Pharmacogenetics of statins: achievements, whole-genome analyses and future perspectives

Statins are the most commonly prescribed class of drug worldwide and therapy is highly effective in reducing low-density lipoprotein cholesterol levels and cardiovascular events. However, there is large variability in clinical response to statin treatment. Recent research provides evidence that genetic variation contributes to this variable response to statin treatment. Until recently, pharmacogenetic studies have used mainly candidate gene approaches to investigate these effects. Since candidate gene studies explain only a small part of the observed variation and results have often been inconsistent, genome-wide association (GWA) studies may be a better approach. In this paper the most important candidate gene studies and the first published GWA studies assessing statin response are discussed. Moreover, we describe the PHASE study, an EU-funded GWAS study that will investigate the genetic variation responsible for the variation in response to pravastatin in a large randomized clinical trial.

KEYWORDS: cardiovascular disease, pharmacogenetics, statins

Pharmacogenetic studies investigating variable lipid-lowering response after statin therapy

Pharmacogenetic studies are performed to assess whether genetic variation accounts for the variability in clinical response to drug therapy. Meaningful candidate genes for investigating statin response are genes that belong to lipid metabolism, inflammation, thrombosis and endothelial function as well as pharmacodynamic target genes, disease-modifying genes and genes involved in uptake, distribution and metabolism of statins (see Figures 1 & 2 for the pharmacodynamic and pharmacokinetic statin pathways) [5]. More than 40 candidate genes have been described with respect to the variable effect of statins in lipid-lowering abilities, and the variable effect on the risk of clinical end points including myocardial infarctions and cardiovascular death [6].

HMGCR is the rate-limiting enzyme in cholesterol synthesis. Statins are competitive inhibitors of HMGCR and therefore this gene is an interesting target for pharmacogenetic studies. The largest reported pharmacogenetic study investigating genetic variation in various candidate genes was performed in 1536 participants of the PRINCE study [7]. One hundred and forty eight SNPs in ten candidate genes known to be involved in cholesterol synthesis and statin metabolism were investigated. After correcting for multiple testing, two common intronic SNPs (chromosome 5 position 74726928 and

Cardiovascular disease is the leading cause of death in industrialized countries [1]. The HMG-CoA reductase (HMGCR) inhibitors, also known as statins, are the most prescribed class of drug worldwide and are widely used in the prevention of cardiovascular disease. Statin therapy is generally associated with a low-density lipoprotein (LDL)-cholesterol lowering up to 55% [2] and a reduction of cardiovascular events by 20–30% [3]. Despite the clinical effectiveness of statins, there is large variability in clinical response to statin treatment. For example, within the PROSPER trial, a large randomized clinical trial assessing the effectiveness of pravastatin in the elderly, compliance with study medication was high, yet 13.3% of the subjects allocated to pravastatin did not reach 10% LDL-cholesterol lowering after 36 months of pravastatin treatment [4]. Many studies from the past years provide evidence that genetic factors contribute to this interindividual variation in drug response [5,6]. The genetic variation associated with lipid lowering in response to statin therapy has been investigated mainly by previous pharmacogenetic studies. Relatively little is known about the genetic variation associated with variability in clinical events and side effects in response to statin therapy. The aim of this paper is to give an overview of the literature on candidate gene studies and the more recently performed genome-wide association (GWA) studies of pharmacogenetics of statins and to introduce the pharmacogenetic study of PHASE.
Genetic variation of APOE, and in particular the ε2/ε3/ε4 variants (coded by rs7412 and rs429358), have been investigated extensively [8]. ApoE has various roles in lipid and lipoprotein metabolism and thus a clear impact on plasma lipid and lipoprotein levels. It has been shown in many studies that the APOE ε4 and ε2 alleles associate with higher and lower concentrations of lipids and lipoproteins compared with the major allele of one of the SNPs. Interestingly, no differences in baseline lipid levels were seen between the genotypes.

Figure 1. Pharmacokinetic pathways of statins.
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of total cholesterol, LDL-cholesterol and ApoB, respectively, compared with the ε3 allele [9–11]. The results of studies on APOE SNPs and statin therapy response are equivocal, which has been summarized in a review by Nieminen et al. [8]. Several studies report less effect of statins in ε4 carriers for lowering cholesterol levels, compared with ε3 carriers, whereas carriers of the ε2 allele have a larger reduction of cholesterol levels during statin therapy compared with ε3 carriers. Nevertheless, several studies found no significant associations for APOE SNPs and lipid levels during statin therapy [8]. A recent meta-analysis did also not confirm the association between APOE SNPs and lipid response during statin therapy [12].

**Figure 2. Pharmacodynamic pathways of statins.**
FA: Fatty acid; HDL: High-density lipoprotein; IDL: Intermediate-density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low-density lipoprotein.
Reproduced from McDonagh et al. [55]. Used with permission of PharmGKB and Stanford University.
Other pharmacogenetic studies have investigated genetic variation in LDL-cholesterol related candidate genes, for example the LDL-receptor gene [13]. However, most of the results from studies investigating changes in lipid levels and cardiovascular event responses are difficult to interpret, because of strong influences of the genetic variation on baseline lipid levels [5]. In addition, several genes have been investigated for their pharmacokinetic and dynamic influences on statins. Two of those genes are SLCO1B1, the gene encoding the solute carrier OATP1B1 influx and ABCB1 efflux transporter. Kesktalo et al. showed that ABCB1 haplotypes (rs1045642, rs2032582 and rs1128503) affected the pharmacokinetics of the active acid forms of simvastatin and atorvastatin [14]. In vivo pharmacokinetic studies have shown associations between SLCO1B1 variations and statin plasma concentrations. Those pharmacokinetic features could only be translated to cholesterol-lowering abilities in small in vivo pharmacodynamic studies, results from larger studies are contradictory [15]. Other genes involved in the metabolism of statins are for example CYP3A4 and ABCG2 but research has not been able to produce definitive results to show the possible role of these genes in the pharmacogenetics of statins [16].

Pharmacogenetic studies investigating variation in clinical events after statin therapy

KIF6 is a member of the molecular motor superfamilly involved in intracellular transport of several important molecules, including mRNA [17]. Several studies have shown an association between the Trp719Arg (rs20455) SNP in the KIF6 gene and coronary heart disease [18–23]. Furthermore, analyses in four large clinical trials have shown a substantially increased benefit of statin therapy in carriers of this SNP compared with noncarriers [18,19,24]. In the WOSCOPS study, a primary prevention statin trial, the absolute risk reduction of coronary heart disease by statin therapy was 5.5% in carriers of the SNP compared with 0.1% in noncarriers [19]. In the secondary prevention trials PROSPER, CARE and PROVE IT–TIMI 22, the absolute risk reduction by statin therapy ranged from 5 to 10% in carriers of the SNP compared with 0.4–1.2% in noncarriers. The end points of interest in those studies were, respectively: coronary events, myocardial infarction and death or major cardiovascular events [18,19,24]. However, those results are equivocal; an accompanying editorial in the Journal of the American College of Cardiology expressed their doubts about the validity of those studies [25]. Moreover, a recent meta-analysis of 19 case–control studies (in total 17,000 coronary artery disease (CAD) cases and 39,369 controls) reported no association between the KIF6 SNP and the risk of clinical CAD [17]. Furthermore, within the 18,348 participants from the HPS study, the KIF6 SNP was not associated with the risk of incidental vascular events among placebo-treated participants, and reductions in the risk of vascular events during statin therapy were similar across KIF6 genotypes [26].

Another gene that has been analyzed comprehensively is the CETP gene. CETP is involved in cholesterol metabolism by transporting cholesterol esters back into the liver and functions to transport triglycerides from LDL and very LDL to high-density lipoprotein (HDL) cholesterol [27]. The most investigated SNP in CETP is the Taq1B variant (rs708272), a SNP in the first intron of the CETP gene. Initial studies associated the B2B2 genotype of the CETP gene with lower CETP levels [28], higher HDL-cholesterol levels, and a lower risk of progression of CAD, compared with the B1B1 CETP genotype [29–32]. However, when patients with the B1B1 genotype were treated with statins, they showed a lower progression of CAD compared with B2B2 carriers. In addition, long-term results from the REGRESS study, the first study to report the possible pharmacogenetic interactions between the CETP SNP and statin treatment [33], demonstrated significantly higher 10-year mortality in statin-treated male B2 carriers, compared with carriers of the B1B1 genotype [34]. Therefore, although untreated B2B2 patients have a lower risk of CAD progression, statin treatment is more beneficial in patients with the B1B1 genotype, denying the initial advantage of the B2 allele in CAD. A large meta-analysis including 13,677 subjects confirmed the association between the Taq1B SNP and HDL-cholesterol levels and the risk of CAD, but the interaction between the SNP and statin therapy could not be confirmed [35].

SNPs in the APOE gene have also been assessed in relation to progression of coronary heart disease during statin therapy [8]. Gerdes et al. analyzed data of 5.5 years of follow-up from 966 Danish and Finnish myocardial infarction survivors enrolled in the Scandinavian Simvastatin Survival Study (4S) [36]. Carriers of the APOE ε4 allele had nearly twofold higher mortality compared with
noncarriers of the ε4 allele during simvastatin therapy. However, the results found in the 4S trial are equivocal; analyses in almost 8000 participants from the Rotterdam Study and in 815 men in the REGRESS study could not confirm the pharmacogenetic effect of statins on cardiovascular end points [37,38].

Two other genes investigated in relation to statin therapy and clinical events are SLCO1B1 and ABCB1. Peters et al. tested 24 tagging SNPs in the two genes in 668 cases with myocardial infarction and 1217 controls from the population-based PHARMO study [39]. They found two SNPs within ABCB1 (rs3789244 and rs1922242) to interact significantly with statin therapy. In addition, they observed a non-significant interaction between the SLCO1B1*1A haplotype and statin treatment; odds ratio (OR) homozygote carriers 0.49 (95% CI: 0.34–0.79) compared with 0.31 (95% CI: 0.24–0.41) for heterozygous or noncarriers of the *1A allele [39].

**GWA studies**

As shown in the previous paragraphs, various studies have assessed the association between genetic polymorphisms and response to statin therapy. At least two reviews have given an elaborate overview of the pharmacogenetic candidate genes and their genotype effects related to statin therapy [5,6]. Table 1 shows a brief overview of the most important investigated candidate genes in their relation to efficiency and clinical effectiveness after statin therapy. Based on these data, candidate genes regulating pharmacokinetic and pharmacodynamic properties of statins appear to be the most promising target genes (see Figure 1 & 2). Although the genetic variation in these pharmacokinetic and pharmacodynamic pathways has received much attention over the past years, it only explains a small part of the observed variation, and the results are often inconsistent. Therefore, it is now important to investigate which other genetic pathways are responsible for the remaining genetic variation in statin response.

GWA studies are another approach to investigate pharmacogenetic effects. Unlike candidate gene approaches, GWA studies can link multiple SNPs to drug response with no a priori assumptions, thereby facilitating new discoveries. At present, only three GWA studies investigating genetic variants and variation in response to statin therapy have been published, focusing on lipid-lowering and adverse effects of statin therapy [40–42]. The first published GWA study on statin response was performed in the TNT study. This study used a combination of a genome-wide and candidate gene approach. Using only the GWA study of 1984 individuals, no SNPs were significantly associated with statin response genome-wide. However, by analyzing the candidate genes in the genotyped participants, they found the SNP rs7412 in the APOE gene significantly associated with statin response (p = 3.65 × 10⁻⁸). The rs7412 SNP was not present on the platform used for the GWA scan used in the TNT study and was not in linkage disequilibrium with any of the SNPs in the GWA scan [40].

The second published GWA study on statin response was a meta-analysis performed in approximately 4000 subjects from three statin trials; The CAP trial, the PRINCE study and the TNT study. An association between the gene CLMN, encoding calmin, and the reduction in total cholesterol levels after statin treatment was observed. The function of calmin is unknown and has not been implicated in cholesterol metabolism before. The combined analysis of these three studies found an 84% posterior probability that the CLMN SNP (rs8014194) was genuinely associated with statin-mediated change in total cholesterol (p = 1.9 × 10⁻⁹). On average across the three studies, carriers with two copies of the minor allele of rs8014194 had a 3% lower total cholesterol reduction compared with noncarriers [42]. Nevertheless, further (functional) studies are needed to replicate this finding and explore the function of calmin.

The third published GWA study investigated genetic variation in relation to statin-induced myopathy, an adverse side effect of statin therapy. This GWA study included 85 subjects with definite or incipient myopathy and 90 controls, all taking 80 mg simvastatin daily. The SNP rs4363657 located within the SLCO1B1 gene, which encodes the polypeptide OATP1B1, which mediates the hepatic uptake of most statins, was found to be associated with myopathy (p = 4.1 × 10⁻⁴). The noncoding rs4363657 SNP was in nearly complete linkage disequilibrium with the nonsynonymous rs4149056 SNP (Val174Ala; r² = 0.97), which was also located within the SLCO1B1 gene and has been linked to statin metabolism. The OR for myopathy was 4.3 per copy of the rs4363657 C allele (minor allele frequency: 0.13) and 17.4 in homozygote carriers of the CC variant compared with homozygote carriers of the common variant (TT) [41]. To date, this is the strongest effect, resulting from a pharmacogenetic effect, in relation to response to statin therapy. Brunham et al. have performed a study aiming to replicate the
The association of rs4149056 and statin-induced myopathy in a cohort of patients using various statin types [43]. In this small study, including 25 cases of severe statin-induced myopathy and 83 controls, the SNP was not associated with myopathy in the complete group. However, stratifying patients by statin type, the SNP was significantly associated with myopathy in simvastatin users (OR: 3.2, p = 0.042), but not in atorvastatin treated patients (OR: 4.5, p = 0.48). These different results between different statin types indicate the presence of possible statin type-specific pharmacogenetic effects.

**Pharmacogenetic study of statin in the elderly at risk, a large GWA study**

Results of pharmacogenetic candidate gene studies are often inconsistent and explain only a small part of the observed variation in statin

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<th>Table 1. Overview of a selection of the genes investigated for associations with statin treatment and several outcomes.</th>
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**CAD:** Coronary artery disease; **CV:** Cardiovascular; **GWA:** Genome-wide association; **LDLC:** Low-density lipoprotein cholesterol; **MI:** Myocardial infarction; **TC:** Total cholesterol.
response. Furthermore, GWA studies enable detection of novel and less obvious genes. Since there are currently only three GWA studies on statin response published with only very low subject numbers, we have initiated the PHASE study [44]. PHASE is a EU sponsored GWA study on the participants of the PROSPER study [4] investigating genetic variations responsible for the individual variation in drug response. The PROSPER study provides a good population to study pharmacogenetics. First, PROSPER was an investigator-driven, prospective, multinational and randomized placebo-controlled trial including 5804 subjects aged 70–82 years at baseline of whom more than 50% were female. Plasma levels of LDL-cholesterol as well as other levels of plasma lipoproteins were measured at baseline and prospectively during follow-up for a mean of 3.2 years (range 2.8–4.0). Second, within the PHASE study, 557,192 SNPs in 5244 subjects are available for analysis, and to maximize the availability of genetic data and coverage of the genome those SNPs have been imputed up to 2.5 million SNPs. A GWA study for LDL-cholesterol was used as proof-of-principle analysis in the PROSPER/PHASE study. With this GWA study, five of the previously discovered genetic associations with LDL-cholesterol were confirmed and this shows that we are able to detect genetic effects within the elderly participants of PROSPER [44].

The large number of statistical tests performed in a GWA analysis requires large sample sizes to provide adequate statistical power to detect small effect sizes. For this purpose it is necessary to cooperate with other studies. To investigate genetic loci affecting statin response and adverse effects, the PHASE study is involved in the Genomic Investigation of Statin Therapy (GIST) consortium. GIST is a large international consortium formed to conduct a combined meta-analysis of GWA and replication studies, including several randomized controlled trials of statin therapy and several non-trial cohorts of statin recipients with GWA data. Most of the large randomized controlled statin trials are participating in the GIST consortium, including the PROSPER/PHASE study, the CAP trial, the PRINCE study, the TNT study, the CARDS study [45] and the ASCOTT trial [46]. Together, these studies provide data for approximately 10,000 statin-treated subjects. The nontrial cohorts involved in the GIST consortium are the cohorts of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium [47]. The studies from CHARGE are supplemented with the MESA study [48], the HABC study [49], HVH study [50], the GoDARTs study [51] and the biobank of the Vanderbilt University (BioVu) [52]. With those studies participating in GIST there is observational data available of approximately 10,000 statin-treated subjects. The first plan for the GIST consortium is to conduct a meta-analysis of GWA studies and replication studies to identify novel loci influencing statin response. To achieve this aim, every participating study will perform their own analysis assuming an additive genetic model. The regression analysis will be performed with the expected allele dosages to overcome the uncertainty of imputing SNPs. The meta-analysis will be performed with the random effects model. To correct for multiple testing, we will use a p-value threshold of $5.0 \times 10^{-8}$ for statistical significance. To conclude, with the GIST consortium we have data of approximately 20,000 statin-treated subjects, which gives the opportunity to assess the genetic variation responsible for the variable response to statin treatment in a large consortium.

### Future perspective

Over previous years, substantive effort has been made in investigating the pharmacogenetics of the variable response to statin treatment. With the GWA analysis performed in the PHASE study and the meta-analysis in the GIST consortium we hope to identify novel genes and pathways involved in the variation in statin response. Expanding the knowledge about the genes and pathways associated with the variation in statin response might lead to substantial improvements in the use of cardiovascular drug therapy, through selection of the most appropriate drug therapy based on an individual’s genetic make-up [53,54]. With the results of the PHASE study and the GIST consortium, we aim to identify nonresponders or subjects who will experience adverse effects by their genetic variation.

However, with the GWA study performed in the PROSPER/PHASE study and the GIST consortium, only common variants associated with statin response will be detected. Within the PROSPER/PHASE study we will perform an exome sequencing study to also identify rare variants associated with statin response. High responders to statin therapy will be compared with low or nonresponders in order to find the biological pathways involved in pharmacogenetics of statin therapy. Moreover, epigenetic studies will be executed to investigate the epigenetic mechanisms involved in the interindividual
variation in response to statin treatment. All genetic or epigenetic variation identified by these studies will be further tested in a clinical setting to investigate their use in clinical practice.

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Executive summary

Background
* Although statins are clinically highly effective, there is large variability in clinical response to statin treatment.

Pharmacogenetic studies investigating variable lipid-lowering response after statin therapy
* Pharmacogenetic candidate gene studies have revealed several genetic variants associated with variation in response to statin therapy. Important genes involved in the lipid-lowering response to statin therapy include the HMGCR and APOE genes. However, those results are equivocal and further studies are needed to explore the role of those genes.

Pharmacogenetic studies investigating variation in clinical events after statin therapy
* Important genes involved in the variation in clinical events after statin therapy include the APOE, CETP, KIF6, ABCB1 and SLCO1B1 genes. However, those results are equivocal and further studies are needed to explore the role of those genes.

Genome-wide association studies
* Genome-wide association studies are a promising approach to investigate other genetic pathways that are responsible for the variation in statin therapy outcome.

Pharmacogenetic study of statins in the elderly at risk: a large genome-wide association study
* With the PHASE study in collaboration with the GIST consortium, we will be able to assess the genetic variation responsible for the individual variation in response to statin therapy.

References
Papers of special note have been highlighted as:
* of interest
** of considerable interest
Pharmacogenetics of statins: achievements, whole-genome analyses & future perspectives


Maitland-van der Zee AH, Stricker BH, Kungel OH et al. The effectiveness of hydroxy-methylglutaryl coenzyme A reductase inhibitors (statins) in the elderly is not influenced by apolipoprotein E genotype. Pharmacogenetics 12(8), 647–653 (2002).


** Genome-wide association (GWA) analysis of lipid-lowering response to statins in the TNT cohort.


** GWA study showing a strong association between SLCO1B1 variants and statin-induced myopathy.


** GWA analysis of lipid-lowering response to statins in the CAP, PRINCE and TNT trials.


* A description of the methods and a proof-of-principle analysis performed in the PROSPER/PHASE study.


