department of Epidemiology, Pharmacoepidemiology Unit, Erasmus Medical Center, Rotterdam, The Netherlands
d Complex Genetics Section, Department of Biomedical Genetics, University Medical Center Utrecht, Utrecht, The Netherlands

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## ABSTRACT

Genetic variability within the *SLCO1B1* and *ABCB1* transporter genes has been associated with modification of statin effectiveness in cholesterol management. We conducted a case-control study using a population-based registry of pharmacy records linked to the hospital discharge records. Within a hypercholesterolemic cohort we included 668 myocardial infarction cases and 1,217 controls. We tested 24 tagging SNPs and found two SNPs within *ABCB1* (rs3789244, p 0.01; rs1922242, p 0.01) to interact with statin treatment. In addition, we found a non-significant haplotype-treatment interaction (p 0.054). The odds ratio for subjects homozygous for *SLCO1B1**1A was 0.49 (95%CI 0.34-0.71) compared to 0.31 (95% CI 0.24-0.41) for heterozygous or non-carriers of the *1A allele. This is the first study to show that common genetic variability within the *SLCO1B1* and *ABCB1* genes is associated with the modification of the effectiveness of statins in the prevention of the clinical outcome myocardial infarction.

## INTRODUCTION

The efficacy of statins has been well-established in randomized controlled trials. Nonetheless, there is high interindividual variability in response to statins.[1] Among many candidate genes, genetic variation in the genes encoding the solute carrier organic anion transporter family, member 1B1 (OATP1B1) influx and ATP-binding cassette sub-family B member 1 (ABCB1) efflux transporters partly accounts for the variability in response to statins.[2-13] Organic anion transporters polypeptides (OATPs) are membrane transport proteins mediating active influx of a range of substrates.[14] OATPs are expressed in the intestinal wall, liver, kidneys and brain-blood barrier which can influence metabolism, distribution and elimination of drugs. The OATP1B1 is located in the membrane of hepatocytes and plays important role in the transport of statins.[9] Efflux transporters, most of which belong to the ATP binding-cassette (ABC) superfamily, actively transport substrates outside of a cell.[15] The ABCB1 transporter (a.k.a. P-glycoprotein (Pg-P), or multidrug resistance protein (MDR1)) was first discovered in chemotherapy-resistant tumor cells. *ABCB1* is also expressed in normal tissues (such as the intestinal epithelium, liver) where it is involved in the efflux of drugs and toxic substances. Statins have been shown to be a substrate of the ABCB1 transporter.[16]

The OATP1B1 transporter is encoded by the *SLCO1B1* gene, with the two most extensively investigated single nucleotide polymorphisms (SNPs) being 388A>G (rs2306283) and 521T>C (rs4149056). These define the common haplotypes conventionally designated *1A, *1B, *5 and *15.[9] Altered hepatic uptake of statins due to genetic variability in the *SLCO1B1* gene has been associated with higher plasma concentrations of statins,[9] reduced lipid response,[9] and increased risk of statin-induced myopathy.[6 17]

Within the *ABCB1* gene, three common polymorphisms – 1236C>T, 2677G>T and 3435C>T – appear to be associated with the variability in ABCB1 transporter functioning.[15] It has been reported that these polymorphisms were linked to the changes in absorption and disposition of a variety of drugs.[15] However, the exact role that the genetic variability in the *ABCB1* gene plays in the expression and functioning of *ABCB1* (and, subsequently, in the pharmacokinetics and pharmacodynamics of substrates) is not well-understood.

Results from pharmacogenetic studies on statins investigating the *ABCB1* gene remain inconclusive.[1 18 19]

Although it has been demonstrated that genetic variability within the *ABCB1* and *SLCO1B1* gene affects statin pharmacokinetics and lipid response to statins, the impact of the gene-treatment interactions between *ABCB1* and *SLCO1B1* and statins on clinically important outcomes such as myocardial infarction has not previously been studied. Consequently, the aim of our study was to investigate whether common genetic variability in the *SLCO1B1* and *ABCB1* gene modifies the effectiveness of statins in the prevention of myocardial infarction.

## METHODS

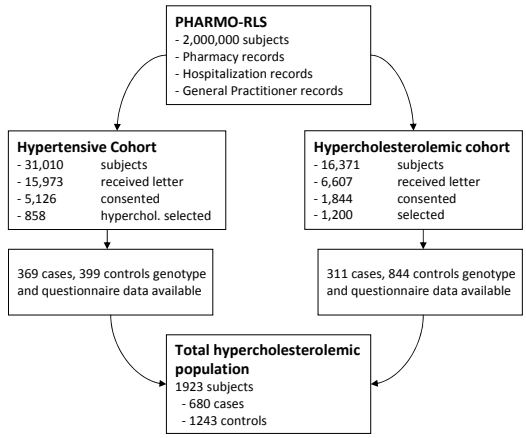
### *Design and Setting*

Participants were enrolled from the population-based Pharmaco-Morbidity Record Linkage System (PHARMO, www.pharmo.nl).[20] PHARMO has been linking drug dispensing histories from a representative sample of Dutch community pharmacies to the national registrations of hospital discharges (National Medical Registration, LMR) since 1985 on a continuous basis. Currently, the base population of PHARMO covers approximately 2,000,000 community-dwelling inhabitants of several population-defined areas in The Netherlands. Approval for this study was obtained from the Medical Ethics Committee of the University Medical Center Utrecht, The Netherlands.

### *Case and Control definition*

The total study population was based on two equivalent pharmacogenetic nested case-control studies to assess whether genetic variability in the *SLCO1B1* and *ABCB1* genes modifies the effect of statins on the risk of MI (Figure 1). The first study was designed to investigate the pharmacogenetics of statin therapy. Briefly, patients with at least one prescription for a cholesterol lowering drug (irrespective of total cholesterol level), or who had total cholesterol of more than 5.0 mmol/l, were selected from the PHARMO database. From this cohort, patients hospitalized for MI (International Classification of Diseases (ICD)-9 code 410) were included as cases if they were registered in PHARMO for at least one year. The index date was defined as the date of hospitalization for the first MI. Subsequently, patients were excluded if they were younger than 18 years, if they had had a previous MI, or if the genotyping assay failed. Controls met the same eligibility criteria as the cases, but had not developed a MI. Controls were matched to the cases on age (± two years), sex, and region. They were assigned the same index date as the cases to whom they were matched. Initially, we randomly selected 12 control participants for each case, using risk set sampling, in order to include three matched controls to each case (assuming a response rate of approximately 30%). A higher-than-expected response rate, combined with the high cost of the saliva collecting kits, led us to decrease the number of matched controls to six.

***Figure 1.*** Study population procedure.




The second study was designed to investigate the pharmacogenetics of antihypertensive drugs and was described in detail elsewhere.[21] This study included patients with at least one prescription for antihypertensive drugs in the three months prior to the date of MI or index date for matched controls. From this study, we selected the patients diagnosed with the hypercholesterolemia (self-reported, or ever-use of cholesterol lowering drugs according to pharmacy records). We validated self-reported hypercholesterolemia by using total cholesterol measurements before the index date that were available for the 22% of the patients with a self-reported diagnosis of hypercholesterolemia. 88% of these patients had at least one total cholesterol measurement that exceeded 5.0 mmol/l. Subsequently, all cases and controls that met the inclusion criteria for this study with sufficient amounts of DNA were retained. For each case, we included one matched control, if available, or selected at random, if more than one control was available. Additional controls for unmatched cases were also randomly sampled.

***Recruitment of participants***

Participants were recruited through community pharmacies, where they received a letter in which the purpose of the study was explained. They were asked to return an informed consent form and a filled-out questionnaire. After the participant had consented to participate in the study, he/she was sent an Oragene collection kit (hypercholesterolemic cohort), or the three cotton swabs and tubes containing buffer (hypertensive cohort),[22] to collect saliva. All participants were explicitly asked to consent for the collection, storage and genotyping of the DNA material.

***Ascertainment of exposure to statins (and other drugs)***

Coded pharmacy records were used to ascertain exposure to statins (and other drugs). In PHARMO, complete pharmacy records were available as of 1991, including the day

of delivery, daily dose, and durations of therapy. To characterize exposure to statins, we assessed the effect of different cumulative defined daily doses (DDD (http://www.whocc.no/atcddd/)) (cumulative DDD cut-off points of 90, 180, 360, and 720 DDD) on the odds ratios (OR) for the association between use of statins and risk of MI. Our data showed that statins were not effective in reducing the risk of MI in patients exposed to a cumulative dose of 180 DDD or less. The effectiveness of statins in patients exposed for more than 180 days but less than 360 days did not differ from a cumulative exposure for more than 360 or 720 days. Therefore, participants were considered exposed when the cumulative DDD of statin use was more than 180, whereas participants with the cumulative DDD of 180 or less (including 0 DDDs) were considered as the reference group.
For each patient we identified all prescriptions for concomitant drug use. The theoretical end date of a prescription was calculated using information on the daily dose instruction and the quantity dispensed. We considered a patient a current user when the index date was between the start and end date of a prescription. Past users were patients who were not current users, but had used the drug prior to the index date.

***Assessment of potential confounding factors and effect modifiers***
For both studies, self-reported data on smoking, hypertension, hypercholesterolemia, diabetes mellitus, use of alcohol, diet, history of cardiovascular diseases (CVDs), family history of CVDs, ethnicity, weight and height before the index date was assessed using similar questionnaires. Furthermore, information from the general practice and laboratory registrations was available for part of the population. In few cases, regardless of questionnaire data, hypercholesterolemia and diabetes status were coded as "yes, medication" if the participants were using antidiabetic or cholesterol lowering drugs. Ischemic heart disease (IHD) was defined as "yes" if a participant was hospitalized for an IHD or ever used nitrates.

***SNP selection***
To assess all common SNPs for *SLCO1B1* and *ABCB1*, we selected tagging SNPs within 200 bp (up- and downstream) with a minor-allele frequency (MAF) higher than 0.2 and a $r^2 > 0.8$ using a web-based tool called QuickSNP version 1.1 (HapMap release 21,[23] US residents with northern and western European ancestry (CEU individuals), being arguably a good fit to the Dutch population[24]).[25] Illumina SNPs with the designability scores lower than 0.4 (1.1 = best validated) or failure codes (http://www.illumina.com/Documents/products/technotes/technote_goldengate_design.pdf) were either substituted with a SNP in linkage disequilibrium (LD) or, if no such SNP were available, removed from the SNP list, resulting in a final set of 25 SNPs.

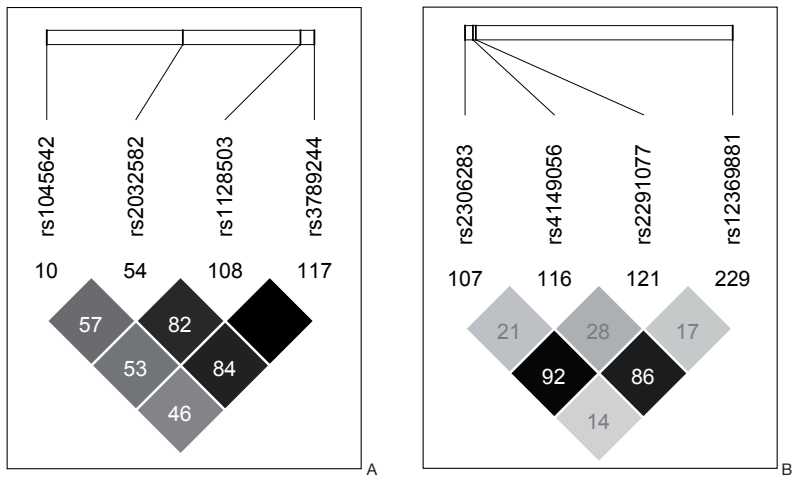
***Genotyping***
DNA was extracted according to the manufacturer's instructions (http://www.dnagenotek.com/techsupport_documents.htm). Samples with a DNA concentration higher than 100 ng per ml were diluted to the 50 ng per ml (required by the Illumina Golden-Gate assay). For each individual participating in the study, a total of 25 SNPs were genotyped using the Golden-Gate assay on an Illumina BeadStation 500 GX (Illumina Inc. San Diego, CA, USA). Genotype calls of all SNPs were individually examined for their resulting quality. SNPs with

a low signal, poor clustering, deviation from Hardy–Weinberg equilibrium (HWE) (≤0.01) or a high number of missing genotypes (>10%) were excluded.

***Haplotype construction***

Software package PHASE 2.1 was used to reconstruct haplotypes.[26] For both genes, there were too many low-frequency haplotypes and too few individuals for robust reconstruction. Therefore, we relied on the known common haplotypes. For *ABCB1*, the most common haplotype included the 1236C>T (rs1128503), 2677G>T (rs2032582) and 3435C>T (rs1045642) polymorphisms,[5] which are in LD.[27] The best proxy to tag the haplotype was a single SNP rs3789244. The LD between the three haplotype SNPs and rs3789244 can be found in Figure 2A, including the $r^2$ values. Using rs3789244 as a proxy for rs1045642 or the haplotype results in misclassification of approximately 15-18% of the genotypes. Most common *SLCO1B1* haplotypes include 388A>G (rs2306283) and 521T>C (rs4149056) polymorphisms,[9] which were best tagged by rs2291077 ($r^2$ = 0.92) and rs12369881 ($r^2$ = 0.86), respectively (Figure 2B). Therefore, the common haplotypes, designated as *1A, *1B, *5 and *15, were obtained by proxy of the rs2291077/rs12369881 genotype (TG, AG, TA and AA, respectively).

***Figure 2.*** Linkage Disequilibrium (LD) plots with $r^2$ value. (A) *ABCB1* rs3789244, 1236C>T (rs1128503), 2677G>T (rs2032582), and 3435C>T (rs1045642). (B) *SLCO1B1* rs2291077, rs12369881, 388A>G (rs2306283), and 521T>C (rs4149056). Source: Haploview, CEU population.




***Statistical methods***

Logistic regression (LR) analysis was used to study the association between statins and the risk of MI, and to adjust for potential confounders. Matching variables --- age, sex, region, and index date --- were included in our statistical model. The inclusion of potential confounders in the LR model was motivated by the assessment of the influence

of each potential confounder on the OR for the association between use of statins and risk of MI. The potential confounding factors that we considered were as follows: use of different cardiovascular drugs (antihypertensive drugs, platelet aggregation inhibitors, anticoagulants, other cholesterol-lowering drugs, and organic nitrates), use of alcohol, physical activity, family history of CVD, and other factors assessed by the questionnaire. Only covariates IHD and use of calcium channel blockers showed at least a 5% change in the regression coefficient (beta) for statin use; therefore, they were included in the LR model. We estimated the multiplicative synergy index (SI), which is the ratio of the OR in those with the variant to the OR in those without the variant.[28] For the significant pharmacogenetic associations, ORs were calculated separately in the strata defined by genotype. For each SNP, HWE was tested using a $X^2$ goodness-of-fit test. Analyses were performed using SPSS statistical software version 16.0.

Subsequently, q-values (the positive false discovery rate (pFDR), analogue of the p-value) were calculated for each gene-treatment interaction that was tested (24 SNPs and four *SLCO1B1* haplotypes) to account for multiple testing.[29]

## RESULTS

The data collection procedure is summarized in figure 1. For the original hypercholesterolemic cohort ('first study' - see methods section), 9,764 patients could not be approached for various reasons (death of a patient, pharmacy did not participate, amount of controls per case was decreased, or patient was untraceable due to change in the community pharmacy computer information system). For the genotyping assay, out of 1,844 consenting subjects, all cases were selected (n = 315), accompanied by the matched controls and a random sample of unmatched controls, to bring the total population to 1,200 (approximately three controls per case). After exclusion of patients that donated an insufficient amount of DNA, patients of which the genotyping results did not pass quality control (QC), and patients with a self-reported ethnicity other than Caucasian, 307 cases and 831 controls were included from the hypercholesterolemic cohort. From the original hypertensive study ('second study' - see methods section), we selected 429 hypercholesterolemic cases and 429 hypercholesterolemic controls. After excluding patients already included from the hypercholesterolemic cohort study, patients for whom the genotyping did not pass QC, and patients with a self-reported ethnicity other than Caucasian, we were able to amass 361 cases and 386 controls.

The baseline characteristics for the 668 cases and 1,217 controls are shown in table 1. Smoking and BMI status were statistically different between cases and controls. Furthermore, ischemic heart disease was associated with an increased risk of MI whereas current use of other cholesterol-lowering drugs was associated with a decreased risk (table 1). Current use of beta blockers and calcium channel blockers was more frequently seen in cases than controls, which is due to the inclusion of non antihypertensive users sampled from the first study. The baseline characteristics of the participants from the hypertension and hypercholesterolemia pharmacogenetic study were similar for most variables. By definition, the most obvious difference was hypertension status.

***Table 1.*** Baseline characteristics according to case control status.

| | | Case n=668 | % | Control n=1217 | % | p |
|---|---|---|---|---|---|---|
| Gender | Female | 171 | 25.6% | 285 | 23.4% | 0.290 |
| Age (years) | Mean (sd) | 63.0 (9.9) | | 62.3 (9.4) | | 0.097 |
| Body Mass Index at ID | >30 kg/m2 | 140/607 | 23.1% | 193/1104 | 17.5% | 0.005 |
| Familial History CVD | Yes, <60 | 135/637 | 21.2% | 214/1167 | 18.3% | 0.103 |
| | Yes, >60 | 266/637 | 41.8% | 464/1167 | 39.8% | |
| Diabetes Status | Yes, no medication | 71/658 | 10.8% | 90/1203 | 7.5% | 0.052 |
| | Yes, medication | 75/658 | 11.4% | 144/1203 | 12.0% | |
| Smoking Status | Current | 152/624 | 24.4% | 195/1135 | 17.2% | 0.001 |
| | Past | 283/624 | 45.4% | 573/1135 | 50.5% | |
| Alcohol Status | No use | 118/646 | 18.3% | 162/1188 | 13.6% | 0.062 |
| | <=1 | 237/646 | 36.7% | 432/1188 | 36.4% | |
| | >1 - <2 | 170/646 | 26.3% | 328/1188 | 27.6% | |
| | >2 | 121/646 | 18.7% | 266/1188 | 22.4% | |
| Physical Activity | > 4 hrs a week | 491/645 | 76.1% | 939/1191 | 78.8% | 0.180 |
| Cumulative DDD use statins | >180 DDD | 218 | 32.6% | 646 | 53.1% | <0.001 |
| Type of Statin | Atorvastatin | 44/218 | 20.2% | 164/646 | 25.4% | <0.001 |
| | Pravastatin | 35/218 | 16.1% | 110/646 | 17.0% | |
| | Simvastatin | 125/218 | 57.3% | 335/646 | 51.9% | |
| | Other | 14/218 | 6.4% | 37/646 | 5.7% | |
| Ischemic Heart Disease | Yes | 211 | 31.6% | 268 | 22.0% | <0.001 |
| **Antihypertensives** | | | | | | |
| Calcium Channel Blockers | Current use | 142 | 21.3% | 193 | 15.9% | 0.003 |
| Diuretics | Current use | 82 | 12.3% | 171 | 14.1% | 0.279 |
| Beta Blockers | Current use | 275 | 41.2% | 415 | 34.1% | 0.002 |
| Ace Inhibitors | Current use | 140 | 21.0% | 273 | 22.4% | 0.459 |
| AT2 Receptor Antagonists | Current use | 48 | 7.2% | 110 | 9.0% | 0.165 |
| **Other drugs** | | | | | | |
| Other Cholesterol Lowering drugs | Current use | 14 | 2.1% | 46 | 3.8% | 0.046 |
| Insulin | Ever use | 30 | 4.5% | 54 | 4.4% | 0.957 |
| Oral Antidiabetics | Current Use | 52 | 7.8% | 96 | 7.9% | 0.936 |
| Platelet Aggregation Inhibitors | Current Use | 219 | 32.8% | 438 | 36.0% | 0.162 |
| Coumarins | Current Use | 37 | 5.5% | 76 | 6.2% | 0.537 |

Abbreviations: AT2 = Angiotensin 2; DDD = Defined Daily Dosage

The risk of MI was significantly lower among participants exposed to statins (adjusted OR 0.36, 95%CI 0.29-0.45). A total of 25 SNPs were genotyped, of which one did not pass quality control (*SLCO1B1* rs4149026). Except for *SLCO1B1* rs4149034 (p=0.04), all *ABCB1* and *SLCO1B1* SNPs were in HWE (Tables 2 and 3). The risk of MI did not differ among patients carrying the various *ABCB1* and *SLCO1B1* genotypes (data not shown). Table 2 and 3 show the SIs for the SNP-treatment interactions between statins and the SNPs for respectively the *ABCB1* and *SLCO1B1* gene. In addition, the ORs for the effectiveness of statins in reducing the risk of MI for each genotype stratum can be found in table 2 and 3. Two SNPs in the *ABCB1* gene were found to modify the effectiveness of statins. Q values for both significant gene-treatment interactions were 0.16. None of the 14 SNPs in the *SLCO1B1* showed a significant interaction with statin treatment. Only the interaction between *SLCO1B1* rs4149050 and statin treatment showed borderline significance (p 0.063).

***Table 2.*** Association between the use of statins and the incidence of myocardial infarction stratified by *ABCB1* genotype.

| | | # | % | OR[a] (95% CI) | OR[b] (95% CI) | SI[b] (95% CI) | p | q |
|---|---|---|---|---|---|---|---|---|
| rs10246878 | GG | 1095 | 58.3% | 0.45 (0.35-0.60) | 0.39 (0.30-0.52) | (ref) | 0.33 | 0.72 |
| | AG | 667 | 35.5% | 0.41 (0.29-0.58) | 0.34 (0.24-0.50) | 0.90 (0.59-1.39) | 0.64 | |
| | AA | 116 | 6.2% | 0.20 (0.08-0.54) | 0.15 (0.05-0.46) | 0.49 (0.19-1.28) | 0.14 | |
| | -- | 7 | | | | | | |
| rs10264990 | AA | 829 | 44.0% | 0.51 (0.37-0.69) | 0.45 (0.33-0.63) | (ref) | 0.09 | 0.32 |
| | AG | 836 | 44.4% | 0.32 (0.23-0.44) | 0.26 (0.18-0.36) | 0.66 (0.43-1.00) | 0.06 | |
| | GG | 218 | 11.6% | 0.60 (0.33-1.12) | 0.49 (0.25-0.94) | 1.17 (0.60-2.29) | 0.65 | |
| | -- | 2 | | | | | | |
| rs13229143 | GG | 494 | 26.2% | 0.44 (0.29-0.66) | 0.39 (0.25-0.59) | (ref) | 0.27 | 0.68 |
| | CG | 958 | 50.8% | 0.39 (0.29-0.52) | 0.32 (0.24-0.44) | 0.92 (0.57-1.50) | 0.75 | |
| | CC | 432 | 22.9% | 0.53 (0.35-0.81) | 0.45 (0.29-0.70) | 1.40 (0.79-2.48) | 0.26 | |
| | -- | 1 | | | | | | |
| rs13233308 | GG | 514 | 27.3% | 0.43 (0.29-0.64) | 0.36 (0.24-0.55) | (ref) | 0.08 | 0.32 |
| | AG | 952 | 50.6% | 0.34 (0.25-0.46) | 0.27 (0.20-0.38) | 0.80 (0.49-1.30) | 0.37 | |
| | AA | 417 | 22.1% | 0.70 (0.45-1.09) | 0.62 (0.39-0.99) | 1.44 (0.82-2.56) | 0.21 | |
| | -- | 2 | | | | | | |
| rs4148734 | GG | 930 | 49.4% | 0.43 (0.32-0.58) | 0.39 (0.29-0.53) | (ref) | 0.91 | 0.74 |
| | AG | 768 | 40.8% | 0.40 (0.29-0.55) | 0.31 (0.22-0.44) | 0.92 (0.60-1.41) | 0.70 | |
| | AA | 184 | 9.8% | 0.47 (0.23-0.94) | 0.37 (0.17-0.78) | 1.03 (0.50-2.13) | 0.93 | |
| | -- | 3 | | | | | | |
| rs6961665 | AA | 473 | 25.2% | 0.46 (0.30-0.70) | 0.33 (0.21-0.53) | (ref) | 0.12 | 0.37 |
| | AC | 940 | 50.0% | 0.33 (0.24-0.44) | 0.29 (0.21-0.40) | 0.84 (0.51-1.38) | 0.48 | |
| | CC | 466 | 24.8% | 0.60 (0.40-0.91) | 0.54 (0.35-0.83) | 1.40 (0.80-2.47) | 0.24 | |
| | -- | 6 | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| rs3789244 | AA | 629 | 33.4% | 0.51 (0.36-0.73) | 0.39 (0.27-0.58) | (ref) | **0.01** | 0.16 |
| | AC | 912 | 48.4% | 0.29 (0.21-0.40) | 0.25 (0.18-0.35) | 0.68 (0.43-1.07) | 0.10 | |
| | CC | 342 | 18.2% | 0.79 (0.49-1.27) | 0.71 (0.43-1.17) | 1.49 (0.84-2.66) | 0.18 | |
| | -- | 2 | | | | | | |
| rs1922242 | AA | 605 | 32.2% | 0.62 (0.43-0.89) | 0.57 (0.39-0.83) | (ref) | **0.01** | 0.16 |
| | AT | 912 | 48.6% | 0.30 (0.22-0.40) | 0.23 (0.17-0.32) | 0.51 (0.32-0.81) | 0.00 | |
| | TT | 361 | 19.2% | 0.54 (0.34-0.87) | 0.44 (0.27-0.73) | 0.92 (0.52-1.62) | 0.76 | |
| | -- | 7 | | | | | | |
| rs1989830 | GG | 793 | 42.1% | 0.47 (0.34-0.65) | 0.40 (0.29-0.57) | (ref) | 0.63 | 0.72 |
| | AG | 854 | 45.4% | 0.39 (0.29-0.54) | 0.33 (0.24-0.46) | 0.81 (0.53-1.25) | 0.34 | |
| | AA | 235 | 12.5% | 0.41 (0.22-0.74) | 0.33 (0.17-0.63) | 0.86 (0.44-1.68) | 0.66 | |
| | -- | 3 | | | | | | |
| rs2235015 | CC | 1208 | 64.1% | 0.44 (0.34-0.57) | 0.36 (0.27-0.48) | (ref) | 0.93 | 0.74 |
| | AC | 593 | 31.5% | 0.41 (0.28-0.59) | 0.35 (0.24-0.52) | 0.98 (0.63-1.51) | 0.92 | |
| | AA | 84 | 4.5% | 0.30 (0.09-0.96) | 0.24 (0.07-0.88) | 0.81 (0.27-2.42) | 0.70 | |
| | -- | | | | | | | |

[a]adjusted for sex, index date, age and region
[b]adjusted for sex, index date, age, region, ischemic heart disease and use of calcium channel blockers
-- = missing, OR = Odds Ratio, SI = Synergy Index

***Table 3.*** Association between the use of statins and the incidence of myocardial infarction stratified by *SLCO1B1* genotype

| | | # | % | OR[a] (95% CI) | OR[b] (95% CI) | SI[b] (95% CI) | p | q |
|---|---|---|---|---|---|---|---|---|
| rs11045812 | GG | 496 | 26.4% | 0.48 (0.32-0.72) | 0.38 (0.25-0.59) | (ref) | 0.79 | 0.74 |
| | AG | 909 | 48.5% | 0.39 (0.29-0.53) | 0.34 (0.25-0.47) | 0.87 (0.53-1.41) | 0.57 | |
| | AA | 471 | 25.1% | 0.42 (0.28-0.64) | 0.37 (0.24-0.57) | 1.00 (0.57-1.75) | 0.99 | |
| | -- | 9 | | | | | | |
| rs11045889 | TT | 784 | 41.6% | 0.44 (0.32-0.61) | 0.38 (0.27-0.54) | (ref) | 0.86 | 0.74 |
| | AT | 876 | 46.5% | 0.39 (0.29-0.53) | 0.33 (0.24-0.45) | 0.89 (0.58-1.37) | 0.59 | |
| | AA | 223 | 11.8% | 0.58 (0.31-1.08) | 0.40 (0.20-0.81) | 0.98 (0.50-1.93) | 0.96 | |
| | -- | 2 | | | | | | |
| rs12369881 | GG | 1320 | 70.3% | 0.44 (0.35-0.56) | 0.37 (0.29-0.48) | (ref) | 0.56 | 0.72 |
| | AG | 518 | 27.6% | 0.41 (0.27-0.63) | 0.36 (0.23-0.56) | 0.90 (0.57-1.45) | 0.67 | |
| | AA | 40 | 2.1% | 0.13 (0.20-0.82) | 0.02 (0.00-0.41) | 0.45 (0.10-2.08) | 0.30 | |
| | -- | 7 | | | | | | |
| rs12578392 | AA | 668 | 35.5% | 0.44 (0.31-0.63) | 0.39 (0.27-0.56) | (ref) | 0.63 | 0.72 |
| | AG | 920 | 48.9% | 0.38 (0.28-0.51) | 0.32 (0.23-0.44) | 0.85 (0.54-1.32) | 0.46 | |
| | GG | 295 | 15.7% | 0.63 (0.36-1.08) | 0.45 (0.25-0.82) | 1.09 (0.59-2.02) | 0.79 | |
| | -- | 2 | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| rs1463565 | GG | 522 | 27.9% | 0.41 (0.27-0.61) | 0.31 (0.20-0.48) | (ref) | 0.71 | 0.72 |
| | CG | 922 | 49.2% | 0.42 (0.31-0.57) | 0.37 (0.27-0.50) | 1.19 (0.73-1.93) | 0.48 | |
| | CC | 429 | 22.9% | 0.45 (0.29-0.70) | 0.40 (0.25-0.62) | 1.25 (0.70-2.20) | 0.45 | |
| | -- | 12 | | | | | | |
| rs16923519 | AA | 1268 | 67.3% | 0.44 (0.34-0.57) | 0.38 (0.29-0.50) | (ref) | 0.86 | 0.74 |
| | AG | 553 | 29.4% | 0.41 (0.28-0.60) | 0.34 (0.23-0.51) | 0.88 (0.56-1.38) | 0.59 | |
| | GG | 63 | 3.3% | 0.26 (0.07-1.06) | 0.08 (0.01-0.62) | 0.91 (0.30-2.78) | 0.87 | |
| | -- | 1 | | | | | | |
| rs2291077 | TT | 627 | 33.4% | 0.52 (0.36-0.74) | 0.47 (0.32-0.68) | (ref) | 0.21 | 0.58 |
| | AT | 913 | 48.7% | 0.38 (0.29-0.52) | 0.32 (0.23-0.44) | 0.64 (0.35-1.16) | 0.14 | |
| | AA | 336 | 17.9% | 0.40 (0.24-0.66) | 0.29 (0.17-0.51) | 0.70 (0.45-1.10) | 0.12 | |
| | -- | 9 | | | | | | |
| rs2417954 | GG | 785 | 41.9% | 0.39 (0.29-0.54) | 0.37 (0.26-0.51) | (ref) | 0.78 | 0.74 |
| | AG | 850 | 45.4% | 0.43 (0.31-0.59) | 0.34 (0.25-0.48) | 1.03 (0.67-1.59) | 0.90 | |
| | AA | 237 | 12.7% | 0.49 (0.27-0.88) | 0.39 (0.21-0.73) | 1.26 (0.66-2.42) | 0.48 | |
| | -- | 13 | | | | | | |
| rs3829307 | AA | 1045 | 55.5% | 0.43 (0.32-0.56) | 0.39 (0.29-0.52) | (ref) | 0.70 | 0.72 |
| | AT | 710 | 37.7% | 0.39 (0.28-0.55) | 0.29 (0.20-0.42) | 0.87 (0.57-1.33) | 0.52 | |
| | TT | 128 | 6.8% | 0.54 (0.23-1.26) | 0.42 (0.17-1.05) | 1.18 (0.52-2.72) | 0.69 | |
| | -- | 2 | | | | | | |
| rs4149034 | GG | 851 | 45.2% | 0.45 (0.33-0.62) | 0.42 (0.30-0.58) | (ref) | 0.70 | 0.72 |
| | AG | 800 | 42.5% | 0.38 (0.28-0.52) | 0.30 (0.21-0.42) | 0.83 (0.54-1.28) | 0.40 | |
| | AA | 232 | 12.3% | 0.46 (0.26-0.83) | 0.33 (0.17-0.64) | 0.91 (0.47-1.74) | 0.76 | |
| | -- | 2 | | | | | | |
| rs4149035 | GG | 675 | 35.9% | 0.36 (0.25-0.51) | 0.30 (0.21-0.43) | (ref) | 0.42 | 0.72 |
| | AG | 927 | 49.3% | 0.45 (0.33-0.60) | 0.39 (0.28-0.53) | 1.29 (0.83-2.01) | 0.26 | |
| | AA | 279 | 14.8% | 0.57 (0.33-0.99) | 0.42 (0.23-0.78) | 1.42 (0.75-2.67) | 0.28 | |
| | -- | 4 | | | | | | |
| rs4149050 | AA | 1138 | 60.4% | 0.50 (0.38-0.65) | 0.42 (0.32-0.56) | (ref) | 0.06 | 0.32 |
| | AG | 653 | 34.7% | 0.36 (0.25-0.52) | 0.30 (0.20-0.44) | 0.64 (0.41-0.99) | 0.05 | |
| | GG | 92 | 4.9% | 0.17 (0.06-0.50) | 0.13 (0.04-0.42) | 0.46 (0.17-1.26) | 0.13 | |
| | -- | 2 | | | | | | |
| rs4149057 | GG | 792 | 42.1% | 0.48 (0.35-0.65) | 0.41 (0.29-0.57) | (ref) | 0.59 | 0.72 |
| | AG | 873 | 46.4% | 0.41 (0.30-0.56) | 0.34 (0.24-0.47) | 0.81 (0.53-1.25) | 0.35 | |
| | AA | 217 | 11.5% | 0.37 (0.19-0.71) | 0.30 (0.15-0.60) | 0.79 (0.40-1.55) | 0.49 | |
| | -- | 3 | | | | | | |
| rs7137060 | GG | 1215 | 64.5% | 0.42 (0.33-0.55) | 0.37 (0.28-0.49) | (ref) | 0.48 | 0.72 |
| | AG | 597 | 31.7% | 0.38 (0.26-0.56) | 0.31 (0.20-0.46) | 0.88 (0.57-1.37) | 0.58 | |
| | AA | 72 | 3.8% | 0.95 (0.30-2.97) | 0.56 (0.15-2.08) | 1.67 (0.60-4.66) | 0.33 | |
| | -- | 1 | | | | | | |

[a]adjusted for sex, index date, age and region
[b]adjusted for sex, index date, age, region, ischemic heart disease and use of calcium channel blockers
-- = missing, OR = Odds Ratio, SI = Synergy Index

Tagging SNP inferred haplotype frequencies for *SLCO1B1* were 56.9% for *1A, 27.1% for *1B, 0.7% for *5 and 15.2% for *15. Table 4 gives an overview of the interactions between the *SLCO1B1* gene haplotypes and treatment with statin therapy. For *SLCO1B1**1A we found a non significant interaction (p=0.054) when comparing homozygous *1A carriers to heterozygous *1A carriers and *1A non-carriers combined. The adjusted OR for statin effectiveness in subjects homozygous for *1A was 0.49 (95%CI 0.34-0.71) compared to 0.31 (95%CI 0.24-0.41) for heterozygous or non-carriers of the *1A allele, resulting in a SI of 1.52 (95%CI 0.99 - 2.34, q value 0.32).

***Table 4.*** Association between the use of statins and the incidence of myocardial infarction stratified by *SLCO1B1* haplotype derivative

| | | # | % | OR[a] (95% CI) | OR[b] (95% CI) | SI[b] (95% CI) | p | q |
|---|---|---|---|---|---|---|---|---|
| *1A | 0 | 340 | 18.0% | 0.41 (0.25-0.67) | 0.29 (0.17-0.51) | (ref) | 0.15 | |
| | 1 | 942 | 50.0% | 0.38 (0.28-0.51) | 0.31 (0.23-0.43) | 1.06 (0.60-1.86) | 0.85 | |
| | 2 | 603 | 32.0% | 0.54 (0.38-0.78) | 0.49 (0.34-0.71) | 1.59 (0.87-2.89) | 0.13 | |
| | 0 or 1 | 1282 | 68.0% | 0.38 (0.30-0.49) | 0.31 (0.24-0.41) | (ref) | | |
| | 2 | 603 | 32.0% | 0.54 (0.38-0.78) | 0.49 (0.34-0.71) | 1.52 (0.99-2.34) | **0.05** | 0.32 |
| *1B | 0 | 1014 | 53.8% | 0.47 (0.36-0.63) | 0.43 (0.32-0.57) | (ref) | 0.44 | 0.72 |
| | 1 | 720 | 38.2% | 0.35 (0.25-0.49) | 0.28 (0.19-0.40) | 0.78 (0.51-1.19) | 0.25 | |
| | 2 | 151 | 8.0% | 0.43 (0.21-0.89) | 0.29 (0.13-0.66) | 0.73 (0.34-1.56) | 0.42 | |
| *5 | 0 | 1896 | 100.6% | 0.43 (0.35-0.53) | 0.36 (0.29-0.45) | (ref) | | |
| | 1 or 2 | 27 | 1.4% | 0.13 (0.01-1.53) | 0.10 (0.01-1.32) | 0.38 (0.04-3.91) | 0.41 | 0.72 |
| *15 | 0 | 1349 | 71.6% | 0.43 (0.34-0.55) | 0.37 (0.28-0.47) | (ref) | 0.45 | 0.72 |
| | 1 | 500 | 26.5% | 0.43 (0.280.66) | 0.37 (0.240.58) | 0.96 (0.60-1.54) | 0.86 | |
| | 2 | 36 | 1.9% | 0.14 (0.02-0.83) | 0.02 (0.00-0.49) | 0.36 (0.07-1.77) | 0.21 | |

[a]adjusted for sex, index date, age and region
[b]adjusted for sex, index date, age, region, ischemic heart disease and use of calcium channel blockers
OR = Odds Ratio, SI = Synergy Index

## DISCUSSION

We found two significant gene-treatment interactions that include the *ABCB1* rs3789244 and *ABCB1* rs1922242 polymorphism. For *ABCB1*, homozygous and heterozygous carriers of the rs3789244 wild-type allele experienced a greater risk reduction of MI from statin therapy compared to homozygous variant allele carriers. The interaction with *ABCB1* rs1922242 represents a model in which heterozygote carriers respond better than homozygous carriers of either the wild-type or variant allele, and could therefore reflect a spurious interaction.

Most of what is known about the *ABCB1* gene relates to the SNPs that are in LD with our intronic tagging SNP rs3789244 (Figure 2A). The exact role of the other tagging SNPs, if any, on ABCB1 functioning is unknown. Interestingly, of the ten *ABCB1* tagging SNPs, we found an interaction between the rs3789244 and the use of statins. Due to LD, the minor frequency allele *ABCB1* rs3789244 C correlates strongly with minor frequency alleles of ABCB1 1236C>T (rs11285030), 2677G>T (rs2032582) and, to a lesser extent, with 3435C>T (rs1045642) (Figure 2A), jointly contributing to the most common haplotypes 1236C – 2677G – 3435C (CGC) and 1235T – 2677T – 3435T (TTT).[27 30] The silent *ABCB1* 3435C>T polymorphism affects the timing of co-translational folding and has been shown to alter the substrate specificity.[31] Substrates for ABCB1 include all statins.[16 32] The pharmacokinetics of several statins has been reported to be different among *ABCB1* genotypes.[5] For atorvastatin acid and simvastatin acid, areas under the plasma-concentration-time curves (AUCs) were 55% and 60%, respectively --- both significantly higher among homozygous haplotype *ABCB1* TTT carriers.[5] Lower ABCB1 activity in subjects carrying the *ABCB1* 3435T genotype and/or *ABCB1* TTT haplotype may enhance intestinal reabsorption and impair biliary excretion of statins resulting in higher hepatic exposure. Consequently, three studies reported a better lipid response to atorvastatin (women only)[4] and simvastatin[3 13] in subjects carrying variant genotypes. However, several other studies could not confirm such an association,[18 19] while Thompson et al. demonstrated wild-type carriers of the *ABCB1* 2677G genotype to respond better to atorvastatin treatment compared to variant allele carriers.[12] We found carriers of the corresponding *ABCB1* rs3789244 C allele treated with statins to benefit less (compared to homozygous *ABCB1* rs3789244 A allele carriers) which is more in line with findings reported by Thompson et al.[12] It should be mentioned that the assumed higher hepatic exposure and better lipid response to statin therapy of patients with the *ABCB1* 3435T and/or 1236T, 2677T, 3435T (TTT) genotype do not necessarily translate directly into a decreased MI risk, as statins exert pleiotropic effects that - independently from HMG-CoA reductase inhibition and cholesterol lowering - are not intrinsically related to hepatic exposure.[33 34]

In addition, we report a pharmacogenetic interaction of statin use with *ABCB1* rs1922242. In our data, the $r^2$ between *ABCB1* rs1922242 and rs3789244 was 0.55 suggesting moderate LD. This intronic SNP has been reported to be associated with several baseline lipid parameters in another study.[35] Although an independent study should confirm the interaction with *ABCB1* rs1922242, the lack of a gene-dose-effect could suggests a false positive association.

For the *SLCO1B1* gene, the *SLCO1B1* 388A>G and 521T>C polymorphisms have previously been associated with increased and decreased hepatocellular statin transport,[9]

respectively. We did not find significant link between the use of statins and tagging SNPs rs2291077 (for 388A>G, rs2032582) and rs12369881 (for 521T>C, rs4149056) (Figure 2B). However, haplotype analysis revealed non significant interaction showing a smaller beneficial effect of statins for homozygous carriers of the *1A allele compared to the heterozygous or *1A non-carriers. Carrying a variant allele of SNP rs2291077 and/or rs12369881 might lead to a larger risk reduction in response to statins, compared to the wild type. The *SLCO1B1* 521T>C variant allele impairs hepatocellular statin uptake,[9] and has been associated with reduced lipid response to statins[2 6 11] and higher risk of myopathy.[6 17] On the other hand, *SLCO1B1* 388A>G has been suggested to enhance hepatocellular statin uptake[7 8] and has been associated with a better lipid response to statins.[6] Finally, the *SLCO1B1* rs4149050 could be of importance for the pharmacogenetics of statin. Carriers of one rs4149050 minor frequency allele seem to respond better than non-carriers, whereas the small group of homozygous minor frequency allele carriers show a substantial risk reduction. This SNP is in LD with rs4149036 ($r^2$=0.91), of which the minor frequency allele has been associated with a more beneficial triglyceride level response compared to the more common T allele.[12] The effect of both intronic SNPs rs4149050 and rs4149036 on OATP1B1 functioning has not been studied.

The OR in the present study seems to be relatively low compared to the relative risk of 0.73 obtained in the meta-analysis of randomized controlled trials,[36] although a recent population-based cohort study including 229,918 participants reported effectiveness up to 58% mortality reduction in the primary prevention group.[37] A possible explanation is that patients that are more conscious of their health status may be more willing to receive statin treatment, which indicates a healthy user effect. Nonetheless, the study was designed to assess effect modification of statin therapy by genetic variability in the *ABCB1* and *SLCO1B1* genes, not to estimate the actual risk reduction achieved by statins. Furthermore, Figure 1 shows differences in response rates among both original studies, which may introduce selection bias. Van Wieren et al. showed that there is little difference between the characteristics of responding and non-responding patients.[21]

The present study has several limitations. First, we considered all the statins as one homogenous group, although it has been shown that affinities for the transporters of interest may differ between statin types.[32] Our study population was not sufficiently large to stratify the analyses by the statin type. According to the record of the latest prescriptions, nearly 78% of the exposed subjects in our study were using either atorvastatin or simvastatin, and approximately 17% pravastatin. *SLCO1B1* 521T>C polymorphism has a marked effect on the pharmacokinetics of simvastatin (acid) (3.2-fold increase in AUC) and atorvastatin (2.4-fold increase), and an intermediate effect on pravastatin (1.9-fold increase).[10 38 39] Furthermore, *ABCB1* haplotypes have been shown to affect the pharmacokinetics of atorvastatin and simvastatin acid.[5] Based on this *in vivo* data, pooling all the statins into one category does not seem to present a major problem. Second, a tagging SNP approach introduces uncertainty with respect to the strength of the relationship with the known causative ones and reduces the power of the study to identify interactions with those SNPs. Based on the Hapmap CEPH individuals, the correlation of *ABCB1* rs3789244 with the possibly causal variant *ABCB1* C3435T is relatively weak (Figure 2A). Also, reconstruction of the *SLCO1B1* haplotypes with tagging SNPs (and reconstruction of haplotypes in general) will necessarily lead to some errors. Nevertheless, *SLCO1B1* haplotype frequencies in

our study are actually in line with the previously reported frequencies.[5] Finally, the choice for the 0.2 MAF cut-off was based on an a priori power calculation with 80% power to detect a SI of 2 (or 0.5). Although our study covers the common genetic variability within the candidate genes, we could miss potentially significant SNPs with lower frequency as past statin pharmacogenetic studies have implicated SNPs with lower MAF and higher penetrance.

A strength of this study is the availability of hospital and (complete) pharmacy records. Statin exposure was defined based on pharmacy records. Although a surrogate for whether patients actually took their medicine properly, pharmacy records have been shown to be a reliable measure.[40] Secondly, we were able to include a large number of patients compared to previous comparable efforts. Furthermore, we comprehensively covered the common genetic variability in the transporter genes by using tagging SNPs. In addition, q values were calculated to address the multiple testing issue. For the significant interactions the q value was approximately 0.2, suggesting possibly one to be a false positive.[41] The most important strength of our study is that it assessed the impact of gene-treatment interactions between *ABCB1*, *SLCO1B1*, and statins on the clinically essential MI outcome instead of surrogate phenotypes. Of note, our study included only survivors of a MI, and no fatal MI cases. In conclusion, this study suggests that common genetic variants in the ABCB1 gene may be important for the clinical outcome of therapy with statins. These results should however be interpreted carefully as there is limited knowledge about these variants. The pharmacogenomic interactions reported with *SLCO1B1* rs4149050 and *ABCB1* rs1922242 should be considered hypothesis generating, whereas those in high LD with known causal variants (*ABCB1* rs3789244, *SLCO1B1* haplotype, rs2291077, and rs12369881) should be considered hypothesis testing. Currently, the clinical value of our findings seem limited since statin treatment provided meaningful benefit in all genotype groups, and the findings first should be replicated in independent populations. Importantly, these findings should be followed up in the future studies, from both the biological, (mechanistic) and epidemiological perspective.

## REFERENCES

1. Peters BJ, Klungel OH, de Boer A, Maitland-van der Zee AH. Genetic determinants of response to statins. Expert Rev Cardiovasc Ther 2009;7(8):977-83.
2. Couvert P, Giral P, Dejager S, Gu J, Huby T, Chapman MJ, et al. Association between a frequent allele of the gene encoding OATP1B1 and enhanced LDL-lowering response to fluvastatin therapy. Pharmacogenomics 2008;9(9):1217-27.
3. Fiegenbaum M, da Silveira FR, Van der Sand CR, Van der Sand LC, Ferreira ME, Pires RC, et al. The role of common variants of ABCB1, CYP3A4, and CYP3A5 genes in lipid-lowering efficacy and safety of simvastatin treatment. Clin Pharmacol Ther 2005;78(5):551-8.
4. Kajinami K, Brousseau ME, Ordovas JM, Schaefer EJ. Polymorphisms in the multidrug resistance-1 (MDR1) gene influence the response to atorvastatin treatment in a gender-specific manner. Am J Cardiol 2004;93(8):1046-50.
5. Keskitalo JE, Kurkinen KJ, Neuvoneni PJ, Niemi M. ABCB1 haplotypes differentially affect the pharmacokinetics of the acid and lactone forms of simvastatin and atorvastatin. Clin Pharmacol Ther 2008;84(4):457-61.
6. Link E, Parish S, Armitage J, Bowman L, Heath S, Matsuda F, et al. SLCO1B1 variants and statin-induced myopathy--a genomewide study. N Engl J Med 2008;359(8):789-99.

7. Maeda K, Ieiri I, Yasuda K, Fujino A, Fujiwara H, Otsubo K, et al. Effects of organic anion transporting polypeptide 1B1 haplotype on pharmacokinetics of pravastatin, valsartan, and temocapril. Clin Pharmacol Ther 2006;79(5):427-39.
8. Mwinyi J, Johne A, Bauer S, Roots I, Gerloff T. Evidence for inverse effects of OATP-C (SLC21A6) 5 and 1b haplotypes on pravastatin kinetics. Clin Pharmacol Ther 2004;75(5):415-21.
9. Niemi M. Role of OATP transporters in the disposition of drugs. Pharmacogenomics 2007;8(7):787-802.
10. Niemi M, Pasanen MK, Neuvonen PJ. SLCO1B1 polymorphism and sex affect the pharmacokinetics of pravastatin but not fluvastatin. Clin Pharmacol Ther 2006;80(4):356-66.
11. Tachibana-Iimori R, Tabara Y, Kusuhara H, Kohara K, Kawamoto R, Nakura J, et al. Effect of genetic polymorphism of OATP-C (SLCO1B1) on lipid-lowering response to HMG-CoA reductase inhibitors. Drug Metab Pharmacokinet 2004;19(5):375-80.
12. Thompson JF, Man M, Johnson KJ, Wood LS, Lira ME, Lloyd DB, et al. An association study of 43 SNPs in 16 candidate genes with atorvastatin response. Pharmacogenomics J 2005;5(6):352-8.
13. Becker ML, Visser LE, van Schaik RH, Hofman A, Uitterlinden AG, Stricker BH. Common genetic variation in the ABCB1 gene is associated with the cholesterol-lowering effect of simvastatin in males. Pharmacogenomics 2009;10(11):1743-51.
14. Hagenbuch B, Meier PJ. The superfamily of organic anion transporting polypeptides. Biochim Biophys Acta 2003;1609(1):1-18.
15. Cascorbi I. Role of pharmacogenetics of ATP-binding cassette transporters in the pharmacokinetics of drugs. Pharmacol Ther 2006;112(2):457-73.
16. Chen C, Mireles RJ, Campbell SD, Lin J, Mills JB, Xu JJ, et al. Differential interaction of 3-hydroxy-3-methylglutaryl-coa reductase inhibitors with ABCB1, ABCC2, and OATP1B1. Drug Metab Dispos 2005;33(4):537-46.
17. Voora D, Shah SH, Spasojevic I, Ali S, Reed CR, Salisbury BA, et al. The SLCO1B1*5 genetic variant is associated with statin-induced side effects. J Am Coll Cardiol 2009;54(17):1609-16.
18. Mangravite LM, Krauss RM. Pharmacogenomics of statin response. Curr Opin Lipidol 2007;18(4):409-14.
19. Mangravite LM, Thorn CF, Krauss RM. Clinical implications of pharmacogenomics of statin treatment. Pharmacogenomics J 2006;6(6):360-74.
20. Herings RM, Bakker A, Stricker BH, Nap G. Pharmaco-morbidity linkage: a feasibility study comparing morbidity in two pharmacy based exposure cohorts. J Epidemiol Community Health 1992;46(2):136-40.
21. van Wieren-de Wijer DB, Maitland-van der Zee AH, de Boer A, Stricker BH, Kroon AA, de Leeuw PW, et al. Recruitment of participants through community pharmacies for a pharmacogenetic study of antihypertensive drug treatment. Pharm World Sci 2009;31(2):158-64.
22. van Wieren-de Wijer DB, Maitland-van der Zee AH, de Boer A, Stricker BH, Kroon AA, de Leeuw PW, et al. Recruitment of participants through community pharmacies for a pharmacogenetic study of antihypertensive drug treatment. Pharm World Sci 2008.
23. Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA, et al. A second generation human haplotype map of over 3.1 million SNPs. Nature 2007;449(7164):851-61.
24. Lao O, Lu TT, Nothnagel M, Junge O, Freitag-Wolf S, Caliebe A, et al. Correlation between genetic and geographic structure in Europe. Curr Biol 2008;18(16):1241-8.
25. Grover D, Woodfield AS, Verma R, Zandi PP, Levinson DF, Potash JB. QuickSNP: an automated web server for selection of tagSNPs. Nucleic Acids Res 2007;35(Web Server issue):W115-20.
26. Stephens M, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet 2003;73(5):1162-9.
27. Kim RB, Leake BF, Choo EF, Dresser GK, Kubba SV, Schwarz UI, et al. Identification of functionally variant MDR1 alleles among European Americans and African Americans. Clin Pharmacol Ther 2001;70(2):189-99.

28. Khoury MJ, Flanders WD. Nontraditional epidemiologic approaches in the analysis of gene-environment interaction: case-control studies with no controls! Am J Epidemiol 1996;144(3):207-13.
29. Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proc Natl Acad Sci U S A 2003;100(16):9440-5.
30. Aarnoudse AJ, Dieleman JP, Visser LE, Arp PP, van der Heiden IP, van Schaik RH, et al. Common ATP-binding cassette B1 variants are associated with increased digoxin serum concentration. Pharmacogenet Genomics 2008;18(4):299-305.
31. Kimchi-Sarfaty C, Oh JM, Kim IW, Sauna ZE, Calcagno AM, Ambudkar SV, et al. A "silent" polymorphism in the MDR1 gene changes substrate specificity. Science 2007;315(5811):525-8.
32. Neuvonen PJ, Backman JT, Niemi M. Pharmacokinetic comparison of the potential over-the-counter statins simvastatin, lovastatin, fluvastatin and pravastatin. Clin Pharmacokinet 2008;47(7):463-74.
33. Arnaud C, Braunersreuther V, Mach F. Toward immunomodulatory and anti-inflammatory properties of statins. Trends Cardiovasc Med 2005;15(6):202-6.
34. Glynn RJ, Danielson E, Fonseca FAH, Genest J, Gotto AM, Jr., Kastelein JJP, et al. A Randomized Trial of Rosuvastatin in the Prevention of Venous Thromboembolism. N Engl J Med 2009:NEJMoa0900241.
35. Jeannesson E, Siest G, Bastien B, Albertini L, Aslanidis C, Schmitz G, et al. Association of ABCB1 gene polymorphisms with plasma lipid and apolipoprotein concentrations in the STANISLAS cohort. Clin Chim Acta 2009;403(1-2):198-202.
36. Cheung BM, Lauder IJ, Lau CP, Kumana CR. Meta-analysis of large randomized controlled trials to evaluate the impact of statins on cardiovascular outcomes. Br J Clin Pharmacol 2004;57(5):640-51.
37. Shalev V, Chodick G, Silber H, Kokia E, Jan J, Heymann AD. Continuation of statin treatment and all-cause mortality: a population-based cohort study. Arch Intern Med 2009;169(3):260-8.
38. Pasanen MK, Fredrikson H, Neuvonen PJ, Niemi M. Different effects of SLCO1B1 polymorphism on the pharmacokinetics of atorvastatin and rosuvastatin. Clin Pharmacol Ther 2007;82(6):726-33.
39. Pasanen MK, Neuvonen M, Neuvonen PJ, Niemi M. SLCO1B1 polymorphism markedly affects the pharmacokinetics of simvastatin acid. Pharmacogenet Genomics 2006;16(12):873-9.
40. Lau HS, de Boer A, Beuning KS, Porsius A. Validation of pharmacy records in drug exposure assessment. J Clin Epidemiol 1997;50(5):619-25.
41. Smith NL, Hindorff LA, Heckbert SR, Lemaitre RN, Marciante KD, Rice K, et al. Association of genetic variations with nonfatal venous thrombosis in postmenopausal women. JAMA 2007;297(5):489-98.

# CHAPTER 4.2

# VARIANTS OF *ADAMTS1* MODIFY THE EFFECTIVENESS OF STATINS IN REDUCING THE RISK OF MYOCARDIAL INFARCTION

Bas J.M. Peters[a]
Andrei S. Rodin[b]
Olaf H. Klungel[a]
Bruno H.Ch. Stricker[c]
Anthonius de Boer[a]
Anke-Hilse Maitland-van der Zee[a]

a Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, Utrecht, The Netherlands
b Human Genetics Center, School of Public Health, University of Texas Health Science Center, Houston, TX, USA
c Department of Epidemiology and Biostatistics, Pharmaco-epidemiology Unit, Erasmus Medical Centre, Rotterdam, The Netherlands

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## ABSTRACT

To investigate the influence of tagging SNPs within candidate genes involved in the putative anti-inflammatory effects of statins on the effectiveness of statins in reducing the risk of myocardial infarction (MI), we conducted a case-control study in a population-based registry of pharmacy records linked to hospital discharge records (PHARMO). Cases and controls were selected from within a hypercholesterolemic cohort. Cases were hospitalized for MI whereas controls were not. Logistic regression analysis was used to investigate pharmacogenetic interactions.

The study population included 668 cases and 1,217 controls. We genotyped 84 SNPs in 24 genes. The effectiveness of statins was found to be modified by seven SNPs in three genes. Five out of six SNPs that were selected in the *ADAMTS1* gene were associated with a modified response to statins, three of which were in strong linkage disequilibrium. The strongest interaction was found for *ADAMTS1* rs402007. Homozygous carriers of the variant allele had most benefit from statins (adjusted odds ratio (OR) 0.10, 95%CI 0.03-0.35), compared to heterozygous (OR 0.43, 95%CI 0.24-0.51) and homozygous wild-type carriers (OR 0.49, 95%CI 0.32-0.57).

Consistent with previous findings, polymorphisms within the *ADAMTS1* gene influenced the effectiveness of statins in reducing the risk of MI. Other pharmacogenetic interactions with SNPs in the *TNFRSF1A* and *ITGB2* genes were established and the confirmation will be pursued in future studies.

## INTRODUCTION

HMG-CoA reductase inhibitors (statins) are widely used to reduce the risk of coronary artery disease (CAD). The efficacy of statins has been well established in many randomized controlled trials, demonstrating average risk reductions of 27% and 15% for major coronary events and all-cause mortality, respectively.[1] The primary mechanism by which statins reduce the risk of CAD is lowering low density lipoprotein cholesterol (LDLc) in plasma. Nonetheless, the beneficial effects of statins seem to go beyond just the cholesterol lowering effects, given that both normocholesterolemic patients and patients in the early treatment stage benefit from treatment.[2] Furthermore, the efficacy of statins in reducing clinically relevant outcomes has been shown to be superior to other cholesterol lowering drugs despite a comparable effect on LDLc, also suggesting LDLc independent effects.[2] Recently, the JUPITER (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) trial demonstrated that rosuvastatin significantly reduced the incidence of major cardiovascular events in healthy men and women with low-density lipoprotein (LDL) cholesterol levels of less than 130 mg per deciliter (3.4 mmol per liter) and inflammatory biomarker high-sensitivity C-reactive protein (CRP) levels of 2.0 mg per liter or higher.[3]

Inflammation is involved in every stage of the atherogenesis process. The early stage of atherogenesis is characterized by leukocyte (monocyte) adhesion and subsequent penetration of the artery wall. The inflammatory response that emerges involves a variety of cytokines, resulting in the formation of an atheromatous plaque, consisting of foam cells, lipids, smooth-muscle cells, collagen, T cells, macrophages, and mast cells.[4 5] Additionally,

inflammation contributes to the instability of the plaque.[2] Plaque rupture might occur, and exposure of smooth-muscle cells, macrophages and collagen to the bloodstream then results in platelet activation, adhesion, and aggregation, and the activation of the coagulation cascade. Ultimately, a thrombus is formed that can (either completely or partially) occlude an artery, resulting in clinical events such as myocardial infarction (MI).[4 5] Several anti-inflammatory mechanisms by which statins influence the atherosclerosis process and thereby contribute to the risk reduction of cardiovascular events have been described.[2] These mechanisms include improvement of endothelial function, reduction of the arterial inflammatory response, increase of the plaque's stability and inhibition of thrombosis.[6]

It has been shown that there is individual variation in response to statins[7] and pharmacogenetic research has shown that part that variation can be explained with genetics.[8] Therefore, the aim of this study was to investigate the potential effect of tagging SNPs located within the candidate genes known to be associated with the anti-inflammatory property of statins on the effectiveness of statins in reducing the risk of MI.

## METHODS

### *Design and Setting*

Participants were enrolled from the population-based Pharmaco-Morbidity Record Linkage System (PHARMO, www.pharmo.nl). PHARMO links drug dispensing histories from a representative sample of Dutch community pharmacies to the national registration of hospital discharges (Dutch National Medical Registry).

Patients who received a prescription for an antihypertensive drug,[9] and/or had hypercholesterolemia (prescription for a cholesterol-lowering drug or total cholesterol > 5.0 mmol/l),[10] were selected from the PHARMO database for pharmacogenetic studies on antihypertensive drugs[9] and statins[10] respectively. From this cohort, a nested case-control study was designed with all hypercholesterolemic (prescription for a cholesterol-lowering drug, total cholesterol >5.0 mmol/l, or self-reported hypercholesterolemia) participants.[10] Patients hospitalized for MI (International Classification of Diseases (ICD)-9 code 410) were included as cases if they were registered in PHARMO for at least one year and were older than 18 years. The index date was defined as the date of hospitalization for the first MI. Controls met the same eligibility criteria as cases, but had not developed MI. Controls were matched to cases on age, sex, and region and assigned the same index date as the case to whom they had been matched. Participants were contacted through community pharmacies, where they received a letter in which the purpose of the study was explained. They were asked to return an informed consent form and a filled-out questionnaire. After the participant had consented to participate in the study, he/she was sent material for saliva collection. All participants were explicitly asked to consent for the collection, storage and genotyping of the DNA material. Approval for this study was obtained from the Medical Ethics Committee of the University Medical Center Utrecht, The Netherlands.

### *Ascertainment of exposure to statins (and other drugs)*

Coded pharmacy records were used to ascertain exposure to statins (and other drugs). In PHARMO, complete pharmacy records were available as of 1991, including the day of

delivery, daily dose, and durations of therapy. To define exposure to statins, we assessed the effect of different cumulative defined daily doses (DDD) (cumulative DDD cut-off points of 90, 180, 360, and 720 DDD) on the odds ratios (ORs) for the association between use of statins and risk of MI. The DDD is the dose per day for a drug used for its main indication in adults defined by the WHO.[11] Our data showed that statins were not effective in reducing the risk of MI in patients exposed to a cumulative dose of 180 DDD or less. The effectiveness of statins in patients exposed for more than 180 days but less than 360 days did not differ from a cumulative exposure for more than 360 or 720 days. Participants were considered exposed when the cumulative DDD of statin use was more than 180, whereas participants with the cumulative DDD of 180, less than 180, or no use were considered the reference group.

For each patient we identified all prescriptions for concomitant drug use. The theoretical end date of a prescription was calculated using information on the daily dose instruction and the quantity dispensed. We considered a patient a current users when the index date was between the start and end date of a prescription. Past users were patients who were no current users, but have used the drug prior to the index date.

***Assessment of potential confounding factors and effect modifiers***

Self-reported data on smoking, hypertension, hypercholesterolemia, diabetes mellitus, use of alcohol, diet, history of cardiovascular diseases (CVDs), family history of CVDs, weight and height was assessed using questionnaires. Furthermore, information from the general practice and laboratory registrations was available for part of the population. In case of a discrepancy between community pharmacy data and questionnaire data, community pharmacy data was the primary source for defining hypercholesterolemia and diabetes status. Ischemic heart disease (IHD) was defined as "yes" if a participant was hospitalized for an IHD or ever used nitrates.

***DNA collection and DNA extraction***

Part of patients were send three cotton swabs and tubes containing buffer to collect buccal cell samples as described elsewhere.[12] Other participants[13] were sent an Oragene collection kit and donor instructions provided by the manufacturer (DNA Genotek, Ottawa, Canada). DNA was extracted according to the manufacturer's instructions (http://www.dnagenotek.com/techsupport_documents.htm). Samples with a DNA concentration higher than 100 ng per ul were diluted to the Illumina Golden-Gate assay required 50 ng per ul.

***Candidate gene selection and SNP selection***

We selected a total of 24 genes that are involved in the anti-inflammatory pathway of statins. Table 1 gives a brief description of the reported associations with cardiovascular disease and/or use of statins. To assess all common SNPs for each gene, we selected tagging SNPs within 200 bp (up- and downstream) with a minor-allele frequency (MAF) higher than 0.2 and a $r^2 > 0.8$ using a web-based tool called QuickSNP version 1.1 (HapMap release 21,[14] US residents with northern and western European ancestry (CEU individuals)).[15] Additionally, dbSNP non-synonymous coding SNPs (MAF higher than 0.2) and previously (pharmaco-)genetically associated SNPs were included. Illumina SNP designability scores lower than 0.4 (1.1 = best validated) or failure codes (http://www.

illumina.com/downloads/GoldenGateDesign_TechNote.pdf) were either substituted with a SNP in linkage disequilibrium (LD) or, if no such SNP were available, removed from the SNP list, resulting in a final set of 93 SNPs.

***Genotyping***

For each individual participating in the study, high throughput SNP genotyping was performed with the 384 SNP Illumina Golden Gate assay (BeadArray technology) using the Sentrix Array Matrix platform (Illumina Inc, San Diego, CA, USA).[16] Illumina Beadstudio software, was used for automated genotype clustering and calling. Genotype calls of all SNPs were individually examined for their resulting quality. SNPs with a low signal, poor clustering, deviation from Hardy–Weinberg equilibrium (HWE) (≤0.01) or a high number of missing genotypes (>10%) were excluded. Out of 384, a total of 93 SNPs were genotyped for this study.

***Statistical methods***

Logistic regression (LR) analysis was used to study the association between statins (>180 DDD of statin versus reference group) and the risk of MI, and to adjust for potential confounders. Matching variables --- age, sex, region, and index date --- were included in our statistical model. A model adjusted for only the matching variables will be referred to as unadjusted. The inclusion of potential confounders in the LR model was motivated by the assessment of the influence of each potential confounder on the OR for the association between use of statins and risk of MI. The potential confounding factors that we considered were as follows: use of different cardiovascular drugs (antihypertensive drugs, platelet aggregation inhibitors, anticoagulants, other cholesterol-lowering drugs, and organic nitrates), use of alcohol, physical activity, family history of CVD, and other factors assessed by the questionnaire. Only covariates IHD and use of calcium channel blockers showed at least a 5% change in the regression coefficient (beta) for statin use; therefore, they were included in the LR model. We estimated the multiplicative synergy index (SI), which is the ratio of the OR in those with the variant to the OR in those without the variant.[17] For the significant (unadjusted or adjusted) pharmacogenetic associations, ORs were calculated separately in the strata defined by genotype. For each SNP, HWE was tested using a $X^2$ goodness-of-fit test. Analyses were performed using SPSS statistical software version 16.0.

Subsequently, q values (the positive false discovery rate (pFDR) analogue of the p value) were calculated for each gene-treatment interaction that was tested to account for multiple testing.[18]

## RESULTS

We were able to include 668 cases and 1,217 controls. The clinical characteristics can be found in table 2. A detailed description of how the number of cases and controls was arrived at can be found elsewhere.[10] All participants were non-Hispanic whites. Questionnaire-derived variables smoking and BMI were associated with the case-control status. A higher percentage of cases had an ischemic heart disease compared to controls. In addition, as a result of the inclusion criteria and sampling, the use of the antihypertensive drugs

***Table 1.*** Candidate genes in the study

| Gene | SNPs (n) | Relation with Statins/Cardiovascular Disease | Reference |
|---|---|---|---|
| *ADAMTS1* | 6 | In males homozygous for the *ADAMTS1* 227Pro allele, treatment with pravastatin reduced the odds of fatal coronary disease or nonfatal MI by 77% compared with 25% in subjects without this allele or heterozygous. | 22 |
| *ADAMTS17* | 1 | *ADAMTS17* is involved in atherosclerosis. SNP rs1994016 was associated with CAD in GWS. | 31 32 |
| *CASP1* | 2 | Statins augment LPS-induced IL-1B release post-translationally, by inducing caspase-1 activity. | 33 34 |
| *CCL2* | 1 | Statin treatment of endothelial cells significantly attenuates monocyte chemotactic protein 1, which is involved in monocyte recruitment | 26 |
| *CDKN2A* | 3 | This gene has been associated with increased risk of CAD. | 35 36 |
| *CRP* | 1 | A common *CRP* haplotype was associated with modified reduction in CRP levels in response to pravastati | 37 38 |
| *FCAR* | 1 | Risk reduction of MI in response to pravastatin was greater in *FCAR* 92Asn allele carriers compared to non-carriers | 39 |
| *ICAM1* | 2 | Ligand for integrin leukocyte receptor LFA-1. Statins affect *ICAM1* expression in stimulated endothelial cells and monocytes. | 26 |
| *IFNG* | 3 | Statins inhibit induction of MHC-II expression by IFN-γ and thereby repress MHC-II-mediated T-cell activation. | 40 |
| *IL10* | 3 | Polymorphisms in IL10 promoter region have been associated with incidence vascular events. Statins increase IL-10 levels. | 41-43 |
| *IL1B* | 4 | Improved coronary function in non-carriers of the *IL1B* A2 only. | 44 |
| *IL6* | 3 | Greatest response to pravastatin in CC carriers of the *IL6* -174G>C with respect to the CHD outcome. An interaction between *IL6* genotypes and response of plasma Lp[a] levels to fluvastatin treatment was reported. | 45 46 |
| *IL8* | 1 | Statin treatment of endothelial cells significantly attenuates IL-8, which is involved in neutrophil recruitment. | 23 26 |
| *ITGA4* | 14 | Major integrin receptor is present on leukocytes. Statins block integrin expression and integrin activation in leukocytes. | 23 26 |
| *ITGB2* | 17 | Major integrin receptor is present on leukocytes. Statins block integrin expression and integrin activation in leukocytes. | 23 26 |
| *OLR1* | 5 | *OLR1* (*LOX1*) genetic variants influence cardiovascular risk reduction induced by statins. | 47 |
| *MMP3* | 2 | Pravastatin did not reduce risk of clinical events in 5A5A carriers of MMP 5A/6A, whereas risk decreased by half for the other genotypes. | 48 |
| *NFKB1* | 7 | Statins inhibit the activation of NF-kB. | 26 |
| *NOS3* | 4 | Myocardial perfusion improved in pravastatin-treated subjects with ba genotype of *NOS3* 4a/b, but not in bb carriers. | 49 |
| *SERPINE1* | 1 | Pravastatin prevented *PAI-1* expression/secretion mainly in endothelial cells with the 4G/4G SERPINE1 4G/5G polymorphism | 50 |
| *TLR4* | 3 | Gly allele carriers of *TLR4* Asp299Gly benefited more (in terms of cardiovascular events) in response to statins, compared to non-carriers. | 51 52 |
| *TNFa* | 2 | Statins interfere with the expression of cytokines in endothelial cells and monocytes. | 23 26 |
| *TNFRSF1A* | 2 | (See above) | |
| *VCAM1* | 5 | Ligand to integrin receptors LFA-1 and VLA4. Statins affect the expression of cell adhesion molecules, including VCAM-1 | 26 |

calcium channel blockers, diuretics, angiotensin II receptor antagonists, and beta blockers were associated with case-control status. The risk of MI was significantly lower among participants exposed to statins than in participants not exposed to statins (adjusted OR 0.36, 95%CI 0.29-0.45). Out of the 93 SNPs, 4 SNPs were excluded (*IL1B* rs1143643, *IL1B* rs16944, *NOS3* rs1800783 and *NOS3* rs3918166) because of low signal or poor clustering and 5 SNPs (*TNFa* rs1800629, *ITGB2* rs170963, *ITGB2* rs2235133, *OLR1* rs3741860 and *NOS3* rs1799983) were excluded because of deviation from HWE (≤0.01) and/or high number of missing genotypes (>10%). A total of 84 SNPs were tested for their interaction with statin treatment on a multiplicative scale. All SNPs (rs numbers) with corresponding genes, genotypes, frequencies, HWE, SI and p and q values can be found in supplemental table I (SI). The p values for the adjusted SI were used to rank the SNPs. Table 3 depicts the gene-treatment interactions with a p value less than 0.05 for either the adjusted SI, unadjusted SI or both, with the most significant interaction listed first. In addition to the SI, ORs for the effectiveness of statins in reducing the risk of MI for each of the genotype strata are included in the table.

Among the SNPs that showed a significant interaction (table 3), we found five out of six SNPs that were selected in the *ADAMTS1* gene and two SNPs that were selected in the *TNFRSF1A* and *ITGB2* gene. Within the *ADAMTS1*, the strongest pharmacogenetic interaction was found with rs402007 (adjusted p=0.021). Although homo- and heterozygous rs402007 G allele carriers did benefit from statin treatment (adjusted $OR_{GG}$ 0.43, 95%CI 0.32-0.57 and adjusted $OR_{CG}$ 0.35, 95%CI 0.24-0.51 respectively), homozygous carriers of the variant C allele had most benefit from statin treatment (adjusted $OR_{CC}$ 0.10, 95%CI 0.03-0.35). Q values calculated with the adjusted p-values of the SI ranged from 0.50 to 0.60 for the interactions in table 3, suggesting that half of the interactions were chance findings.

***Table 2.*** Clinical characteristics by case control status.

| | | Case n=668 | % | Control n=1217 | % | p |
|---|---|---|---|---|---|---|
| Gender | Female | 171 | 25.6% | 285 | 23.4% | 0.290 |
| Age (years) | Mean (sd) | 63.0 (9.9) | | 62.3 (9.4) | | 0.097 |
| Body Mass Index at ID | >30 kg/m2 | 140/607 | 23.1% | 193/1104 | 17.5% | 0.005 |
| Familial History CVD | Yes, <60 | 135/637 | 21.2% | 214/1167 | 18.3% | 0.103 |
| | Yes, >60 | 266/637 | 41.8% | 464/1167 | 39.8% | |
| Diabetes Status | Yes, no medication | 71/658 | 10.8% | 90/1203 | 7.5% | 0.052 |
| | Yes, medication | 75/658 | 11.4% | 144/1203 | 12.0% | |
| Smoking Status | Current | 152/624 | 24.4% | 195/1135 | 17.2% | 0.001 |
| | Past | 283/624 | 45.4% | 573/1135 | 50.5% | |
| Use of alcohol per day (glasses) | No use | 118/646 | 18.3% | 162/1188 | 13.6% | 0.062 |
| | <=1 | 237/646 | 36.7% | 432/1188 | 36.4% | |
| | >1 - <2 | 170/646 | 26.3% | 328/1188 | 27.6% | |
| | >2 | 121/646 | 18.7% | 266/1188 | 22.4% | |
| Physical Activity | > 4 hrs a week | 491/645 | 76.1% | 939/1191 | 78.8% | 0.180 |
| Cumulative DDD use statins | >180 DDD | 218 | 32.6% | 646 | 53.1% | <0.001 |
| | 1-180 DDD | 75 | 11.2% | 121 | 9.9% | |
| Type of Statin | Atorvastatin | 44/218 | 20.2% | 164/646 | 25.4% | <0.001 |
| | Pravastatin | 35/218 | 16.1% | 110/646 | 17.0% | |
| | Simvastatin | 125/218 | 57.3% | 335/646 | 51.9% | |
| | Other | 14/218 | 6.4% | 37/646 | 5.7% | |
| Ischemic Heart Disease | Yes | 211 | 31.6% | 268 | 22.0% | <0.001 |
| **Antihypertensives** | | | | | | |
| Calcium Channel Blockers | Current use | 142 | 21.3% | 193 | 15.9% | <0.001 |
| | Past Use | 87 | 13.0% | 115 | 9.4% | |
| Diuretics | Current use | 82 | 12.3% | 171 | 14.1% | 0.044 |
| | Past Use | 70 | 10.5% | 89 | 7.3% | |
| Beta Blockers | Current use | 275 | 41.2% | 415 | 34.1% | 0.002 |
| | Past Use | 108 | 16.2% | 181 | 14.9% | |
| Ace Inhibitors | Current use | 140 | 21.0% | 273 | 22.4% | 0.660 |
| | Past Use | 63 | 9.4% | 104 | 8.5% | |
| ATII Receptor Antagonists | Current use | 48 | 7.2% | 110 | 9.0% | 0.021 |
| | Past Use | 26 | 3.9% | 24 | 2.0% | |
| **Other drugs** | | | | | | |
| Other Cholesterol Lowerers | Current use | 14 | 2.1% | 46 | 3.8% | 0.124 |
| | Past Use | 25 | 3.7% | 40 | 3.3% | |
| Insulin | Ever use | 30 | 4.5% | 54 | 4.4% | 0.957 |
| Oral Antidiabetics | Current Use | 52 | 7.8% | 96 | 7.9% | 0.993 |
| | Past Use | 13 | 1.9% | 23 | 1.9% | |
| Platelet Aggregation Inhibitors | Current Use | 219 | 32.8% | 438 | 36.0% | 0.293 |
| | Past Use | 64 | 9.6% | 100 | 8.2% | |
| Coumarins | Current Use | 37 | 5.5% | 76 | 6.2% | 0.382 |
| | Past Use | 41 | 6.1% | 58 | 4.8% | |

Abbreviations: ATII = Angiotensin II; DDD = Defined Daily Dosage

***Table 3.*** Statistically significant pharmacogenetic interactions (p value < 0.05) with SIs, p and q values, and ORs for each genotype group, ranked by p value of the synergy index.

| Gene | SNP | G | # | HWE | SI* (95% CI) | p | SI** (95% CI) | p | q | OR* (95% CI) | OR** (95% CI) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ADAMTS1 | rs402007 | CC | 103 | | (ref) | 0.013 | (ref) | 0.021 | 0.50 | 0.10 (0.03-0.34) | 0.10 (0.03-0.35) |
| | | CG | 618 | 0.26 | 4.41 (1.32-14.72) | 0.016 | 3.73 (1.11-12.57) | 0.033 | | 0.43 (0.30-0.62) | 0.35 (0.24-0.51) |
| | | GG | 1075 | | 5.60 (1.71-18.30) | 0.004 | 4.94 (1.50-16.27) | 0.009 | | 0.49 (0.38-0.65) | 0.43 (0.32-0.57) |
| | | -- | 89 | | | | | | | | |
| ADAMTS1 | rs422381 | AA | 107 | | (ref) | 0.012 | (ref) | 0.022 | 0.50 | 0.08 (0.03-0.29) | 0.09 (0.03-0.30) |
| | | AG | 656 | 0.41 | 5.14 (1.55-17.05) | 0.007 | 4.35 (1.30-14.53) | 0.017 | | 0.45 (0.32-0.64) | 0.36 (0.25-0.52) |
| | | GG | 1117 | | 5.91 (1.82-19.19) | 0.003 | 5.19 (1.59-17.00) | 0.006 | | 0.46 (0.35-0.60) | 0.40 (0.30-0.53) |
| | | -- | 5 | | | | | | | | |
| ITGB2 | rs3746972 | AA | 1294 | | (ref) | 0.027 | (ref) | 0.026 | 0.50 | 0.45 (0.35-0.57) | 0.37 (0.29-0.49) |
| | | AG | 525 | 0.39 | 0.68 (0.43-1.06) | 0.090 | 0.68 (0.43-1.08) | 0.105 | | 0.34 (0.23-0.52) | 0.30 (0.20-0.45) |
| | | GG | 61 | | 3.06 (0.96-9.72) | 0.058 | 3.28 (1.01-10.63) | 0.048 | | 1.43 (0.36-5.69) | 1.51 (0.31-7.28) |
| | | -- | 5 | | | | | | | | |
| ADAMTS1 | rs229041 | CC | 595 | | (ref) | 0.029 | (ref) | 0.028 | 0.50 | 0.66 (0.46-0.94) | 0.56 (0.39-0.82) |
| | | CG | 929 | 0.84 | 0.64 (0.41-1.01) | 0.053 | 0.65 (0.41-1.03) | 0.065 | | 0.38 (0.28-0.52) | 0.33 (0.24-0.45) |
| | | GG | 356 | | 0.47 (0.26-0.85) | 0.012 | 0.46 (0.25-0.83) | 0.010 | | 0.25 (0.15-0.43) | 0.20 (0.12-0.35) |
| | | -- | 5 | | | | | | | | |
| TNFRSF1A | rs4149576 | AA | 301 | | (ref) | 0.034 | (ref) | 0.032 | 0.50 | 0.63 (0.38-1.05) | 0.55 (0.32-0.94) |
| | | AG | 938 | 0.17 | 0.48 (0.27-0.85) | 0.011 | 0.47 (0.27-0.84) | 0.010 | | 0.37 (0.27-0.49) | 0.30 (0.22-0.42) |
| | | GG | 640 | | 0.66 (0.36-1.19) | 0.168 | 0.64 (0.35-1.17) | 0.148 | | 0.45 (0.32-0.64) | 0.39 (0.27-0.56) |
| | | -- | 6 | | | | | | | | |
| ADAMTS1 | rs436525 | AA | 890 | | (ref) | 0.042 | (ref) | 0.038 | 0.56 | 0.48 (0.36-0.65) | 0.42 (0.31-0.58) |
| | | AG | 800 | 0.47 | 0.82 (0.54-1.24) | 0.347 | 0.78 (0.51-1.19) | 0.250 | | 0.42 (0.31-0.58) | 0.34 (0.24-0.48) |
| | | GG | 194 | | 0.37 (0.17-0.81) | 0.012 | 0.37 (0.17-0.80) | 0.012 | | 0.21 (0.10-0.44) | 0.18 (0.08-0.39) |
| | | -- | 1 | | | | | | | | |
| ADAMTS1 | rs420742 | AA | 1059 | | (ref) | 0.037 | (ref) | 0.053 | 0.60 | 0.49 (0.37-0.64) | 0.42 (0.31-0.56) |
| | | AG | 597 | 0.25 | 0.81 (0.52-1.25) | 0.331 | 0.78 (0.50-1.21) | 0.262 | | 0.44 (0.30-0.62) | 0.35 (0.24-0.52) |
| | | GG | 98 | | 0.24 (0.08-0.74) | 0.013 | 0.27 (0.09-0.83) | 0.022 | | 0.12 (0.04-0.38) | 0.12 (0.04-0.39) |
| | | -- | 131 | | | | | | | | |

*adjusted for sex, index date, age and region
**adjusted for sex, index date, age, region, use of calcium channel blockers and ischemic heart disease
-- = missing; G = genotype; HWE = Hardy-Weinberg Equilibrium, SI = synergy index; OR = odds ratio;
CI = Confidence Interval

## DISCUSSION

In our current study, the effectiveness of statins in reducing the risk of MI was found to be influenced by six SNPs in three genes after adjusting for confounding factors (table 3). The significant interactions were dominated by SNPs within the *ADAMTS1* gene. The strongest interaction was found for rs402007 in the *ADAMTS1* gene, for which the benefit of statin treatment was greatest in homozygous carriers of the variant rs402007 C allele (adjusted $OR_{CC}$ 0.10, 95%CI 0.03-0.35) compared to homo- and heterozygous rs402007 G allele carriers (adjusted $OR_{GG}$ 0.43, 95%CI 0.32-0.57 and adjusted $OR_{CG}$ 0.35, 95%CI 0.24-0.51 respectively).

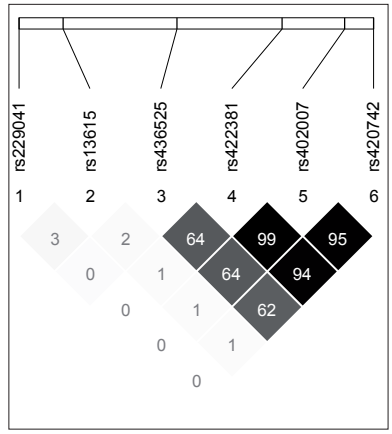
A disintegrin and metallopeptidase with thrombospondin motif type I (ADAMTS-1), encoded by *ADAMTS1*, belongs to the ADAMTS family of metallopeptidases. ADAMTS-1 plays a role in atherosclerosis, mainly because it has been shown to cleave the proteoglycan versican, a prominent component of the extracellular matrix (ECM), expressed by vascular smooth muscle cells (VSMCs).[19 20] Versican cleavage is thought to increase intima permeability for VSMCs migration and to destabilize the fibrous cap. Furthermore, versican can be found in atherosclerotic plaques and is involved in inflammation and thrombus formation.[19 20]

The most frequently investigated polymorphism in the *ADAMTS1* gene is the $^{Ala}227^{Pro}$ (rs428785) polymorphism, with unknown functional consequences.[21] Nonetheless, the homozygous $227^{Pro}$ allele carriers have been shown to have a higher risk of coronary heart disease (CHD),[21] fatal coronary disease, and nonfatal MI.[22] In addition, both the Cholesterol and Recurrent Events trial (CARE) and the West of Scotland Coronary Prevention Study (WOSCOPS) revealed a pharmacogenetic interaction with statins.[22] In both independent trials, the effectiveness of pravastatin in reducing the risk of fatal coronary disease or nonfatal MI was much higher in homozygous carriers of the $227^{Pro}$ allele (77%) compared to non-carriers or heterozygotes (25%).[22]

It has been shown that the *ADAMTS1* tagging SNP rs402007 genotyped in this study serves as an excellent proxy for *ADAMTS1* $^{Ala}227^{Pro}$ because of strong linkage disequilibrium (LD).[22] In keeping with the results from the CARE and WOSCOPS trial, we also find *ADAMTS1* $227^{Pro}$ allele carriers (assessed with tagging SNP rs402007) to benefit much more from statin treatment compared to the heterozygotes or non-carriers. Of note, our study only included cases with nonfatal MI, whereas the CARE and WOSCOPS trials also included fatal coronary disease. Although the underlying mechanism is unknown, Sabatine et al. propose a possible explanation for the observed gene-treatment interaction. The *ADAMTS1* $227^{Pro}$ allele promotes atherosclerosis, contributes to plaque instability, and increases the risk of CHD.[21 22] Assuming that statins - similar to matrix metallopeptidases (MMPs) activity - reduce ADAMTS-1 activity, one could hypothesize that plaque stabilization by statins may be of greater importance for homozygous *ADAMTS1* $227^{Pro}$ allele carriers compared to heterozygotes or non-carriers.[22] Moreover, inhibition of ADAMTS-1 by statins might be enhanced in homozygous *ADAMTS1* $227^{Pro}$ allele carriers.[22]

In addition to *ADAMTS1* rs402007, we genotyped three tagging SNPs (rs422381, rs436525 and rs229041) and two SNPs in the untranslated region (UTR) (rs13615 and rs420742). Although *ADAMTS1* rs422381, rs436525, and rs420742 also seem to modify statin effectiveness, these SNPs are very likely to represent the same effect as *ADAMTS1* rs402007, given the high degree of LD (figure 1).

***Figure 1.*** Linkage Disequilibrium Plot of SNPs in *ADAMTS1*




One additional independent *ADAMTS1* SNP was found to be pharmacogenetically associated: *ADAMTS1* rs229041 which is located in the 5'near gene region. It is unknown if (and how) this SNP affects ADAMTS-1 activity and the effectiveness of statins. Nonetheless, this region contains transcription- and translation- regulating sequences and might, therefore, be of great importance.

Other SNPs that appear among those declared significant (table 3) include the intronic tagging SNPs *TNFRSF1A* rs4149576 and *ITGB2* rs3746972. The *TNFRSF1A* gene encodes the p55 tumor necrosis factor (TNF) receptor (TNFR) and mediates the majority of TNF responses. Statins have been shown to interfere with the expression of TNF alpha in monocytes.[23] Increased plasma TNF alpha has been shown to be associated with a higher risk of first-time cardiovascular disease.[24] In addition, in a murine model, p55 TNFR expression was shown to promote atherosclerosis by enhancing expression of endothelial adhesion molecules.[25] Although statins appear to modify the TNF alpha response, and genetics might influence this process, the exact mechanism is unknown. The present study is the first statin pharmacogenetic study that included this gene.

Also *ITGB2* rs3746972 has never been subject of statin pharmacogenetic research before. The *ITGB2* encodes integrin beta 2. Statins have been shown to block integrin expression and integrin activation in leukocytes.[23 26] rs3746972 has not been studied before, and the functional consequence and further implications are unknown.

The present study has several limitations. First, inherent to an observational study on the effectiveness of statins is the introduction of confounding by indication. As more severely ill patients are more likely to be treated with a statin, they could be overrepresented in the statin treated group. On the other hand, patients that are more conscious of their health status may be more willing to receive statin treatment, which would indicate a healthy user effect. The magnitude of the effectiveness of statins in our study would suggest a healthy user effect rather than bias as a result of confounding by indication. Most importantly, confounding by indication does not influence the SI measure that was

used to detect gene-treatment interactions. Second, the choice of 0.2 as the MAF cut-off is an important limitation. Our study is likely to miss potentially significant SNPs, as past statin pharmacogenetic studies have also implicated SNPs with lower MAF and higher penetrance. Nonetheless, our study covers the common genetic variability within a candidate gene with a MAF of more than 0.2. The choice for the 0.2 MAF cut-off was based on a power calculation with 80% power to detect a SI of 2 (or 0.5). Third, unfortunately, nine SNPs did not pass QC and therefore we were not able to cover much of the variability in some important candidate genes for statin pharmacogenetics such as *IL1B* and *NOS3*.
One could debate the choice of candidate genes in our study. At the time of choosing candidate gene (2006), we searched literature to identify inflammation genes that have previously been shown to interact with statin therapy and genes that play an important role in atherosclerosis. Nonetheless, some important statin pharmacogenes such as *CXCL5* are omitted from the list.[27 28] Future studies should include these high priority candidate gene.
Furthermore, we considered all statins as one homogenous group, although it has been shown that anti-inflammatory properties may differ between statin types.[29] Additional analyses showed that our study population was not sufficiently large to stratify the analyses by the statin type. Finally, for *ADAMTS1*, our findings were shown to be in keeping with previous findings from the CARE and WOSCOPS trials,[22] but for the pharmacogenetic associations in genes that have not been studied before, we do not have an opportunity for a replication study. Therefore, these results should be considered hypothesis generating and taken with caution.
An important strength of our study is the uniqueness of having centralized system to define exposure (availability of the community pharmacy records) and outcomes (hospital records). Statin exposure was defined based on pharmacy records, which validity to measure drug exposure has been shown to be good.[30] Furthermore, we covered virtually all common genetic variability in most of the candidate genes by using tagging SNPs. Another strength of our study is its reasonably large sample size, although (pharmaco)genetic studies in complex diseases necessarily demand a much larger number of patients to detect small effects. When testing 84 SNPs for a pharmacogenetic interaction, multiple testing increases the possibility of chance findings (spurious associations). To evaluate this, we calculated the q values --- suggesting approximately half of the significant associations to be false discoveries. Finally, our study assessed the impact of gene-treatment interactions on the clinically important (endpoint) outcome MI, instead of surrogate parameters.
In conclusion, consistent with previous findings,[22] we report tagging SNPs in LD with the *ADAMTS1* $^{Ala}227^{Pro}$ polymorphism to influence the effectiveness of statins in reducing the risk of MI. Furthermore, we report additional common genetic variability in the 5' region around the *ADAMTS1* gene to be important for the pharmaogenetics of statins. Although homozygous carriers of tagging SNPs in LD with the *ADAMTS1* $^{Ala}227$ allele benefit the least from statin therapy, ours (and previous)[22] findings show that statin therapy is also effective in reducing the risk of MI/CHD in patients homozygous for *ADAMTS1* $^{Ala}227$. Therefore, the clinical value of our findings seem limited since statin treatment provided meaningful benefit in both *ADAMTS1* genotype groups and the other significantly interacting SNP genotype groups. Nonetheless, the *ADAMTS1* $^{Ala}227^{Pro}$ genotype should be considered as a marker when assessing cardiovascular risk and pharmacological intervention with statins. Patients homozygous for *ADAMTS1* $227^{Pro}$ allele could benefit from statin therapy with a

lower cardiovascular risk profile compared to wild type carriers. Furthermore, pinpointing the causal variants in the *ADAMTS1* gene and elucidating the exact mechanism of the *ADAMTS1* statin interaction will give a better understanding of the pathophysiology of CHD and might lead to discovery of new drug targets --- therefore, it should have a high priority in future research. Additionally, confirmation of the pharmacogenetic associations with SNPs in the *TNFRSF1A* and *ITGB2* genes should be a priority for the future studies.

## REFERENCES

1. Cheung BM, Lauder IJ, Lau CP, Kumana CR. Meta-analysis of large randomized controlled trials to evaluate the impact of statins on cardiovascular outcomes. Br J Clin Pharmacol 2004;57(5):640-51.
2. Jain MK, Ridker PM. Anti-inflammatory effects of statins: clinical evidence and basic mechanisms. Nat Rev Drug Discov 2005;4(12):977-87.
3. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM, Jr., Kastelein JJ, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med 2008;359(21):2195-207.
4. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 2005;352(16):1685-95.
5. Yeghiazarians Y, Braunstein JB, Askari A, Stone PH. Unstable angina pectoris. N Engl J Med 2000;342(2):101-14.
6. Rosenson RS, Tangney CC. Antiatherothrombotic properties of statins: implications for cardiovascular event reduction. JAMA 1998;279(20):1643-50.
7. Simon JA, Lin F, Hulley SB, Blanche PJ, Waters D, Shiboski S, et al. Phenotypic predictors of response to simvastatin therapy among African-Americans and Caucasians: the Cholesterol and Pharmacogenetics (CAP) Study. Am J Cardiol 2006;97(6):843-50.
8. Peters BJ, Klungel OH, de Boer A, Maitland-van der Zee AH. Genetic determinants of response to statins. Expert Rev Cardiovasc Ther 2009;7(8):977-83.
9. van Wieren-de Wijer DB, Maitland-van der Zee AH, de Boer A, Kroon AA, de Leeuw PW, Schiffers P, et al. Interaction between the Gly460Trp alpha-adducin gene variant and diuretics on the risk of myocardial infarction. J Hypertens 2009;27(1):61-8.
10. Peters BJM, Rodin AS, Klungel OH, van Duijn CM, Stricker BHC, Slot Rvt, et al. Pharmacogenetic interactions between ABCB1 and SLCO1B1 tagging SNPs and the effectiveness of statins in the prevention of myocardial infarction. Pharmacogenomics 2010;11(8):1065-1076.
11. WHO. Collaboration Centre for Drug Statistics Methodology. Complete ATC index. Oslo, 2010. (http://www.whocc.no/atc_ddd_index/ [accessed July 27th 2010]).
12. van Wieren-de Wijer DB, Maitland-van der Zee AH, de Boer A, Stricker BH, Kroon AA, de Leeuw PW, et al. Recruitment of participants through community pharmacies for a pharmacogenetic study of antihypertensive drug treatment. Pharm World Sci 2008.
13. Peters BJM, Rodin AS, Klunge lOH, Stricker BH, de Boer A, Maitland-van der Zee AH. Pharmacogenetic interactions between ABCB1 and SLCO1B1 tagging SNPs and the effectiveness of statins in the prevention of myocardial infarction. Pharmacogenomics 2010;August (in press).
14. Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA, et al. A second generation human haplotype map of over 3.1 million SNPs. Nature 2007;449(7164):851-61.
15. Grover D, Woodfield AS, Verma R, Zandi PP, Levinson DF, Potash JB. QuickSNP: an automated web server for selection of tagSNPs. Nucleic Acids Res 2007;35(Web Server issue):W115-20.
16. Fan JB, Chee MS, Gunderson KL. Highly parallel genomic assays. Nat Rev Genet 2006;7(8):632-44.
17. Khoury MJ, Flanders WD. Nontraditional epidemiologic approaches in the analysis of gene-environment interaction: case-control studies with no controls! Am J Epidemiol 1996;144(3):207-13.

18. Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proc Natl Acad Sci U S A 2003;100(16):9440-5.
19. Jonsson-Rylander AC, Nilsson T, Fritsche-Danielson R, Hammarstrom A, Behrendt M, Andersson JO, et al. Role of ADAMTS-1 in atherosclerosis: remodeling of carotid artery, immunohistochemistry, and proteolysis of versican. Arterioscler Thromb Vasc Biol 2005;25(1):180-5.
20. Wight TN, Merrilees MJ. Proteoglycans in atherosclerosis and restenosis: key roles for versican. Circ Res 2004;94(9):1158-67.
21. Morrison AC, Bare LA, Chambless LE, Ellis SG, Malloy M, Kane JP, et al. Prediction of coronary heart disease risk using a genetic risk score: the Atherosclerosis Risk in Communities Study. Am J Epidemiol 2007;166(1):28-35.
22. Sabatine MS, Ploughman L, Simonsen KL, Iakoubova OA, Kirchgessner TG, Ranade K, et al. Association between ADAMTS1 matrix metalloproteinase gene variation, coronary heart disease, and benefit of statin therapy. Arterioscler Thromb Vasc Biol 2008;28(3):562-7.
23. Weitz-Schmidt G. Statins as anti-inflammatory agents. Trends Pharmacol Sci 2002;23(10):482-6.
24. Cesari M, Penninx BW, Newman AB, Kritchevsky SB, Nicklas BJ, Sutton-Tyrrell K, et al. Inflammatory markers and onset of cardiovascular events: results from the Health ABC study. Circulation 2003;108(19):2317-22.
25. Xanthoulea S, Thelen M, Pottgens C, Gijbels MJ, Lutgens E, de Winther MP. Absence of p55 TNF receptor reduces atherosclerosis, but has no major effect on angiotensin II induced aneurysms in LDL receptor deficient mice. PLoS One 2009;4(7):e6113.
26. Greenwood J, Mason JC. Statins and the vascular endothelial inflammatory response. Trends Immunol 2007;28(2):88-98.
27. Zineh I, Aquilante CL, Langaee TY, Beitelshees AL, Arant CB, Wessel TR, et al. CXCL5 gene polymorphisms are related to systemic concentrations and leukocyte production of epithelial neutrophil-activating peptide (ENA-78). Cytokine 2006;33(5):258-63.
28. Zineh I, Beitelshees AL, Welder GJ, Hou W, Chegini N, Wu J, et al. Epithelial neutrophil-activating peptide (ENA-78), acute coronary syndrome prognosis, and modulatory effect of statins. PLoS One 2008;3(9):e3117.
29. Weitz-Schmidt G, Welzenbach K, Brinkmann V, Kamata T, Kallen J, Bruns C, et al. Statins selectively inhibit leukocyte function antigen-1 by binding to a novel regulatory integrin site. Nat Med 2001;7(6):687-92.
30. Lau HS, de Boer A, Beuning KS, Porsius A. Validation of pharmacy records in drug exposure assessment. J Clin Epidemiol 1997;50(5):619-25.
31. Wagsater D, Bjork H, Zhu C, Bjorkegren J, Valen G, Hamsten A, et al. ADAMTS-4 and -8 are inflammatory regulated enzymes expressed in macrophage-rich areas of human atherosclerotic plaques. Atherosclerosis 2008;196(2):514-22.
32. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447(7145):661-78.
33. Montero MT, Hernandez O, Suarez Y, Matilla J, Ferruelo AJ, Martinez-Botas J, et al. Hydroxymethylglutaryl-coenzyme A reductase inhibition stimulates caspase-1 activity and Th1-cytokine release in peripheral blood mononuclear cells. Atherosclerosis 2000;153(2):303-13.
34. Kuijk LM, Mandey SH, Schellens I, Waterham HR, Rijkers GT, Coffer PJ, et al. Statin synergizes with LPS to induce IL-1beta release by THP-1 cells through activation of caspase-1. Mol Immunol 2008;45(8):2158-65.
35. Samani NJ, Erdmann J, Hall AS, Hengstenberg C, Mangino M, Mayer B, et al. Genomewide association analysis of coronary artery disease. N Engl J Med 2007;357(5):443-53.
36. Schunkert H, Gotz A, Braund P, McGinnis R, Tregouet DA, Mangino M, et al. Repeated replication and a prospective meta-analysis of the association between chromosome 9p21.3 and coronary artery disease. Circulation 2008;117(13):1675-84.

37. Albert MA, Danielson E, Rifai N, Ridker PM. Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. JAMA 2001;286(1):64-70.
38. Suk HJ, Zee RY, Cook NR, Chasman DI, Ridker PM. A common haplotype within the CRP gene predicts lower CRP levels and greater change in CRP levels in response to pravastatin therapy. Circulation 2004;110 Supplement III(17):III-426.
39. Iakoubova OA, Tong CH, Chokkalingam AP, Rowland CM, Kirchgessner TG, Louie JZ, et al. Asp92Asn polymorphism in the myeloid IgA Fc receptor is associated with myocardial infarction in two disparate populations: CARE and WOSCOPS. Arterioscler Thromb Vasc Biol 2006;26(12):2763-8.
40. Kwak B, Mulhaupt F, Myit S, Mach F. Statins as a newly recognized type of immunomodulator. Nat Med 2000;6(12):1399-402.
41. Li JJ, Fang CH. Effects of 4 weeks of atorvastatin administration on the antiinflammatory cytokine interleukin-10 in patients with unstable angina. Clin Chem 2005;51(9):1735-8.
42. Li JJ, Li YS, Fang CH, Hui RT, Yang YJ, Cheng JL, et al. Effects of simvastatin within two weeks on anti-inflammatory cytokine interleukin 10 in patients with unstable angina. Heart 2006;92(4):529-30.
43. Trompet S, Pons D, AJ DEC, Slagboom P, Shepherd J, Blauw GJ, et al. Genetic variation in the interleukin-10 gene promoter and risk of coronary and cerebrovascular events: the PROSPER study. Ann N Y Acad Sci 2007;1100:189-98.
44. Lehtimaki T, Laaksonen R, Janatuinen T, Vesalainen R, Nuutila P, Mattila K, et al. Interleukin-1B genotype modulates the improvement of coronary artery reactivity by lipid-lowering therapy with pravastatin: a placebo-controlled positron emission tomography study in young healthy men. Pharmacogenetics 2003;13(10):633-9.
45. Basso F, Lowe GD, Rumley A, McMahon AD, Humphries SE. Interleukin-6 -174G>C polymorphism and risk of coronary heart disease in West of Scotland coronary prevention study (WOSCOPS). Arterioscler Thromb Vasc Biol 2002;22(4):599-604.
46. Elghannam H, Tavackoli S, Ferlic L, Gotto AM, Jr., Ballantyne CM, Marian AJ. A prospective study of genetic markers of susceptibility to infection and inflammation, and the severity, progression, and regression of coronary atherosclerosis and its response to therapy. J Mol Med 2000;78(10):562-8.
47. Puccetti L, Pasqui AL, Bruni F, Pastorelli M, Ciani F, Palazzuoli A, et al. Lectin-like oxidized-LDL receptor-1 (LOX-1) polymorphisms influence cardiovascular events rate during statin treatment. Int J Cardiol 2007;119(1):41-7.
48. de Maat MP, Jukema JW, Ye S, Zwinderman AH, Moghaddam PH, Beekman M, et al. Effect of the stromelysin-1 promoter on efficacy of pravastatin in coronary atherosclerosis and restenosis. Am J Cardiol 1999;83(6):852-6.
49. Kunnas TA, Lehtimaki T, Laaksonen R, Ilveskoski E, Janatuinen T, Vesalainen R, et al. Endothelial nitric oxide synthase genotype modulates the improvement of coronary blood flow by pravastatin: a placebo-controlled PET study. J Mol Med 2002;80(12):802-7.
50. Roncal C, Orbe J, Belzunce M, Rodriguez JA, Paramo JA. The 4G/5G PAI-1 polymorphism influences the endothelial response to IL-1 and the modulatory effect of pravastatin. J Thromb Haemost 2006;4(8):1798-803.
51. Boekholdt SM, Agema WR, Peters RJ, Zwinderman AH, van der Wall EE, Reitsma PH, et al. Variants of toll-like receptor 4 modify the efficacy of statin therapy and the risk of cardiovascular events. Circulation 2003;107(19):2416-21.
52. Holloway JW, Yang IA, Ye S. Variation in the toll-like receptor 4 gene and susceptibility to myocardial infarction. Pharmacogenet Genomics 2005;15(1):15-21.

# CHAPTER 4.3

# THE *PCSK9* E670G VARIANT AND REDUCED STATIN EFFECTIVENESS

Bas J.M. Peters[a]
Helmi Pett[b]
Olaf H. Klungel[a]
Bruno H.Ch. Stricker[c,d,e]
Bruce M. Psaty[f]
Nicole L. Glazer[f]
Kerri L. Wiggins[f]
Josh C. Bis[f]
Anthonius de Boer[a]
Anke-Hilse Maitland-van der Zee[a]

a Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, Utrecht, The Netherlands
b University of Helsinki, Helsinki, Finland
c Department of Epidemiology, Erasmus MC, Rotterdam, The Netherlands
d Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands
e Drug Safety Unit, Inspectorate for Health Care, The Hague, The Netherlands
f Cardiovascular Health Research Unit, Departments of Medicine, Epidemiology, and Health Services, University of Washington, Seattle, WA; Group Health Research Institute, Group Health Cooperative, Seattle, WA.

Submitted

## ABSTRACT

Although the efficacy of statins of has been well established, genetic variability has been shown to affect statin responsiveness. The aim of this study was to investigate the genetic influence of tagging SNPs within candidate genes involved in the cholesterol lowering pathway of statins on the effectiveness of statins in reducing the risk of the outcome myocardial infarction (MI). Participants from the Utrecht Cardiovascular Pharmacogenetics (UCP) studies were enrolled from a population-based registry of pharmacy records linked to hospital discharge records (PHARMO). Patients who received a prescription for an antihypertensive drug, and/or had hypercholesterolemia (prescription for a cholesterol-lowering drug or total cholesterol >5.0 mmol/l), were selected from the PHARMO database. We designed a nested case-control study in which cases were hospitalized for MI and controls were not. Patients were contacted through their community pharmacies. For this study, only hypercholesterolemic participants were selected. Logistic regression analysis was used to investigate pharmacogenetic interactions. The Heart and Vascular Health Study (HVH) was used to replicate findings from UCP. The study population included 668 cases and 1,217 controls. We selected 231 SNP of which 209 SNPs in 27 genes involved in the cholesterol lowering pathway passed quality control and were tested. Ten SNPs in eight genes were found to influence the effectiveness of statins in UCP, of which the most significant interaction was found with *SCARB1* rs4765615. Five out of ten statistically significant SNPs were available in the HVH study for replication. None of the HVH findings reached statistical significance. Carriers of the *PCSK9* rs505151 variant allele had less benefit from statin treatment in UCP, a finding that was replicated in the HVH study, although the formal test for interaction was not statistically significant.
In conclusion, variant allele carriers *PCSK9* E670G polymorphism do not seem to benefit/have less benefit from statin treatment and may benefit from more aggressive lipid-lowering treatment. Furthermore, confirmation of interactions reported with other SNPs in *SCARB1*, *PCSK9*, and *LIPC* should be pursued.

## INTRODUCTION

To reduce the risk of cardiovascular events, statins are among the most prescribed drugs worldwide. Although the efficacy has been well established in clinical trials,[1] interindividual differences in response exist.[2] Besides non-genetic factors such as age, concomitant drug use, and co-morbidities, it has been well recognized that variability in statin-related genes contribute to differences in response to statins. These include both genes in the lipid and non-lipid pathways.[2] Statins' foremost pharmacological action is the competitive inhibition of HMG-CoA reductase, the first enzyme and rate-limiting step in the cholesterol biosynthesis cascade. Subsequently, there is an increase in hepatic low-density lipoprotein (LDL) receptors resulting in increased LDL clearance from the blood stream. Although the *HMGCR* gene, encoding HMG-CoA reductase, is an important candidate gene for the pharmacogenomics of statins, a range of other cholesterol pathway related genes may be of importance for statin responsiveness. These include genes that are involved in the hepatic cholesterol metabolism or metabolism and transport of plasma lipoproteins. Well known examples of such genes that have previously been subject of pharmacogenomic research

are *LDLR*, encoding LDL receptor, *CETP*, encoding cholesteryl ester transfer protein and *APOE*, encoding apolipoprotein E.[2] Most pharmacogenetic studies have investigated the cholesterol lowering response to statins as opposed to clinically important outcomes such as myocardial infarction (MI). Therefore, the aim of this study was to investigate the genetic influence of tagging SNPs within candidate genes involved in the cholesterol lowering pathway of statins on the effectiveness of statins in reducing the risk of MI.

## METHODS

***Design and Setting***

This study was performed in part of the UCP study.[3 4] Participants from the Utrecht Cardiovascular Pharmacogenetics (UCP) studies were enrolled from the population-based Pharmaco-Morbidity Record Linkage System (PHARMO, www.pharmo.nl). PHARMO links drug dispensing histories from a representative sample of Dutch community pharmacies to the national registration of hospital discharges (Dutch National Medical Registry).

First, patients who received a prescription for an antihypertensive drug,[3] and/or had hypercholesterolemia (prescription for a cholesterol-lowering drug or total cholesterol > 5.0 mmol/l),[4] were selected from the PHARMO database for pharmacogenetic studies on antihypertensive drugs[3] and statins[4] respectively. From this cohort, a nested case-control study was designed using hospital discharge records. Patients hospitalized for MI (International Classification of Diseases (ICD)-9 code 410) were included as cases if they were registered in PHARMO for at least one year and were older than 18 years. The index date was defined as the date of hospitalization for the first MI. Controls met the same eligibility criteria as the cases, but had not developed MI. Participants were contacted through community pharmacies, where they received a letter in which the purpose of the study was explained. They were asked to return an informed consent form and a filled-out questionnaire. After the participant had consented to participate in the study, he/she was sent material for saliva collection. All participants were explicitly asked to consent for the collection, storage and genotyping of the DNA material. Approval for this study was obtained from the Medical Ethics Committee of the University Medical Center Utrecht, The Netherlands. For this study, all hypercholesterolemic (prescription for a cholesterol-lowering drug, total cholesterol >5.0 mmol/l, or self-reported hypercholesterolemia) participants were selected from the UCP study. In detail, the case-control ratios for sampling from the nested case-control study on the antihypertensive drugs[3] and statins[4] were one to one and one to three respectively.

***Ascertainment of exposure to statins (and other drugs)***

Coded pharmacy records were used to ascertain exposure to statins (and other drugs). In PHARMO, complete pharmacy records were available as of 1991, including the day of delivery, daily dose, and durations of therapy. To define exposure to statins, we assessed the association of different cumulative defined daily doses (DDD) with the risk of MI. The DDD is the dose per day for a drug used for its main indication in adults. Participants were considered exposed when the cumulative DDD of statin use was more than 180, whereas participants with the cumulative DDD of 180, less than 180, or no use were considered the reference group.For each patient we identified all prescriptions for concomitant drug use.

The projected end date of a prescription was calculated using information on the daily dose instruction and the quantity dispensed. We considered a patient a current user when the index date was between the start and end date of a prescription. Past users were patients who were not current users, but had used the drug prior to the index date.

***Assessment of potential confounding factors and effect modifiers***

Questionnaires were used to assess CVD risk factors such as smoking, hypertension, hypercholesterolemia, diabetes mellitus, use of alcohol, diet, history of cardiovascular diseases (CVDs), family history of CVDs, weight and height. Furthermore, information from the general practitioner files and laboratory registrations were available for part of the population. In case of a discrepancy between community pharmacy data and questionnaire data, community pharmacy data was the primary source for defining hypercholesterolemia and diabetes status. Ischemic heart disease (IHD) was defined as "yes" if a participant was hospitalized for an IHD or ever used nitrates.

***DNA collection and DNA extraction***

Part of patients were send three cotton swabs and tubes containing buffer to collect buccal cell samples as described elsewhere.[5] Other participants were sent an Oragene collection kit and donor instructions provided by the manufacturer (DNA Genotek, Ottawa, Canada).[4] DNA was extracted according to the manufacturer's instructions (http://www.dnagenotek.com/techsupport_documents.htm). Samples with a DNA concentration higher than 100 ng per µl were diluted to the Illumina Golden-Gate assay required 50 ng per µl.

***Candidate gene selection and SNP selection***

We selected a total of 231 SNPs in 27 genes that are involved in the cholesterol lowering pathway of statins. To assess common variation in each gene, we selected tagging SNPs within 200 bp (up- and downstream) with a minor-allele frequency (MAF) higher than 0.2 (based on a power calculation with 80% power to detect a SI of 2 or 0.5) and a $r^2$>0.8 using a web-based tool called QuickSNP version 1.1 (HapMap release 21,[6] U.S. residents with northern and western European ancestry (CEPH individuals)).[7] Additionally, dbSNP8 nonsynonymous coding SNPs (MAF higher than 0.2) and previously (pharmaco-)genetically associated SNPs were included. Illumina SNP designability scores lower than 0.4 (1.1 = best validated) or failure codes (http://www.illumina.com/downloads/GoldenGateDesign_TechNote.pdf) were either substituted with a SNP in linkage disequilibrium (LD) or, if no such SNP were available, removed from the SNP list, resulting in a final set of 231 SNPs.

***Genotyping***

For each individual participating in the study, SNPs were genotyped using the custom GoldenGate assay on an Illumina BeadStation 500 GX (Illumina Inc. San Diego, CA, USA). Genotype calls of all SNPs were individually examined for their resulting quality. SNPs with a low signal, poor clustering, deviation from Hardy–Weinberg equilibrium (HWE) (≤0.01) or a high number of missing genotypes (>10%) were excluded.

***Replication study (Heart and Vascular Health Study (HVH))***

The setting for the replication study was a large integrated health care system in Washington

State, called Group Health Cooperative (GHC). The data were from an ongoing case-control study of incident MI and stroke cases with a shared control group and has been described elsewhere.[9 10] The study was approved by the human subjects committee at GHC, and all study participants provided an informed consent. All study participants were GHC members aged 30-79 years. We selected MI cases and controls if they had a prescription for a cholesterol-lowering drug or total cholesterol measurement of >5.0 mmol/l. Cases were hospitalized for a non-fatal incident MI, identified from computerized hospital discharge abstracts and billing records.[9 10] Controls were a random sample of GHC members frequency matched to MI cases on age, sex, and calendar year of identification. The index date for MI cases was the date of admission for the first acute MI, whereas controls were assigned a computer-generated random date within the calendar year for which they had been selected. Medication use was ascertained using computerised GHC pharmacy records. Definitions of drug exposure matched the definitions from the UCP study. Eligibility and risk factor information were collected by trained medical record abstractors from a review of the GHC medical record using only data available prior to the index date and through a telephone interview. A venous blood sample was collected from all consenting subjects, and DNA was extracted from white blood cells using standard procedures. Genotype data was available from two sources. Part of the genotype data was available from the Illumina 370CNV BeadChip system. Imputation was performed using BIMBAM with reference to HapMap CEU using release 22, build 36 using one round of imputations and the default expectation-maximization warm-ups and runs. In addition, genotype data was available from an Illumina (Illumina Inc, San Diego California) GoldenGate custom panel using BeadArray® technology. SNPs that showed a significant interaction ($p<0.05$) with statin treatment in UCP were identified in the HVH study genotype data. Five of these SNPs had genotype data available from the Illumina 370CNV BeadChip system and/or the Illumina GoldenGate custom panel. For these SNPs, if a subject had genotype results available from both Illumina methods, the GoldenGate panel results were preferentially selected. One SNP had genotype data available only from the Illumina GoldenGate panel (n=865). All remaining SNPs had genotype data available only from the Illumina 370CNV BeadChip system (n=2,446). SNPs from the Illumina 370 CNV BeadChip system were chosen with a lower cut-off for the RSQR (or OEvar) score of 0.6. The RSQR denotes the average of the observed-to-expected variance ratio of any SNP, which indicates deviation from HWE and quality of imputation.

***Statistical methods***

The same analysis was applied to the UCP and HVH study. Logistic regression (LR) analysis was used to study the association between statins and the risk of MI, and to adjust for potential confounders. Matching variables --- age, sex, region, and index date --- were included in our statistical model. The inclusion of potential confounders in the LR model was motivated by the assessment of the influence of each potential confounder on the OR for the association between use of statins and risk of MI. The potential confounding factors that we considered were as follows: use of different cardiovascular drugs (antihypertensive drugs, platelet aggregation inhibitors, anticoagulants, other cholesterol-lowering drugs, and organic nitrates), use of alcohol, physical activity, family history of CVD, and other factors assessed by the questionnaire. Only covariates IHD and the use of calcium channel

blockers showed at least a 5% change in the regression coefficient (beta) for statin use; therefore, they were included in the LR model. We estimated the multiplicative synergy index (SI), which is the ratio of the OR in those with the variant to the OR in those without the variant.[11] For the significant (unadjusted or adjusted) pharmacogenetic associations, ORs were calculated separately in the strata defined by genotype. Heterozygotes and homozygotes for the variant allele of the *PCSK9* E670G (rs505151) polymorhpism were combined into one group, because of a low frequency homozygous variant allele carriers. For each SNP, HWE was tested using a $X^2$ goodness-of-fit test. Analyses were performed using SPSS statistical software version 16.0. Subsequently, q values (the positive false discovery rate (pFDR) analogue of the p value) were calculated for each gene-treatment interaction that was tested in UCP to account for multiple testing.[12]

## RESULTS

The total UCP study population included 1,885 individuals, of which 668 were MI cases and 1,217 controls. Table 1 describes the clinical characteristics of the population according to case control status. The well known cardiovascular risk factors smoking (current), a BMI of more than 30 kg/$m^2$, and the presence of IHD were more frequently seen in cases compared to controls. Current use of other non-statin cholesterol-lowering drugs was associated with a decreased risk of MI. Current use of beta-blockers and calcium channel blockers was more frequently seen in cases than controls, which is due to oversampling of nonantihypertensive users in the control group as described in the methods section.[13]

Out of the 231 selected SNPs, 209 passed quality control and were tested for an interaction with statin treatment. The LR analysis revealed ten SNPs in eight genes that significantly ($p<0.05$) interacted with statin treatment (table 2), either with or without adjustment for the additional confounding factors or both. *SCARB1* rs4765615 showed the most significant interaction, with a more beneficial effect of statins for GG and AG carriers (OR 0.30, 95% CI 0.22-0.42 and OR 0.30, 95% CI 0.18-0.50 respectively) as compared to AA carriers (OR 0.64, 95% CI 0.41-0.98). The *PCSK9*, and *ABCG5* gene were both represented by two SNPs among the significant interactions. The only nonsynonymous SNP that was found to interact with statin treatment, was *PCSK9* rs505151, for which variant allele carriers had no significant benefit from statin treatment (OR 0.63, 95% CI 0.30-1.32) compared to homozygous wildtype carriers who did benefit (OR 0.36, 95% CI 0.28-0.45). The five other SNPs that appeared to be implicated in the pharmacogenetics of statins were found in the *LRP1*, *LIPC*, *ABCA1*, *SOAT1*, and *PPARG* gene. The q value for the interaction with *SCARB1* rs4765615 and *PCSK9* rs10888896 was 0.19 and 0.24 respectively, whereas the q-value of the interactions with the other eight SNPs within the significant results was 0.57. The SIs for all of the SNPs can be found in Table II of the Supplementary data.

Out of the 10 SNPs that were found to modify the effectiveness of statins in the UCP study, the *PCSK9* SNP was genotyped in the HVH study using the Illumina GoldenGate panel only and the other nine were genotyped in the HVH study using the Illumina 370CNV BeadChip system only. Four of the nine had an RSQR score more than 0.6, and five had an RSQR score less than 0.6 and were therefore not included in the analysis. None of the five interactions tested in HVH showed a significant interaction (table 2). Nonetheless, similarly to the results from the UCP study, *PCSK9* rs505151 variant allele carriers had no significant benefit from statin treatment (OR 1.05, 95% CI 0.18-6.26), whereas homozygous wildtype

carriers did (OR 0.61, 95% CI 0.39-0.94). In addition, the point estimates and directionality of the non-significant HVH results for the *LIPC* rs16940379 interaction resemble the UCP results in which homozygous wildtype allele carriers appear to respond better to statin treatment compared to homozygous variant allele carriers.

***Table 1.*** Baseline characteristics UCP by case control status.

| | | Case n=668 | % | Control n=1217 | % | p |
|---|---|---|---|---|---|---|
| Gender | Female | 171 | 25.6% | 285 | 23.4% | 0.290 |
| Age (years) | Mean (sd) | 63.0 (9.9) | | 62.3 (9.4) | | 0.097 |
| Body Mass Index at ID | >30 kg/m2 | 140/607 | 23.1% | 193/1104 | 17.5% | 0.005 |
| Familial History CVD | Yes, <60 | 135/637 | 21.2% | 214/1167 | 18.3% | 0.103 |
| | Yes, >60 | 266/637 | 41.8% | 464/1167 | 39.8% | |
| Diabetes Status | Diabetes, no medication | 71/658 | 10.8% | 90/1203 | 7.5% | 0.052 |
| | Diabetes, medication | 75/658 | 11.4% | 144/1203 | 12.0% | |
| Smoking Status | Current | 152/624 | 24.4% | 195/1135 | 17.2% | 0.001 |
| | Past | 283/624 | 45.4% | 573/1135 | 50.5% | |
| Alcohol Status (consumptions) | No use | 118/646 | 18.3% | 162/1188 | 13.6% | 0.062 |
| | <=1 | 237/646 | 36.7% | 432/1188 | 36.4% | |
| | >1 - <2 | 170/646 | 26.3% | 328/1188 | 27.6% | |
| | >2 | 121/646 | 18.7% | 266/1188 | 22.4% | |
| Physical Activity | > 4 hrs a week | 491/645 | 76.1% | 939/1191 | 78.8% | 0.180 |
| Cumulative DDD use statins | >180 DDD | 218 | 32.6% | 646 | 53.1% | <0.001 |
| Type of Statin | Atorvastatin | 44/218 | 20.2% | 164/646 | 25.4% | <0.001 |
| | Pravastatin | 35/218 | 16.1% | 110/646 | 17.0% | |
| | Simvastatin | 125/218 | 57.3% | 335/646 | 51.9% | |
| | Other | 14/218 | 6.4% | 37/646 | 5.7% | |
| Ischemic Heart Disease | Yes | 211 | 31.6% | 268 | 22.0% | <0.001 |
| **Antihypertensives** | | | | | | |
| Calcium Channel Blockers | Current use | 142 | 21.3% | 193 | 15.9% | 0.003 |
| Diuretics | Current use | 82 | 12.3% | 171 | 14.1% | 0.279 |
| Beta Blockers | Current use | 275 | 41.2% | 415 | 34.1% | 0.002 |
| Ace Inhibitors | Current use | 140 | 21.0% | 273 | 22.4% | 0.459 |
| AT2 Receptor Antagonists | Current use | 48 | 7.2% | 110 | 9.0% | 0.165 |
| **Other drugs** | | | | | | |
| Non-statin Cholesterol Lowering drugs | Current use | 14 | 2.1% | 46 | 3.8% | 0.046 |
| Insulin | Ever use | 30 | 4.5% | 54 | 4.4% | 0.957 |
| Oral Antidiabetics | Current Use | 52 | 7.8% | 96 | 7.9% | 0.936 |
| Platelet Aggregation Inhibitors | Current Use | 219 | 32.8% | 438 | 36.0% | 0.162 |
| Coumarins | Current Use | 37 | 5.5% | 76 | 6.2% | 0.537 |

Abbreviations: ATII = Angiotensin II; DDD = Defined Daily Dosage

## DISCUSSION

In this population-based retrospective case-control study, we tested 209 SNPs in 27 genes involved in the cholesterol lowering pathway and found ten SNPs in eight genes to influence the effectiveness of statins in UCP, of which the most significant interaction was found with *SCARB1* rs4765615. Also genetic variability within the *PCSK9*, *LIPC*, *LRP1*, *ABCG5*, *ABCA1*, *PPARG*, and *SOAT1* genes were found to affect statin effectiveness. Five out of ten statistically significant SNPs were available in the HVH study for replication. None of the HVH findings reached statistical significance. Carriers of the *PCSK9* rs505151 variant allele had less benefit from statin treatment in UCP, a finding that was replicated in the HVH study, although the formal test for interaction was not statistically significant.

The highly significant interaction with *SCARB1* rs4765615 showed that homozygous carriers of the A allele did not benefit from statin treatment compared to those carrying one or two G alleles. Scavenger receptor class B member 1, encoded by *SCARB1*, functions as a receptor for high-density lipoprotein and plays an important role in the reverse cholesterol transport (RCT). Although the gene is associated with RCT and high density lipoprotein (HDL) cholesterol, recently a genetic variant other than *SCARB1* rs16940379 has been shown to affect the LDL cholesterol response to atorvastatin.[14] Despite the unknown underlying mechanism of this gene treatment interaction, this and one previous[14] study indicate a role for *SCARB1* in the response to statins. In the field of the pharmacogenomics of statins, genetic variability of *SCARB1* should be investigated in future studies. For *SCARB1* rs16940379 and four other SNPs that were found to interact with statin treatment in UCP, the imputation quality of the HVH study data was too poor and the SNPs were not used. Besides the interaction with *SCARB1* rs16940379 and *PCSK9* rs10888896 (which will be discussed hereafter), these interactions are generally characterized by high q-values, no well defined known functional SNP that is in LD with the interacting tagging SNP, and/or models that lack of gene-dose effect, suggesting that these findings are likely to be false positives.

*PCSK9*, encoding proprotein convertase subtilisin/kexin type 9, was found to be the third locus involved in autosomal dominant hypercholesterolemia (ADH).[15] PCSK9 promotes the degradation LDLR and overexpression has been shown to lead to higher LDL cholesterol (LDLc) levels, whereas loss-of-function mutations leads to lower LDLc.[16] Low LDL-c levels, caused by nonsense and missense mutations of *PCSK9*, have been shown to be substantially protective against CHD.[17] A range of mutations are known that affect the expression and activity of *PCSK9*.[18] The mutations that cause severe hypercholesterolemia are rare. However, there more common mutations have also been shown to affect lipid levels and possibly the effectiveness of statins. In 2005, Chen et al. found a relatively common nonsynonymous polymorphism *PCSK9* E670G to be an independent marker for higher plasma LDL-c levels.[19] The *PCSK9* E670G polymorphism was included in the current study. The findings of Chen et al. have been confirmed in some,[20-22] but not all[23-25] subsequent studies assessing the effect of *PCSK9* E670G on baseline LDLc levels. Norata et al. also showed an association with increased carotid artery intima media thickness,[22] whereas others did not show an association between *PCSK9* E670G and CAD/CHD/vascular disease risk.[21 24 25] In turn, it has been suggested that individuals carrying *PCSK9* loss-of-function polymorphisms have an increased lipid response to statins,[26] whereas

gain-of-function *PCSK9* polymorphisms have been shown to result in a decreased lipid response to statin therapy.[27 28]

*PCSK9* E670G has been investigated with respect to statin responsiveness in two studies. The PROSPER trial (40 mg pravastatin versus placebo) including almost 6,000 elderly subjects (mean age 75 years) could not reveal a significant difference in LDLc response or CHD risk reduction between carriers and non-carriers of the variant.[24] The Treating to New Targets (TNT) trial, carriers of the 670G allele were found to have a significantly smaller decrease in LDLc levels in response to statin treatment.[29] Similar to the results of the TNT study and the observations that *PCSK9* gain-of-function variants have a deleterious effect on statin responsiveness,[27 28] we show that carriers of the 670G allele have a better response to statin treatment in UCP (significant) and HVH (not significant). Nonetheless, the magnitude of the observation by the TNT study (1.8 mg/dl less LDLc reduction *PCSK9* 670G carriers) does not reflect the large effect of *PCSK9* E670G found in this study, suggesting a partially lipid independent mechanism behind this gene treatment interaction. Mechanistically, statins decrease the endogenous cholesterol biosynthesis by inhibition of 3-hydroxy-3-methylglutaryl–coenzyme A, which leads to transcriptional activation of both *LDLR* and *PCSK9*.[16] Although *PCSK9* counteracts the statin induced increased LDLR activity, the net result of statin treatment is reduction in plasma LDL cholesterol. In the case of *PCSK9* E670G polymorphism, it can be hypothesized that the net result of statin treatment is no longer beneficial due to its gain-of-function nature.

In addition, our tagging SNP approach revealed a second – more significant – *PCSK9* SNP that affected the response to statins. *PCSK9* rs10888896 resides in the first intron of the *PCSK9* gene and has not been extensively researched. No effect of this SNP on LDLc reduction was found in the Treating to New Targets (TNT) trial.[29] Possibly, the variant allele of the *PCSK9* is in LD with a (recessive) gain-of-function polymorphism, because our results suggest that homozygous wild-type variant carriers have no benefit from statin treatment. We did not test this SNP in the HVH population because the SNP was not genotyped and no SNPs were available that met the RSQR requirement. As this gene is of great interest for statin responsiveness, the effect of *PCSK9* rs10888896 should be assessed in future studies. None of the results from the UCP study showed a statistically significant interaction in the HVH study (table 2). Besides *PCSK9* E670G, only the interaction with *LIPC* rs16940379 showed a similar trend in the HVH study as was found in UCP study. In both UCP and HVH, homozygous variant allele carriers of *LIPC* rs16940379 appear to have no or less effect compared to hetero- and/or homozygous wild-type carriers. Hepatic lipase, encoded by *LIPC*, may affect statin responsiveness through its involvement in modulation of LDL size and density, which in turn is has been shown to affect the risk of CHD.[30] Although no data are available on the role of rs16940379 on hepatic lipase activity, our results indicate a role for *LIPC* in the pharmacogenomics of statins.

The present study has several limitations. For statistical power reasons, we assessed only SNPs with a MAF cut-off of 0.2, the consequences of which are that this study is likely to miss potentially important SNPs. Nevertheless, our study covers the common genetic variability within the selected candidate genes completely. Furthermore, we considered all statins and all dosage regimes as a homogenous group. Although, all statins share the primary working mechanism by which they lower cholesterol, it has been shown that there are differences between the different statins.[31 32] The sample size of the current study does

| Gene | SNP | UCP G | # | HWE | SI**(95% CI) | p | q | OR*(95% CI) | OR**(95% CI) | HVH RSQR |
|---|---|---|---|---|---|---|---|---|---|---|
| SCARB1 | rs4765615 | AA | 452 | | (ref) | 0.001 | 0.19 | 0.80 (0.53-1.20) | 0.63 (0.41-0.97) | 0.23 |
| | | AG | 837 | 0.54 | 0.39 (0.24-0.65) | 0.000 | | 0.33 (0.24-0.46) | 0.29 (0.21-0.41) | |
| | | GG | 411 | | 0.54 (0.30-0.99) | 0.045 | | 0.38 (0.24-0.61) | 0.31 (0.19-0.51) | |
| | | -- | 185 | | | | | | | |
| PCSK9 | rs10888896 | CC | 119 | | (ref) | 0.003 | 0.24 | 1.44 (0.65-3.18) | 1.38 (0.60-3.16) | 0.18 |
| | | CG | 658 | 0.12 | 0.23 (0.10-0.55) | 0.001 | | 0.37 (0.26-0.54) | 0.32 (0.22-0.47) | |
| | | GG | 1105 | | 0.26 (0.11-0.60) | 0.002 | | 0.40 (0.31-0.53) | 0.34 (0.25-0.45) | |
| | | -- | 3 | | | | | | | |
| ABCG5 | rs4245786 | AA | 1099 | | (ref) | 0.016 | 0.57 | 0.56 (0.43-0.73) | 0.46 (0.35-0.61) | 0.08 |
| | | AG | 692 | 0.23 | 0.53 (0.34-0.82) | 0.005 | | 0.26 (0.18-0.37) | 0.24 (0.16-0.34) | |
| | | GG | 93 | | 0.64 (0.25-1.65) | 0.355 | | 0.49 (0.19-1.24) | 0.38 (0.13-1.12) | |
| | | -- | 1 | | | | | | | |
| ABCG5 | rs1864815 | AA | 834 | | (ref) | 0.022 | 0.57 | 0.60 (0.44-0.81) | 0.48 (0.35-0.67) | 0.68 |
| | | AT | 835 | 0.61 | 0.55 (0.36-0.85) | 0.007 | | 0.29 (0.21-0.40) | 0.25 (0.18-0.36) | |
| | | TT | 198 | | 0.61 (0.30-1.25) | 0.178 | | 0.38 (0.20-0.74) | 0.42 (0.21-0.84) | |
| | | -- | 18 | | | | | | | |
| LIPC | rs16940379 | GG | 986 | | (ref) | 0.031 | 0.57 | 0.47 (0.35-0.62) | 0.38 (0.28-0.51) | 0.82 |
| | | CG | 749 | 0.68 | 0.72 (0.47-1.11) | 0.138 | | 0.32 (0.23-0.45) | 0.28 (0.20-0.40) | |
| | | CC | 149 | | 2.02 (0.93-4.40) | 0.078 | | 0.83 (0.38-1.82) | 0.81 (0.36-1.79) | |
| | | -- | 1 | | | | | | | |
| ABCA1 | rs4149264 | CC | 1192 | | (ref) | 0.034 | 0.57 | 0.52 (0.40-0.68) | 0.44 (0.34-0.58) | |
| | | CG | 607 | 0.67 | 0.58 (0.37-0.89) | 0.014 | | 0.27 (0.19-0.40) | 0.23 (0.15-0.34) | 0.35 |
| | | GG | 82 | | 1.25 (0.47-3.34) | 0.658 | | 0.54 (0.19-1.51) | 0.50 (0.16-1.58) | |
| | | -- | 4 | | | | | | | |
| PPARG | rs2972164 | GG | 585 | | (ref) | 0.034 | 0.57 | 0.42 (0.26-0.65) | 0.35 (0.22-0.57) | 1.00 |
| | | AG | 897 | 0.14 | 0.54 (0.34-0.86) | 0.010 | | 0.33 (0.24-0.45) | 0.28 (0.20-0.39) | |
| | | AA | 395 | | 0.75 (0.43-1.31) | 0.312 | | 0.60 (0.42-0.85) | 0.49 (0.34-0.72) | |
| | | -- | 8 | | | | | | | |
| PCSK9 | rs505151 | AA | 1632 | | (ref) | | | 0.42 (0.33-0.52) | 0.36 (0.28-0.45) | NI† |
| | | G | 158 | 0.23 | 2.11 (1.04-4.27) | 0.038 | 0.57 | 0.91 (0.46-1.79) | 0.63 (0.30-1.32) | |
| | | -- | 95 | | | | | | | |
| LRP1 | rs715948 | GG | 908 | | (ref) | 0.04 | 0.57 | 0.17 (0.08-0.37) | 0.17 (0.08-0.37) | 1.05 |
| | | AG | 794 | 0.7 | 1.10 (0.72-1.69) | 0.65 | | 0.49 (0.36-0.67) | 0.44 (0.31-0.60) | |
| | | AA | 181 | | 0.41 (0.19-0.88) | 0.022 | | 0.45 (0.33-0.60) | 0.36 (0.26-0.49) | |
| | | -- | 2 | | | | | | | |
| SOAT1 | rs2493121 | AA | 217 | | (ref) | 0.047 | 0.57 | 0.20 (0.11-0.39) | 0.19 (0.10-0.38) | |
| | | AT | 832 | 0.75 | 2.36 (1.19-4.67) | 0.014 | | 0.49 (0.36-0.68) | 0.43 (0.31-0.60) | 0.51 |
| | | TT | 824 | | 2.08 (1.05-4.15) | 0.037 | | 0.44 (0.32-0.61) | 0.36 (0.25-0.50) | |
| | | -- | 12 | | | | | | | |

| # | HWE | SI** (95% CI) | p | OR* (95% CI) | OR** (95%CI) |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| | | | | | |
| 1071 | | (ref) | 0.82 | 0.96 (0.70-1.33) | 0.80 (0.57-1.13) |
| 1104 | 0.646 | 1.11 (0.73-1.70) | 0.61 | 0.99 (0.72-1.36) | 0.83 (0.60-1.17) |
| 271 | | 1.21 (0.60-2.42) | 0.60 | 1.23 (0.62-2.44) | 0.98 (0.47-2.04) |
| 0 | | | | | |
| 168 | | (ref) | 0.45 | 0.88 (0.66-1.18) | 0.69 (0.51-0.95) |
| 939 | 0.637 | 1.32 (0.86-2.01) | 0.20 | 1.15 (0.81-1.63) | 1.02 (0.70-1.46) |
| 1339 | | 1.38 (0.59-3.22) | 0.39 | 1.38 (0.54-3.53) | 1.43 (0.52-3.93) |
| 0 | | | | | |
| | | | | | |
| 507 | | (ref) | 0.89 | 1.06 (0.73-1.54) | 0.96 (0.64-1.43) |
| 1176 | 0.48 | 0.93 (0.59-1.47) | 0.76 | 1.00 (0.73-1.37) | 0.79 (0.57-1.11) |
| 763 | | 0.88 (0.51-1.52) | 0.64 | 1.02 (0.64-1.64) | 0.84 (0.50-1.39) |
| 0 | | | | | |
| 788 | | (ref) | 0.56 | 0.69 (0.46-1.04) | 0.61 (0.39-0.94) |
| 76 | 0.621 | 1.43 (0.43-4.73) | 0.55 | 1.47 (0.38-5.71) | 1.05 (0.18-6.26) |
| 1 | | | | | |
| 271 | | (ref) | 0.93 | 0.95 (0.70-1.29) | 0.79 (0.57-1.09) |
| 1011 | 0.058 | 0.99 (0.65-1.51) | 0.96 | 1.06 (0.76-1.47) | 0.90 (0.63-1.28) |
| 1164 | | 0.87 (0.44-1.75) | 0.71 | 1.12 (0.54-2.31) | 0.86 (0.39-1.86) |
| 0 | | | | | |
| | | | | | |

***Table 2.*** Significant interactions in UCP and replication results from the HVH study.

For each gene and SNP, the number of subjects for each genotype is given, together with the HWE. The SI was used to test for the interaction between the genotype and statin treatment. For each interaction, the p value of the heterozygote group denotes whether there is statistically significant difference in statin effectiveness between heterozygote carriers and the homozygous reference group. Similarly, the p value of the homozygous variant allele group denotes whether there is statistically significant difference in statin effectiveness between homozygous variant carriers and the homozygous reference group. The upper p value for each interaction denotes whether there is an overall difference in the effectiveness of statins between the three genotype groups (with two degrees of freedom). The q value gives an estimate of the proportion of false discoveries among the statistically significant results. For each genotype stratum, the OR reflects the effectiveness of statins within the specific genotype group. The RSQR is a measure for the quality of an imputed SNP in the HVH (cut-off=0.6).

-- = missing genotype
OR = odds ratio
SI = synergy index
* = adjusted for age, sex, region, index date
** = adjusted for age, sex, region, index date, use of calcium channel blockers, and ischemic heart disease
G = genotype
# = number of participants
HWE = Hardy-Weinberg equilibrium
lower = lower limit of the 95% confidence interval
upper = upper limit of the 95% confidence interval
p = p-value for the interaction
q = q-value for the interaction
NI = not imputed
RSQR= the average of the observed by expected variance ratio
† = HVH study data only available from the Illumina GoldenGate panel

not allow to study the interaction between individual statins and genetic variability. Also, our replication study has two limitations. First, five out of ten SNPs that were found to interact with statins in UCP were not available in the HVH study, or had a low imputation score. Second, imputation of rs1864815, rs16940379, and rs2972164 gives uncertainty about the true genotype and thereby lowers the statistical power to detect an interaction. A strength of the current study is the availability of a replication study that used the same study design (case-control, exposure definition, outcome, data analysis) as the UCP study. In addition, a centralized system to define exposure (availability of the community pharmacy records) and outcomes (hospital records) was available. Statin exposure was defined based on pharmacy records, which validity to measure drug exposure has been shown to be good.[33] Testing a large number of variables, the possibility of chance findings (spurious associations) increases. We addressed the issue of multiple testing by calculation of q values that suggested that a large proportion of our significant interaction were false discoveries. Finally, our study assessed the impact of gene-treatment interactions on the clinically important (endpoint) outcome MI, instead of surrogate parameters.

We show that the *PCSK9* E670G polymorphism is of great importance for the effectiveness of statins in reducing the risk of MI because carriers of one or two variant alleles do not benefit from statin treatment. *PCSK9* gain-of-function variant carriers have been shown to have high untreated and treated cholesterol levels, but a similar percentage LDLc fall from statin treatment compared to wild-type carriers.[27] Therefore, from a clinical perspective, carriers of *PCSK9* 670G variant allele may benefit from more aggressive lipid-lowering treatment.

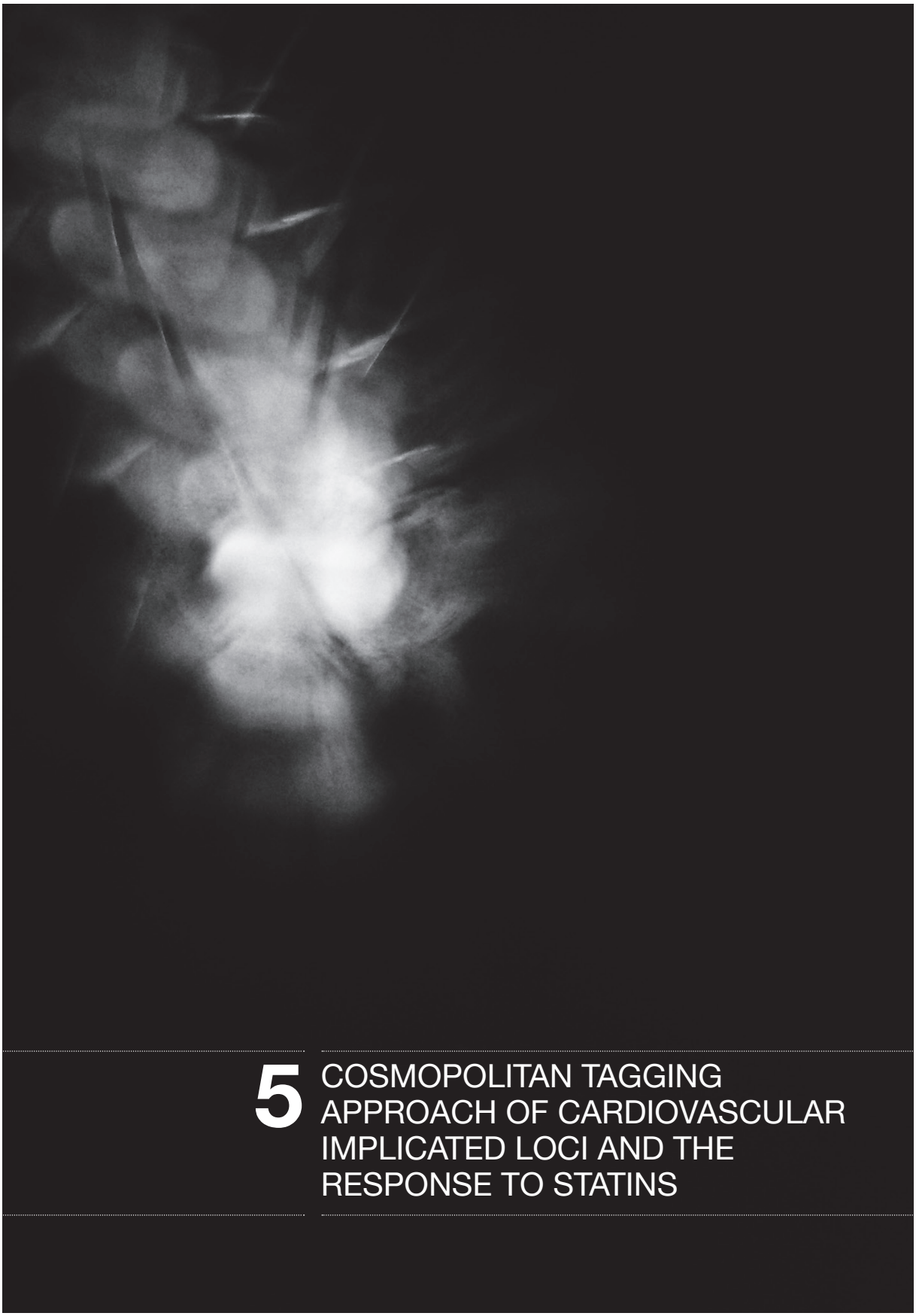
In conclusion, variant allele carriers *PCSK9* E670G polymorphism do not seem to benefit/have less benefit from statin treatment and confirmation of the pharmacogenetic associations with *LIPC* rs16940379, *SCARB1* rs16940379, and *PCSK9* rs10888896 should be subject of future research to pinpoint possible causal variants that affect statin responsiveness.

## REFERENCES

1. Cheung BM, Lauder IJ, Lau CP, Kumana CR. Meta-analysis of large randomized controlled trials to evaluate the impact of statins on cardiovascular outcomes. Br J Clin Pharmacol 2004;57(5):640-51.
2. Peters BJ, Klungel OH, de Boer A, Maitland-van der Zee AH. Genetic determinants of response to statins. Expert Rev Cardiovasc Ther 2009;7(8):977-83.
3. van Wieren-de Wijer DB, Maitland-van der Zee AH, de Boer A, Kroon AA, de Leeuw PW, Schiffers P, et al. Interaction between the Gly460Trp alpha-adducin gene variant and diuretics on the risk of myocardial infarction. J Hypertens 2009;27(1):61-8.
4. Peters BJM, Rodin AS, Klungel OH, van Duijn CM, Stricker BHC, Slot Rvt, et al. Pharmacogenetic interactions between ABCB1 and SLCO1B1 tagging SNPs and the effectiveness of statins in the prevention of myocardial infarction. Pharmacogenomics 2010;11(8):1065-1076.
5. van Wieren-de Wijer DB, Maitland-van der Zee AH, de Boer A, Stricker BH, Kroon AA, de Leeuw PW, et al. Recruitment of participants through community pharmacies for a pharmacogenetic study of antihypertensive drug treatment. Pharm World Sci 2008.
6. Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA, et al. A second generation human haplotype map of over 3.1 million SNPs. Nature 2007;449(7164):851-61.

7. Grover D, Woodfield AS, Verma R, Zandi PP, Levinson DF, Potash JB. QuickSNP: an automated web server for selection of tagSNPs. Nucleic Acids Res 2007;35(Web Server issue):W115-20.
8. Sherry ST, Ward MH, Kholodov M, Baker J, Phan L, Smigielski EM, et al. dbSNP: the NCBI database of genetic variation. Nucleic Acids Res 2001;29(1):308-11.
9. Psaty BM, Heckbert SR, Atkins D, Lemaitre R, Koepsell TD, Wahl PW, et al. The risk of myocardial infarction associated with the combined use of estrogens and progestins in postmenopausal women. Arch Intern Med 1994;154(12):1333-9.
10. Psaty BM, Heckbert SR, Koepsell TD, Siscovick DS, Raghunathan TE, Weiss NS, et al. The risk of myocardial infarction associated with antihypertensive drug therapies. JAMA 1995;274(8):620-5.
11. Khoury MJ, Flanders WD. Nontraditional epidemiologic approaches in the analysis of gene-environment interaction: case-control studies with no controls! Am J Epidemiol 1996;144(3):207-13.
12. Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proc Natl Acad Sci U S A 2003;100(16):9440-5.
13. Peters BJM, Rodin AS, Klunge lOH, Stricker BH, de Boer A, Maitland-van der Zee AH. Pharmacogenetic interactions between ABCB1 and SLCO1B1 tagging SNPs and the effectiveness of statins in the prevention of myocardial infarction. Pharmacogenomics 2010;August (in press).
14. Cerda A, Genvigir FD, Arazi SS, Hirata MH, Dorea EL, Bernik MM, et al. Influence of SCARB1 polymorphisms on serum lipids of hypercholesterolemic individuals treated with atorvastatin. Clin Chim Acta 2010;411(9-10):631-7.
15. Abifadel M, Varret M, Rabes JP, Allard D, Ouguerram K, Devillers M, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nat Genet 2003;34(2):154-6.
16. Horton JD, Cohen JC, Hobbs HH. Molecular biology of PCSK9: its role in LDL metabolism. Trends Biochem Sci 2007;32(2):71-7.
17. Cohen JC, Boerwinkle E, Mosley TH, Jr., Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. N Engl J Med 2006;354(12):1264-72.
18. Ding K, Kullo IJ. Molecular population genetics of PCSK9: a signature of recent positive selection. Pharmacogenet Genomics 2008;18(3):169-79.
19. Chen SN, Ballantyne CM, Gotto AM, Jr., Tan Y, Willerson JT, Marian AJ. A common PCSK9 haplotype, encompassing the E670G coding single nucleotide polymorphism, is a novel genetic marker for plasma low-density lipoprotein cholesterol levels and severity of coronary atherosclerosis. J Am Coll Cardiol 2005;45(10):1611-9.
20. Evans D, Beil FU. The E670G SNP in the PCSK9 gene is associated with polygenic hypercholesterolemia in men but not in women. BMC Med Genet 2006;7:66.
21. Hsu LA, Teng MS, Ko YL, Chang CJ, Wu S, Wang CL, et al. The PCSK9 gene E670G polymorphism affects low-density lipoprotein cholesterol levels but is not a risk factor for coronary artery disease in ethnic Chinese in Taiwan. Clin Chem Lab Med 2009;47(2):154-8.
22. Norata GD, Garlaschelli K, Grigore L, Raselli S, Tramontana S, Meneghetti F, et al. Effects of PCSK9 variants on common carotid artery intima media thickness and relation to ApoE alleles. Atherosclerosis 2008;208(1):177-82.
23. Kotowski IK, Pertsemlidis A, Luke A, Cooper RS, Vega GL, Cohen JC, et al. A spectrum of PCSK9 alleles contributes to plasma levels of low-density lipoprotein cholesterol. Am J Hum Genet 2006;78(3):410-22.
24. Polisecki E, Peter I, Robertson M, McMahon AD, Ford I, Packard C, et al. Genetic variation at the PCSK9 locus moderately lowers low-density lipoprotein cholesterol levels, but does not significantly lower vascular disease risk in an elderly population. Atherosclerosis 2008;200(1):95-101.
25. Scartezini M, Hubbart C, Whittall RA, Cooper JA, Neil AH, Humphries SE. The PCSK9 gene R46L variant is associated with lower plasma lipid levels and cardiovascular risk in healthy U.K. men. Clin Sci (Lond) 2007;113(11):435-41.

26. Berge KE, Ose L, Leren TP. Missense mutations in the PCSK9 gene are associated with hypocholesterolemia and possibly increased response to statin therapy. Arterioscler Thromb Vasc Biol 2006;26(5):1094-100.
27. Humphries SE, Whittall RA, Hubbart CS, Maplebeck S, Cooper JA, Soutar AK, et al. Genetic causes of familial hypercholesterolaemia in patients in the UK: relation to plasma lipid levels and coronary heart disease risk. J Med Genet 2006;43(12):943-9.
28. Naoumova RP, Tosi I, Patel D, Neuwirth C, Horswell SD, Marais AD, et al. Severe hypercholesterolemia in four British families with the D374Y mutation in the PCSK9 gene: long-term follow-up and treatment response. Arterioscler Thromb Vasc Biol 2005;25(12):2654-60.
29. Thompson JF, Hyde CL, Wood LS, Paciga SA, Hinds DA, Cox DR, et al. Comprehensive whole-genome and candidate gene analysis for response to statin therapy in the Treating to New Targets (TNT) cohort. Circ Cardiovasc Genet 2009;2(2):173-81.
30. Zambon A, Deeb SS, Pauletto P, Crepaldi G, Brunzell JD. Hepatic lipase: a marker for cardiovascular disease risk and response to therapy. Curr Opin Lipidol 2003;14(2):179-89.
31. Jones P, Kafonek S, Laurora I, Hunninghake D. Comparative dose efficacy study of atorvastatin versus simvastatin, pravastatin, lovastatin, and fluvastatin in patients with hypercholesterolemia (the CURVES study). Am J Cardiol 1998;81(5):582-7.
32. Weitz-Schmidt G. Statins as anti-inflammatory agents. Trends Pharmacol Sci 2002;23(10):482-6.
33. Lau HS, de Boer A, Beuning KS, Porsius A. Validation of pharmacy records in drug exposure assessment. J Clin Epidemiol 1997;50(5):619-25.



# 5 COSMOPOLITAN TAGGING APPROACH OF CARDIOVASCULAR IMPLICATED LOCI AND THE RESPONSE TO STATINS

# CHAPTER 5.1

# A CARDIOVASCULAR GENE-CENTRIC 50 K SNP ARRAY AND THE RESPONSE TO STATINS: A PILOT STUDY

Bas J.M. Peters
Olaf H. Klungel
Anthonius de Boer
Anke-Hilse Maitland-van der Zee

Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, Utrecht, The Netherlands

## ABSTRACT

Several genetic markers have been identified by both candidate gene studies and (three) genome-wide association studies (GWAS) that were shown to affect statin response in more than one study and many others have been proposed, but not reproduced. Although a GWAS approach is of great value, it is limited by relatively low statistical power. The aim of the current pilot study was to investigate modification of the effectiveness of statins in reducing the risk of myocardial infarction (MI) by SNPs represented on a cardiovascular gene-centric 50K SNP array.

We conducted a case-control study using a population-based registry of pharmacy records linked to the hospital discharge records. Within a hypercholesterolemic cohort we included 664 myocardial infarction cases and 390 controls. Out of over 50 K SNPs covering approximately 2,000 loci, we tested 18,011 SNPs that passed quality control and had a minimum allele frequency of 0.2 or more for the interaction with statin treatment. The most significant interactions were found with *RBP1* rs295488 and *STARD13* rs2764625 showing p values of $3.42*10^{-5}$ and $5.32*10^{-5}$ respectively, and q values of 0.46 (both), whereas the Bonferroni corrected p value threshold was set at $2.8*10^{-6}$. Among the interactions with a p value smaller than 0.001, *RHOBTB1* (encoding Rho-related BTB domain-containing protein 1) was most closely related to the working mechanism of statins.

In conclusion, no SNPs reached statistical significance and a future meta-analysis within a existing consortium should reveal whether the most significant genes (*RPB1* and *STARD13*) and candidate gene *RHOBTB1* are genuine interactions or represent false positive findings.

## INTRODUCTION

Statins reduce low density lipoprotein cholesterol (LDLc) and are widely prescribed to reduce the risk of cardiovascular disease (CVD). Genetic factors have been shown to influence the response to statin treatment.[1] Previous efforts to investigate the pharmacogenetics of statin treatment include many candidate gene studies,[2] as well as three genome-wide association studies (GWAS).[3 4] So far, several genetic markers have been identified that were shown to affect statin response in more than one study and many other have been proposed, but not reproduced.[2] Although a GWAS approach is of great value, it is limited by relatively low statistical power (due to multiple testing) to detect small effects that are often seen in complex diseases such as CVD. Also, the costs and incomplete coverage in the HapMap samples are limitations of a GWAS approach.[5]

Informed by GWAS of vascular and inflammatory disease, expression quantitative trait loci implicated in atherosclerosis, pathway based approaches and comprehensive literature searching, a custom 50 K SNP genotyping array (IBC array) was designed by investigators from the Institute of Translational Medicine and Therapeutics (ITMAT), Broad Institute, and National Heart Lung and Blood Institute (NHLBI) supported Candidate-gene Association Resource (CARe) Consortium.[5] General advantages of the IBC array over a GWAS include (i) assessment of only potentially relevant loci, (ii) greater density of markers within those loci, (iii) relatively cheap, and (iv) usage of the IBC array in a large consortium gives the opportunity to replicate findings or initialize a meta-analysis.

The aim of the current preliminary study was to investigate modification of the effectiveness

of statins in reducing the risk of myocardial infarction (MI) by SNPs represented on a cardiovascular gene-centric 50K SNP array.[5]

## METHODS

Description of the study design that was used for this study has been described elsewhere.[6] Briefly, participants from the Utrecht Cardiovascular Pharmacogenetics (UCP) studies were enrolled from the population-based Pharmaco-Morbidity Record Linkage System (PHARMO, www.pharmo.nl). PHARMO links drug dispensing histories from a representative sample of Dutch community pharmacies to the national registration of hospital discharges (Dutch National Medical Registry).

Patients who received a prescription for an antihypertensive drug,[7] or had hypercholesterolemia (prescription for a cholesterol-lowering drug or total cholesterol >5.0 mmol/l),[6] were selected from the PHARMO database for pharmacogenetic studies on antihypertensive drugs[7] and statins[6] respectively. From this cohort, a nested case-control study was designed with all hypercholesterolemic (prescription for a cholesterol-lowering drug, total cholesterol >5.0 mmol/l, or self-reported hypercholesterolemia) participants. Patients hospitalized for MI (International Classification of Diseases (ICD)-9 code 410) were included as cases if they were registered in PHARMO for at least one year and were older than 18 years. The index date was defined as the date of hospitalization for the first MI. Controls met the same eligibility criteria as the cases, but had not developed MI. Participants were contacted through community pharmacies. All participants were explicitly asked to consent for the collection, storage and genotyping of the DNA material. Approval for this study was obtained from the Medical Ethics Committee of the University Medical Center Utrecht, The Netherlands.

Coded pharmacy records were used to ascertain exposure to statins (and other drugs). In PHARMO, complete pharmacy records were available as of 1991, including the day of delivery, daily dose, and durations of therapy. Participants were considered exposed to statins when the cumulative DDD[8] of statin use was more than 180, whereas participants with the cumulative DDD of 180, less than 180, or no use were considered the reference group. For each patient we identified all prescriptions for concomitant drug use, and coded each patient as a current, past, or no usage of a certain drug. Questionnaires were used to assess CVD risk factors such as smoking, hypertension, hypercholesterolemia, diabetes mellitus, use of alcohol, diet, history of cardiovascular diseases (CVDs), family history of CVDs, weight and height. Patients were send three cotton swabs and tubes containing buffer to collect buccal cell samples or an Oragene collection kit (DNA Genotek, Ottawa, Canada).[6]

Genotyping was performed using the Illumina (Illumina Inc. San Diego, CA, USA) IBC Candidate Gene array, version 3, representing 53,831 SNPs.[5] Genotype calls of all SNPs were individually examined for their resulting quality. The quality control (QC) criteria consisted of exclusion of SNPs with a less than 95% call rate. Individuals with a SNP call rate less than 95%, related samples, contaminated samples and population outliers were excluded using PLINK and EIGENSTRAT.[9] For the analysis, we excluded SNPs with a minimum allele frequency (MAF) of less than 0.2 and/or a Hardy-Weinberg Equilibrium $<1*10^{-7}$.

Logistic regression (LR) analysis was used to study the association between statins and

the risk of MI, and to adjust for potential confounders. Matching variables --- age, sex, region, and index date --- were included in each statistical model. Covariates IHD, family history of CVD, hypertension status, and the use of ACE inhibitors showed at least a 10% change in the regression coefficient (beta) for statin use; therefore, they were included in the LR model. We estimated the multiplicative synergy index (SI), which is the ratio of the OR in those with the variant to the OR in those without the variant [10]. The genotype data was coded as 0, 1, and 2 for each allele present (allelic). Analyses were performed using PLINK. The Bonferroni corrected (18,000 tests) p value for statistical significance was set at $2.8*10^{-6}$. In addition, q values (the positive false discovery rate (pFDR) analogue of the p value) were calculated for each gene-treatment interaction that was tested in UCP to account for multiple testing.[11]

For the interactions with a p value smaller than 0.001, a webtool called Gene Relationships Across Implicated Loci (GRAIL) was used to identify nearby genes (based on a linkage disequilibrium (LD) $r^2$ of 0.5).[12]. Furthermore, WGA viewer was used to identify possible functional SNPs in LD ($r^2$ 0.8) with SNPs interacting with statin treatment ($p < 0.001$).

## RESULTS

We were able to include 664 hypercholesterolemic MI cases and 390 hypercholesterolemic controls. The clinical characteristics of all participants by case control status can be found in table 1. The IBC array version 3 comprised 53,831 SNPs of which a total of 49,771 SNPs passed QC, and a total of 18,011 SNPs passed QC had a MAF of 0.2 or more. These SNPs were tested for the interaction with statin treatment. Table 2 gives an overview of all pharmacogenomic interactions with a p value of less than 0.001, representing a total of 24 SNPs and 10 different genes. GRAIL identified 19 additional nearby genes (table 2). No functional SNPs were found to be in LD with any of the SNPs listed in table 2. None of the pharmacogenomic interactions met the Bonferroni corrected p value threshold of $2.8*10^{-6}$. The most significant interactions with statin treatment were found with *RBP1* rs295488 and *STARD13* rs2764625 showing p values of $3.42*10^{-5}$ and $5.32*10^{-5}$ respectively, and q values of 0.46 (both).

***Table 1.*** Clinical characteristics by case control status

| | | Case (n=664) Count | % | Control (n=390) Count | % |
|---|---|---|---|---|---|
| Gender | Female | 183 | 27.6% | 102 | 26.2% |
| Age | Year (SD) | 63.1 (9.9) | | 63.2 (9.4) | |
| BMI | >30 | 134/604 | 22.2% | 54/356 | 15.2% |
| Family history of CVD | Yes, <60 | 64/641 | 10.0% | 2/382 | 0.5% |
| | Yes, >60 | 144/641 | 22.5% | 26/382 | 6.8% |
| Self reported diagnosis hypertension | | 466/654 | 71.3% | 321/686 | 83.2% |
| Diabetes status | Yes, no medication | 69/654 | 10.6% | 32/389 | 8.2% |
| | Yes, medication | 76/654 | 11.6% | 49/389 | 12.6% |
| Smoking status | Current | 157/619 | 25.4% | 55/358 | 15.4% |
| | Past | 281/619 | 45.4% | 185/358 | 51.7% |
| Use of alcohol per day (glasses) | No use | 118/631 | 18.7% | 52/290 | 17.9% |
| | <=1 | 242/631 | 38.4% | 94/290 | 32.4% |
| | >1 - <2 | 167/631 | 26.5% | 78/290 | 26.9% |
| | >2 | 104/631 | 16.5% | 66/290 | 22.8% |
| Physical Activity Leisure | > 4 hrs a week | 469/642 | 73.1% | 292/389 | 75.1% |
| Calcium Channel Blockers | Current Use | 144 | 21.7% | 90 | 23.1% |
| | Past Use | 88 | 13.3% | 59 | 15.1% |
| Diuretics | Current Use | 89 | 13.4% | 81 | 20.8% |
| | Past Use | 70 | 10.5% | 50 | 12.8% |
| Beta Blockers | Current Use | 276 | 41.6% | 185 | 47.4% |
| | Past Use | 107 | 16.1% | 75 | 19.2% |
| ACE inhibitors | Current Use | 131 | 19.7% | 132 | 33.8% |
| | Past Use | 63 | 9.5% | 49 | 12.6% |
| ATII antagonists | Current Use | 50 | 7.5% | 40 | 10.3% |
| | Past Use | 27 | 4.1% | 7 | 1.8% |
| Oral Antidiabetics | Current Use | 52 | 7.8% | 34 | 8.7% |
| | Past Use | 16 | 2.4% | 9 | 2.3% |
| Platelet Aggregation Inhibitors | Current Use | 201 | 30.3% | 142 | 36.4% |
| | Past Use | 78 | 11.7% | 34 | 8.7% |
| Coumarins | Current Use | 32 | 4.8% | 28 | 7.2% |
| | Past Use | 48 | 7.2% | 37 | 9.5% |
| Statins | 0-180 DDD | 445 | 67.0% | 206 | 52.8% |
| | >180 DDD | 219 | 33.0% | 184 | 47.2% |
| Ischemic Heart Disease | | 257 | 38.7% | 125 | 32.1% |

ATII = Angiotensin II; ACE = Angiotensin Converting Enzyme; CVD = Cardiovascular Disease

***Table 2.*** Interactions between IBC array SNPs and statin treatment with a p value smaller than 0.001.

| Gene | CHR | SNP | BP | Allele | # | SI* | P | SI** | p | q | Nearby genes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *RBP1* | 3 | rs295488 | 140722013 | A | 1054 | 2.308 | 6.56E-05 | 2.482 | 3.42E-05 | 0.46 | *COPB2, NMNAT3, MRPS22, RBP2* |
| *STARD13* | 13 | rs2764625 | 32684615 | C | 1050 | 0.4237 | 0.00014 | 0.381 | 5.32E-05 | 0.46 | |
| *VEPH1* | 3 | rs2305619 | 158637555 | A | 1054 | 2.058 | 0.00050 | 2.307 | 0.00012 | 0.63 | *CCNL1, PTX3* |
| *VEPH1* | 3 | rs2120243 | 158630262 | A | 1054 | 1.938 | 0.00141 | 2.262 | 0.0001 | 0.63 | *CCNL1, PTX3* |
| *STARD13* | 13 | rs2764626 | 32676469 | G | 1053 | 0.4641 | 0.00058 | 0.4163 | 0.00021 | 0.63 | |
| *TNNT3* | 11 | rs2734498 | 1899790 | G | 1047 | 0.4686 | 0.00040 | 0.4369 | 0.00026 | 0.63 | *LSP1* |
| *STARD13* | 13 | rs2858126 | 32679381 | C | 1054 | 0.4766 | 0.00080 | 0.4262 | 0.00027 | 0.63 | |
| no gene | 5 | rs4912894 | 139217146 | A | 1052 | 0.5072 | 0.00097 | 0.4643 | 0.00039 | 0.63 | *PSD2, NRG2* |
| *PIK3CB* | 3 | rs2595932 | 139878274 | A | 1053 | 1.986 | 0.00088 | 2.15 | 0.00043 | 0.63 | *FAM62C, MRAS, FAIM, TXNDC6, CEP70* |
| *NRG2* | 5 | rs197197 | 139329099 | C | 1051 | 2.027 | 0.00108 | 2.187 | 0.00054 | 0.63 | |
| *LGALS9* | 17 | rs3763959 | 22981461 | A | 1052 | 0.5287 | 0.00185 | 0.474 | 0.00054 | 0.63 | *KSR1, NOS2A* |
| *KCNE1* | 21 | rs1007295 | 34760937 | G | 1054 | 0.4477 | 0.00015 | 0.4671 | 0.00056 | 0.63 | |
| no gene | 7 | rs1922086 | 155708987 | A | 1054 | 0.4626 | 0.00037 | 0.4589 | 0.00057 | 0.63 | |
| no gene | 3 | rs295470 | 140696593 | G | 1054 | 2.074 | 0.00057 | 2.137 | 0.00060 | 0.63 | *COPB2, MRPS22, RBP2, RBP1* |
| no gene | 4 | rs546829 | 75175236 | A | 1051 | 1.989 | 0.00102 | 2.118 | 0.00063 | 0.63 | *MTHFD2L, CXCL3, CXCL2* |
| no gene | 13 | rs2066219 | 68428665 | G | 1043 | 0.4505 | 0.00129 | 0.4126 | 0.00064 | 0.63 | |
| *PIK3CB* | 3 | rs361088 | 139865891 | C | 1054 | 1.912 | 0.00143 | 2.078 | 0.00066 | 0.63 | *FAM62C, MRAS, FAIM, TXNDC6, CEP70* |
| *RHOBTB1* | 10 | rs7913431 | 62352314 | G | 1054 | 0.4911 | 0.00054 | 0.482 | 0.00067 | 0.63 | |
| *PIK3CB* | 3 | rs2197387 | 139960328 | G | 1049 | 1.923 | 0.00123 | 2.054 | 0.00074 | 0.63 | *FAM62C, MRAS, FAIM, TXNDC6, CEP70* |
| *MTHFD1L* | 6 | rs6557106 | 151320417 | G | 1053 | 2.036 | 0.00075 | 0.4531 | 0.00075 | 0.63 | |
| *RBP1* | 3 | rs2071388 | 140719373 | G | 1054 | 2.036 | 0.00075 | 2.101 | 0.00077 | 0.63 | *COPB2, MRPS22, RBP2* |
| no gene | 4 | rs1837559 | 75177957 | G | 1054 | 1.985 | 0.00105 | 2.087 | 0.00080 | 0.63 | *MTHFD2L, CXCL3, CXCL2* |
| *RHOBTB1* | 10 | rs957854 | 62367648 | G | 1054 | 0.4975 | 0.00068 | 0.4892 | 0.00086 | 0.65 | |
| *PIK3CB* | 3 | rs361063 | 139887068 | C | 1053 | 1.9 | 0.00154 | 2.026 | 0.00095 | 0.69 | FAM62C, MRAS, FAIM, TXNDC6, CEP70 |

CHR = Chromosome, BP = Physical position (base-pair), Allele = Tested allele (minor allele by default), # = Number of participants, SI = Synergy index, * = Adjusted for age, gender, index date, and region, ** = Adjusted for age, gender, index date, region, IHD, family history of CVD, hypertension status, and the use of ACE inhibitors

## DISCUSSION

In this case control study on the pharmacogenomics of statins, out of 18,000 SNPs from a cardiovascular gene-centric genotyping array with a MAF of 0.2, we did not identify any SNPs that reached the statistical significance p value threshold of $2.8*10^{-6}$. Suggestive p values lower than $5.4*10^{-5}$ were found for the *RPB1* and *STARD13* gene. These interactions both showed a q value of 0.46.

Although there are several genes that have repeatedly been shown to affect the response to statins pharmacokinetically and pharmacodynamically,[2 13] no well known candidate genes were found among the most significant interacting genes (or nearby genes). One gene in table 2 could be considered as a candidate gene, namely *RHOBTB1*. This gene encodes Rho-related BTB domain-containing protein 1 and belongs to the Rho family of the small GTPase superfamily. Statins have been shown to influence the Rho pathway, a mechanism that is believed to contribute to the non-lipid beneficial effect of statins.[14 15] Inhibition of HMG-CoA reductase by statins does not only reduce endogenous cholesterol biosynthesis, it also prevents biosynthesis of isoprenoids, which results in reduced prenylation of GTPases such as Rho. This may lead to reduced leukocyte adhesion and fibrinolytic activity, as well as induced eNOS accumulation in endothelial cells.[14 15] Therefore, genetic variability within *RHOBTB1* may in fact be important for statin responsiveness. Nevertheless, the interaction with *RHOBTB1* was not statistically significant and could very well represent a false positive finding.

The most obvious explanation for not finding any significant interactions, is the fact that our study is underpowered. These results should therefore be considered preliminary. The q values suggest that a proportion of the interactions found in table 2 are not chance findings. To increase the statistical power to elucidate which of these interactions are genuine, a large-scale study on the pharmacogenomics of statins should be conducted. Efforts are currently ongoing to gather partners through a consortium that genotyped the IBC array for a large amount of individuals who have been phenotyped for cardiovascular diseases[5] to study statin responsiveness. Furthermore, potentially important SNPs could have been missed because only SNPs with a MAF cut-off of 0.2 were tested for statistical power reasons. Also SNPs that may be important for the pharmacogenomics of statins are possibly not included on the chip. For example, a combined analysis of genome-wide association (GWA) results from three trials found the *CLMN* rs8014194 to influence statin efficacy,[3] a SNP that was not available on the IBC chip.

Besides the advantages of the IBC chip genotyping approach over a candidate gene or GWAS approach, an important strength of our study is the uniqueness of having centralized system to define exposure (availability of the community pharmacy records) and outcomes (hospital records). Statin exposure was defined based on pharmacy records, which validity to measure drug exposure has been shown to be good.[16] Importantly, a well defined exposure measure is essential for a study on the pharmacogenomics of statins. Therefore, a meta-analysis within the aforementioned consortium will be restricted to those that have well documented data on drug exposure. In conclusion, no SNPs reached statistical significance for the interaction with statins in this pilot study. A future meta-analysis utilizing the IBC consortium should reveal whether the most significant genes (*RPB1* and *STARD13*) and candidate gene *RHOBTB1* are genuine interactions or represent false positive findings.

# REFERENCES

1. Barber MJ, Mangravite LM, Hyde CL, Chasman DI, Smith JD, McCarty CA, et al. Genome-wide association of lipid-lowering response to statins in combined study populations. PLoS One;5(3):e9763.
2. Peters BJ, Klungel OH, de Boer A, Maitland-van der Zee AH. Genetic determinants of response to statins. Expert Rev Cardiovasc Ther 2009;7(8):977-83.
3. Barber MJ, Mangravite LM, Hyde CL, Chasman DI, Smith JD, McCarty CA, et al. Genome-wide association of lipid-lowering response to statins in combined study populations. PLoS One 2010;5(3):e9763.
4. Thompson JF, Hyde CL, Wood LS, Paciga SA, Hinds DA, Cox DR, et al. Comprehensive whole-genome and candidate gene analysis for response to statin therapy in the Treating to New Targets (TNT) cohort. Circ Cardiovasc Genet 2009;2(2):173-81.
5. Keating BJ, Tischfield S, Murray SS, Bhangale T, Price TS, Glessner JT, et al. Concept, design and implementation of a cardiovascular gene-centric 50 k SNP array for large-scale genomic association studies. PLoS One 2008;3(10):e3583.
6. Peters BJM, Rodin AS, Klungel OH, van Duijn CM, Stricker BHC, Slot Rvt, et al. Pharmacogenetic interactions between ABCB1 and SLCO1B1 tagging SNPs and the effectiveness of statins in the prevention of myocardial infarction. Pharmacogenomics 2010;11(8):1065-1076.
7. van Wieren-de Wijer DB, Maitland-van der Zee AH, de Boer A, Kroon AA, de Leeuw PW, Schiffers P, et al. Interaction between the Gly460Trp alpha-adducin gene variant and diuretics on the risk of myocardial infarction. J Hypertens 2009;27(1):61-8.
8. WHO. Collaboration Centre for Drug Statistics Methodology. Complete ATC index. Oslo, 2010. (http://www.whocc.no/atc_ddd_index/ [accessed July 27th 2010]).
9. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet 2006;38(8):904-9.
10. Khoury MJ, Flanders WD. Nontraditional epidemiologic approaches in the analysis of gene-environment interaction: case-control studies with no controls! Am J Epidemiol 1996;144(3):207-13.
11. Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proc Natl Acad Sci U S A 2003;100(16):9440-5.
12. Raychaudhuri S, Plenge RM, Rossin EJ, Ng AC, Purcell SM, Sklar P, et al. Identifying relationships among genomic disease regions: predicting genes at pathogenic SNP associations and rare deletions. PLoS Genet 2009;5(6):e1000534.
13. Peters BJM, Klungel OH, de Boer A, Stricker BH, Maitland-van der Zee AH. Pharmacogenetics of cardiovascular drug therapy. Clin Cases Miner Bone Metab. 2009;6(1):55-65.
14. Jain MK, Ridker PM. Anti-inflammatory effects of statins: clinical evidence and basic mechanisms. Nat Rev Drug Discov 2005;4(12):977-87.
15. Rikitake Y, Liao JK. Rho GTPases, statins, and nitric oxide. Circ Res 2005;97(12):1232-5.
16. Lau HS, de Boer A, Beuning KS, Porsius A. Validation of pharmacy records in drug exposure assessment. J Clin Epidemiol 1997;50(5):619-25.

# 6 GENETICS OF STATIN-INDUCED MYOPATHY

# CHAPTER 6.1

# PHARMACOGENOMIC INSIGHTS INTO TREATMENT AND MANAGEMENT OF STATIN-INDUCED MYOPATHY

Bas J.M. Peters[a]
Olaf H. Klungel[a]
Frank L. Visseren[b]
Anthonius de Boer[a]
Anke-Hilse Maitland-van der Zee[a]

a Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, Utrecht, The Netherlands
b Department of Vascular Medicine, University Medical Centre Utrecht, The Netherlands

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## ABSTRACT

Although generally well tolerated, the most common adverse drug reaction from statin therapy is myopathy. This article reviews the current pharmacogenomic knowledge of statin-induced myopathy. Furthermore, we will discuss the importance of recent pharmacogenetic advances for the treatment and management of statin-induced myopathy. Variation in the *SLCO1B1* gene is associated with increased incidence of statin-induced myopathy, particularly with simvastatin and less so with other statins. If different pharmacokinetic enzymes and transporters are responsible for susceptibility to myopathy, this may explain differences in the occurrence of statin-induced myopathy in individual patients. Genotyping in patients suffering from statin-induced myopathy may help to personalize the choice of statin for the lowest chance of developing myopathy.

## INTRODUCTION

To reduce morbidity and mortality associated with heart disease, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) inhibitors (statins) are used by millions of patients worldwide. The primary mechanism by which statins reduce the risk of coronary artery disease (CAD) involves lowering low density lipoprotein cholesterol (LDLc) in plasma. Although generally well tolerated, the most common adverse drug reaction (ADR) from statin therapy is myopathy, symptoms of which can range from myalgia (mild fatigue and muscle pain without raised creatine kinase (CK))[1] to life-threatening rhabdomyolysis (muscle symptoms associated with marked CK elevations, typically substantially more than 10 times the upper limit of normal).[1] Among 20 randomized trials, the incidence of minor muscle pains was 190 per 100,000 person years, whereas rhabdomyolysis incidence was reported to be 1.6 per 100,000 person years.[2] In the ambulatory setting, the incidence of hospitalized rhabdomyolysis per 100,000 person years for monotherapy with atorvastatin, pravastatin, or simvastatin was shown to be 4.4.[3] In contrast to the incidence of minor muscle pains in clinical trials, observational studies have reported considerably higher numbers of statin-associated myalgia cases.[4 5]

Currently, there is no consensus of the exact definition of statin-induced myopathy (SIM).[6] The mechanism behind SIM is poorly understood, but a number of (arguable) underlying mechanisms have been proposed, including isoprenoid and coenzyme Q10 depletion, low cholesterol content myocyte skeletal membrane related instability, and mitochondrial dysfunction. SIM is of great clinical importance because (i) mild ADRs in patients on life-long statin treatment lower the quality of life, and (ii) patients may discontinue statin therapy because of intolerance.

Numerous factors have been proposed which may increase the risk of SIM, including older age, female sex, low BMI, excessive alcohol use and drug interactions (for example, concomitant use of fibrates, cyclosporine, protease inhibitors, macrolide antibiotics and amiodarone).[6] Recent advances in pharmacogenomic research have revealed important genetic factors which contribute to the risk of ADRs. Clinically important examples of gene drug interactions are (i) coumarins, genetic variation in *VKORC1*, *CYP2C9*, and the risk of bleeding, (ii) abacavir, *HLA-B**5701, and hypersensitivity and (iii) irinotecan, *UGT1A1**28 and neutropenia. This article reviews the current pharmacogenomic knowledge of SIM.

Furthermore, we will discuss the importance of recent pharmacogenetic advances for the treatment and management of SIM.

***Genetic predisposition to statin induced myopathy***

Several studies have been conducted to investigate the contribution of genetic variability to the risk of statin induced myopathy. Pharmacokinetically-related genes are obvious candidates, since the exposure to a particular statin is much higher for a patient who is a poor metabolizer for a certain cytochrome P-450 (CYP450) enzyme, compared to an intermediate, extensive or rapid metabolizer. For example, the pharmacokinetics of fluvastatin depends on the *CYP2C9* genotype, with a 3-fold difference in the active enantiomer and an even greater difference in the inactive enantiomer.[7] A case report in 2004/2005 suggested genetic variability within the *CYP2C8* gene to be causative of a cerivastatin- (withdrawn from market) associated case of rhabdomyolysis.[8 9] The relationship between *CYP3A4* and *CYP3A5* gene polymorphisms and atorvastatin-induced muscle damage was investigated in a case-control study with 68 cases and 69 controls.[10] Muscle damage (measured as degree of serum CK elevation) was greatest in patients on atorvastatin treatment and homozygous for the *CYP3A5**3 allele. Another study, including 136 patients with SIM (taking either atorvastatin and simvastatin) and 296 controls, investigated the relationship between 388 common SNPs (most of them within CYP coding genes) and elevated CK or myalgia, and reported an association between the *CYP2D6**4 allele and atorvastatin-induced myopathy.[11] Interestingly, the results extended to muscle events induced by simvastatin, which is not known to be metabolized by *CYP2D6*.

Drug transporters that mediate the uptake and elimination of statins have recently gained interest. The hepatocellular influx transporter OATP1B1 (encoded by the SLCO1B1 gene) and intestinal and hepatocellular efflux transporter ABCB1 (*ABCB1* gene) have been shown to affect the pharmacokinetics of statins.[12-14]

The pharmacokinetically different profile of statins between *SLCO1B1* genotypes has also been shown to affect the risk of myopathy. The SEARCH Collaborative Group study conducted a genome-wide association study (GWAS) in 85 myopathy cases and 90 controls who were all taking 80mg of simvastatin once daily.[15] Only a noncoding SNP (rs4363657) within the *SLCO1B1* gene showed a strong association with myopathy. This SNP is in strong linkage disequilibrium with the nonsynonymous rs4149056 SNP, which had previously been associated with altered statin pharmacokinetics.[13 14] For each copy of the variant allele there was approximately a 4-times higher risk of myopathy. Importantly, this finding was replicated in a trial with subjects treated with 40mg simvastatin once daily.[15] The STRENGTH study investigated the genetics of four CYP genes and the *SLCO1B1* gene in relation to SIM (use of simvastatin, atorvastatin and pravastatin were included). Not only did they confirm the findings from the SEARCH study, they also reported an association between the *SLCO1B1* risk allele and myalgia symptoms without CK elevation for simvastatin and atorvastatin (weaker), but not for pravastatin treatment.[16]

An additional potentially important, but very rare, SNP in the *SLCO1B1* gene is 1628T>G. This novel mutation was discovered by a Japanese group in a patient with pravastatin induced myopathy,[17] and was shown to reduce transporter activity of OATP1B1.[18] In another study, the TTT (or TAT) haplotype of the *ABCB1* 1236C>T, 2677G>A/T, or 3435C>T polymorphisms was more frequently seen in the simvastatin-treated group

without myalgia.[19] This observation may seem surprising as simvastatin (acid) area under the plasma-concentration-time curve (AUCs) has been shown to be 60% higher among homozygous haplotype *ABCB1* TTT carriers.[12]

Several underlying diseases may increase the risk of developing myopathy during statin therapy. These diseases include exercise intolerance disorders (such as McArdle disease or carnitine palmitoyltransferase (CPT) II- deficiency), malignant hyperthermia, coenzyme Q10 deficiency, and gene expression abnormalities (such as over-expression of major histocompatibility complex class I (MHC-I)).[20] The evidence that these diseases lead to an increased risk of myopathy in combination with statin use is not always clear. However, for example, McArdle disease and CPT II deficiency have been shown to be more common in statin myopathy patients.[21] Both disorders are very rare (prevalence of McArdle 1:100,000 individuals,[22] and CPT II deficiency 1:300,000 individuals).[21]. These genetic or acquired diseases might be asymptomatic in patients starting statin therapy and statins may then act as unmasking agents.[23] These underlying diseases might therefore be one reason why certain patients develop myopathy during statin use. However, because of the rarity of the diseases it does not seem appropriate to test patients for these diseases before initiating statin use. If serum CK is still high after discontinuation of statins, the physician should pursue further diagnostic evaluations for the detection of neuromuscular disorders.[23]

***New insights into treatment and management***

With the increasing number of patients being treated with (high dose) statins to meet the stringent cholesterol levels as advocated in (inter)national guidelines, SIM becomes more common in absolute numbers. Clinically, mild myopathic symptoms may lead to poor adherence to statin treatment and lower clinical benefit, and extreme myopathy manifests as the rare but life threatening event rhabdomyolysis. Therefore it is also important to evaluate the role of the pharmacogenetic interactions in the prediction of the more common myalgia. This is a scientific challenge because of the difficulty of defining the phenotype, but is very valuable because the incidence of myalgia complaints is relatively high among statin users.

Besides genetics, other considerations should be taken into account when prescribing statins. For example, female sex was shown to be a risk factor for developing adverse effects during statin therapy.[16] This may be due to the fact that the bodyweight of females is lower than that of males and therefore lower doses for females should be considered.[16] It is also of utmost importance that the use of other drugs by the patient is considered. Patients that use CYP3A4 inhibitors (such as fibrates, protease inhibitors, amiodarone, and cyclosporine) should not use simvastatin, but should preferably be treated with a statin that is metabolized by other enzymes (such as fluvastatin or atorvastatin).[24]

In this review we gave an overview of the studies that aimed to unravel the contribution of genetic variability to the risk of SIM. Before addressing the clinical implications of these findings, it is important to note that there seems to be a common pattern of (pharmaco) genomic associations that cannot be confirmed by others.[25] Reasons for inconsistent findings between studies include differences in study population, different outcome definitions, statistical power issues, and chance findings due to testing many genetic variables. Many studies have been published which examine genetic markers for the prediction of statin efficacy.[26] However very few genetic interactions have been confirmed,

and the clinical utility of the interactions is very low, because the differential efficacy caused by genes is small.[26] With this in mind, the current evidence is most compelling for the *SLCO1B1* gene since this association has been confirmed in different studies[15] [16] and the findings are corroborated by previously conducted genetic pharmacokinetic studies.[14] However, variation in other pharmacokinetic enzymes/drug transporters might also be of importance for one or more statins, and therefore statin-specific studies should be conducted to confirm these findings as well. It would be helpful for this process if the complete metabolic route for all statins were elucidated, but unfortunately this is not the case; the pathway is very complex and involves numerous transporters, CYP450 enzymes, and phase II enzymes.

The robust association between the *SLCO1B1* gene and SIM may warrant genetic testing in clinical practice, since it is increasingly clear that there might be an important genetic component in the prediction of the development of SIM during statin treatment. However, it is important to realize that there are significant differences between the statins concerning their route of hepatic uptake. Lipophilic statins such as fluvastatin can enter the liver via passive diffusion, while hydrophilic statins need active transportation. Importantly, fluvastatin has been shown to have a pharmacokinetic profile independent of *SLCO1B1* genotype.[27] In studies investigating the pharmacogenetics of SIM, simvastatin (and to a lesser extent atorvastatin) seems to be an important substrate for the OATP1B1 transporter. Patients with the rs4149056 polymorphism in the *SLCO1B1* gene should therefore preferably be treated with another statin (such as fluvastatin). Because there are differences in pharmacokinetics and in susceptibility for drug transporters between statins, the genetic variation predicting the side-effects of these drugs might be different for each type of statin. Genotyping all patients before initiating therapy is not cost-effective at present because the risk of developing severe side effects such as myopathy and rhabdomyolysis is low, but genotyping costs will probably decrease with time, changing the situation. In the future, genotyping before the start of therapy could be helpful in choosing the right statin for the individual patient in such a way that the patient has a very low chance of developing statin-induced myopathy.

## REFERENCES

1. Pasternak RC, Smith SC, Jr., Bairey-Merz CN, Grundy SM, Cleeman JI, Lenfant C. ACC/AHA/NHLBI Clinical Advisory on the Use and Safety of Statins. Stroke 2002;33(9):2337-41.
2. Law M, Rudnicka AR. Statin safety: a systematic review. Am J Cardiol 2006;97(8A):52C-60C.
3. Graham DJ, Staffa JA, Shatin D, Andrade SE, Schech SD, La Grenade L, et al. Incidence of hospitalized rhabdomyolysis in patients treated with lipid-lowering drugs. JAMA 2004;292(21):2585-90.
4. Bruckert E, Hayem G, Dejager S, Yau C, Begaud B. Mild to moderate muscular symptoms with high-dosage statin therapy in hyperlipidemic patients--the PRIMO study. Cardiovasc Drugs Ther 2005;19(6):403-14.
5. Nichols GA, Koro CE. Does statin therapy initiation increase the risk for myopathy? An observational study of 32,225 diabetic and nondiabetic patients. Clin Ther 2007;29(8):1761-70.
6. Joy TR, Hegele RA. Narrative review: statin-related myopathy. Ann Intern Med 2009;150(12):858-68.
7. Kirchheiner J, Kudlicz D, Meisel C, Bauer S, Meineke I, Roots I, et al. Influence of CYP2C9 polymorphisms on the pharmacokinetics and cholesterol-lowering activity of (-)-3S,5R-fluvastatin and (+)-3R,5S-fluvastatin in healthy volunteers. Clin Pharmacol Ther 2003;74(2):186-94.

8. Ishikawa C, Ozaki H, Nakajima T, Ishii T, Kanai S, Anjo S, et al. A frameshift variant of CYP2C8 was identified in a patient who suffered from rhabdomyolysis after administration of cerivastatin. J Hum Genet 2004;49(10):582-5.
9. Ozaki H, Ishikawa CT, Ishii T, Toyoda A, Murano T, Miyashita Y, et al. Clearance rates of cerivastatin metabolites in a patient with cerivastatin-induced rhabdomyolysis. J Clin Pharm Ther 2005;30(2):189-92.
10. Wilke RA, Moore JH, Burmester JK. Relative impact of CYP3A genotype and concomitant medication on the severity of atorvastatin-induced muscle damage. Pharmacogenet Genomics 2005;15(6):415-21.
11. Frudakis TN, Thomas MJ, Ginjupalli SN, Handelin B, Gabriel R, Gomez HJ. CYP2D6*4 polymorphism is associated with statin-induced muscle effects. Pharmacogenet Genomics 2007;17(9):695-707.
12. Keskitalo JE, Kurkinen KJ, Neuvoneni PJ, Niemi M. ABCB1 haplotypes differentially affect the pharmacokinetics of the acid and lactone forms of simvastatin and atorvastatin. Clin Pharmacol Ther 2008;84(4):457-61.
13. Niemi M. Role of OATP transporters in the disposition of drugs. Pharmacogenomics 2007;8(7):787-802.
14. Pasanen MK, Neuvonen M, Neuvonen PJ, Niemi M. SLCO1B1 polymorphism markedly affects the pharmacokinetics of simvastatin acid. Pharmacogenet Genomics 2006;16(12):873-9.
15. Link E, Parish S, Armitage J, Bowman L, Heath S, Matsuda F, et al. SLCO1B1 variants and statin-induced myopathy--a genomewide study. N Engl J Med 2008;359(8):789-99.
16. Voora D, Shah SH, Spasojevic I, Ali S, Reed CR, Salisbury BA, et al. The SLCO1B1*5 genetic variant is associated with statin-induced side effects. J Am Coll Cardiol 2009;54(17):1609-16.
17. Morimoto K, Oishi T, Ueda S, Ueda M, Hosokawa M, Chiba K. A novel variant allele of OATP-C (SLCO1B1) found in a Japanese patient with pravastatin-induced myopathy. Drug Metab Pharmacokinet 2004;19(6):453-5.
18. Furihata T, Satoh N, Ohishi T, Ugajin M, Kameyama Y, Morimoto K, et al. Functional analysis of a mutation in the SLCO1B1 gene (c.1628T>G) identified in a Japanese patient with pravastatin-induced myopathy. Pharmacogenomics J 2009;9(3):185-93.
19. Fiegenbaum M, da Silveira FR, Van der Sand CR, Van der Sand LC, Ferreira ME, Pires RC, et al. The role of common variants of ABCB1, CYP3A4, and CYP3A5 genes in lipid-lowering efficacy and safety of simvastatin treatment. Clin Pharmacol Ther 2005;78(5):551-8.
20. Vladutiu GD. Genetic predisposition to statin myopathy. Curr Opin Rheumatol 2008;20(6):648-55.
21. Vladutiu GD, Simmons Z, Isackson PJ, Tarnopolsky M, Peltier WL, Barboi AC, et al. Genetic risk factors associated with lipid-lowering drug-induced myopathies. Muscle Nerve 2006;34(2):153-62.
22. Haller RG. Treatment of McArdle disease. Arch Neurol 2000;57(7):923-4.
23. Tsivgoulis G, Spengos K, Karandreas N, Panas M, Kladi A, Manta P. Presymptomatic neuromuscular disorders disclosed following statin treatment. Arch Intern Med 2006;166(14):1519-24.
24. Mantel-Teeuwisse AK, Klungel OH, Schalekamp T, Verschuren WM, Porsius AJ, de Boer A. Suboptimal choices and dosing of statins at start of therapy. Br J Clin Pharmacol 2005;60(1):83-9.
25. Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K. A comprehensive review of genetic association studies. Genet Med 2002;4(2):45-61.
26. Peters BJ, Klungel OH, de Boer A, Maitland-van der Zee AH. Genetic determinants of response to statins. Expert Rev Cardiovasc Ther 2009;7(8):977-83.
27. Niemi M, Pasanen MK, Neuvonen PJ. SLCO1B1 polymorphism and sex affect the pharmacokinetics of pravastatin but not fluvastatin. Clin Pharmacol Ther 2006;80(4):356-66.

# CHAPTER 6.2

# THE *SLCO1B1* GENE AND DISCONTINUATION OF STATIN TREATMENT

Bas J.M. Peters[a]
Harm C.J. Geers[a]
Matthijs L. Becker[b,c]
Albert Hofman[b]
Andre G. Uitterlinden[b,f]
Patrick C. Souverein[a]
Olaf H. Klungel[a]
Bruno H.Ch. Stricker[b,d,e]
Anthonius de Boer[a]
Anke-Hilse Maitland-van der Zee[a]

a Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, Utrecht, The Netherlands
b Department of Epidemiology, Erasmus MC, Rotterdam, The Netherlands
c Department of Hospital Pharmacy, Erasmus MC, Rotterdam, The Netherlands
d Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands
e Drug Safety Unit, Inspectorate for Health Care, The Hague, The Netherlands
f Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands

## ABSTRACT

Although generally well tolerated, the most common adverse drug reaction (ADR) from statin therapy is myopathy. Carriers of the *SLCO1B1* T521C variant allele have been shown to have higher plasma concentrations of most statins and an increased risk of statin induced myopathy (SIM). Whether the *SLCO1B1* T521C variant allele also increases the risk of discontinuation is unknown.

All incident users of statins from the Utrecht Cardiovascular Pharmacogenetics studies and Rotterdam study were selected. Statin treatment episodes were constructed for each patient to define the duration of a dispensed prescription. Patients were followed starting at the dispensing date of the first prescription of any statin until the end of the study period, discontinuation of statin use, death, or no longer registered in database (e.g. moved), whichever came first. A Cox Proportional Hazards model was used to assess the effect of the *SLCO1B1* rs12369881 (UCP)/ rs1871395 (Rotterdam) on discontinuation incidence.

We identified 942 (UCP) and 1911 (Rotterdam) incident statin users. In UCP, an increased risk of statin discontinuation was found for variant allele carriers (HR 1.25, 95%CI 1.02-1.54), which was more pronounced in women compared to men and in participants starting with a high dose compared to a low starting dose. In Rotterdam, we did not observe an effect of the *SLCO1B1* polymorphism on statin discontinuation (HR 0.95, 95%CI 0.85 – 1.07). Also the analysis by gender, age, or starting dose of statin could not reveal an association.

In conclusion, compatible with previous findings we found a small increase in risk of statin discontinuation for variant allele carriers in UCP (rs12369881), but not in the Rotterdam study (rs1871395). Statins with low affinity for *SLCO1B1* should be favoured in *SLCO1B1* T521C variant allele carriers at high risk for statin associated ADRs (e.g. high doses) to increase the safety of statin treatment, quality of life for patients on life-long treatment, and clinical benefit due to better adherence.

## INTRODUCTION

To reduce morbidity and mortality associated with heart disease, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) inhibitors (statins) are used by millions of patients worldwide. Two studies on primary prevention with follow-up of 36 and 48 months reported discontinuation rates of 74.6%[1] and 61%[2] respectively. Studies on secondary prevention reported lower discontinuation rates of 34.5%[3] and 18%,[4] after follow-up of 72 and 84 months.

Although generally well tolerated, the most common adverse drug reaction (ADR) from statin therapy is myopathy, symptoms of which can vary from myalgia to life-threatening rhabdomyolysis. In contrast to the very low incidence of rhabdomyolysis,[5 6] observational studies have reported considerably high incidences of statin-associated myalgia (7.9-10.5%).[7 8] Statin induced myopathy (SIM) and other ADRs are of great clinical importance because (1) they lower quality of life for patients on statin treatment, and (2) may lead to discontinuation of statin therapy due to intolerance.

Numerous factors have been proposed which may increase the risk of SIM and other ADRs, including an older age, female sex, low BMI, excessive alcohol use and several drug interactions.[9] In addition, recent advances in pharmacogenomic research have revealed an

important genetic factor that contributes to the risk of SIM and other ADRs. The *SLCO1B1* T521C (rs4149056) has been shown to increase the risk of statin associated mild myalgia[10] and severe myopathy.[11] The *SLCO1B1* gene encodes OATP1B1, which mediates active hepatic influx of most statins.[12] Indeed, it has been repeatedly shown that the SLCO1B1 variant allele considerably increases the plasma concentration of most statins.[13] Because SIM and other ADRs are likely to be an important reason for statin discontinuation,[14] the aim of our study was to investigate the influence of the *SLCO1B1* T521C polymorphism on the risk of discontinuation of statin treatment.

## METHODS

Participants from Utrecht Cardiovascular Pharmacogenetics (UCP) study Participants from the UCP studies were enrolled from the population-based Pharmaco-Morbidity Record Linkage System (PHARMO, www.pharmo.nl).[15] PHARMO has linked drug dispensing histories from a representative sample of Dutch community pharmacies to the national registration of hospital discharges (Dutch National Medical Registry) since 1985.
Patients who received a prescription for an antihypertensive drug,[16] or had hypercholesterolemia (prescription for a cholesterol-lowering drug or total cholesterol >5.0 mmol/l),[17] were selected from the PHARMO database for pharmacogenetic studies on antihypertensive drugs and statins respectively. From this cohort, nested case-control studies were designed of which all hypercholesterolemic (prescription for a cholesterol-lowering drug, total cholesterol >5.0 mmol/l, or self-reported hypercholesterolemia) participants that were registered in PHARMO for at least one year and were older than 18 years.[17] Participants were contacted through their community pharmacies between 2002 and 2008. After the participant had consented to participate in the study and filled in a questionnaire, (s)he was sent an Oragene collection kit[17] or three cotton swabs and tubes containing buffer to collect saliva for genotyping purposes.[16] All participants were explicitly asked to consent for the collection, storage and genotyping of the DNA material. Information on all filled prescriptions from January 1st 1985 until October 31st 2006 was available and included the product name of the drug, the Anatomical Therapeutical Chemical (ATC) code, the amount dispensed, the prescribed dosage regimen and the date of dispensing.[18]

***Participants from replication study: Rotterdam Study***
The Rotterdam Study is a prospective population-based cohort study of 7,983 Caucasians aged 55 years and older in the suburb Ommoord in Rotterdam. Participants were invited between 1989 and 1993, and 1999 and 2001, and have been continuously followed since then. All participants of the Rotterdam Study gave written informed consent. Ethical approval was obtained from the Medical Ethical Committee of the Erasmus MC. The rationale, ethical approval and design of this study have been described before.[19] The seven pharmacies in Ommoord dispense the prescriptions of more than 99% of all participants. Information on all filled prescriptions from January 1st 1991 until December 31st 2009 was available and included the product name of the drug, the ATC code, the amount dispensed, the prescribed dosage regimen and the date of dispensing.[18]

***Sample, follow-up, and study outcome***

For both studies, we selected all incident users of statins, defined as having a first statin prescription with no statin or other cholesterol lowering drug prescription in the 365 (1 year) plus 90 (to account for refill) days before the first statin prescription. Patients were excluded if the *SLCO1B1* genotype was not available. Subsequently, statin treatment episodes were constructed for each patient to define the duration of a dispensed prescription. The duration of a prescription was based on the amount of tablets dispensed and the prescribed dosage regimen. The theoretical end date of each prescription equals the dispensing date plus the estimated duration of drug use. If a prescription from the same ATC5 group (statins) was collected prior to the theoretical end date of a previous prescription, the number of overlapping days was added to the theoretical end date of the subsequent statin prescription.

Follow up started at the dispensing date of the first prescription of any statin. Participants were followed until October 31st 2006 (UCP), December 31st 2009 (Rotterdam), discontinuation of statin use, death, or no longer registered in database (e.g. moved), whichever came first. Discontinuation was defined as the end of the last treatment episode or a treatment gap of at least 60 days after the theoretical end date of the treatment episode. This definition has previously been used to study statin discontinuation.[2 3 20]

***Confounders and effect modifiers***

Age, gender, and the prescribed dose of the first prescription (as DDD)[18] were included as potential confounders. Because higher doses of statin are more likely to be associated with ADRs and because older patients and women are more susceptible to statin associated ADRs compared to men,[10 11] the prescribed dose of the first prescription, gender, and age were tested for effect modification.

***SNP selection and Genotyping***

For the UCP study, *SLCO1B1* rs12369881 polymorphism genotyping was performed using the Golden-Gate assay on an Illumina BeadStation 500 GX (Illumina Inc.,San Diego, USA). This tagging SNP was selected for a candidate gene study on the effectiveness of statins,[17] and is in high linkage disequilibrium (LD) with *SLCO1B1* T521C (rs4149056) ($r^2$=0.81).[21] Rs12369881 was genotyped using the Golden-Gate assay on an Illumina BeadStation (Illumina Inc. San Diego, CA, USA). More information about DNA collection, purification, and genotyping in this study can be found elsewhere.[17]

For the Rotterdam study, genome-wide genotyping was performed using the Infinium II HumanHap 550K Genotyping BeadChip® version 3 (Illumina, San Diego, CA, USA). For this study we selected the *SLCO1B1* rs1871395 tagging polymorphism, which is in strong LD ($r^2$=1.0) with *SLCO1B1* T521C. More information about DNA collection, purification, and genotyping in this study can be found elsewhere.[22]

***Statistical methods***

A $\chi^2$ test was used to test for deviations from Hardy Weinberg equilibrium (HWE). A Cox Proportional Hazards model was used to assess the effect of the *SLCO1B1* variant on discontinuation incidence. The heterozygous and homozygous carriers of the variant allele were grouped because of low power in the homozygous variant allele group. Stratified

analyses were performed based on gender, age, and the starting dose of the first prescription for a statin. In addition, we did a sensitivity analysis to investigate the effect of the *SLCO1B1* tagging SNPs on statin discontinuation for simvastatin and atorvastatin only (highest affinity for SLCO1B1).[13] Also, we considered a cut-off follow-up time of one, two, and three years. Finally, we also assessed the composite outcome of discontinuation, dose reduction, or switching of statins.

## RESULTS

In UCP, we identified 942 incident statin users of whom genotype data was available. Baseline characteristics according to genotype status can be found in table 1. *SLCO1B1* rs12369881 was in HWE (p = 0.64) and genotype frequencies were 653, 265, and 24 for GG, GA, and AA, respectively. The mean follow-up time was 2.9 years (SD 2.2). In the total follow-up, out of 942 incident statin users, 401 (42.6%) patients discontinued statin therapy during the study period.

Out of 289 *SLCO1B1* rs12369881 A allele carriers, 139 (48.1%) discontinued during follow-up, whereas only 262 (40.1%) out of 653 GG genotype carriers discontinued statin treatment. The Kaplan-Meier Curve for the association between the *SLCO1B1* rs12369881 polymorphism and discontinuation of statin treatment can be found in Figure 1. The risk of discontinuation of statin treatment was statistically significant higher in patients hetero- or homozygous for the variant allele of *SLCO1B1* rs12369881 compared to wild-type carriers (adjusted hazard ratio (HR) 1.25; 95% CI 1.02-1.54) (table 2). Also for the composite outcome discontinuation, dose reduction, or switching of statins, the same statistically significant association was seen. The effect of *SLCO1B1* rs12369881 was more pronounced in patients starting with a higher dose when considering the point HR estimates (table 2). Stratification of the analysis by gender showed that the point estimate for the risk of discontinuation among females was higher (adjusted HR 1.50; 95% CI 0.97-2.29) as compared to males (adjusted HR 1.19; 95% CI 0.94-1.51). Women starting at a DDD of >1 but <2 had the highest risk of discontinuation of statin treatment (HR 2.89; 95% CI 1.31-6.39). Furthermore, we did not find age to modify the effect of *SLCO1B1* rs12369881 on statin discontinuation and we did not find a stronger association for simvastatin and atorvastatin only (data not shown).

In the Rotterdam study, we identified 1,911 incident statin users of whom genotype data was available. Baseline characteristics according to genotype status can be found in table 1. *SLCO1B1* rs1871395 was in HWE (p=0.21). The percentage of statin users that discontinued during follow up was identical for homozygous wild-type carriers of the *SLCO1B1* rs11871395 polymorphism as for variant allele carriers (73.3%). The risk of discontinuation for variant allele carriers did not differ from homozygous wild-type carriers (adjusted HR 0.95; 95% CI 0.85-1.07). For the composite outcome no association was found either. Also, no differences in the risk of discontinuation between *SLCO1B1* genotypes were observed in subpopulations based on gender and/or starting dose of statin treatment (table 2). Neither the analysis with atorvastatin and simvastatin only nor the analysis stratified by age revealed an association between *SLCO1B1* rs11871395 and the risk of statin discontinuation (data not shown).

***Table 1.*** Baseline characteristics by *SLCO1B1* polymorphism status

| | UCP (rs12369881) Wild-type (GG) | Variant (AG or AA) | Rotterdam (rs11871395) Wild-type (AA) | Variant (AG or GG) |
|---|---|---|---|---|
| N | 653 | 289 | 1374 | 539 |
| Gender Male (%) | 492 (75.3%) | 228 (78.9%) | 648 (47,2%) | 248 (46.0%) |
| Average age (years (sd)) | 63.1 (9.8) | 61.6 (10.1) | 69.7 (8.1) | 70.1 (7.5) |
| Starting dose (sd) (DDD) | 1.7 (1.1) | 1.6 (1.1) | 1.3 (0.8) | 1.3 (0.8) |
| Discontinuation | 262 (40.1%) | 139 (48.1%) | 1007 (73.3%) | 395 (73.3%) |
| Follow-up (sd) (year) | 2.93 (2.3) | 2.86 (2.2) | 3.5 (3.9) | 3.7 (3.9) |
| Simvastatin | 282 (43.2%) | 124 (42.9%) | 855 (62.2%) | 315 (58.4%) |
| Pravastatin | 132 (20.2%) | 66 (22.8%) | 166 (12.1%) | 78 (14.5%) |
| Atorvastatin | 179 (27.4%) | 76 (26.3%) | 250 (18.2%) | 108 (20.0%) |
| Other | 60 (9.2%) | 23 (8.0%) | 103 (7.5%) | 38 (7.1%) |

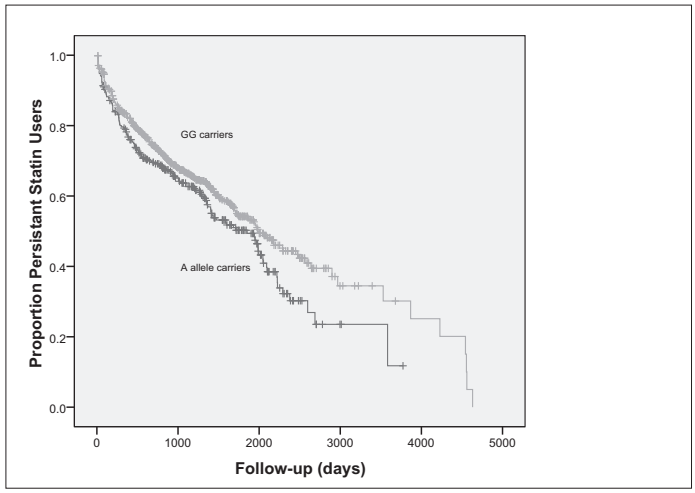
DDD = Defined Daily Dosage; sd = standard deviation; N = number of participants

***Table 2.*** The association between the *SLCO1B1* genotype and the risk of statin discontinuation

| | Starting Dose | UCP HR* | adj. HR* | 95% CI | p value | Rotterdam HR* | adj. HR* | 95% CI | p value |
|---|---|---|---|---|---|---|---|---|---|
| All | all doses | 1.25 | 1.25 | (1.02-1.54) | 0.035 | 0.96 | 0.95 | (0.85 – 1.07) | 0.410 |
| | <= 1 DDD | 1.11 | 1.10 | (0.80-1.52) | 0.559 | 0.98 | 0.98 | (0.84 – 1.14) | 0.744 |
| | >1-<2 DDD | 1.33 | 1.36 | (0.91-2.03) | 0.139 | 0.95 | 0.95 | (0.73 – 1.23) | 0.677 |
| | >= 2 DDD | 1.35 | 1.40 | (0.96-2.03) | 0.079 | 0.91 | 0.90 | (0.70 – 1.16) | 0.427 |
| Males | all doses | 1.19 | 1.19 | (0.94-1.51) | 0.143 | 0.91 | 0.90 | (0.76 – 1.08) | 0.254 |
| | <= 1 DDD | 1.12 | 1.12 | (0.78-1.60) | 0.554 | 0.94 | 0.94 | (0.74 – 1.19) | 0.596 |
| | >1-<2 DDD | 1.07 | 1.07 | (0.67-1.72) | 0.780 | 0.82 | 0.80 | (0.53 – 1.20) | 0.275 |
| | >= 2 DDD | 1.39 | 1.41 | (0.92-2.12) | 0.120 | 0.94 | 0.93 | (0.65 – 1.33) | 0.685 |
| Females | all doses | 1.48 | 1.50 | (0.97-2.29) | 0.066 | 0.99 | 0.99 | (0.84 – 1.15) | 0.854 |
| | <= 1 DDD | 1.05 | 1.05 | (0.51-2.14) | 0.900 | 1.00 | 0.99 | (0.81 – 1.21) | 0.938 |
| | >1-<2 DDD | 2.70 | 2.89 | (1.31-6.39) | 0.009 | 1.11 | 1.09 | (0.77 – 1.55) | 0.628 |
| | >= 2 DDD | 1.44 | 1.46 | (0.68-3.13) | 0.327 | 0.87 | 0.88 | (0.62 – 1.25) | 0.461 |

* Homozygote wild-type genotype as reference genotype
CI = Confidence Interval, HR = Hazard Ratio
Adj HR = Hazard Ratio adjusted for age, sex, and starting dose (if applicable)
DDD = defined daily dosage

***Figure 1.*** Kaplan-Meier Curve for the association in UCP between the *SLCO1B1* rs12369881 polymorphism and discontinuation of statin treatment




## DISCUSSION

In this cohort study, we found that patients starting statin treatment and carrying a variant allele of the *SLCO1B1* rs12369881 polymorphism had a slightly higher risk of discontinuation compared to wild-type carriers. This risk was more pronounced as the starting dose increased and for females. A replication study conducted in a prospective, population-based cohort study could not reveal similar associations.

The loss-of-function *SLCO1B1* T521C polymorphism has been shown to have a marked effect on the pharmacokinetics of simvastatin (acid) (3.2-fold increase in area under the curve (AUC)) and atorvastatin (2.4-fold increase), and an intermediate effect on pravastatin (1.9-fold increase).[12 23-25] Because statin intolerance is dose dependent, this polymorphism causes an increased susceptibility to statin intolerance.[10] including both SIM and other ADRs. The SEARCH Collaborative Group study conducted a genome-wide association study (GWAS) in 85 myopathy cases and 90 controls who were all taking 80mg of simvastatin once daily.[11] Only a noncoding SNP (rs4363657) within the *SLCO1B1* gene showed a strong association with myopathy. *SLCO1B1* encodes the OATP1B1 transporter that mediates the hepatic uptake of statins. This associated SNP is in strong linkage disequilibrium with the nonsynonymous *SLCO1B1* T521C polymorphism (rs4149056, *5). For each copy of the variant allele there was approximately a 4-times higher risk of myopathy. Importantly, this finding was replicated in a trial with subjects treated with 40 mg simvastatin once daily.[11] The STRENGTH study also investigated the genetics of the *SLCO1B1* gene in relation to statin intolerance (use of simvastatin, atorvastatin and pravastatin were included). Their results were in line with the findings from the SEARCH study, reporting an association

between the *SLCO1B1* risk allele and statin related ADRs (including SIM) for simvastatin and atorvastatin (weaker), but not for pravastatin treatment.[10]

In line with these findings, we showed in the UCP that the increased risk of ADRs associated with the *SLCO1B1* T521C variant allele also translates in a dose dependent higher risk of statin treatment discontinuation. As previously shown in both the SEARCH and STRENGTH study, women are more susceptible to statin related ADRs.[10] Compatible with this observation, although non significant, the point estimates suggest a more pronounced genetic effect on the risk of discontinuation in women as compared to men (table 2). The STRENGTH study reports that carriers of the *SLCO1B1* T521C variant allele are at a 2-fold relative risk of mild statin induced side effects.[10] Therefore, a smaller effect of the *SLCO1B1* tagging SNP on the risk of statin discontinuation was to be expected, given that the risk of mild statin induced side effects will not always lead to statin discontinuation. Using the same definitions for exposure and discontinuation status as in the UCP study, the replication study in the Rotterdam study did not show an association between the *SLCO1B1* rs11871395 tagging SNP and the risk of statin discontinuation.

Differences between the UCP and Rotterdam study population may help to explain why we did not find an association in the Rotterdam study. The Rotterdam study population has a higher mean age and includes more women than the UCP study, which may be related to differences in co-morbidities and medication use. However, these population characteristics would be expected to result in a larger contribution of the *SLCO1B1* genotype on statin treatment discontinuation. Hence, these population characteristics differences cannot explain the different observations of UCP and the Rotterdam study. Also, no association was found when considering only participants with a high starting dose of statins. Therefore, an overall lower mean starting dose of statin treatment can not explain the difference in results between the UCP and Rotterdam study either. Lastly, the follow-up period was longer and discontinuation rate was remarkably higher for the Rotterdam study compared to UCP, although identical definitions were used. An analysis in the Rotterdam study with a shortened follow-up period similar to UCP did not give different results (data not shown).

The current study has several limitations. First, for both studies, we used a tagging SNP to determine the effect of *SLCO1B1* T521C on the risk of statin treatment discontinuation whereas data of the *SLCO1B1* T521C itself would be preferable. In Rotterdam, there is full LD ($r^2$=1) between the *SLCO1B1* rs11871395 and T521C genotype, indicating a perfect marker for the *SLCO1B1* T521C according to the HapMap project.[21] In UCP, the $r^2$ between *SLCO1B1* rs12369881 and *SLCO1B1* T521C is 0.81, indicating strong LD. Nonetheless, some misclassification will be present, which could affect the magnitude of the association we report (i.e. results may be diluted). Second, although there seems to be a clear relationship between the altered pharmacokinetics due to the *SLCO1B1* T521C genotype and the susceptibility to ADRs,[10 11 13] we investigated the risk of discontinuation based on community pharmacy records. It would be of great value to have information about why patients discontinued statins. Finally, we included all statins in our study although the pharmacokinetic profile is differently affected by *SLCO1B1* T521C genotype for different statins.[13] To rule out the possibility that this genetic association is only true for certain statins, we performed a sensitivity analysis with only incident users of simvastatin and atorvastatin as their pharmacokinetic profile has been shown to be mostly affected by the

*SLCO1B1* T521C genotype.[13] This analysis did not reveal a stronger effect as compared to the results presented in this article (data not shown).

The strengths of this study are that we had complete community pharmacy records available to assess statin use. Pharmacists are the key administrators of pharmacy records, which have been shown to be a cheap and reliable source for estimating discontinuation rates, and for medication use evaluation.[26 27] Furthermore, besides the availability of a replication study, we were able to include a large number of participants in both the UCP (n=942) and Rotterdam study (n=1,911). Also, our study represents the real world setting rather than an experimental setting to asses the impact of the *SLCO1B1* T521C polymorphism on the risk of statin discontinuation.

Previously, it has been shown that the *SLCO1B1* T521C polymorphism has a marked effect on statin plasma concentrations[13] as well as on the risk of myopathy.[10 11] Although we found a small increase in risk of statin discontinuation for variant allele carriers in only one study, our findings endorse the recommendations given by Niemi that high dosing of certain statins (simvastatin, atorvastatin, pitavastatin, rosuvastatin, and pravastatin) should be avoided in patients carrying the variant allele.[13] This is especially true for patient groups that are more susceptible to statin myopathy (e.g. women, high doses, and older age). Increasing safety of high-dose statin treatment using the *SLCO1B1* genotype will increase the quality of life for patients on life-long treatment, and may increase clinical benefit due to better adherence.

In conclusion, patients carrying a variant allele of the *SLCO1B1* T521C polymorphism were shown to have a small increase in the risk of statin treatment discontinuation in one, but not both studies. The interaction was more pronounced among women as compared to men.

## REFERENCES

1. Jackevicius CA, Mamdani M, Tu JV. Adherence with statin therapy in elderly patients with and without acute coronary syndromes. JAMA 2002;288(4):462-7.
2. Perreault S, Blais L, Dragomir A, Bouchard MH, Lalonde L, Laurier C, et al. Persistence and determinants of statin therapy among middle-aged patients free of cardiovascular disease. Eur J Clin Pharmacol 2005;61(9):667-74.
3. Hudson M, Richard H, Pilote L. Parabolas of medication use and discontinuation after myocardial infarction--are we closing the treatment gap? Pharmacoepidemiol Drug Saf 2007;16(7):773-85.
4. Gislason GH, Rasmussen JN, Abildstrom SZ, Gadsboll N, Buch P, Friberg J, et al. Long-term compliance with beta-blockers, angiotensin-converting enzyme inhibitors, and statins after acute myocardial infarction. Eur Heart J 2006;27(10):1153-8.
5. Graham DJ, Staffa JA, Shatin D, Andrade SE, Schech SD, La Grenade L, et al. Incidence of hospitalized rhabdomyolysis in patients treated with lipid-lowering drugs. JAMA 2004;292(21):2585-90.
6. Law M, Rudnicka AR. Statin safety: a systematic review. Am J Cardiol 2006;97(8A):52C-60C.
7. Bruckert E, Hayem G, Dejager S, Yau C, Begaud B. Mild to moderate muscular symptoms with high-dosage statin therapy in hyperlipidemic patients--the PRIMO study. Cardiovasc Drugs Ther 2005;19(6):403-14.
8. Nichols GA, Koro CE. Does statin therapy initiation increase the risk for myopathy? An observational study of 32,225 diabetic and nondiabetic patients. Clin Ther 2007;29(8):1761-70.
9. Joy TR, Hegele RA. Narrative review: statin-related myopathy. Ann Intern Med 2009;150(12):858-68.
10. Voora D, Shah SH, Spasojevic I, Ali S, Reed CR, Salisbury BA, et al. The SLCO1B1*5 genetic variant is associated with statin-induced side effects. J Am Coll Cardiol 2009;54(17):1609-16.

11. Link E, Parish S, Armitage J, Bowman L, Heath S, Matsuda F, et al. SLCO1B1 variants and statin-induced myopathy--a genomewide study. N Engl J Med 2008;359(8):789-99.
12. Niemi M. Role of OATP transporters in the disposition of drugs. Pharmacogenomics 2007;8(7):787-802.
13. Niemi M. Transporter pharmacogenetics and statin toxicity. Clin Pharmacol Ther 2010;87(1):130-3.
14. Kiortsis DN, Giral P, Bruckert E, Turpin G. Factors associated with low compliance with lipid-lowering drugs in hyperlipidemic patients. J Clin Pharm Ther 2000;25(6):445-51.
15. Herings RM, Bakker A, Stricker BH, Nap G. Pharmaco-morbidity linkage: a feasibility study comparing morbidity in two pharmacy based exposure cohorts. J Epidemiol Community Health 1992;46(2):136-40.
16. van Wieren-de Wijer DB, Maitland-van der Zee AH, de Boer A, Kroon AA, de Leeuw PW, Schiffers P, et al. Interaction between the Gly460Trp alpha-adducin gene variant and diuretics on the risk of myocardial infarction. J Hypertens 2009;27(1):61-8.
17. Peters BJM, Rodin AS, Klungel OH, van Duijn CM, Stricker BHC, Slot Rvt, et al. Pharmacogenetic interactions between ABCB1 and SLCO1B1 tagging SNPs and the effectiveness of statins in the prevention of myocardial infarction. Pharmacogenomics 2010;11(8):1065-1076.
18. WHO. Collaboration Centre for Drug Statistics Methodology. Complete ATC index. Oslo, 2010. (http://www.whocc.no/atc_ddd_index/ [accessed July 27th 2010]).
19. Hofman A, Breteler MM, van Duijn CM, Janssen HL, Krestin GP, Kuipers EJ, et al. The Rotterdam Study: 2010 objectives and design update. Eur J Epidemiol 2009;24(9):553-72.
20. Perreault S, Blais L, Lamarre D, Dragomir A, Berbiche D, Lalonde L, et al. Persistence and determinants of statin therapy among middle-aged patients for primary and secondary prevention. Br J Clin Pharmacol 2005;59(5):564-73.
21. The International HapMap Project. Nature 2003;426(6968):789-96.
22. Richards JB, Rivadeneira F, Inouye M, Pastinen TM, Soranzo N, Wilson SG, et al. Bone mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study. Lancet 2008;371(9623):1505-12.
23. Niemi M, Pasanen MK, Neuvonen PJ. SLCO1B1 polymorphism and sex affect the pharmacokinetics of pravastatin but not fluvastatin. Clin Pharmacol Ther 2006;80(4):356-66.
24. Pasanen MK, Fredrikson H, Neuvonen PJ, Niemi M. Different effects of SLCO1B1 polymorphism on the pharmacokinetics of atorvastatin and rosuvastatin. Clin Pharmacol Ther 2007;82(6):726-33.
25. Pasanen MK, Neuvonen M, Neuvonen PJ, Niemi M. SLCO1B1 polymorphism markedly affects the pharmacokinetics of simvastatin acid. Pharmacogenet Genomics 2006;16(12):873-9.
26. Boudreau DM, Doescher MP, Saver BG, Jackson JE, Fishman PA. Reliability of Group Health Cooperative automated pharmacy data by drug benefit status. Pharmacoepidemiol Drug Saf 2005;14(12):877-84.
27. Lau HS, de Boer A, Beuning KS, Porsius A. Validation of pharmacy records in drug exposure assessment. J Clin Epidemiol 1997;50(5):619-25.

# 7 GENERAL DISCUSSION

## SCOPE OF THESIS

Individual variation in the response to drugs is an important clinical problem and may range from a lack of therapeutic effect to serious adverse drug reactions. While (clinical) variables such as disease severity, age, co-morbidities, gender, adherence, drug interactions, and smoking status are potentially important determinants of the response to drugs, genetic heterogeneity in genes encoding proteins related to drug metabolism, disposition and/or targets may be an even more important cause of interindividual variability in the therapeutic effects of drugs.[1,2] Information on the modification of drug effects by genetic polymorphisms may help to identify patients that have less benefit or experience harmful effects of these drugs. Over the past years, the terms pharmacogenetics and pharmagenomics are interchangeably used to define the phenomena that genetic variability can affect the response to a drug. Yet, these terms can be differentiated. Pharmacogenetics generally (i) studies genetic variation related to drug response, (ii) considers one or a few candidate genes, (iii) has a hypothesis testing approach, and (iv) utilizes conventional statistical methods to test the interaction between genetic variability and the response to a given drug. In contrast, pharmacogenomics generally (i) is regarded a broader application of genomic technologies (ii) considers the whole genome, (iii) may consider other than genetic data such as metabolomics, transcriptomics, proteomics, and/or epigenetics data, (iv) has a hypothesis generating approach, and (v) utilizes new statistical and computational methods that are capable of dealing with the large amount of data, possibly from different sources. Nonetheless, these definitions are not definite, and used interchangeably in literature. Therefore, from this point onwards, we use the abbreviation PGx to denote either definition.

According to the World Health Organization (WHO), death from cardiovascular disease (CVD) is the number one leading cause of death worldwide. In 2004, an estimated 17.1 million people died from CVD, of which 7.2 million were defined as Coronary heart disease (CHD) deaths.[3] Improved control of cardiovascular risk factors (lower target levels of low-density lipoprotein (LDL) cholesterol and blood pressure, public smoking ban) over time have accounted for the decrease of myocardial infarction (MI) incidence.[4] However, many patients still experience a MI and other cardiovascular events, despite the availability of a variety of effective drugs for the prevention of MI. This suggests that current pharmacotherapeutic strategies may not be optimal for all patients. Previous findings have shown that genetic variation contributes to the variability in effects of cornerstone pharmacological interventions to reduce the risk of MI.[5] Ideally, the identification of genetic biomarkers that modify the response to drugs gives the opportunity to optimize safety and effectiveness of the currently available drugs in the prevention of CVD. In addition, optimizing pharmacotherapeutic strategies using genetics may ultimately reduce the costs and utilization of health care resources. In the introduction of this thesis, we described the limited amount of promising findings specifically within the field of the PGx of statins and some of the key elements of the methodological challenges that PGx research is faced with. Studies conducted at that time mostly included single SNPs in candidate genes, small sample sizes, surrogate parameters, and conventional statistics. In this thesis, we further contribute to the knowledge about the genetics to the response to statin treatment, as well as to innovation of methods to investigate PGx of statins and other drugs. First,

we discuss our most important findings in the light of what is currently known. Second, we discuss methodological considerations for PGx research and give recommendations for future research. Finally, we elaborate on the clinical implications of our findings, and present the conclusion of this thesis.

## PGX OF STATINS (MAIN FINDINGS)

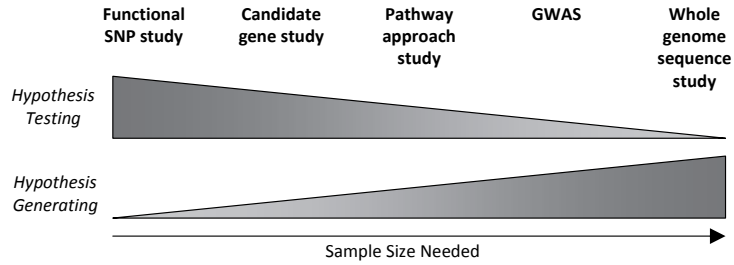
Worldwide, 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase) inhibitors, also known as statins, are among the most prescribed drugs in the prevention of CVD. Statins have been shown to be highly effective in reducing the risk of cardiovascular events.[6] Nonetheless, genetic variability has been shown to affect the response to statins on a pharmacokinetic and pharmacodynamic level (chapter 2.1).[7]

Following a PGx study method described by van Wieren et al.,[8] we were able to collect a large amount of data for a population based PGx case-control study on the genetics of statin response. We identified a range of polymorphisms that modify the effectiveness of statins in reducing the risk of MI.

We tested ten well known SNPs within six genes in two candidate gene studies involving *GNB3* and coagulation factor genes and found *GNB3*, *F5* and *F7* to be important for statin response. The study on the pharmacokinetic genes *ABCB1* and *SLCO1B1* revealed two SNPs in *ABCB1* that affected the response to statins. We investigated 84 SNPs in 24 genes related to the inflammation pathway, which revealed seven SNPs in three genes that influenced statin response. Among other SNPs, we found that carriers of two alleles of a tagging SNP for the *ADAMTS1* $^{Ala}227^{Pro}$ variant allele had much more benefit from statin treatment compared to heterozygous and homozygous wild-type carriers, a finding that is in keeping with the results from Cholesterol and Recurrent Events (CARE) and West of Scotland Coronary Prevention Study (WOSCOPS) trial.[9] Furthermore, we tested 209 SNPs in 27 genes involved in the cholesterol lowering pathway of statin treatment and found ten SNPs in eight genes to be important for statin response. An intronic SNP within the *SCARB1* gene was found to be the most highly significantly interacting SNP. Among other significant SNPs, we found two *PCSK9* variants to affect statin response, one of which being the non-synonymous *PCSK9* E670G (rs505151) polymorphism. We found carriers of one or two variant alleles did not benefit from statin treatment. Mechanistically, we provided a sound biological hypothesis and a replication study revealed an interaction showing compatible directionality, although the measure for the interaction (synergy index) was not statistically significant. Furthermore, we explored genetic data from a cardiovascular gene-centric 50 k SNP array, given rise to a variety of genes possibly implicated in the PGx of statins, findings that need to be investigated in a larger collaborative effort. Finally, variability within the hepatic transporter gene *SLCO1B1* was found to increase the risk of discontinuation of statin treatment in one, but not in a second study.

From a methodological perspective, we used a single SNP candidate gene approach, a pathway approach, and a more genomic approach to study the PGx of statins. In that order, our studies shift from a hypothesis testing to a hypothesis generating approach (figure 1). Additionally, the positive false discovery rate (pFDR) was used to deal with the issue of multiple testing.

***Figure 1.*** The relationship between the approach to capture and test genetic variability in a PGx study, the degree of applicability of the label hypothesis testing versus generating, and the sample size needed for adequate statistical power.




## METHODOLOGICAL CONSIDERATIONS

The recent technological advances shifted genotyping from single SNPs to high throughput genotyping, as reflected by the increasing genetic information that was assessed in chapter 3, 4, and 5. Nonetheless, acquiring more genetic information brings along methodological challenges.

***Study Design***

Within epidemiology and in particular PGx epidemiology, multiple study designs are possible, but not all designs will be either suitable or practical for PGx studies as described in chapter 2.2. In this thesis, besides one study in the lipid lowering arm of the randomized GENHAT trial (chapter 3.2), the primary source for the PGx research presented in this thesis, was an observational case control study enriched with genetic information. The methodological aspects for this type of study design have previously been described.[8] Important advantages of this design over a randomized clinical trial and cohort study are that it (i) is relatively cheap and easy, (ii) is feasible to include a large amount of cases, and (iii) reflects everyday clinical care (as opposed to the experimental setting of a RCT).[8] Besides the observational case control study design enriched with genetic information, future research in the field of PGx may benefit from initiative such as the Mondriaan project that aims to enrich health care databases by linking biobank data.[10] The Biobanking and Biomolecular Resources Research Infrastructure (BBMRI) has been established to support such initiatives (www.bbmri.nl)

Dealing with confounding in epidemiology is a huge challenge and has been subject of many discussions.[11] Besides the conventional confounding in epidemiology, PGx research is faced with other potential confounding such as 'hidden population structure' (or population stratification). This phenomenon is present when genetically incompletely mixed distinct subpopulations exist within the research population. This type of confounding should be addressed in both the study design[12] as well as in the data-analysis.[13] In our study design, we minimized population stratification by sampling cases and controls from the same population and by matching cases and controls on the basis of genetic background

using the surrogate marker geographic proximity. In addition, population stratification was addressed in chapter 5 using EIGENSTRAT,[13] a method that models ancestry differences between cases and controls along continuous axes of variation based on a principal components analysis.

An important consideration for any PGx study design is the definition of the outcome. The choice of the outcome may influence the power to detect a PGx interaction and it can affect the implications of a PGx finding.[14] First, findings from PGx studies that assess surrogate parameters may not directly be predictive for a related clinical outcome. Second, to a certain extend every outcome is characterized by heterogeneity which will affect the probability of detecting a gene-treatment interaction. An example that illustrates the importance of the definition of the outcome is the interaction between hydrochlorothiazide and the alpha-adducin (*ADD1*) gene as described in chapter 2.1. The *ADD1* $^{Gly}460^{Trp}$ polymorphism was shown to modify the blood-pressure response of hydrochlorothiazide in a eight week follow up prospective study involving newly diagnosed mild hypertensive patients.[15] The effect pointed in the direction that was expected based on the known functional consequences of the polymorphism and working mechanism of hydrochlorothiazide.[16] Moreover, this finding was replicated in similar studies.[17 18] Nonetheless, all but one[19] large retrospective studies with MI as the outcome could not show a similar interaction.[20-22] The association of the *ADD1* $^{Gly}460^{Trp}$ polymorphism with altered response to diuretics seems genuine because there is a clear hypothesis with a mechanistic explanation and all positive studies point in the same direction.[5] Given the current knowledge, this interaction seems of no clinical importance.

This example highlights that a PGx finding assessed with a surrogate outcome does not necessarily translate into a similar result when looking at a related clinical outcomes. Also, it can be argued that heterogeneity of the clinical outcomes that were used to investigate the effect of the *ADD1* $^{Gly}460^{Trp}$ polymorphism on hydrochlorothiazide is the reason for not reproducing the results from surrogate outcome studies. For example, the underlying pathophysiological mechanism preceding a MI differs among patients.[23] PGx interactions may therefore differ between subclasses of MI cases that share an underlying pathophysiological mechanism.

In this thesis, we most often used the outcome nonfatal hospitalized MI. Therefore, heterogeneity represented by the underlying mechanism of the outcome can be considered as a limitation of our studies. Although MI may be the result of a range of underlying diseases, such as hypertension, hypercholesterolemia, and diabetes or environmental factors, we assessed the influence of these conditions/risk factors, and adjusted in our statistical models if necessary. In contrast, the strength of our outcome is that it directly allows for evaluation of the effect of a certain polymorphism on the effectiveness of statins in reducing the risk of a clinical relevant outcome, as opposed to a surrogate parameter such as LDL cholesterol reduction.

Besides effectiveness, adverse drug reactions (ADR) are also often used as the outcome of a PGx study. Some of the most well established PGx examples investigate an ADR as the primary outcome.[24 25] Also in this thesis, we indirectly used ADR as an outcome for the PGx of statins. We assessed the effect of the *SLCO1B1* T521C polymorphism on the risk of discontinuation of statin treatment. This polymorphism is known to be associated with a higher susceptibility to statin induced muscle related ADRs.[24 26]

***Assessment of genetic variability: are we genotyping the right loci?***

As described in the previous paragraphs, different approaches were used to assess genetic variability. In chapter 3, we used a single SNP approach in one or few genes. Nonetheless, variability in other genes may well explain part of the interindividual differences in response to statins. Therefore, in chapter 4, we assessed common genetic variability within candidate genes belonging to the most well known pathways of the statin working mechanism. Yet, given the complexity of both human pathophysiology and working mechanism of statins, covering genetic variability within the candidate genes investigated in chapter 4, may still not give us all the answers. In comparison to a genome wide scan, the cardiovascular gene-centric 50 k SNP array used in chapter 5 does not cover the whole genome but comprises only loci that are potentially relevant for a range of cardiovascular, metabolic, and inflammatory syndromes. Relevant loci were chosen based on results from GWAS of vascular and inflammatory disease, expression quantitative trait loci implicated in atherosclerosis, pathway based approaches and comprehensive literature searching.[27]

Besides the selection of candidate genes, it is important to consider what variability to capture within the gene(s) of interest. In chapter 3, we genotyped for each gene one or two relatively well-known common SNPs with mostly functional consequences. A tagging SNP approach was used in chapter 4. Based on linkage disequilibrium (LD) blocks derived from the CEU (Utah residents with ancestry from northern and western Europe) HapMap population, all common genetic variability is captured when genotyping tagging SNPs. In comparison to the tagging SNP approach used in chapter 4, the cardiovascular gene-centric 50 k SNP array has a much better coverage (greater density of genetic markers) of the loci that were selected for the array. In fact, the array includes a high number of rare variants that may have a larger effect on the response to statins compared to more common SNPs. Importantly, the minimum allele frequency (MAF) for each SNP and the number of SNPs included in a study deserve important considerations for statistical power reasons, which we will elaborate on hereinafter.

In addition to the approaches that are presented by the studies in this thesis, other approaches are available and should be considered if resources are available. A genome wide scan could help identify genetic variants in loci that were previously not considered to be a candidate gene for the drug of interest. Within the field of the PGx of cardiovascular drug treatment, several genome wide association studies (GWAS) have been reported. For example, a GWAS on the response to anticoagulant treatment with warfarin did not reveal any genes with major contribution other than *VKORC1* and *CYP2C9* to warfarin responsiveness.[28-30] Three GWAS have been conducted so far on the PGx of statins.[31 32] The first report showed that *APOE*, *HMGCR*, and *PCSK9* influenced statin response in the Treating to New Targets (TNT) study, whereas it failed to identify new loci.[32] The second included the TNT, Cholesterol and Pharmacogenetics (CAP) trial, and the Pravastatin Inflammation/CRP Evaluation (PRINCE) study. Using the combined GWAS analysis from these three clinical trials involving nearly 4,000 individuals treated with statins, they identified a new SNP (rs8014194) in the *CLMN* gene.[31] GWAS may help identify new loci as shown in the PGx of statins, whereas it may also rule out any other significant contribution to interindividual variability to a drug such as the case for warfarin.

In addition to SNPs, other type of genetic variability exists, such as copy number

variants (CNVs) and duplicated regions. More information about these type of variants should also be assessed in future PGx research using sequencing techniques, as they may explain a larger part of the differences in response to drugs[33 34] and because their variability is not well captured using a GWAS approach.[35] Importantly, the approach to assess genetic variability has consequences for the data-analysis strategy and for whether it should be considered a "hypothesis testing" or "hypothesis generating" study.

***Statistical power and data analysis***

To test for PGx interactions in this thesis, logistic regression models including an interaction term were used. Using this approach, the statistical power to detect a PGx interaction depends on several parameters. First, the samples size of the study contributes to the statistical power. Second, it depends on the magnitude of the effect of the polymorphism on drug response. Third, the proportion of patients exposed to the drug of interest is an important parameter that should be considered when designing a study. Fourth, the minimum allele frequency (MAF) of a SNP in the research population contributes to the statistical power of a PGx study. Finally, the statistical power depends on the numbers of tests that are carried out. Choices that are made regarding these parameters will have consequences for the conclusions that can be drawn from the research conducted. These parameters will be discussed in greater detail in the next paragraphs. The sample size is of great importance. Yet, often financial constraints put a limit to the amount of patients that can be included in a study. In this thesis, to collect the primary data source, we used a relatively cheap and easy method to achieve a reasonably sized study population.[8] To increase statistical power, it is of utmost importance that research groups collaborate[36] to replicate findings, or to conduct a joint meta-analysis. The last one of which has been shown to be most powerful.[37] In addition, pharmaceutical industry is systematically collecting PGx data in clinical trials, which will increase chances of collaborating with parties outside the academics in the near future.[38] In most of our studies, the study population included approximately 600-1,300 cardiovascular cases and 400-1,200 controls. This is a reasonable amount, however not sufficient to study rare SNPs, SNPs with small effects, and genome-wide data. Aside from the sample size, reproducing results in an independent population is of major importance. Therefore, we collaborated with other research groups to replicate some of our findings (chapter 4.3, 6.2).

Regarding the MAF cut-off and the magnitude of an effect that one may expect, there is much debate about the value of common versus more rare SNPs in genetic research.[14] One hypothesis suggests that relatively common SNPs do have an effect on a certain outcome, but have such a small effect (low penetrance) that it is not possible to show a statistical association. Another hypothesis suggests that this small effect associated with a common SNP, in fact is caused by a rare SNP with a tremendous effect on the outcome of interest (i.e. common disease). The signal picked up by the common SNP is simply the diluted effect of the rare SNP. Rare SNPs are more likely to have a large effect on disease status compared to common SNPs, as such a polymorphism is subject to selection pressure. This is also true for PGx related SNPs, as genes related to disease maybe implicated in the working mechanism of a drug. An example of which is genetic variability within the *LDLR* gene, encoding the LDL receptor. Rare *LDLR* loss of function mutations cause

familial hypercholesterolemia (FH) which is associated with an increased risk of mortality and a variable response to statins.[39] In addition, rare SNPs not related to disease (e.g. in pharmacokinetic genes) are also more likely to have higher impact because selection pressure also exists for drugs during drug development.

In this thesis, we generally applied a MAF cut-off of 0.2 for the selection of SNPs. This means that the minor allele represents 20% of all the alleles for a given SNP in our study population. Although this threshold was chosen for statistical power reasons, the downside of this cut off is that we are likely to miss the lower frequency polymorphisms. In the meta-analysis of three GWAS studies on the PGx of statins by Barber et al.,[31] the top variants associated with the lipid response to statins all had a MAF of 0.11 or more. This suggests that (i) there is a minor role for rare variants in the PGx of statins, or (ii) the study was underpowered to detect rare variants with intermediate/low penetrance, or (iii) genetic variability important for the PGx of statins was not captured with their genotyping array. The fact that rare variants have not been addressed in this thesis is one of the limitations of our study.

One of the biggest data-analysis challenges in PGx epidemiology lies within dealing with multiple testing, as the number of genetic variables to test with most aforementioned approaches is subject to detection of false positive signals. Whether to adjust for multiple testing depends on whether a study is hypothesis testing or hypothesis generating. The difference between hypothesis testing and generating is not always completely clear and therefore often heavily debated.

In the studies involving the *GNB3* and coagulation factor genes, we tested the hypothesis that single known functional polymorphisms within these genes could act as an effect modifier for the effectiveness of statins (chapter 3). We also conducted studies to investigate the effect of all common genetic variability (using tagging SNPs) in many candidate genes within multiple pathways (pharmacokinetic, inflammation, and cholesterol lowering) on the response to statins, which can be appreciated from both a hypothesis testing and hypothesis generating point of view. Clearly, these genes are all candidate genes, because they are implicated in a pathway or have previously been associated with altered response to statin treatment. Therefore, the hypothesis to test would be that variability within these genes could affect statin effectiveness. Tagging SNPs capture variability within a gene often without any knowledge about the functional consequences of the SNP of interest, or the SNPs that are in linkage disequilibrium with the tagging SNP. Although, a tagging SNP within a candidate gene may be testing the hypothesis that the gene of interest is implicated in the PGx of statins, it gives rise to the hypothesis (hypothesis generating) that a SNP in LD with the tagging SNP has a functional effect on the working mechanism of statins. Results from a cardiovascular gene-centric 50 k SNP array, GWAS, or whole genome sequencing study are mostly hypothesis generating, but can also confirm previously identified markers such as the case with the warfarin GWAS that have been reported.[1 28 30] The more genes, more SNPs in those genes, and the more pathways included in a study, the more a study should be considered as hypothesis generating with all the methodological challenges that such a study brings along such as the issue of multiple testing which has been depicted in figure 1. Opinions differ as to what would be the best approach.[40 41] Clearly, hypothesis-driven science has shown to be very successful over the past decades, whereas the successes of data-driven research has yet to be proven.[41] On the other hand, there is

much we do not understand in medicine, and data-driven research can give new leads for better and new treatments.[40]

A widely accepted penalty for multiple testing is the Bonferroni correction, which lowers the p-value threshold (Type-I error) for significance as the number of tests increases.[42] This correction can be considered too conservative as it is likely to reject true positive interactions when applied to genetic data such as presented in chapter 4 and 5. Also, the Bonferroni correction is too conservative because many SNPs are not independent. To deal with multiple testing, we calculated the positive false discovery rate (pFDR) to assess the proportion of false positives among those declared significant.[43] All of the tests performed in chapter 4 would have been declared statistically not significant if the Bonferroni correction would have been applied. Nonetheless, much more valuable is the estimate of the proportion of false discoveries using the q-value. The q-value is the pFDR derived p value analog adjusted for multiple testing.

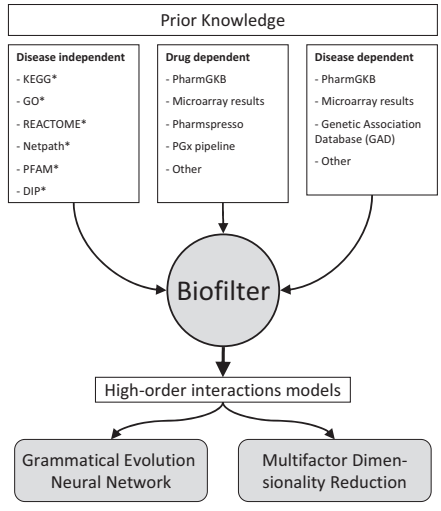
Given the complexity of human (patho)physiology and statin working mechanism, it is very unlikely that the genetic contribution to the interindividual variability in response to statins depends on one single SNP or gene. The opposite is more plausible: statin responsiveness is a function of a network of genes and regulatory sequences. Nonetheless, very few studies in genetics and PGx address epistasis, partly because of the lack of consensus on how to perform this analysis.

A first step to assess the effect of multiple SNPs together rather than one can be taken by using haplotypes instead of genotypes. A haplotype is a combination of SNPs on a single chromatid that is inherited together. An expensive method to obtain haplotype information is direct, laboratory-based haplotyping or genotyping family members to infer the unknown phase. However, statistical methods have been developed to infer haplotypes from genotypes of unrelated individuals. Theoretically, the concept of haplotypes may be more informative than genotypes.[13 44 45] However, statistically inferring haplotypes from genotype data introduces uncertainty and is therefore typically less informative than genotypes. This is especially true when LD is weak, such as the case using a tagging SNP approach.[13] Another example of a common problem with a haplotype analysis is the presence of many low frequency haplotypes. To avoid the penalty of having many degrees of freedom during data-analysis, these are generally combined in one category. Because a tagging SNP approach (chapter 4.1, 4.2, 4.3) is not suitable for haplotype construction, we only had the opportunity to construct predefined haplotypes of two well known polymorphisms in the *SLCO1B1* gene in chapter 4.1. Although the individual SNPs did not show a PGx interaction, the haplotype analysis did.

There are many methods available that surpass the haplotype analysis in assessing the effect of combinations of SNPs on drug response, such as methods described in chapter 2.2. An additional data analysis strategy capable of detecting high-order interactions will be introduced hereafter. This method may especially be valuable because of its flexibility in incorporating prior knowledge. The data analysis pipeline is depicted in Figure 2, the concept of which is largely known as ATHENA.[46] The core of this method is the Biofilter, a tool for knowledge-driven multi-SNP analysis of large scale SNP data. The Biofilter uses biological information about gene-gene relationships, gene-disease, and/or gene-drug relationships to construct multi-SNP models prior to a statistical analysis. Instead of testing the independent effect of each SNP in a genomic dataset, the Biofilter can

explicitly detect and model interactions between a set of SNPs. Thereby, the Biofilter is capable of discovering significant multi-SNP models with nonsignificant main effects that have established biological plausibility. Additionally, it reduces both the computational and statistical burden of exhaustively evaluating all possible multi-SNP models.[47] By default, sources that are used by the Biofilter include six so called disease independent (DI) databases (KEGG, GO, Reactome, Netpath, PFAM, DIP). It assigns a score called the implication index for each model, which is a measure for the degree of knowledge-based support for a given model. It simply represents the sum of the number of data sources that provide evidence of a gene-gene interaction. In addition to the DI databases, the Biofilter allows a researcher to incorporate more databases that contribute to the implication index, such as a disease dependent database (e.g. list of candidate genes for a certain disease). For studies in the field of PGx, drug dependent databases can be included. Three examples of sources that can be used to build a drug dependent databases are: (i) candidate genes from the pharmacogenomics knowledge base (GKB),[48] (ii) genes identified by a PGx text mining tool called pharmspresso,[49] and/or genes identified by the PGx pipeline, a method that ranks genes based on known gene-drug interactions, networks of gene-gene interactions, and available measures of drug-drug similarity.[50]

***Figure 2.*** Data analysis pipeline ATHENA[46]




The models that are constructed by the Biofilter can be tested with grammatical evolution neural networks (GENN) and multifactor dimensionality reduction (MDR). These methods use different approaches. MDR is a nonparametric method suitable for data with a binary outcome that reduces dimensionality by converting two or more variables to a single variable.[51] Each specific high-order model can be tested with MDR. Starting with the models with the highest implication index, the search space can be widened (i.e. models with lower implication index) when poorly predictive models are found. GENN is based on neural networks, a non-linear statistical data-modeling tool that can be used to model complex

relationships between inputs and outputs or to find patterns in data.[46 52] To optimize neural networks and detect PGx models, GENN uses evolutionary computation (EC), which has the benefit that it allows the user to optimize weight, inputs, and architecture (e.g. using the Biofilter models). The genetic algorithm searches for an optimal solution, using a classification error as the fitness function.

Future PGx research will be dealing with incredible amounts of data from unremitting advances in genotyping technology, represented by new initiative such as the 1,000 genomes project. In addition, genetic data should be complemented with data from other disciplines such as epigenomics, transcriptomics, proteomics, and metabolomics. After all, it is not only variability within the genome that affects the response to a drug. Epigenomics defines heritable patterns of gene expression that occur without changes in the DNA sequence. Transcriptomics investigates RNA, which is transcribed from DNA. Proteomics studies the dynamics of the entire set of proteins expressed by a genome, whereas metabolomics studies the metabolites in a biological cell, tissue, organ or organism. Innovative bioinformatical methods such as ATHENA will be needed to identify those patients that respond differently to a certain treatment from the enormous amounts of data from different disciplines.

## CLINICAL IMPLICATIONS

Ultimately, research in PGx aims to predict the response to a drug. The results presented in this thesis do not have direct clinical implications. First, many of the results should be replicated in other independent populations. For now, they should be considered as hypothesis generating. In addition, although the interaction with *ADAMTS1* was shown to be statistically significant and was shown to be in keeping with other studies, our (and previous)[9] findings show that statin treatment is also effective in reducing the risk of MI/CHD in patients homozygous for the disadvantageous *ADAMTS1* $^{Ala}$227 allele. Therefore, the clinical value of this and other findings, seem limited since statin treatment provided meaningful benefit in most genotype groups. Nonetheless, these genotypes could be considered as a marker when assessing cardiovascular risk and pharmacological intervention with statins as patients with a relatively low cardiovascular risk profile could already benefit from statin therapy. In other words, depending on the genotype the risk/benefit assessment may change. Additionally, our findings advocate for a more aggressive lipid lowering strategy for patients carrying a *PCSK9* E670G variant allele although the replication study for this interaction showing compatible did not reach statistical significance. Future independent studies should further contribute to the knowledge about this SNP and statin response, and may warrant clinical implication. Finally, we have shown in the Utrecht Cardiovascular Pharmacogenetics (UCP) studies, but not the Rotterdam study, that there is a small increase in risk of discontinuation of statin treatment for patients carrying a variant allele of the (tagging SNP for) *SLCO1B1* T521C. This result endorses previous recommendations made by Niemi, proposing to avoid certain statins in carriers of the variant allele.[53]

Looking at all PGx findings and their implications, considerable progress has been made over the past years. Two well established examples of PGx interactions in cardiovascular drug treatment are *CYP2C19* and clopidogrel,[5 54] and *CYP2C9*/*VKORC1* and coumarins.[5 55]

Genotype guided dosing for coumarins is currently investigated in clinical trials to assess both benefit and cost-effectiveness.[56] Besides cardiovascular drugs, a variety of PGx interactions have been found to be clinically relevant, among which irinotecan and *UGT1A1*, abacavir and *HLA-B**5701, and 5 fluorouracil and *DPD*. In fact, a study on PGx information provided by labels of the United States Food and Drug Administration (FDA) revealed that almost one fourth of all outpatients received one or more drugs that have PGx information in the label.[57] Although this seems a large proportion, the direct clinical application of this information to date is lagging behind.[58]

Ongoing is the discussion about what level of evidence is needed for PGx findings to be used in clinical practice.[59-61] Although there is call for randomized clinical prospective studies to evaluate the benefit of genotype based treatment decisions, an argument can be made for embracing evidence from case-control studies, cohort studies, and post-hoc analysis of RCTs to justify the use of genetic biomarkers for personalized drug treatment.[60] Frueh argues that groundbreaking new drugs and knowledge about disease mechanisms come from careful observation and experience.[60] Cholesterol, for example, was identified as a marker for CVD through experimental, genetic, and epidemiological research. It were only cholesterol lowering drugs that were tested in RCTs.[60] Analogue to this example, clinical application of a genetic biomarker in pharmacotherapy may be warranted given sufficient evidence from observational research. Providing high quality PGx observational studies to the medical community is therefore of great importance. Following the publication of guidelines for strengthening the reporting of observational epidemiological research (STROBE),[62] recently STREGA was published as an extension of the STROBE guidelines specifically for genetic association studies[63] to improve the quality of both design and reporting of PGx studies.

For a variety of other reasons RCTs cannot be the golden standard for evidence that warrants future implication of PGx information into practice. First of all, it will be unaffordable to conduct RCTs for all new biomarkers in PGx. Second, RCT study designs may be infeasible for many PGx interactions from an ethical, clinical, and/or logistical point of view. Finally, RCTs reflect an experimental setting whereas biomarkers should be evaluated in a real-world clinical environment.[60] However, the current attitude in medicine seems that clinical implication of a PGx interaction only seems warranted with sufficient evidence from trials.

## FUTURE OF PERSONALIZED MEDICINE

The future of personalized medicine can be appreciated from a research and clinical perspective. From a research point of view, recommendations have been provided in the methodological considerations section. An aspect that has not been discussed yet is the use of PGx for drug discovery and development. PGx research does not only identify patient groups that respond different to a drug, it also identifies new potential drug targets. For instance, variability in *PCSK9* was found to alter the response to statins in chapter 4.3. Over the past years, pharmaceutical companies are diving into this new drug target for treating dyslipidemia.[64] Similarly, the discovery of the implication of *ADAMTS1* in statin PGx may lead to the development of a new drug. In addition to the discovery of novel drug targets, PGx should play a role during drug development.[38 65]

With more important PGx discoveries to come, we need to think about how to bridge

the gap between the current state of knowledge about PGx and the clinical application. The topic of evidence has already been discussed. First, it will be of great importance to educate the health care providers that prescribe and dispense the drugs. Although the FDA does provide PGx content in drug labels, this information is worthless if physicians and pharmacists are not equipped to interpret their significance.[66] For the Dutch situation, the Royal Dutch Association for the Advancement of Pharmacy has developed PGx recommendations for 17 drugs.[67] These recommendations have been implemented in computerized pharmacy systems and are ready to assist pharmacists and physicians.[67] However, in many clinical settings genotyping is currently not performed on a routine basis. Referring to the aforementioned discussion about evidence, when genotyping has been implemented in daily practice such as the case in some psychiatric settings, observationally collected data could assist in assessing the benefit from genotype guided clinical decision making. Two US based companies have already started to use a comparable approach of implementing and evaluating genotype guided clinical decisions.[68] In fact, there is an active approach from these companies to provide both the patient and physician information about a certain PGx interaction. This includes what kind of genetic test to use, and concrete advice about the clinical decision to make. Important to consider is the costs that genetic testing brings along. There is reason to believe that payers will have a positive attitude towards PGx testing to improve the quality of drug treatment, as they are currently confronted with a high proportion of patients that is not responding to and therefore not benefiting from the prescribed drug.[69]

## CONCLUSIONS

In this thesis, we report a variety of SNPs in candidate genes and new genes to interact with statin treatment. Some of our results should be considered hypothesis testing, others hypothesis generating. Most important interactions were found with SNPs in the *ADAMTS1*, *PCSK9*, and *SLCO1B1* gene, based on previous findings, the putative mechanism lying behind the interaction, and/or results from a replication study. Extensive investigation of these genes in relation to statin responsiveness may warrant future genotype based individualized statin treatment.

## REFERENCES

1. Daly AK. Genome-wide association studies in pharmacogenomics. Nat Rev Genet 2010;11(4):241-6.
2. Evans WE, Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. Science 1999;286(5439):487-91.
3. World Health Organization.
4. Yeh RW, Sidney S, Chandra M, Sorel M, Selby JV, Go AS. Population trends in the incidence and outcomes of acute myocardial infarction. N Engl J Med 2010;362(23):2155-65.
5. Peters BJM, Klungel OH, de Boer A, Stricker BH, Maitland-van der Zee AH. Pharmacogenetics of cardiovascular drug therapy. Clin Cases Miner Bone Metab. 2009;6(1):55-65.
6. Cheung BM, Lauder IJ, Lau CP, Kumana CR. Meta-analysis of large randomized controlled trials to evaluate the impact of statins on cardiovascular outcomes. Br J Clin Pharmacol 2004;57(5):640-51.

7. Peters BJ, Klungel OH, de Boer A, Maitland-van der Zee AH. Genetic determinants of response to statins. Expert Rev Cardiovasc Ther 2009;7(8):977-83.
8. van Wieren-de Wijer DB, Maitland-van der Zee AH, de Boer A, Stricker BH, Kroon AA, de Leeuw PW, et al. Recruitment of participants through community pharmacies for a pharmacogenetic study of antihypertensive drug treatment. Pharm World Sci 2009;31(2):158-64.
9. Sabatine MS, Ploughman L, Simonsen KL, Iakoubova OA, Kirchgessner TG, Ranade K, et al. Association between ADAMTS1 matrix metalloproteinase gene variation, coronary heart disease, and benefit of statin therapy. Arterioscler Thromb Vasc Biol 2008;28(3):562-7.
10. Klungel OH, Rietveld MC, de Groot MCH, Stolk RP, Postma MJ, Leufkens HG, et al. Abstract 418 of the 26th International Conference on Pharmacoepidemiology & Therapeutic Risk Management, 19–22 August 2010, Brighton, UK. Pharmacoepidemiology and Drug Safety 2010;19(S1):S177-S178.
11. Rothman KJ, Greenland S. Modern Epidemiology. 2nd ed. Greenland: Lippincott-Raven, 1998.
12. Cardon LR, Palmer LJ. Population stratification and spurious allelic association. Lancet 2003;361(9357):598-604.
13. Balding DJ. A tutorial on statistical methods for population association studies. Nat Rev Genet 2006;7(10):781-91.
14. Maher B. Personal genomes: The case of the missing heritability. Nature 2008;456(7218):18-21.
15. Cusi D, Barlassina C, Azzani T, Casari G, Citterio L, Devoto M, et al. Polymorphisms of alpha-adducin and salt sensitivity in patients with essential hypertension. Lancet 1997;349(9062):1353-7.
16. Manunta P, Burnier M, D'Amico M, Buzzi L, Maillard M, Barlassina C, et al. Adducin polymorphism affects renal proximal tubule reabsorption in hypertension. Hypertension 1999;33(2):694-7.
17. Glorioso N, Manunta P, Filigheddu F, Troffa C, Stella P, Barlassina C, et al. The role of alpha-adducin polymorphism in blood pressure and sodium handling regulation may not be excluded by a negative association study. Hypertension 1999;34(4 Pt 1):649-54.
18. Sciarrone MT, Stella P, Barlassina C, Manunta P, Lanzani C, Bianchi G, et al. ACE and alpha-adducin polymorphism as markers of individual response to diuretic therapy. Hypertension 2003;41(3):398-403.
19. Psaty BM, Smith NL, Heckbert SR, Vos HL, Lemaitre RN, Reiner AP, et al. Diuretic therapy, the alpha-adducin gene variant, and the risk of myocardial infarction or stroke in persons with treated hypertension. JAMA 2002;287(13):1680-9.
20. Davis BR, Arnett DK, Boerwinkle E, Ford CE, Leiendecker-Foster C, Miller MB, et al. Antihypertensive therapy, the alpha-adducin polymorphism, and cardiovascular disease in high-risk hypertensive persons: the Genetics of Hypertension-Associated Treatment Study. Pharmacogenomics J 2007;7(2):112-22.
21. Schelleman H, Klungel OH, Witteman JC, Breteler MM, Hofman A, van Duijn CM, et al. Diuretic-gene interaction and the risk of myocardial infarction and stroke. Pharmacogenomics J 2007;7(5):346-52.
22. van Wieren-de Wijer DB, Maitland-van der Zee AH, de Boer A, Kroon AA, de Leeuw PW, Schiffers P, et al. Interaction between the Gly460Trp alpha-adducin gene variant and diuretics on the risk of myocardial infarction. J Hypertens 2009;27(1):61-8.
23. Finn AV, Nakano M, Narula J, Kolodgie FD, Virmani R. Concept of vulnerable/unstable plaque. Arterioscler Thromb Vasc Biol 2010;30(7):1282-92.
24. Link E, Parish S, Armitage J, Bowman L, Heath S, Matsuda F, et al. SLCO1B1 variants and statin-induced myopathy--a genomewide study. N Engl J Med 2008;359(8):789-99.
25. Mallal S, Nolan D, Witt C, Masel G, Martin AM, Moore C, et al. Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. Lancet 2002;359(9308):727-32.
26. Voora D, Shah SH, Spasojevic I, Ali S, Reed CR, Salisbury BA, et al. The SLCO1B1*5 genetic variant is associated with statin-induced side effects. J Am Coll Cardiol 2009;54(17):1609-16.

27. Keating BJ, Tischfield S, Murray SS, Bhangale T, Price TS, Glessner JT, et al. Concept, design and implementation of a cardiovascular gene-centric 50 k SNP array for large-scale genomic association studies. PLoS One 2008;3(10):e3583.
28. Cooper GM, Johnson JA, Langaee TY, Feng H, Stanaway IB, Schwarz UI, et al. A genome-wide scan for common genetic variants with a large influence on warfarin maintenance dose. Blood 2008;112(4):1022-7.
29. Takeuchi F, McGinnis R, Bourgeois S, Barnes C, Eriksson N, Soranzo N, et al. A genome-wide association study confirms VKORC1, CYP2C9, and CYP4F2 as principal genetic determinants of warfarin dose. PLoS Genet 2009;5(3):e1000433.
30. Teichert M, Eijgelsheim M, Rivadeneira F, Uitterlinden AG, van Schaik RH, Hofman A, et al. A genome-wide association study of acenocoumarol maintenance dosage. Hum Mol Genet 2009;18(19):3758-68.
31. Barber MJ, Mangravite LM, Hyde CL, Chasman DI, Smith JD, McCarty CA, et al. Genome-wide association of lipid-lowering response to statins in combined study populations. PLoS One 2010;5(3):e9763.
32. Thompson JF, Hyde CL, Wood LS, Paciga SA, Hinds DA, Cox DR, et al. Comprehensive whole-genome and candidate gene analysis for response to statin therapy in the Treating to New Targets (TNT) cohort. Circ Cardiovasc Genet 2009;2(2):173-81.
33. Funke S, Timofeeva M, Risch A, Hoffmeister M, Stegmaier C, Seiler CM, et al. Genetic polymorphisms in GST genes and survival of colorectal cancer patients treated with chemotherapy. Pharmacogenomics 2010;11(1):33-41.
34. Ouahchi K, Lindeman N, Lee C. Copy number variants and pharmacogenomics. Pharmacogenomics 2006;7(1):25-9.
35. Stranger BE, Forrest MS, Dunning M, Ingle CE, Beazley C, Thorne N, et al. Relative impact of nucleotide and copy number variation on gene expression phenotypes. Science 2007;315(5813):848-53.
36. Psaty BM, Hofman A. Genome-wide association studies and large-scale collaborations in epidemiology. Eur J Epidemiol 2010.
37. Skol AD, Scott LJ, Abecasis GR, Boehnke M. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. Nat Genet 2006;38(2):209-13.
38. Roses AD. Pharmacogenetics in drug discovery and development: a translational perspective. Nat Rev Drug Discov 2008;7(10):807-17.
39. Chaves FJ, Real JT, Garcia-Garcia AB, Civera M, Armengod ME, Ascaso JF, et al. Genetic diagnosis of familial hypercholesterolemia in a South European outbreed population: influence of low-density lipoprotein (LDL) receptor gene mutations on treatment response to simvastatin in total, LDL, and high-density lipoprotein cholesterol. J Clin Endocrinol Metab 2001;86(10):4926-32.
40. Golub T. Counterpoint: Data first. Nature 2010;464(7289):679.
41. Weinberg R. Point: Hypotheses first. Nature 2010;464(7289):678.
42. Shaffer JP. Multiple Hypothesis Testing. Annual Review of Psychology 1995;46:561-584.
43. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society, Series B (Methodological) 1995;57(1):289–300.
44. Vormfelde SV, Brockmoller J. On the value of haplotype-based genotype-phenotype analysis and on data transformation in pharmacogenetics and -genomics. Nat Rev Genet 2007;8(12).
45. Balding DJ. Reply: On the value of haplotype-based genotype-phenotype analysis and on data transformation in pharmacogenetics and -genomics. Nat Rev Genet 2007;8(12).
46. Holzinger ER, Buchanan CC, Dudek SM, Torstenson EC, Turner SD, Ritchie MD. Initialization Parameter Sweep in ATHENA: Optimizing Neural Networks for Detecting Gene-Gene Interactions in the Presence of Small Main Effects. GECCO '10, July 7-11, 2010, Portland, Oregon, USA. 2010.
47. Bush WS, Dudek SM, Ritchie MD. Biofilter: a knowledge-integration system for the multi-locus analysis of genome-wide association studies. Pac Symp Biocomput 2009:368-79.

48. Klein TE, Chang JT, Cho MK, Easton KL, Fergerson R, Hewett M, et al. Integrating genotype and phenotype information: an overview of the PharmGKB project. Pharmacogenetics Research Network and Knowledge Base. Pharmacogenomics J 2001;1(3):167-70.
49. Garten Y, Altman RB. Pharmspresso: a text mining tool for extraction of pharmacogenomic concepts and relationships from full text. BMC Bioinformatics 2009;10 Suppl 2:S6.
50. Hansen NT, Brunak S, Altman RB. Generating genome-scale candidate gene lists for pharmacogenomics. Clin Pharmacol Ther 2009;86(2):183-9.
51. Hahn LW, Ritchie MD, Moore JH. Multifactor dimensionality reduction software for detecting gene-gene and gene-environment interactions. Bioinformatics 2003;19(3):376-82.
52. Motsinger-Reif AA, Fanelli TJ, Davis AC, Ritchie MD. Power of grammatical evolution neural networks to detect gene-gene interactions in the presence of error. BMC Res Notes 2008;1:65.
53. Niemi M. Transporter pharmacogenetics and statin toxicity. Clin Pharmacol Ther 2010;87(1):130-3.
54. Ellis KJ, Stouffer GA, McLeod HL, Lee CR. Clopidogrel pharmacogenomics and risk of inadequate platelet inhibition: US FDA recommendations. Pharmacogenomics 2009;10(11):1799-817.
55. Schalekamp T, de Boer A. Pharmacogenetics of oral anticoagulant therapy. Curr Pharm Des 2010;16(2):187-203.
56. van Schie RM, Wadelius MI, Kamali F, Daly AK, Manolopoulos VG, de Boer A, et al. Genotype-guided dosing of coumarin derivatives: the European pharmacogenetics of anticoagulant therapy (EU-PACT) trial design. Pharmacogenomics 2009;10(10):1687-95.
57. Frueh FW, Amur S, Mummaneni P, Epstein RS, Aubert RE, DeLuca TM, et al. Pharmacogenomic biomarker information in drug labels approved by the United States food and drug administration: prevalence of related drug use. Pharmacotherapy 2008;28(8):992-8.
58. Novelli G, Borgiani P, Ciccacci C, Di Daniele N, Sirugo G, Papaluca Amati M. Pharmacogenomics: role in medicines approval and clinical use. Public Health Genomics 2010;13(5):284-91.
59. Cascorbi I. The promises of personalized medicine. Eur J Clin Pharmacol 2010;66(8):749-54.
60. Frueh FW. Back to the future: why randomized controlled trials cannot be the answer to pharmacogenomics and personalized medicine. Pharmacogenomics 2009;10(7):1077-81.
61. Vandenbroucke JP. Observational research, randomised trials, and two views of medical science. PLoS Med 2008;5(3):e67.
62. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Lancet 2007;370(9596):1453-7.
63. Little J, Higgins JP, Ioannidis JP, Moher D, Gagnon F, von Elm E, et al. Strengthening the reporting of genetic association studies (STREGA): an extension of the strengthening the reporting of observational studies in epidemiology (STROBE) statement. J Clin Epidemiol 2009;62(6):597-608 e4.
64. Pollex RL, Joy TR, Hegele RA. Emerging antidyslipidemic drugs. Expert Opin Emerg Drugs 2008;13(2):363-81.
65. Sofat R, Hingorani AD, Smeeth L, Humphries SE, Talmud PJ, Cooper J, et al. Separating the mechanism-based and off-target actions of cholesteryl ester transfer protein inhibitors with CETP gene polymorphisms. Circulation 2010;121(1):52-62.
66. Frueh FW, Goodsaid F, Rudman A, Huang SM, Lesko LJ. The need for education in pharmacogenomics: a regulatory perspective. Pharmacogenomics J 2005;5(4):218-20.
67. Swen JJ, Wilting I, de Goede AL, Grandia L, Mulder H, Touw DJ, et al. Pharmacogenetics: from bench to byte. Clin Pharmacol Ther 2008;83(5):781-7.
68. Frueh FW. Real-world clinical effectiveness, regulatory transparency and payer coverage: three ingredients for translating pharmacogenomics into clinical practice. Pharmacogenomics 2010;11(5):657-60.
69. Epstein RS, Frueh FW, Geren D, Hummer D, McKibbin S, O'Connor S, et al. Payer perspectives on pharmacogenomics testing and drug development. Pharmacogenomics 2009;10(1):149-51.

# 8 SUMMARY SAMENVATTING

# SUMMARY

Cardiovascular drugs such as platelet aggregation inhibitors, oral anticoagulants, antihypertensives and cholesterol lowering drugs are abundantly prescribed to reduce risk of cardiovascular disease. Treatment with these (and other) drugs can be ineffective, and/or might be accompanied by adverse drug reactions (ADRs). Ineffectiveness and susceptibility to ADRs on a patient level can partially be explained by factors such as gender, age, diet, concomitant drug use and environmental factors. To a certain extent, genetic variability may contribute to the variability in response to these cardiovascular drugs. In **Chapter 2.1** we review the current evidence for genetic variability and differences in response to the aforementioned drug classes. Clearly, there are some well established examples such as coumarin treatment and the variability within the genes coding for metabolizing liver enzyme CYP2C9 and vitamin K reductase complex subunit 1. Although several genetic markers have been identified that were shown to affect cardiovascular drug response in more than one study, many more genetic markers have been proposed, but not replicated. Numerous explanations have been proposed to contribute to the lack of reproducibility of many findings. In **Chapter 2.2**, we described some of the methodological and statistical issues within study design, gene and single nucleotide polymorphism (SNP) selection and data analysis that should be considered in future pharmacogenomic research. We elaborate on some of the pros and cons of a study that considers a small amount of genetic variation (candidate gene approach) versus a study that considers most variation in the human genome (genome-wide association study (GWAS) approach). Furthermore, we discussed conventional data analysis as well as several innovative approaches that deal with testing many variables (SNPs) and the human biological complexity in different ways.
Following Chapter 2.1, we further contributed to the knowledge about genetic determinants of the effectiveness of statins in reducing the risk of cardiovascular events in **Chapter 3**, **Chapter 4**, and **Chapter 5**. For these studies, we used data from the Genetics of Hypertension Associated Treatment (GenHAT) trial and data from an observational case control study enriched with genetic information (Utrecht Cardiovascular Pharmacogenetics studies (UCP)). The advantages of the latter study design were the relatively low costs and efforts, the feasibility to include a large amount of cases, and that it reflects everyday clinical care. Following **Chapter 2.2**, we used different methodological approaches for the studies described in **Chapter 3**, **Chapter 4**, and **Chapter 5**.
Chapter 3 focuses on one or two Single Nucleotide Polymorphisms (SNPs) within a candidate gene for effect modification of statin effectiveness. Because the *GNB3* C825T polymorphism has been shown to affect lipid parameters, atherosclerosis progression, and incidence of myocardial infarction (MI), we assessed whether the effectiveness of statins in reducing the risk of MI was modified by the *GNB3* C825T polymorphism in **Chapter 3.1**. We found that patients carrying one or two variant alleles of the *GNB3* C825T polymorphism had less benefit from statin treatment compared to homozygous wild-type carriers.
Statins have been shown to influence the expression of coagulation factors II, V, VII, XII and XIII. In **Chapter 3.2**, we investigated whether variants genes coding for these coagulation factors had an effect on the efficacy of pravastatin in reducing the risk of cardiovascular events a large randomized clinical trial. We showed that both the *F5* $^{Arg}506^{Gln}$ G>A (rs6025) and *F7* $^{Arg}353^{Gln}$ G>A (rs6046) polymorphism had an effect on pravastatin efficacy.

**Chapter 4** describes studies that consider all common variability in candidate genes within a certain pathway for the pharmacogenetics of statins, rather than a single candidate gene approach as described in **Chapter 3**. The pathways that were researched in **Chapter 4.1**, **Chapter 4.2**, and **Chapter 4.3** are the pharmacokinetic, anti-inflammatory, and cholesterol lowering pathway, respectively. Genetic variability within the *SLCO1B1* and *ABCB1* transporter genes have been associated with modification of statin effectiveness in cholesterol management, but were not subject of a pharmacgenetic study with respect to cardiovascular events so far. Previously implicated variants in *SLCO1B1* for statin responsiveness were not confirmed although we reported a non-significant haplotype-treatment interaction (p 0.054). Two variants in *ABCB1* (rs3789244, p 0.01; rs1922242, p 0.01) were shown to have an effect on statin treatment, *ABCB1* rs3789244 being an arguably good proxy for a well know haplotype involving the 1236C>T, 2677G>T and 3435C>T variants. In **Chapter 4.2**, we investigated the influence of 84 tagging SNPs within 24 candidate genes involved in the putative anti-inflammatory effects of statins on the effectiveness of statins in reducing the risk of myocardial infarction (MI). Consistent with previous findings, we found that homozygous carriers of the variant allele of a tagging SNP (rs402007) for *ADAMTS1* $^{Ala}227^{Pro}$ had most benefit from statins (adjusted odds ratio (OR) 0.10, 95%CI 0.03-0.35), compared to heterozygous (OR 0.43, 95%CI 0.24-0.51) and homozygous wild-type carriers (OR 0.49, 95%CI 0.32-0.57). Finally, a study on 209 SNPs in 27 genes involved in the cholesterol lowering pathway revealed ten SNPs in eight genes of importance for statin effectiveness, of which the most significant interaction was found with *SCARB1* rs4765615. Furthermore, we showed that carriers of the *PCSK9* E670G (rs505151) variant allele had less benefit from statin treatment, a finding that was replicated in another study, although the formal test for interaction was not statistically significant.

Subsequently, we studied a larger amount of genetic variability with respect to statin response in **Chapter 5.1**. A total of 18,011 SNPs with a minimum allele frequency of 0.2 represented in a cardiovascular gene-centric 50K SNP array were tested for their influence on statin responsiveness. This pilot study did not show any interactions that met the Bonferroni corrected p value of $2.8*10^{-6}$. *RBP1* rs295488 and *STARD13* rs2764625 showed p values of $3.42*10^{-5}$ and $5.32*10^{-5}$, respectively, and q values of 0.46 (both). Among the interactions with a p value smaller than 0.001, *RHOBTB1* was most closely related to the working mechanism of statins.

Although statins are generally well tolerated, muscular side effects are often experienced by patients. **Chapter 6.1** reviewed the literature on genetic determinants of statin induced myopathy and their implication. The T521C variant in the *SLCO1B1* gene encoding a hepatic influx transporter was previously found to increase the susceptibility to statin induced myopathy. Therefore, in **Chapter 6.2**, tagging SNPs for this variant were subject of a study on discontinuation in two independent populations, an event that is in line with suffering from ADRs. Compatible with previous findings we found a small increase in risk of statin discontinuation for variant allele carriers in UCP, but not the Rotterdam study.

Finally, **Chapter 7** provides a general discussion of our findings in a broader perspective, including the implications for clinical practice and recommendations for future research.

# SAMENVATTING

Cardiovasculaire geneesmiddelen zoals bloedplaatjesaggregatieremmers, orale anticoagulantia, antihypertensiva en cholesterolverlagende geneesmiddelen worden meestal voorgeschreven om het risico op hart- en vaatziekten te verlagen. Behandeling met deze (maar ook andere) geneesmiddelen is soms niet effectief en/of kan gepaard gaan met het optreden van bijwerkingen. Dit gebrek aan effectiviteit en de gevoeligheid voor bijwerkingen kunnen gedeeltelijk worden verklaard door factoren zoals geslacht, leeftijd, dieet, gebruik van andere geneesmiddelen en omgevingsfactoren. Tot op zekere hoogte kunnen ook genetische verschillen tussen patiënten bijdragen aan hoe een patiënt reageert op geneesmiddelen. Onderzoek naar deze genetische verschillen in relatie tot de werking van geneesmiddelen is het terrein van de farmacogenetica.

In **Hoofdstuk 2.1** geven we een overzicht van wat er bekend is over genetische variabiliteit en de verschillen in respons op de cardiovasculaire geneesmiddelen. Er zijn een aantal farmacogenetische voorbeelden te noemen waarvan het bewijs sterk is, zoals bij orale anticoagulantia (coumarines) die worden toegepast bij patiënten met een verhoogde neiging tot bloedstolling. Veel observationele studies hebben laten zien dat varianten in het gen *VKORC1*, dat betrokken is bij de bloedstolling, en het gen *CYP2C9*, dat codeert voor het leverenzym dat verantwoordelijk is voor de afbraak (metabolisme) van coumarines, een grote invloed hebben op de benodigde dosering en mate van bloedstolling. Naast dit voorbeeld zijn er verschillende genetische markers ontdekt die in meerdere studies belangrijk zijn gebleken voor de respons van een bepaald cardiovasculair geneesmiddel. Het bewijs voor het bestaan van een farmacogenetische relatie is in de meeste gevallen echter veel beperkter omdat (1) de genetische variant slechts in één studie is onderzocht of omdat (2) verschillende studies uiteenlopenede resultaten laten zien. Voor deze uiteenlopende resultaten zijn een aantal verklaringen te geven. Deze hebben te maken met de studie-opzet (bijvoorbeeld hoeveel patiënten zijn ingesloten), de keuze van de genetische varianten, etcetera. Meer hierover is te lezen in **Hoofdstuk 2.2**. Het genoom bevat onze erfelijke informatie in de vorm van DNA wat opgebouwd is uit circa drie miljard baseparen (nucleotidenparen). Combinaties van deze baseparen vormen een code voor de productie van een eiwit. Het uitrollen van het DNA uit slecht één lichaamscel zal resulteren in een twee meter lang lint. Het overgrote deel van de variatie in ons DNA wordt bepaald door Single Nucleotide Polymorhpisms (SNPs), waarvan er miljoenen bekend zijn. Het kan zijn dat een SNP geen enkel gevolg heeft voor het eiwit dat ontstaat, maar er kan ook een structureel ander eiwit ontstaan. Daarnaast kan een SNP leiden tot meer of minder van een bepaald eiwit. De hoeveelheid genetische variatie die wordt bestudeert in relatie tot de werking van een geneesmiddel (farmacogenetica), kan variëren van één enkele SNP (kandidaat SNP/gen benadering) tot wel één miljoen SNPs (genome-wide association study (GWAS) benadering). In **Hoofdstuk 2.2** beschrijven we enkele van de methodologische en statistische problemen bij het ontwerpen van een farmacogenetische studie, waaronder het selecteren van SNPs en genen. Deze keuze zal gevolgen hebben voor zowel de analyse van de data als de gehele studie-opzet. Bij het bestuderen van duizenden varianten in het DNA zal immers de kans op toevalsbevindingen sterk toenemen. We bediscussiëren zowel conventionele data-analyse methoden als innovatieve benaderingen die op verschillende

manieren geschikt zijn om om te gaan met het testen van een grote hoeveelheid SNPs en de complexiteit van het menselijk lichaam.
Statines zijn geneesmiddelen die worden ingezet om het risico op hart- en vaatziekten te verminderen door verlaging van het cholesterol. In **Hoofdstuk 3**, **Hoofdstuk 4** en **Hoofdstuk 5** worden studies beschreven die bijgedragen aan de kennis over het effect van genetische variabiliteit op de effectiviteit van statines in het reduceren van het risico op een cardiovasculaire gebeurtenis zoals een hartinfarct. Voor deze studies hebben we voornamelijk gegevens gebruikt uit een grote database met daarin medische gegevens van een groot aantal inwonders van Nederland (PHARMO). De geselecteerde patiënten voor deze studie zijn uitgenodigd door hun apotheek om mee te doen door een vragenlijst in te vullen en speeksel te doneren voor DNA-analyse. We hebben gebruik gemaakt van een case-controle studie-opzet waarbij cases hypercholesterolemie (hoog cholesterol) patiënten zijn die een hartinfarct hebben gehad en controles hypercholesterolemie patiënten zonder doorgemaakt hartinfarct. De voordelen van een dergelijke studie-opzet waren de relatief lage arbeidsintensiteit en lage kosten, de haalbaarheid veel patiënten in te sluiten en de overeenkomsten met de dagelijkse klinische praktijk.
In navolging van **Hoofdstuk 2.2** hebben we verschillende methodologische benaderingen gebruikt zoals beschreven in **Hoofdstuk 3**, **Hoofdstuk 4** en **Hoofdstuk 5**. In **Hoofdstuk 3** hebben we ons geconcentreerd op hoe één of twee SNPs in een gen de effectiviteit van statines beïnvloeden. Kandidaat genen zijn genen waarvoor we een hypothese hebben over hoe ze betrokken zijn bij de werking van een geneesmiddel. Omdat men eerder een associatie tussen het *GNB3* C825T polymorfisme en een verandering van de cholesterolhuishouding, atherosclerose progressie en incidentie van een hartinfarct heeft gevonden hebben wij in **Hoofdstuk 3.1** onderzocht of de effectiviteit van statines verschillend is tussen patiënten met of zonder de variant van het *GNB3* C825T polymorfisme. Uit onze studie bleek dat dragers van één of twee variant allelen (CT of TT) van het *GNB3* C825T polymorfisme minder baat van de behandeling met statines hadden vergeleken met dragers van twee wildtype ('normale') allelen. Volgens bevindingen van andere onderzoekers hebben statines invloed op de expressie van bloedstollingsfactoren II, V, VII, XII en XIII. In **Hoofdstuk 3.2** hebben we in een grote gerandomiseerde klinische trial bekeken of variabiliteit in genen die coderen voor deze factoren een effect hebben op de werking van pravastatine in de reductie van het risico op een cardiovasculaire gebeurtenis. In onze studie beïnvloedde zowel het F5 $^{Arg}506^{Gln}$ G>A (rs6025) als het *F7* $^{Arg}353^{Gln}$ G>A polymorfisme de effectiviteit van pravastatine.
**Hoofdstuk 4** beschijft een drietal studies die niet één enkele SNP (zoals in **Hoofdstuk 3**), maar alle frequent voorkomende genetische variabiliteit in kandidaat genen bekijken in relatie tot de effectiviteit van statines. De genen die in **Hoofdstuk 4.1**, **Hoofdstuk 4.2** en **Hoofdstuk 4.3** zijn bestudeerd zijn respectievelijk betrokken bij farmacokinetiek (opname, verdeling, afbraak en uitscheiding van een geneesmiddel), de ontstekingsremmende en cholesterolverlagende werking van statines. In **Hoofdstuk 4.1** zijn 25 varianten in de genen *SLCO1B1* en *ABCB1* onderzocht. Deze twee genen coderen voor transporters die betrokken zijn bij de opname van een statine vanuit de darmen en bij de opname in de levercel waar een statine primair zijn werk doet. Genetische variatie in deze genen kan de effectiviteit beïnvloeden omdat een verminderde of toegenomen activiteit van het eiwit dat is afgelezen kan resulteren in een veranderde opname en verdeling van de statine

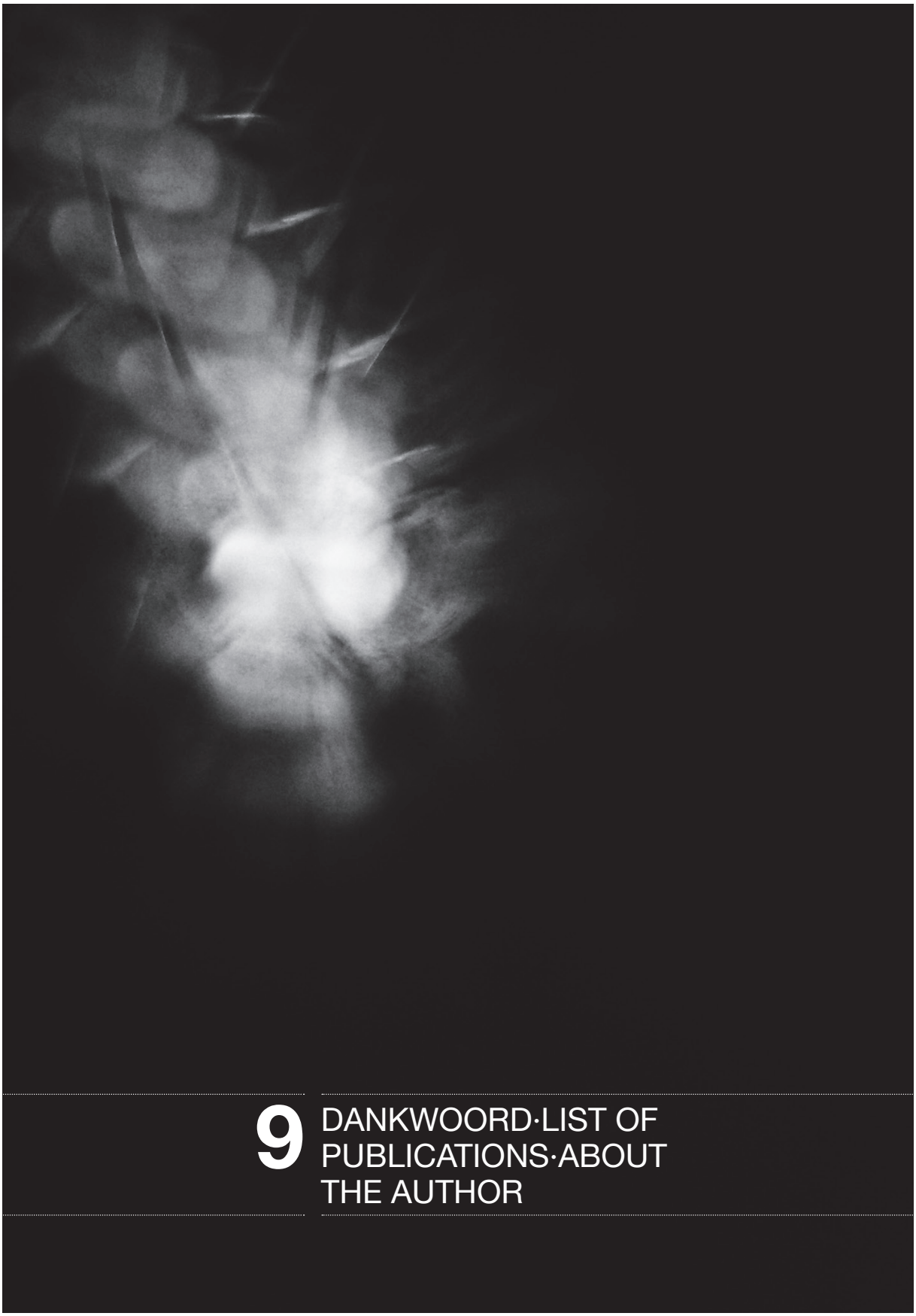
over het lichaam. Er zijn twee varianten in het *ABCB1* gen gevonden die de effectiviteit van statines beïnvloeden. Statines verlagen niet alleen het cholesterol, maar hebben ook een positief effect op het vasculaire ontstekingproces dat vaak aanwezig is bij patiënten met atherosclerose (aderverkalking). In **Hoofdstuk 4.2** hebben we 84 varianten in 24 genen bestudeerd die betrokken zijn bij dit ontstekingsproces. In overeenstemming met bevindingen van andere onderzoekers hebben we gevonden dat patiënten die twee varianten dragen van de *ADAMTS1* $^{Ala}227^{Pro}$ SNP het meeste baat hebben bij behandeling met statines (odds ratio (OR) 0.10, 95% betrouwbaarheidsinterval (BI) 0.03-0.35) in vergelijking met patiënten die één (OR 0.43, 95% BI 0.24-0.51) of geen (OR 0.49, 95% BI 0.32-0.57) variant dragen.

Het laatste **Hoofdstuk 4.3** omvat 209 SNPs in 27 genen betrokken bij de cholesterolhuishouding. Hiervan bleken tien SNPs in acht genen de effectiviteit van statines statistisch significant te beïnvloeden, waarvan de meest significante interactie met *SCARB1* rs4765615. Daarnaast hebben we in twee studies (waarvan één statistisch significant) laten zien dat patiënten met de bekende *PCSK9* E670G variant minder baat hebben bij behandeling met statines ($OR_{EG/GG}$ 0.63, 95% BI 0.30-1.32 versus $OR_{EE}$ 0.36, 95% BI 0.28-0.45).

Vervolgens hebben we in **Hoofdstuk 5.1** een totaal van 18.011 SNPs bekeken. Deze SNPs liggen in genen die in tegenstelling tot de genen uit **Hoofdstuk 3** en **4** een kleinere kans hebben om geïmpliceerd te zijn voor de farmacogenetica van statines. De reden daarvoor is dat deze varianten niet per se in genen liggen die direct gerelateerd zijn aan de werking van statines. Wel zijn al deze genen cardiovasculair gerelateerd. De insteek van deze studie is dan ook fundamenteel anders. Waar we eerder meer een hypothese-testende benadering hebben gekozen, beschrijft dit Hoofdstuk een hypothese-genererende studie. Varianten die worden ontdekt in onbekende genen genereren immers de hypothese dat een gen belangrijk is voor het werkingsmechanisme van statines. Doordat er zoveel verschillende testen worden uitgevoerd bestaat er een grote kans op vals positieve bevindingen. Ondanks dat *RBP1* rs295488 en *STARD13* rs2764625 een interactie met statines lieten zien met een p-waarde van $3.42 \cdot 10^{-5}$ and $5.32 \cdot 10^{-5}$, is dit niet voldoende significant volgens de conservatieve Bonferroni correctie voor het uitvoeren van 18,011 testen. Een andere correctie, genaamde de positive False Discovery Rate (pFDR), suggereert echter dat slechts één van deze interacties een toevalsbevinding zou zijn als gevolg van 'multiple testing'. Onder de meest significante bevindingen was het *RHOBTB1* gen het enige gen dat mechanistisch een duidelijke relatie heeft met statines. Vervolgstudies moeten uitwijzen welke van deze bevindingen daadwerkelijk waar zijn.

Zoals eerder genoemd kan genetische variabiliteit niet alleen invloed hebben op de effectiviteit van een geneesmiddel zoals bestudeerd in **Hoofdstuk 3**, **4** en **5** maar ook op de gevoeligheid voor bijwerkingen. Over het algemeen worden statines goed verdragen. De meest gerapporteerde bijwerkingen van statines zijn spiergerelateerd. In **Hoofdstuk 6.1** geven we een overzicht van de kennis over genetische varianten en de gevoeligheid voor statine-geïnduceerde myopathie. De variant die in een aantal studies belangrijk werd gevonden is de T521C variant in het *SLCO1B1* gen. Dragers van het C allel hebben een grotere gevoeligheid voor bijwerkingen van behandeling met een statine. Daarom is specifiek deze variant in twee verschillende populaties bestudeerd in relatie tot het risico op het stoppen van de statine behandeling in **Hoofdstuk 6.2**. In overeenstemming met

eerdere beinvindingen vonden we een kleine toename in risico op stoppen voor patiënten met de genetische variant in één van de twee populaties.
Tenslotte omvat **Hoofdstuk 7** een algemene discussie van onze resultaten in een breder perspectief. We hebben hierbij de klinische implicaties en aanbevelingen voor toekomstig onderzoek besproken. We hebben onder andere aangegeven dat gerandomiseerde klinische trials niet per definitie de gouden standaard hoeven te zijn die klinische implicatie van een farmacogenetische interactie rechtvaardigd.



# 9 DANKWOORD·LIST OF PUBLICATIONS·ABOUT THE AUTHOR

# DANKWOORD

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# LIST OF PUBLICATIONS

Peters B, Maitland-van der Zee AH. Pharmacogenomic importance of pravastatin. Pharmacogenomics 2008;9(9):1207-10.

Peters BJ, Maitland-van der Zee AH, Stricker BH, van Wieren-de Wijer DB, de Boer A, Kroon AA, et al. Effectiveness of statins in the reduction of the risk of myocardial infarction is modified by the GNB3 C825T variant. Pharmacogenet Genomics 2008;18(7):631-6.

Peters BJ, Klungel OH, de Boer A, Stricker BH, Maitland-van der Zee AH. Pharmacogenetics of cardiovascular
drug therapy. Clin Cases Miner Bone Metab. 2009;6(1):55-65.

Peters BJ, Klungel OH, de Boer A, Maitland-van der Zee AH. Genetic determinants of response to statins. Expert Rev Cardiovasc Ther 2009;7(8):977-83.

Maitland-van der Zee AH, Peters BJ, Lynch AI, Boerwinkle E, Arnett DK, Cheng S, et al. The effect of nine common polymorphisms in coagulation factor genes (F2, F5, F7, F12 and F13) on the effectiveness of statins: the GenHAT study. Pharmacogenet Genomics 2009;19(5):338-44.

Peters BJ, Klungel OH, Visseren FL, de Boer A, Maitland-van der Zee AH. Pharmacogenomic insights into treatment and management of statin-induced myopathy. Genome Med 2009;1(12):120.

Peters BJ, Rodin AS, de Boer A, Maitland-van der Zee AH. Methodological and statistical issues in pharmacogenomics. J Pharm Pharmacol 2010;62(2):161-6.

Peters BJ, Rodin AS, Klungel OH, van Duijn CM, Stricker BH, van't Slot R, et al. Pharmacogenetic interactions between ABCB1 and SLCO1B1 tagging SNPs and the effectiveness of statins in the prevention of myocardial infarction. Pharmacogenomics 2010;11(8):1065-76.

Peters BJ, Rodin AS, Klungel OH, Stricker BH, de Boer A, Maitland-van der Zee AH. Variants of ADAMTS1 modify the effectiveness of statins in reducing the risk of myocardial infarction. Pharmacogenet Genomics 2010;20(12).

# ABOUT THE AUTHOR

Bas Peters was born in Veghel, The Netherlands, on September 28th 1982. In 2000, he completed secondary school at the Zwijsen College in Veghel. Subsequently, he studied pharmacy at Utrecht University. In 2005, he obtained his Master's degree in pharmacy followed by his pharmacist degree in December 2006. The same month, Bas started his PhD at the division of Pharmacoepidemiology and Clinical Pharmacology. In January 2011, he will continue his carreer at the St. Antonius Ziekenhuis as a hospital pharmacist trainee.