6 Genetic Polymorphisms and Response to HMG-CoA Reductase Inhibitors

Anke-Hilse Maitland-van der Zee, PharmD, PhD,
Olaf H. Klungel, PharmD, PhD,
and Anthonius de Boer, MD, PhD

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SUMMARY

Coronary artery disease is among the leading causes of death worldwide. Clinical trials show a protective effect of statins against coronary artery disease. The mean risk reductions for subjects using statins compared with placebo found in these trials is about 30%. These are average reductions for all patients included in the trials. Important factors in interpreting the variability in outcome of drug therapy include the patient's health profile, prognosis, disease severity, quality of drug prescribing, compliance with prescribed pharmacotherapy, and the genetic profile of the patient. This chapter aims to give an overview of the known polymorphisms that have an influence on the effects of statins in the general population. The expectation is that in the future, a subject's genotype may determine whether he/she will be treated with statins or not. Determining the genotype will not deny therapy to a subject, but will help in the decision as to which therapy suits the patient best.

Key Words: Pharmacogenetics; HMG-CoA reductase inhibitor; Apolipoprotein ε; ACE insertion deletion; CETP; Stromelysin-1; β fibrinogen gene; lipoprotein lipase; hepatic lipase; platelet glycoprotein; toll-like receptor 4; interleukin-1B.

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INTRODUCTION

Cardiovascular disease is one of the leading causes of death, especially in developed countries. Several risk factors for cardiovascular disease have now been well established, especially cigarette smoking, hypertension, lipid disorders, and diabetes mellitus. Pharmacotherapeutic interventions such as the use of aspirin and other antithrombotic drugs, antihypertensive drugs, and cholesterol-lowering therapy probably have contributed to the gradual decline in cardiovascular disease over the last decades *(1,2)*. Statins are inhibitors of hydroxy-methylglutaryl coenzyme A (HMG-CoA) reductase, which inhibits cholesterol production in the hepatocyte. This increases the synthesis of low-density lipoprotein (LDL) receptors and thereby lowers cholesterol. In recent years, several landmark trials have been published that establish the efficacy of statin therapy in primary and secondary prevention *(3–7)*. Treatment with statins over a 5-yr period was associated with a statistically significant reduction in mortality and in the number of patients experiencing a heart attack or a stroke, or undergoing a revascularization procedure. The risk reduction for coronary artery disease for subjects using statins was approx 30%. These reductions, however, are average effects for all patients included in the trials; in subpopulations these effects may differ.

Important factors in interpreting variability in the outcome of drug therapy include the patient's overall health, prognosis, disease severity, quality of drug prescribing, compliance with prescribed pharmacotherapy, and the genetic profile of the patient *(8,9)*. In the large trials, several subgroups have been analyzed. In the Cholesterol and Recurrent Events (CARE) trial, the effect of pravastatin on the rates of major coronary events was greater among women (–46% [95% CI –22 to –62%]) than among men (–20% [95% CI –8 to –30%]) *(3)*. In the other trials there were no significant differences between men and women. Other subgroup analyses included age groups; comorbidity such as hypertension, diabetes mellitus, previous myocardial infarction, and smoking; and pretreatment plasma lipid levels, but these yielded no statistically significant differences *(3–7,10,11)*. In the Scandinavian Simivastatin Survival study (4S), measurements of the serum cholestanol concentration revealed a subgroup of patients in whom coronary events were not reduced by simvastatin treatment. The reduction in relative risk increased gradually from 0.62 to 1.17 in the quarters of distribution of cholestanol at baseline. Thus, patients with high baseline synthesis of cholesterol seem to be responders whereas those with low synthesis of cholesterol are nonresponders *(12)*.

PHARMACOGENETICS

Pharmacogenetics and pharmacogenomics are emerging disciplines that focus on genetic determinants of drug response at the levels of single genes or the entire human genome, respectively *(13)*. Polymorphisms may influence drug response in three ways *(14)*. The first way is through pharmacokinetic interactions, caused by genetically based differences in drug absorption, disposition, metabolism and excretion. An important role is played by polymorphisms in the cytochrome P450 (CYP) enzymes. In addition, there are pharmacodynamic gene–drug interactions that involve gene products expressed as receptors or signal transduction molecules, which are relevant to the pharmacodynamics of drugs. After entering the circulation each drug interacts with numerous proteins and multiple types of receptors. These proteins determine the site of action and the pharmacological response. The third possibility of influencing drug response is through genes

that are in the causal pathway of disease and are able to modify the effects of drugs. For example, differences in the effects of statins may be measured on cholesterol levels. However, because statins have certain effects that are independent of cholesterol lowering, such as effects on blood pressure, coagulation, cell proliferation, immune function, and macrophage metabolism *(15,16)*, the effects on cholesterol levels alone may not accurately predict the effects on cardiovascular morbidity and mortality *(17)*. Another way to measure the effects of statins is the rate of progression of coronary atherosclerosis measured by coronary angiography. When a difference in response is measured on these endpoints it is not known whether this drug resistance also translates into a lack of effect on important clinical outcomes (fatal and nonfatal coronary disease).

This chapter aims to give an overview of the currently known polymorphisms that influence the effects of statins. This chapter is an update of a previous publication on this subject *(18)*.

PHARMACOKINETIC INTERACTIONS

Although several studies have been published recently on possible pharmacokinetic interactions with statins, currently no useful polymorphisms for the prediction of the pharmacokinetics (and thereby predictions of efficacy and adverse effects) are available. Statins are highly extracted by the liver. The CYP enzyme system plays an important part in the metabolism of statins. Most statins (lovastatin, simvastatin, and atorvastatin) are predominantly metabolized by CYP3A4 *(19,20)*. For this enzyme, no functional polymorphisms have been described so far *(21)*.

Simvastatin is given orally as a prodrug and is converted to the active form simvastatin hydroxy acid. Simvastatin is not only metabolized by CYP3A4, but also by CYP2D6 and CYP2C9 *(22)*. Several studies have been published on the role of the CYP2D6 polymorphism in the efficacy of simvastatin. The in vivo activity of the CYP2D6 enzyme is characterized by extreme individual variability. The variability in the rate of metabolism by CYP2D6 is more than 100-fold, which is genetically determined *(23)*. To date, 30 polymorphisms in the *CYP2D6* gene have been described, resulting in classification of the phenotype into three categories *(24)*: subjects with normal activity (extensive metabolizers), subjects with low or absent activity (slow metabolizers), and subjects with high activity (ultraprapid metabolizers). It appears that genotyping for the most common defective alleles will predict the CYP2D6 phenotype with a high amount of certainty *(24)*. In 1997, Nordin et al. found in a study of 10 healthy volunteers that subjects who were ultrarapid metabolizers might need higher dosages of simvastatin to be effective *(25)*. Mulder et al. studied 88 patients, with primary and secondary hypercholesterolemia, to investigate the link between polymorphism of CYP2D6 and the efficacy and tolerability of simvastatin treatment. Mulder et al. found results in line with the results of Nordin et al. Adverse effects were more common in slow metabolizers and the efficacy in lowering cholesterol levels was also higher in this group *(26)*. Geisel et al. did not find such an association in a study of 41 patients with primary hypercholesterolemia *(27)*. Several reasons for the different results were discussed. Mulder et al. included patients with secondary hypercholesterolemia and neglected to analyze several nonfunctional alleles and therefore, may have misclassified subjects. On the other hand, the study of Mulder et al. included more patients and therefore, might have had a better chance of finding significant differences *(27,28)*. Because only a few small studies have been performed, larger studies are necessary to assess whether this interaction is relevant.

Polymorphisms in CYP2C9 have been shown to influence response to warfarin *(29)*. Because fluvastatin is also metabolized via this isoform, studies assessing a possible interaction between fluvastatin and CYP2C9 polymorphisms might be of interest *(21)*. Kirchheiner et al. investigated the pharmacokinetics and the cholesterol-lowering activity of fluvastatin in 24 healthy subjects who took 40 mg fluvastatin for 2 wk *(30)*. The pharmacokinetics depended on the CYP2C9 genotype, but differences in plasma concentrations were not reflected in cholesterol *(30)*. Larger studies in hypercholesterolemic patients are needed to confirm these results.

Another pharmacokinetic interaction was published with pravastatin. Pravastatin is not significantly metabolized by the CYPP450 system *(31)*. Pravastatin is rapidly absorbed from the upper region of the small intestine, and then taken up efficiently from the circulation by the liver through organic anion transporting polypeptide C (OATP-C), a sodium independent bile transporter *(32)*. A number of single nucleotide polymorphisms have been identified that changed the in vitro transport capability *(33)*. Nishizato et al. investigated the significance of polymorphisms in the *OATP-C* and *OAT3* genes in a Japanese population ($n = 120$) with regard to the disposition kinetics of pravastatin. They provide evidence that the *OATP-C* gene polymorphism contributes to in vivo activity of OATP-C and thereby to differences in the pharmacokinetics of pravastatin. This might lead to differences in the efficacy and safety of pravastatin. Because of the small number of subjects in this study, these results should be confirmed in larger studies *(32)*.

All results in the pharmacokinetic studies discussed in this section are still debated and must be further investigated. Alternative candidate genes for pharmacokinetic interactions include the *CYP3A4* gene, the ABC transporter-like *MDR1* gene, or the glucuronyl transferase gene, because these genes are also involved in the excretion an biotransformation of HMG-CoA reductase inhibitors *(21,34)*.

GENES IN CAUSAL PATHWAY OF DISEASE

Cholesteryl Ester Transfer Protein Polymorphism

Reverse Cholesterol Transport

The presence of a polymorphism in the cholesteryl ester transfer protein *(CETP)* gene (which is also called *Taq1B*) is associated with alterations in lipoprotein levels and high-density lipoprotein (HDL) concentrations. The CETP enzyme has a central role in reverse cholesterol transport (RCT), whereby cholesterol from peripheral tissues is transported back to the liver, where it is preferentially excreted into bile. There are several proteins crucial for RCT. These proteins include the ATP-binding cassette transporter 1 gene (ABC_1 transporter), lecithin cholesterol acyl transferase (LCAT), CETP, hepatic lipase (HL), and scavenger receptor type BI (SR-BI) *(35)*.

The *ABC_1* gene product has been provisionally termed cholesterol-efflux regulatory protein (CERP). CERP is involved in the transfer of free cholesterol and phospholipids (PLs) form macrophages to apoA-I. Mutations in the *ABC_1* gene cause plasma HDL deficiency and premature atherosclerosis *(36,37)*.

Nascent HDL particles, mainly consisting of apo-A1 and PLs, are acceptors for free cholesterol. As soon as these HDL discs have absorbed free cholesterol, the LCAT enzyme converts this cholesterol to cholesterol esters, which increase HDL size from disc to spherical. CETP is then capable of transporting these cholesterol esters to very low-density lipoprotein (VLDL) or LDL. In exchange, CETP converts triglycerides (TGs)

back to HDL. VLDL and, consequently, LDL transport these cholesterol esters back to the liver, where they are taken up through the LDL receptor system. Conversely, in the other pathway, HL hydrolyzes the TGs in HDL *(35)*, which renders HDL a prime candidate for direct uptake into the liver by the SR-BI receptor

CETP Polymorphism

The presence of the polymorphism in the first intron of the *CETP* gene is referred to as TaqIB. People with the B1 allele have higher CETP concentrations and, because cholesterol esters are extracted from HDL, lower HDL-cholesterol concentrations. CETP levels are highest in subjects with two copies of the B1 allele (B1B1) and lowest in persons with the B2B2 genotype. The mechanism by which the CETP polymorphism may affect CETP activity is not known. It is unlikely that this polymorphism, which is located in an intron, represents a functional mutation. The most plausible explanation is that this polymorphism is in almost complete linkage disequilibrium with a functional polymorphism in the promotor region of the *CETP* gene, the CETP/–629 polymorphism *(38,39)*. This –629 polymorphism, corresponding to a C/A substitution at position –629, exerts a significant effect on transcriptional activity.

Kuivenhoven et al. observed a significant genotype-dependent association of the CETP TaqIB polymorphism with the progression of coronary atherosclerosis in the placebo group; carriers of the B1B1 genotype had the highest CETP concentrations, the lowest HDL-cholesterol (C) concentrations and the fastest progression of coronary atherosclerosis *(40)*. Ordovas et al. found that CETP activity was decreased in men and women with the B2 allele. The HDL concentration for both men and women was lowest in the B1B1 group. For men carrying the B2 allele, the odds ratio for prevalent coronary heart disease was 0.70 (95% CI 0.50–0.98), but after adjustment for HDL-C levels and other known risk factors, the odds ratio was no longer significantly different (0.74 [95% CI 0.46-1.16]). No significant protective effects of this genotype were observed in women *(41)*. There is some controversy in the literature as to whether CETP inhibition is beneficial in preventing ischemic heart disease. Several studies in Japanese populations support the results from Kuivenhoven et al., which showed that CETP deficiency is potentially antiatherogenic and may be associated with an increased life span *(42,43)*. Other studies find an increased risk of ischemic heart disease in subjects with low CETP concentrations even though they have higher HDL-C concentrations *(44–48)*. Van Venrooij et al. found in a 30-wk randomized trial in 217 unrelated diabetic subjects that subjects with the B1B1 genotype or the CC carriers (which were tightly concordant in their study [$p < 0.001$]) had a more atherogenic lipid profile (lower HDL, higher TGs). They also showed that these subjects responded better to atorvastatin with respect to reduction of CETP mass and elevation of HDL-C concentrations *(49)*.

In addition, Kuivenhoven et al. found no difference in plasma lipoprotein response to pravastatin between the different genotypes, but the B2B2 genotype (about 16% of all Caucasian men) with low CETP and high HDL concentrations did not respond to pravastatin in terms of decreased disease progression 40. Furthermore, in a case-control study, Fumeron et al. showed that HDL-C levels were only increased in subjects with the B2B2 genotype after ingesting at least 25 g of alcohol per day *(50)*. In the cohort of Kuivenhoven et al., alcohol consumption did not affect the association between the CETP genotype and the angiographic outcome in the control group, nor in the group treated with pravastatin *(51)*. In the WOSCOPS trial (one of the primary prevention trials) the same

association between the B2B2 genotype and cardiovascular events was found. Homozygotes for the B2B2 allele had a higher HDL-C and a 30% reduction of suffering a cardiovascular event compared to B1B1 homozygotes. This association was primarily found in nonsmokers, not in smokers. However, no interaction was observed between the *CETP* genotype and pravastatin treatment *(52)*. They also typed the –631 C/A, –629 C/A, I405V, and D442G CETP polymorphisms but they did not find significant association with risk. Haplotype analysis did not add to the information given by the individual polymorphisms *(52)*. Blankenberg et al. found in the Atherogene study, a cohort study including 1303 patients who had at least one stenosis greater than 30% in a major coronary artery diagnosed with coronary angiography, that in patients with coronary artery disease, the CETP –629 A allele (which is almost completely concordant with the B2 allele of the Taq IB polymorphism) had a strong protective effect on future mortality from cardiovascular causes. Furthermore, a statistically significant interaction between the CETP –629 C/A polymorphism and statin treatment on cardiovascular mortality was found. The benefit of statin treatment was restricted to subjects with the –629 C allele. In these patients, cardiovasular mortality was reduced by about 50% in those taking statin medication, whereas no effect was observed in patients carrying the –629 A allele *(53)*.

Stromelysin-1 Polymorphism

Matrix metalloproteinases (such as stromelysin) have important roles in connective tissue remodeling during tissue repair, cell migration, angiogenesis, tissue morphogenesis, and growth *(54–57)*. These physiological processes require a tightly controlled balance between matrix metalloproteinases and their specific tissue inhibitors. Disruption of this balance may lead to various pathological states. Low tissue levels of these metalloproteinases are associated with atheroscerosis *(54)*. A common polymorphism in the promotor sequence of the stromelysin-1 gene exists, with one allele having a run of six adenosines (6A) and the other of five adenosines (5A). The prevalence of the two alleles 5A and 6A was found to be 51% and 49% in a sample of 354 healthy individuals in the United Kingdom *(54)*. This promotor variant affects transcription. In vitro studies have shown that genotypes with a 5A allele expressed higher activity of stromelysin-1 than the 6A allele in both cultured fibroblasts and vascular smooth muscle cells *(54)*. Compared with other genotypes, individuals homozygous for the 6A allele would have lower stromelysin-1 levels in their arterial walls because of lower transcription level, and this might therefore favor deposition of extracellular matrix in the atherosclerotic lesions. This may lead to the development of an atherosclerotic plaque with a thick cap, and result in a more rapid progression of angiographically defined stenosis *(58)*.

In the REGRESS trial of pravastatin vs placebo, de Maat et al. compared male patients with coronary artery disease with 5A5A, 5A6A, and 6A6A genotypes of the stromelysin polymorphism, with respect to relevant clinical events such as myocardial infarction (fatal or nonfatal), mortality of coronary heart disease, percutaneous transluminal coronary angioplasty or coronary artery bypass graft surgery, stroke and transient ischemic attack, and overall mortality *(59)*. Patients in the placebo group with the 5A6A or 6A6A genotypes had a higher 2-yr cumulative incidence of one of these clinical events than patients with the 5A5A genotype (26% and 12%, respectively; $p = 0.03$). In the pravastatin group, the risk of clinical events in patients with 5A6A or 6A6A genotypes was lower (14%) than in patients on placebo, whereas it was unchanged in those with a 5A5A genotype (17%) (*see* Table 1). These effects were independent of the effects of pravastatin on lipid levels *(59)*.

Table 1
Polymorphisms That Might Influence the Efficacy of Statin Therapy

Polymorphism	*Effect*	*References*
Cytochrome P450 2D6	Slow metabolizers may have higher efficacy and more side effects of simvastatin	*25–28*
Cytochrome P450 2C9	No effect found with fluvastatin	*30*
OATP-C	Disposition kinetics of pravastatin were different.	*32*
CETP Taq IB and –629 C/A	Subjects with the B2B2 or with –629 AA allele had no effect from statin therapy on reduction of angiographically defined CHD and on reduction of cardiovascular events.	*40,51–53*
Stromelysin-1	Subjects with the 5A5A genotype had no effects of pravastatin on incidence of clinical events.	*59*
β-fibrinogene –455 G/A and TaqI	Subjects with the –455 AA genotype had a greater efficacy of statins.	*67*
Apolipoprotein E	Subjects with the ε4 allele have the least cholesterol lowering effect with statins, but they have the same or even a better effect of statins (compared with the ε2 and ε3 allele) in reducing CHD.	*72,74–85*
Lipoprotein lipase gene	Subjects with the Asp9Asn mutation had a greater efficacy of statins on angiographic parameters.	*91*
ACE insertion deletion	Results for this polymorphism are conflicting.	*78,100–103*
Hepatic lipase –514 C/T	Subjects with the TT genotype did not have a regression of angiographically determined atherosclerosis after 2.5 y of intensive lipid-lowering therapy.	*108*
Platelet Glycoprotein	Statin therapy reduced the increased rate of restenosis associated with the $P1^{A2}$ allele.	*100,110*
Toll-like receptor 4	Carriers of the 229 Gly had a much stronger cardiovascular risk reduction, but the interaction was not found in reduction of lipid parameters.	*117*
Interleukin-1B	Coronary function improves after 6 mo of statin therapy in subjects with the A2- allele but not in subjects with the A2+ allele.	*118*

OATP, organic anion transporting polypeptide; CETP, cholesteryl ester transfer protein; ACE, angiotensin-coverting enzyme; CHD, coronary heart disease.

–455G/A and Taq I Polymorphisms of the β-Fibrinogen Gene

Several epidemiological studies have reported a strong associaton between elevated plasma levels of fibrinogen and an increased risk of myocardial infarction *(60–65)* and stroke *(66)*. Because fibrinogen is an acute phase protein, an increased plasma fibrinogen level may reflect the inflammatory state of the vascular wall and may thus be related to cardiovascular risk *(64)*. The three chains of fibrinogen are encoded by three different genes *(68)*. Polymorphisms of the α gene (*Taq I*) and of the β gene (*–455G/A*) are associated with differences in plasma levels of fibrinogen.

De Maat et al. found in The REGRESS trial significantly higher fibrinogen levels (3.9 g/L [95% CI 3.2–4.8]) in subjects who where homozygous for the rare –455A allele (the

frequency was 0.21%), whereas the homozygotes for the common -455G allele and the heterozygotes had comparable fibrinogen levels (3.2 g/L [95% CI 3.0–3.3], and 3.1 g/L [95% CI 2.9–3.3], respectively) *(67)*. In the placebo group, subjects with the –455AA had a significantly greater progression of coronary artery disease, as reflected by angiographic variables (mean segment diameter decrease 0.24 ± 0.45), than patients with the –455GG and the –455GA genotypes (mean segment diameter decrease 0.09 ± 0.39 and 0.10 ± 0.39, respectively). In patients receiving pravastatin, this difference was not observed. These results suggest that pravastatin is capable of offsetting the deleterious effects of the –455AA genotype of the β-fibrinogen gene *(67)*.

Apolipoprotein-ε4

Several studies have investigated the relation between apolipoprotein-ε (apoε) genotypes and the LDL cholesterol response to HMG-CoA reductase inhibitors in hypercholesterolemic patients. Human apoε is a genetically polymorphic protein defined by three alleles—ε2, ε3, and ε4—at a single gene locus on chromosome 19; these code for three isoforms (ε2, ε3, and ε4) that differ by an amino acid substitution at residues 112 and 158, and thus determine the six genotypes resulting from the combination of any two of them *(69)*. Apoε allele frequencies in the normal population were assessed in 2457 subjects in the Framingham Offspring study and were 8%, 78%, and 14% *(70)*, respectively, for ε2, ε3, and ε4; Corbo et al. investigated the worldwide distribution of the apoε polymorphism and discovered that the ε3 allele is the most frequent in all human populations and that its frequency is always negatively correlated with that of ε4 *(71)*. The ε4 allele occurs relatively frequently in human populations where foraging still exists or food supplies are scarce or qualitatively poor. Carrying this allele would be favorable, because this allele promotes intestinal cholesterol uptake and increases plasma cholesterol levels which otherwise would be too low *(71)*.

The polymorphism of the apoε genotype influences hepatic cholesterol content because lipoproteins with the ε4 isoform are taken up with greater affinity than those with the common ε3 isoform, which in turn are cleared more efficiently than those with the ε2 isoform. Accelerated lipoprotein clearance by the liver leads to a downregulation of hepatocyte LDL receptors. This, in addition to increased intestinal uptake, underlies the well-known hypercholesterolemic effect of the ε4 allele, with its attendant high risk of atherosclerosis and cardiovascular mortality *(72)*. On the other hand, lipoproteins containing apoε2 have a reduced binding affinity for the LDL receptor; thus their plasma clearance rate is reduced. This lowers intracellular cholesterol levels and upregulates HMG-CoA reductase synthesis. HMG-CoA reductase inhibitors (statins) may be less effective in reducing cholesterol levels in apoε4 individuals, as they may already have low HMG-CoA reductase activities *(44,73)*. Several studies found a significant interaction between an individuals's apoε genotype and plasma lipoprotein–lipid changes with statin therapy *(74–78)*. In these studies, subjects with the ε2 allele, and sometimes subjects with the ε3 allele, were more likely to respond favorably to statin therapy than subjects with at least one ε4 allele. The decrease in both total cholesterol and LDL cholesterol was larger in subjects with the ε2 allele than in subjects with an ε3 or an ε4 allele. In subjects with the ε3 allele, total cholesterol and LDL cholesterol were reduced more than in subjects with at least one ε4 allele. Five other studies did not find significant differences in the cholesterol lowering effects of statins in the different apoε genotypes *(72,79–82)*. However, in two of these studies there was a trend for greater LDL cholesterol reductions in subjects with the ε2 and ε3 allele *(80,82)*. Two studies reported that,

whereas men displayed a significant gene–drug response interaction, in women the apoE polymorphism did not account for any significant gene–drug interaction *(76,83)*. It was also found in the REGRESS study that ε2 carriers exhibited the largest improvement in lipoprotein levels upon pravastatin treatment, compared with those having ε3 or ε4 alleles, but the efficacy of pravastatin toward angiographic parameters was less pronounced in carriers of the ε2 allele, although this was not statistically significant *(84)*. Importantly, in a substudy from the 4S study myocardial infarction survivors with the ε4 allele proved to have a nearly twofold increased risk of dying in a follow-up period of about 5.5 yr, compared with other patients. Compared with placebo, the relative risk of mortality in subjects treated with simvastatin was 0.33 (95% CI 0.16–0.69) in ε4 carriers and 0.66 (95% CI 0.35–1.24) in other patients (*see* Table 1). The increased risk of death was not accompanied by an increased risk of a major coronary event, and there was also no difference in the efficacy of statin treatment on reducing coronary events *(81)*.

In a hypercholesterolemic subcohort ($n = 3626$) of the Rotterdam study, a large cohort study that included 7983 subjects of 55 yr and older in a suburb of Rotterdam, no differences in effect were found between subjects with and without the ε4 allele in reducing the risk of coronary artery disease and total mortality. The mortality risk in subjects with the ε4 allele was reduced to 0.71 *(85)* after 2 yr of statin treatment, whereas the mortality risk in subjects without the ε4 allele was reduced to 0.91 *(85)*. The difference with the 4S study might be explained by the difference in mean age of both populations. Subjects in the Rotterdam study had a higher mean age and, unlike the subjects in the 4S study, in the untreated subjects there was no increased risk of dying in subjects with the ε4 allele. Perhaps risk factors other than carrying the ε4 allele became more important at a higher age in predicting the risk of dying *(85)* Furthermore, among subjects in the Rotterdam study that started statin therapy, the risk of discontinuing medication within 3 yr was 3.18 times higher in men with the ε4ε4 genotype compared to men with the ε2ε3 genotype *(86)*. This suggests that subjects who are genetically prone to develop hypercholesterolemia showed the highest risk of discontinuation of statin treatment. A possible explanation for this observation is that these men have the least effect on their cholesterol levels. This may lead to the conclusion that measuring cholesterol levels might not be a good way to evaluate the efficacy of statin therapy *(86)*.

Mutations in the Lipoprotein Lipase Gene

Lipoprotein lipase is a multifunctional protein and pivotal enzyme in lipoprotein metabolism. It is anchored to the vascular endothelium where it constitutes the rate-limiting step in the catabolism of TGs in circulating TG-rich lipoproteins *(87,88)*. The contribution of lipoprotein lipase to atherogenesis is significantly influenced by the balance between vessel wall protein (proatherogenic) and plasma activity (antiatherogenic) *(89)*.

Mailly et al. found in their study that the frequency of healthy Dutch, Swedish, English, and Scottish carriers of the Asp_9Asn mutation (Asp substituted by Asn at position 9 in exon 2) was 1.6 to 4.4% *(90)*. The frequency of the carriers was roughly twice as high (range, 4.0–9.8%) in selected subjects with combined hyperlipidemia (elevated plasma levels of cholesterol and TGs) and in patients with angiographically assessed atherosclerosis (REGRESS) *(91)*. Carriers of the mutation more often had a family history of cardiovascular disease and higher TG and lower HDL-C levels than noncarriers.

The reduction of total and LDL cholesterol appeared to be less in patients with the mutation compared to patients without the mutation. However, the differences between

patients with and without the mutation did not differ significantly between the placebo and the pravastatin groups *(91)*. Angiographic results (progression of focal atherosclerosis and percentage diameter stenosis) showed a deleterious effect of the mutation which could be reversed by pravastatin therapy *(91)*. The relative risk of the presence of the Asp9Asn mutation for any clinical event within 2 yr was estimated to be 1.85 (95% CI 0.94–3.66). The effect of pravastatin on the clinical event-free period was not significantly different for patients with and without the mutation *(91)*.

Although the lipid-lowering effect was attenuated in patients carrying the Asp_9Asn mutation, the deleterious effect of this mutant on the progression of atherosclerosis could be reversed by pravastatin.

Two other common polymorphisms in the lipoprotein lipase gene are associated with coronary heart disease. The Ser447X polymorphism is seen in 17–22% of the population. This polymorphism was found to be protective against high TG levels, low HDL-C cholesterol levels, and premature coronary heart disease *(92–94)*. The N291S is seen in 1–7% of the population.This polymorphism is associated with elevated TGs, decreased HDL cholesterol, and, most likely, increased heart disease risk *(93,94)*. It is not yet established if the effects of statins differ in subjects with these polymorphisms.

ACE Deletion-Type Gene

The angiotensin-converting enzyme (ACE) is thought to play an important role in the development of coronary artery disease. In humans, plasma levels of ACE are partly under genetic control. Plasma and cellular levels of ACE are associated with the insertion/deletion (I/D) polymorphism located in intron 16 of the *ACE* gene. DD carriers have about twice the plasma levels of ACE compared with II carriers, whereas heterozygotes have intermediate levels *(95)*. The frequencies of the ACE genotypes differ in various populations. In the control group of the male Caucasian study population of Gardemann et al., the frequencies of II, ID, and DD were 23, 50, and 27%, respectively *(96)*. The angiotensin converting enzyme converts angiotensin I into the bioactive angiotensin II, and is also responsible for the breakdown of bradykinin to kinin degradation products. These processes result in an increased vascular tone, neointimal proliferation, and LDL oxidation, all predisposing to atherosclerosis *(97)*. Many studies have examined the correlation of the different genotypes with various cardiovascular diseases. Cambien et al. were the first to report an association of this deletion polymorphism with an increased risk of coronary artery disease *(98)*. The meta-analysis of Staessen et al. included 145 reports with an overall sample size of 49,959 subjects *(99)*. In comparison with the II reference group, the excess risk in DD homozygotes was 32% for coronary heart disease (CHD; 30 studies) and 45% for myocardial infarction (20 studies). The D allele behaves as a marker of atherosclerotic cardiovascular complications *(99)*. Concerning the influence of the ACE genotype on the effectiveness of statins, however, contradictory results have been published.

Marquez-Vidal et al. found no interaction of the ACE polymorphism and statins on reduction of lipids and lipoproteins in their case-control study *(78)*. In the CARE trial, subjects with the Platelet Pla2 allele ACE D allele carriers had a larger risk reduction than ACE II subjects *(100)*. In the Lipoprotein and Coronary Atherosclerosis study (LCAS) study, subjects with the ACE DD genotype had the strongest reduction of coronary atherosclerosis with pravastatin. The distribution of clinical events among the genotypes

was not clinically significantly different *(101)*. In The REGRESS trial comparing pravastatin with placebo, van Geel et al. found that the DD genotype was associated with a significantly higher incidence of ischemic events. Although pravastatin decreased serum lipids to a similar extent in the *DD* as in the ID and II groups, the beneficial effects of pravastatin on angiographically defined coronary atherosclerosis were apparently blunted by the ACE deletion type *DD* gene *(102)*. Treating patients with the DD genotype with pravastatin seems less effective than treating patients with the other genotypes with pravastatin.

In a hypercholesterolemic cohort in the Rotterdam study ($n = 3624$), an association between the ACE genotype and the effectiveness of statins in reducing coronary events was found. The relative risk reduction in DD subjects was 1.29 (95% CI 0.62–2.60), in ID subjects, 0.87 (95% CI 0.52–1.45), and in II subjects, 0.40 (95% CI 0.15–1.10). In men, this interaction was much stronger than in women *(103)*. In men the relative risk reduction in DD subjects was 1.34 (0.44–4.09), and in II subjects 0.23 (0.04–1.28); in women, the relative risk reduction in DD subjects was 1.27 (0.43–3.81), and in II subjects, 0.72 (0.20–2.56). The gender difference in risk reduction is not easily explained. Other studies showed that genetic variance in the region of the *ACE* gene significantly influenced interindividual blood pressure in males, but not in females *(104,105)*. This suggests that there are gender differences in genetic regulation of the ACE system. There might be an interaction between the ACE genotype and statin therapy in men, but these data need to be confirmed in a larger study.

–514 CT Polymorphism in *HL* Gene

HL is a plasma lipolytic enzyme that plays a major role in the metabolism of LDL cholesterol and HDL cholesterol. HL promotes the conversion of large TG-rich HDL2 to small, dense HDL3 particles. High HL levels are associated with low HDL2 levels. In addition, HL catalyzes the hydrolysis of TGs and PLs of the intermediate density lipoproteins (IDL) and large buoyant LDL to form the more atherogenic small, dense LDL particles *(106)*. Patients with small, dense LDL have an increased risk of coronary artery disease *(107)*. The presence of a C instead of a T at position –514 with respect to the transcription start site of the *HL* gene accounts for 20–30% of the variance in HL activity in men and women *(108)*. The presence of the C allele contributes to increased HL activity, which leads to more atherogenic LDL particles and lower levels of anti-atherogenic HDL cholesterol.

Zambon et al. conducted a study within a small but intense clinical trial *(108)*. They studied 49 dyslipidemic men who were treated for 2.5 yr with intensive lipid-lowering therapy with either lovastatin and colestipol, or niacin and colestipol. The type of lipid-lowering therapy did not affect the association between polymorphism and changes in coronary stenosis. The results from the two different treatment groups were therefore pooled and analyzed together. Homozygous CC patients showed a greater decrease in HL activity and a greater increase in LDL buoyancy with lipid-lowering therapy than subjects with at least one T allele.

Lipid-lowering therapy resulted in a significant improvement of coronary stenosis in CC patients ($\Delta\%_{Sprox}$ –2.1) and to a lesser extent in the TC group ($\Delta\%_{Sprox}$ –1.1), whereas progression of stenosis ($\Delta\%_{Sprox}$ 4.0) was observed in the TT group. Because no distinction was made between the two lipid-lowering therapies, it is not clear if the differences in treatment effect were attributable to statin therapy.

Platelet Glycoprotein

Platelets play a central role in the restenosis process by inducing neointimal proliferation after coronary interventions *(109)*. The glycoprotein IIb/IIIa PI^{A2} polymorphism has been associated with the occurrence of acute coronary syndromes and increased restenosis rates. Statins have been shown to exert potent antiproliferative, anti-inflammatory, and antithrombotic properties, thereby potentially interfering with the major processes of in-stent restenosis. To investigate if statin therapy affects restenosis rates Walter et al. followed 650 patients for 6 mo after coronary stent insertion. Carriers of the PI^{A2}allele (22% of the patients) demonstrated a significantly increased restenosis rate, which was diminished by statin therapy (50.9% vs 28.6%, $p = 0.01$). Patients homozygous for the PI^{A1} allele only had a slight recuction in stenosis rate (27% vs 34%, $p = 0.13$). Moreover, statin therapy was associated with a significant reduction (28.2% vs 49.3%) of myocardial infarction, cardiac death, and target vessel revascularization in the 6 mo after the intervention in patients with the PI^{A2}allele, and only with a minimal reduction in subjects with the PI^{A1} allele (32.1% in statin treated patients and 37.5% in patients without treatment). Statin therapy reduces increased stent restenosis rates and improves clinical outcome following coronary stent implantation in patients bearing the PI^{A2}allele, suggesting that statins interfere with the functional consequence of a genetically determined platelet-mediated risk factor associated with PI^{A2} polymorphism *(110)*.

The clinical effect of the PI^{A} genotype has also been investigated in a substudy of the CARE trial *(100)*.The prevalence of the $PI^{A1.A2}$ genotype was 28.8% in the cases and 23.8% in controls; this was not significantly different. The relative risk of CHD death or nonfatal myocardial infarction in the $PI^{A1.A2}$ genotype group was 1.32 (95% CI 0.99–1.76). This suggests that there is a trend towards a higher relative risk, but it is not statistically significant. In patients with the $PI^{A1.A2}$ genotype, treatment with pravastatin reduced the risk of fatal CHD or nonfatal myocardial infarction by 31%, whereas in patients with the $PI^{A1.A1}$ genotype, treatment with pravastatin only reduced these events with 7%. Of the seven patients with the $PI^{A2.A2}$ genotype in the control group only one experienced a fatal CHD or a myocardial infarction, whereas in the pravastatin group six of the nine $PI^{A2.A2}$ patients experienced such an event. These data suggest that the $PI^{A2.A2}$ genotype is not associated with an increased risk of death resulting from CHD or nonfatal myocardial infarction, and that pravastatin might have a detrimental effect on coronary events in patients with this genotype *(100,111)*. Because of the low sample size in this study, these results should be cautiously interpreted.

Toll-Like Receptor 4

Atherosclerosis is increasingly considered to be a chronic inflammatory disease *(112)*. Lipopolysaccharide (LPS) is a product of Gram-negative microorganisms that activates the immune system. LPS in combination with CD14 serves as a ligand to toll-like receptor 4 (TLR4). The presence of LPS in the circulation is not confined to sepsis, but also occurs in healthy subjects *(113,114)* and is associated with early atherosclerosis *(113)*. Two functional SNPs have been discovered in the *TLR4* gene. These variants, Asp299Gly and Thr399Ile, lead to a blunted immunological response to inhaled LPS *(115)* and to lower levels of proinflammatory cytokines, acute-phase reactants, and soluble adhesion molecules *(116)*. Most importantly, they were associated with reduced extent and progression of angiographically-determined carotid atherosclerosis *(116)*. Boekholdt et al. investigated in the REGRESS study whether the Asp299Gly and the Thr399Ile polymor-

phisms influenced the progression of coronary atherosclerosis and the risk of cardiovascular events *(117)*. There were no significantly differences between genetically defined subgroups with respect to baseline factors, treatment, or in-trial changes of lipid, lipoprotein, or angiographic measurements. Carriership of the 299Gly allele did not significantly affect the risk of cardiovascular events in the entire cohort when compared with noncarriers (11.5% vs 14.9%, $p = 0.58$). However, in the pravastatin group, carriers of the 299Gly allele had a significantly lower risk of cardiovascular events than noncarriers. Among noncarriers, the risk of cardiovascular events was reduced from 18.1% to 11.5%, whereas among carriers of the 229Gly the risk was reduced from 29.6% to 2%. Testing for the interaction between the 299Gly genotype and pravastatin was statistically significant ($p = 0.025$) *(117)*. Analyses were performed to investigate whether the Thr399Ile polymorphism influenced the interaction between 299Gly and pravastatin, but the results did not allow any conclusions because of the low frequency of this genotype *(117)*. This is another observation of an interaction between a genotype and statin treatment in reducing clinical events without an interaction reducing lipid parameters.

Interleukin-1B Genotype

A polymorphism at position –511 of interleukin (IL)-1B gene promotor regulates IL-1B levels, immune and inflammatory responses, and possible atherogenesis. Lehtimäki et al. found in a small trial ($n = 34$) that at baseline, there was no difference in basal or adenosine-stimulated myocardial flow between subjects with the A2+ and subjects with the A2– genotype. After 6 mo of treatment with pravastatin, the adenosine-stimulated myocardial flow increased by 18.0% in subjects with the A2– genotype ($n = 7$), and decreased by 2% in subjects with the A2+ genotype ($n = 7$). In the placebo recipients there were no significant changes compared with the baseline values. Both genotype groups showed a similar decrease in total LDL cholesterol levels. Coronary function improves after 6 mo of statin therapy in subjects with the IL-1B A2– allele but not in those with the A2+ allele *(118)*.

CONCLUSIONS

In this review, we have discussed a number of polymorphisms which might interfere with the effectiveness of statin therapy. Most polymorphisms described in this chapter have an indirect effect on statin response. Only a few of them are in genes encoding for proteins that are involved in the disposition of statins, or in genes-encoding proteins that are direct targets of statin therapy. More proteins are involved in efficacy and metabolism of statins; therefore, more genes are involved, and probably more polymorphisms will influence the efficacy of statin therapy. The use of other techniques in pharmacogenetic and pharmacogenomic research will enable us to compare gene profiles of patients with differences in reactions to drugs. Instead of looking at one single SNP in a gene, haplotypes might better explain the differences in response to drugs. Furthermore, models need to be developed that enable us to look at combinations of polymorphisms at the same time. Because so many genes are involved, probably more polymorphisms have to be determined to predict a patients response to statins.

What is the clinical relevance of the polymorphisms described in this chapter? Some of them might be useful to select patients with a high risk for coronary artery disease (for example, ACE and ApoE). Subjects with that particular genotype might be treated rigorously, especially in the case of Apoε4 carriers, who are genetically prone to develop coronary artery disease and who are at higher risk to discontinue their therapy *(86)*. Other

polymorphisms could predict the effectiveness of statins. For example, carriers of the CETP B2B2 polymorphism or the stromelysin-1 A5A5 polymorphism seem to have no benefit from treatment with statins. Nevertheless, in the study of Kuivenhoven et al., it was only the effect on angiographic progression of coronary disease that was measured, whereas statins do have effects that are independent of cholesterol lowering. Therefore, we do agree with Goldstein *(119)* that at this moment, there is certainly not enough evidence to exclude patients with a certain genotype from statin treatment. However, in the near future, it is likely that genetic information may be used to modify dose and suggest supplemental treatment regimens. Further research in large general population samples and large clinical trials is necessary to assess the importance of these polymorphisms on the effectiveness of statins in the reduction of cardiovascular diseases.

The ethical, legal, and social implications of population-based genotyping are still unresolved and much debated. It is important that distinctions are made between disease susceptibility gene polymorphisms, which provide information about risks of diseases, and pharmacogenetic profiles *(120)*, even though it is not always possible to make this distinction. An example is the Apoε polymorphism. This polymorphism might predict a patient's response to statins *(76)* or the risk for discontinuation of statins *(86)*, but it also predicts a patients risk on developing Alzheimer's disease *(121)*. For such polymorphisms, it might lead to difficult decisions for health care professionals. Is it the task of health care professionals to tell the patient about this risk? The patient has of course the right (not) to know. This information might not only influence the patient, but also members of his family who might carry the same polymorphism. Furthermore, it might not only influence the patient's perception of his health and life, but also his eligibility for healthcare and life insurance. The debate is ongoing in various countries with, so far, an uncertain outcome.

If polymorphisms exist that influence the response to statins, it is also important to evaluate the cost-effectiveness of screening for such genotypes. In a recent study, the cost-effectiveness of screening for the ACE genotype before starting statin therapy was evaluated *(122)*. If the interaction between the ACE genotype and the effectiveness of statin therapy is confirmed in more studies, then screening could save money. The model used in this study can also be used to determine the cost-effectiveness for screening for other polymorphisms.

At present, no single polymorphism has been identified that renders statin treatment ineffective based on clinical outcomes. Therefore, results from large-scale population studies are needed, to complement results from clinical trials and small-scale studies in selected populations.

Possibly, one day a person's genotype might determine if he or she should get a statin or not. Then, determining the genotype will not deny therapy to a subject, but will help in the decision as to which therapy suits the patient best, and potentially increase cost-effectiveness of the treatment

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