DISCOVERY MEDICINE®
www.discoverymedicine.com
ISSN: 1539-6509; eISSN: 1944-7930

Statin Pharmacogenomics: Pursuing Biomarkers for Predicting Clinical Outcomes

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Abstract: Indicated for treating hyperlipidemias and for the prevention of cardiovascular disease (CVD), statins rank among the most commonly prescribed drug classes. While statins are considered to be highly effective in preventing atherosclerotic events, a substantial portion of treated patients still progress to overt CVD. Genetic factors are thought to contribute substantially to treatment outcome. Several candidate genes have been associated with statin dose requirements and treatment outcomes, but a clinically relevant pharmacogenomics test to guide statin therapy has not yet emerged. Here we define basic pharmacogenomics terminology, present strong candidate genes (*CETP*, *HMGCR*, *SLCO1B1*, *ABCB1*, and *CYP3A4/5*), and discuss the challenges in developing much-needed statin pharmacogenomics biomarkers for predicting treatment outcomes. [*Discovery Medicine* 16(86):45-51, August 2013]

Cardiovascular Disease and Statins

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in the United States. More than one in three Americans have some form of CVD, and coronary artery disease (CAD) alone accounts for one in every six deaths and treatment costs exceed 300 billion dollars annually (Gaziano *et al*., 2010). Thus, CVD is one of the most pervasive and expensive disorders facing our nation. The relatively high efficacy and low side effect profile of statins drove them to become the gold standard in preventing adverse cardiac events associated with hyperlipidemia. Large clinical trials have shown that statins reduce the risk of major vascular events in a linear fashion with a 20% risk reduction for every 1 mmol/L decrease in low-density-lipoprotein (LDL) cholesterol (Amarenco and Labreuche, 2009). Twenty-five years after receiving FDA approval, statins remain a cornerstone of the U.S. healthcare system with simvastatin being the third most prescribed drug (IMS Health, 2012). However, responses to statin therapy can be influenced by various factors and are not universal: some patients cannot attain their lipid-reduction goals, some experience atherosclerotic events despite therapy, and some suffer adverse events such as hepatitis or myopathy. This article offers a brief introduction to pharmacogenomics testing, describes a variety of genes that influence statin pharmacology, and characterizes current and future trends in statin pharmacogenomics research. As readers become increasingly familiar with pharmacogenomics research and terminology, the level of detail is increased with each subsequent gene presented in this review. Readers will note also that differences regarding genetic influence exist among statin types and can be attributed to dissimilarities in their chemical structure, potency, and disposition.

Pharmacogenomics

Pharmacogenomics is the study of how genetic factors influence inter-individual variability of drug response. Many patients, clinicians, and scientists may not be entirely familiar with its background and the terminology used in the pharmacogenomic literature. A brief review of the commonly-used terminology follows. The coding sequence of DNA base pairs for a particular gene is the genotype, and a phenotype refers to a trait resulting from the protein product encoded by a gene.

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Examples of phenotypes include blood type, hair color, and the extent to which an individual metabolizes or responds to a certain pharmacotherapy. A gene's name is italicized and often refers to its protein product (e.g., the *CYP3A5* gene encodes for the CYP3A5 enzyme). The genotype is formed from two alleles per autosomal gene, one maternal and one paternal. Homozygotes possess two of the same alleles, and heterozygotes possess two different alleles. The most common allele in a population is referred to as the wild-type, and allele frequencies often vary from one patient population to the next. Most sequence variations are single nucleotide polymorphisms (SNPs, pronounced "snips"). A SNP is a single DNA base pair substitution that may result in a different gene product. SNPs can be classified as structural RNA polymorphisms (srSNPs), regulatory polymorphisms (rSNPs), or polymorphisms in coding regions (cSNPs): srSNPs alter mRNA processing and translation, rSNPs alter transcription, and cSNPs alter protein sequence and function. A haplotype is a combination of alleles or set of SNPs at nearby locations on a chromosome that are inherited together. Tag SNP(s) do not necessarily alter the function of a gene or its protein product. They are representative SNPs associated with other polymorphism(s), known or unknown, which are responsible for functional change(s). Recently, genetic associations with a phenotype have been done on a large scale, with millions of SNPs measured in thousands of subjects. This approach, called a genome-wide association study or GWAS, has revealed countless candidate genes for clinical traits, but only a few have resulted in a practical clinical application. SNPs may by themselves exert a pharmacokinetic effect (i.e., how the body processes the drug), a pharmacodynamic effect (i.e., how the drug affects the body), or both. Often they may act in concert with other genes or other genetic factors (e.g., promoter or enhancer regions). Pharmacodynamic effects can result from a pharmacokinetic effect or can result from variations in a pharmacologic target (e.g., HMG CoA reductase is the target enzyme of statins). Establishing a genotype-phenotype association can involve clinical studies, animal transgenic studies, or molecular and cellular functional assays. A comprehensive approach to pharmacogenomic investigation should include examining the influence of genes involved in drug absorption, transport, metabolism, excretion, and proteins related to mechanism of action. Following this approach, we describe a variety of genes that influence various aspects of statin pharmacology.

Cholesterol Ester Transferase Protein (CETP)

CETP is involved in cholesterol metabolism by transporting cholesteryl esters into the liver and functions to exchange triglycerides from LDL to high-density-lipoprotein (HDL) cholesterol. Variants in *CETP* have been associated with differences in cholesterol levels, cardiovascular outcomes, and response to statin pharmacotherapy. Early studies found carriers of the *Taq* 1B polymorphism in *CETP* to have lower CETP concentrations, higher HDL concentrations, and lower risk of progression of coronary artery disease compared with noncarriers (Freeman *et al*., 1994; Kuivenhoven *et al*., 1998; Willer *et al*., 2008). Unexpectedly, statin-treated male carriers had a higher 10-year mortality rate compared with noncarriers of the *Taq* 1B variant (Regieli *et al*., 2008). These findings suggest that statin treatment may be more beneficial in noncarriers even though untreated carriers have a lower risk of CAD progression than untreated noncarriers. The association between the *Taq* 1B SNP and HDL-cholesterol levels and the risk of CAD was substantiated in a large meta-analysis (n=13,677), but the interaction between the polymorphism and statin therapy was not (Boekholdt *et al*., 2005). Arguably, the *Taq* 1B polymorphism is now regarded as a tag SNP because *in vitro* studies have failed to provide a mechanistic or functional explanation regarding its association with differences in cholesterol levels, cardiovascular risk, and response to statins.

Recently, two regulatory variants have been identified in *CETP,* a SNP in exon 9 enhancing the formation of a non-functional or dominant-negative splice isoform, and SNPs in the promoter and enhancer regions affecting transcription. Both are significantly associated with cholesterol levels and sex-dependent cardiovascular risk (Papp *et al*., 2012). Their associations with response to statin therapy are currently being investigated in several patient cohorts. Further investigation of *CETP* is likely to provide the insight necessary for developing a clinical test capable of identifying individuals not likely to benefit from statin therapy.

HMG CoA Reductase (HMGCR)

As the rate-limiting step of cholesterol synthesis, the HMGCR enzyme is the primary target of statins. By inhibiting HMGCR activity, statin therapy results in decreased intrahepatic cholesterol synthesis. Subsequently, bloodstream-to-liver cholesterol transport is increased, and the resulting reduction of cholesterol concentrations in the bloodstream slows the progression of atherosclerosis. Genetic variation of *HMGCR*, however, can result in significantly attenuated responses to statin pharmacotherapy. One of about ten described haplotypes of *HMGCR*, haplotype 7 (H7) is defined by the following three SNPs: rs17244841, rs3846662, and rs17238540 (Krauss *et al*., 2008). The

rs# is a unique identification number for genetic polymorphisms recorded in the Single Nucleotide Polymorphism Database (dbSNP). The dbSNP is maintained by the National Center for Biotechnology Information (NCBI) in collaboration with the National Human Genome Research Institute (NHGRI). The genetic variation associated with H7 affects alternative splicing of the *HMGCR* mRNA, resulting in decreased sensitivity to statins. In the Pravastatin Inflammation/CRP Evaluation (PRINCE) trial, Cholesterol and Pharmacogenomics (CAP) study, and the Genetics of Diabetes Audit and Research in Tayside Scotland (GoDARTS) database, carriers of the H7 haplotype had a diminished response to statins: Reductions in LDL cholesterol were about 20% less and the likelihood of achieving predefined cholesterol goals was about 50% less than that of noncarriers of H7 (Chasman *et al*., 2004; Donnelly *et al*., 2008; Krauss *et al*., 2008). These findings, however, were not replicated in the Atorvastatin Comparative Cholesterol Efficacy and Safety Study (ACCESS), Assessment of Lescol in Renal Transplantation (ALERT), Prospective Study of Pravastatin in the Elderly at Risk (PROPSER), or Treatment to New Targets (TNT) studies (Thompson *et al*., 2005; Singer *et al*., 2007; Polisecki *et al*., 2008; Thompson *et al*., 2009). Variation of response to statin therapy is better explained by *HMGCR* haplotypes than by genotypes, but the available evidence is insufficient at present to warrant routine testing.

Self-limited, dose-dependent statin-induced muscle toxicity is the most commonly recognized serious adverse outcome associated with statin therapy. Recently, another type of myopathy has been linked to statin use: Immune-mediated necrotizing myopathy (IMNM) is characterized by anti-HMGCR autoantibodies (Mammen *et al*., 2011). The amount of autoantibody is associated with increased creatine kinase (CK) levels and muscle weakness for statin-exposed patients, but not for statin-naive patients afflicted with IMNM. Furthermore, immunotherapy reversed muscle weakness and decreased the levels of CK and autoantibodies only for statin-exposed IMNM patients (Werner *et al*., 2012). As IMNM is a statin-related autoimmune myopathy involving HMCGR, genetic variations in *HMGCR* and genes involved in immunity should be investigated for phenotype-genotype associations regarding the incidence and severity of IMNM.

Solute Carrier Organic Anion Transporter Family, Member 1B1 (SLCO1B1)

As illustrated in Figure 1, SLCO1B1 transports statins and metabolites from the blood stream into the cells of the liver. The primary lipid-lowering action of statins, competitive inhibition of the cholesterol-synthesizing enzyme HMGCR, occurs within hepatocytes. A loss-of-function (LOF) or a decrease-of-function (DOF) variant in *SLCO1B1* should arguably result in lower efficacy. Significant reductions in efficacy, however, have not been shown to be associated with variants of *SLCO1B1* (Peters *et al*., 2010; Yang *et al*., 2010). Variant *SLCO1B1* alleles have demonstrated significant pharmacokinetic influence. The area under the curve of the time-concentration curve (AUC) was approximately double ($n=41$, $p<0.01$) for *SLCO1B1* variant allele carriers compared to homozygous carriers of the wild-type allele (Niemi *et al*., 2004). Homozygous carriers in the Go-DARTS database were approximately three times more likely ($n=4,340$, $p<0.01$) to be statin intolerant, with an increase in serum creatine kinase concentration to above the upper limit of normal or an increase in serum alanine aminotransferase concentration to above 1.5 times the upper limit of normal (Donnelly *et al*., 2010). A GWAS investigation in patients taking simvastatin 80 mg daily found that heterozygous and homozygous *SLCO1B1* variant allele carriers were 4.5 and 16.9 times more likely to have developed myopathy compared to homozygous carriers of the wild-type allele ($n=175$, $p<0.01$ after correction for multiple-hypotheses testing). The odds ratios (OR) were 2.6 and 5.2 for heterozygous and homozygous carriers, respectively, for individuals taking only 40 mg daily (Link *et al*., 2008). In a case-control study ($n=108$) the association was replicated for individuals taking simvastatin (OR: 3.2, $p<0.05$) but not for those taking atorvastatin (OR: 4.5, $p=0.48$) (Brunham *et al*., 2012). Although the association between the *SLCO1B1* variant and simvastatin-related muscle toxicity is robust, the adverse outcome can still occur in the absence of the variant allele. Other potentially deleterious variants in *SLCO1B1* or in other genes may increase systemic exposure of statins, increasing risk of developing muscle-toxicity risk.

ATP-binding Cassette, Sub-family B1 (ABCB1)

As depicted in Figure 1, statins and their metabolites are eliminated via bile and urine. ABCB1 transports statins and metabolites from hepatocytes to bile and from renal cells to urine. The following three SNPs have demonstrated significant direct influence on statin pharmacokinetics and indirect influence on statin pharmacodynamics: rs1128503, rs2032582, and rs1045642. These polymorphisms are also denoted as c.1236C>T, c.2677G>T/A, and c.3435C>T of *ABCB1,* referring to the DNA base substitution and their position in the gene's cDNA sequence. For example, c.1236C>T indicates that the polymorphism has a thymine in place of a cytosine at base position 1236 of the *ABCB1* cDNA

sequence. SNP C3435T alone has also been associated with decreased *ABCB1* mRNA stability, leading to reduced expression of ABCB1 (Wang *et al*., 2005). In combination with SNP 1236 or 2677 or both, SNP 3435 affects codon usage dynamics and timing of cotranslational folding, resulting in alterations in the transporters structure and function. Individuals with the 3-locus genotype pattern (T;T)-(T;T)-(T;T), also referred to as the TTT haplotype, had an AUC that was 60% larger for simvastatin's metabolite (p=0.039) and 55% larger for atorvastatin's metabolite (p<0.025) compared to those with the CGT haplotype (Keskitalo *et al*., 2008). Genotyping for *ABCB1* is not routinely performed; however, investigations that combine *ABCB1* with other influential statin-pharmacokinetic genes (*SLCO1B1, CYP3A*) aim to develop clinically-relevant tests to aid in statin-dose selection and avoid toxicity.

Cytochrome P450, Family 3, Subfamily A (CYP3A)

CYP3A enzymes are involved in the metabolism of nearly half of the most-commonly prescribed medications. Of the statins, they metabolize atorvastatin, lovastatin, and simvastatin. CYP3A4 is the most prominent member of CYP3A, and a DOF allele, *CYP3A4*22*, has recently been characterized. The allele nomenclature for CYP enzymes designates significant SNPs with a number preceded by an * (pronounced "star"). This designation suggests that sufficient evidence exists regarding the functionality, prevalence, and clinical relevance of the polymorphism (Sim, 2013). The *1 designation is typically assigned to the wild-type or "normal functioning" allele, and a polymorphism can be referred to by rs# or by "star" designation. CYP3A4 enzyme level and activity were greater in wild-type homozygous patients than in *CYP3A4*22* allele carriers, and carriers of the DOF allele required only 20-60% (n=235, p<0.05) of the statin dose required by homozygotic wild-type patients taking stable doses of atorvastatin, simvastatin, or lovastatin for optimal lipid control (Wang *et al*., 2011). The steady-state serum concentrations of simvastatin and its active acid metabolite were about 50% higher (n=~800, p<0.05) in *CYP3A4*22* allele carriers compared to wild-type homozygous patients (Kitzmiller J.P., unpublished data). Significant associations between the **22* allele and increased lipid-lowering response to simvastatin have been reported (Elens *et al*., 2011), and *CYP3A4*22* allele carriers may be at a higher risk (n=~300, p=~0.06) for developing statin-

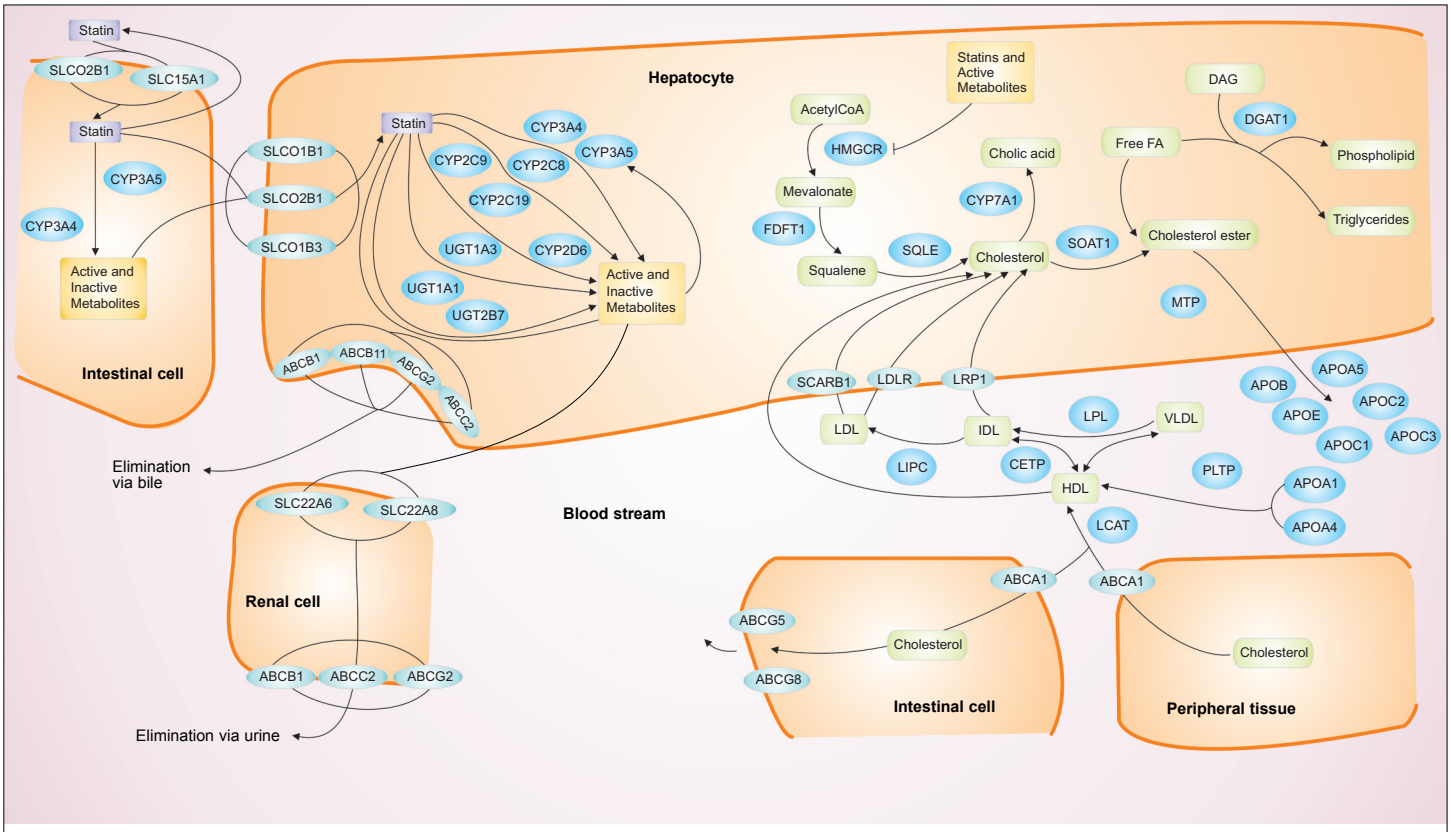

Figure 1. Statin pharmacokinetics and pharmacodynamics. FA, fatty acid; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein. Adapted from pathway diagrams under copyright by PharmGKB; with permission from PharmGKB and Stanford University.

induced myopathy (Kitzmiller J.P., unpublished data). About 7% of Caucasians possess at least one *CYP3A4*22* allele, and the frequency of the DOF allele has not yet been well characterized in non-Caucasian patient populations.

Although *in vitro* studies strongly suggest that CYP3A5 plays only a limited role in statin metabolism (Park *et al*., 2008), some investigators have reported significant associations among *CYP3A5* and statin pharmacokinetics and pharmacodynamics. The *CYP3A5*1* allele is the only allele that produces high levels of full-length CYP3A5 mRNA and functional protein. Approximately a dozen other *CYP3A5* alleles have been described, with *CYP3A5*3* being the most common LOF allele. Simvastatin exposure was higher for *CYP3A5*3* homozygotes compared with *CYP3A5* 1* homozygotes (Kim *et al*., 2007), and diminished lipid-lowering responses have been reported for *CYP3A5 *1* homozygotes (Kivistö *et al*., 2004; Shin *et al*., 2011). Conversely, other findings suggest that *CYP3A5* status does not influence statin pharmacokinetics and pharmacodynamics: Systemic exposure of the biologically-active atorvastatin acid metabolite was not significantly associated with *CYP3A5* status (Willrich *et al*., 2008), and no significant association was observed between *CYP3A5*3* and efficacy or tolerability of simvastatin (Fiegenbaum *et al*., 2005). As shown in Figure 1, other enzymes including CYP2C and Uridine'5 Diphospho-Glucuronosyl Transferase (UGT) are involved in the metabolism of certain statins; however, genetic variation regarding those enzymes seems to be of lesser consequence regarding statin pharmacology and has not been as aggressively investigated.

Current and Future Trends

Retrospective, prospective, case-study, cross-sectional, candidate-gene, and genome-wide approaches have been implemented in a variety of patient populations across the United States and around the world in order to investigate the impact of genetic polymorphisms on statin pharmacotherapy. In addition to the select genes presented in this review several other key genes (*APOE LDL-R*, *PCSK9*, *KIF6*, and *CLMN*) have significant influence on statin pharmacology, and analyses integrating several key genes and their interactions have been reported. A clinically-relevant pharmacogenomic test regarding statins, however, has not yet emerged. Alone no gene has a large enough clinical impact to warrant routine testing. A multi-gene approach involving the integration of a large patient population, however, may eventually provide useful guidance regarding statin pharmacotherapy, similar to that provided for warfarin at warfarindosing.org. The pharmacogenomic data provided at this site provides guidance for warfarin dosing based on clinical factors, concomitant medications, comorbidities, and genetic status of *VKORC1*, *CYP2C9*, *CYP4F2*, and *GGCX*. The popularity of this site among clinicians and patients has increased, and FDA-approved drug labeling of warfarin recommends considering a patient's *CYP2C9* and *VKORC1* status when choosing an initial dose (Bristol-Myers Squibb, 2010). As statin-related genetic research advances, a similar multi-gene approach also encompassing clinical factors may be established to assist clinicians in answering the following fundamental questions: How likely is my patient to benefit from taking a statin? Which statin is best for my patient? What dose should be chosen initially? Does my patient's individual risk for adverse events outweigh their expected benefit? Should my patient be monitored more frequently for adverse events?

Examining current clinical trials involving statins and pharmacogenomics provides some foresight, and ClinicalTrials.gov is a registry and results database of publicly- and privately-supported clinical studies conducted around the world. Registration of trials involving pharmaceutics is mandated or at least strongly recommended by the following governing organizations: U.S. Food and Drug Administration, the World Health Organization, National Institutes of Health, the World Medical Association, and the International Committee of Medical Journal Editors (U.S. National Library of Medicine, 2013). Approximately 1,500 currently-registered trials involve a statin, and 20 of them list a primary genetic component. A random sampling of the remaining registered statin trials suggests that about 10% include genetic factors as a secondary study measurement. The most studied statin is atorvastatin with 576 registered trials, followed by simvastatin, rosuvastatin, pravastatin, fluvastatin, lovastatin, pitavastatin, and cerivastatin with the following numbers of respective registered clinical trials: 422, 282, 128, 58, 53, 49, and 1. Of the registered statin trials 16, 20, 30, and 34% are phase 1, 2, 3, and 4 trials, respectively; 35, 4, 17, and 44% list their funding source as Industry, Federal, Combination, and Other, respectively (U.S. National Library of Medicine, 2013).

Many large genotype-phenotype datasets regarding statin pharmacotherapy exist, and more are developing. Combining these data would provide advantages toward accomplishing the primary goal of statin pharmacogenomics research: development of clinically-meaningful pharmacogenomic tests to guide statin pharmacotherapy. A major challenge in combining these data involves differences in predefined and limit-

ed phenotypic domains. A GWAS approach typically determines associations between the variation of millions of SNPs and a few predefined phenotypes. A newly-developed alternative method, a phenome-wide association study (PheWAS) approach, utilizes all available phenotypic information and all genetic variants in the estimation of associations between genotypes and phenotypes (Pendergrass, Brown-Gentry *et al*., 2011). This approach provides an expanded view of the relationship between genetic variation and networks of phenotypes because a diverse range of phenotypes can be combined and investigated. Flexible and adaptive statistical methods like the PheWAS approach are likely to significantly advance the development of pharmacogenomic applications, but prospective randomized trials evaluating improvement in patient outcomes are likely to remain the gold standard for determining the clinical significance of a pharmacogenomics test.

Arguably, the majority of statin clinical trials and pharmacogenomics research focus on statin efficacy related to lipid-lowering. However, the overall benefits of statin therapy seem to go beyond those expected from changes in lipid levels alone. Recent studies indicate that some of the pleiotropic effects (i.e., effects other than the drug's initial indication) of statins include increasing the availability of endogenous nitric oxide, enhancing plaque stability, improving endothelial function, and decreasing inflammation. Statin's pleiotropic effects are likely to be genetically-influenced, and future statin pharmacogenomics research and clinical investigations should investigate potential genotype-phenotype associations.

Acknowledgments

The authors would like to acknowledge the following NIH grants that supported the authors writing of this review article: K23 GM100372 and U01 GM092655.

Disclosure

The authors report no conflicts of interest.

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