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Review Article

Evaluation of the effect of genetic variation on the relationship between statins, cardiovascular disease and cancer

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Abstract: Statins are a class of medications that are competitive inhibitors of Hydroxy Methyl Glutaryl Co-enzyme A (HMG-CoA) reductase which is the rate-limiting enzyme in the cholesterol bio-synthesis pathway. As a result, statins lower total cholesterol and low density lipoprotein (LDL) cholesterol thus impacting cardiovascular mortality. The downstream effects of statins are not limited to inhibition of cholesterol synthesis alone. Statins have anti-inflammatory effects thought to be important in the setting of acute myocardial infarction which also may be a mechanism involved in anti-carcinogenic properties of statins. Furthermore, statin inhibition of the mevalonate pathway may impact Ras and RhoGTPases that are important in cell proliferation, migration and apoptosis. These alterations may also play a role in the anti-cancer effect of statins. In this article we will review the literature on how genetic variation modifies the effect of statins on the risk of cardiovascular disease and how genetic variation may impact the relationship between statins and the risk of a number of different cancers.

Keywords: Statins, blood lipids, cancer risk, cardiovascular disease, genetic variation

Introduction

Statins are a class of medications that are competitive inhibitors of Hydroxy Methyl Glutaryl Co-enzyme A (HMG-CoA) reductase which is the rate-limiting enzyme in the cholesterol bio-synthesis pathway. As a result, statins lower total cholesterol and low density lipoprotein (LDL) cholesterol thus impacting cardiovascular mortality. The downstream effects of statins are not limited to inhibition of cholesterol synthesis alone. Statins have anti-inflammatory effects thought to be important in the setting of acute myocardial infarction which also may be a mechanism involved in anti-carcinogenic properties of statins. Furthermore, statin inhibition of the mevalonate pathway may impact Ras and RhoGTPases that are important in cell proliferation, migration and apoptosis. These alterations may also play a role in the anti-cancer effect of statins.

Variations in the metabolism of a number of medications have been linked to genetic polymorphisms. One prominent example is warfarin where SNPs in the vitamin K epoxide reductase complex subunit 1 (VKORC1) (the molecular target of warfarin) or genetic variants in enzymes involved in the metabolism of warfarin (cytochrome P-450 e, CYP2C9 and CYP4F2) have been linked to warfarin anticoagulant activity and dosing [1-3]. Variations in SNPs associated with warfarin activity are responsible for up to 40% of dose variance and is an example of how pharmacogenomics has led to a better understanding of clinical dosing of a commonly used medication [4]. Similarly, genetic variation leads to differences in metabolism of other commonly used anti-cancer drugs including irinotecan [5], 6-mercaptopurine [6] and tamoxifen [7] which also have important clinical implications.

In summary, variation in lipid metabolism genes including those that alter HMG-CoA reductase

as well as other genes that modify lipid metabolism may directly or indirectly impact the effect of statins on blood lipid levels as well as the possible effect of statins on cancer risk. In this article we will review the literature on how genetic variation modifies the effect of statins on the risk of cardiovascular disease and how genetic variation may impact the relationship between statins and the risk of a number of different cancers.

Association of lipid metabolism genes with lipid phenotypes

A number of studies have investigated the relationship between polymorphisms in genes involved in lipid metabolism and/or the effect of statins on blood lipid levels [8-13]. Lipid levels are influenced by diet and diurnal variations; however an evaluation of the influence of genetic variation has the potential to provide additional insight into the effects of statins on lipid metabolism which in turn may help to better predict the effect of statins on cardiovascular outcomes.

In the Pravastatin Inflammation/CRP Evaluation (PRINCE) study, Chasman et al. evaluated the relationship between 33 polymorphisms in the 3-hydroxy-3 methylglutaryl-coenzyme A (HMGCR) gene and the effect of pravastatin on blood lipid levels [8]. The team identified two SNPs (rs17244841 and rs17238540) that were significantly associated with a reduced effect of pravastatin on lowering LDL and total cholesterol. Among individuals treated with pravastatin, a single copy of the major allele (A) in rs17244841 was associated with a mean decrease in total cholesterol of 32.8 mg/dL, and for those homozygous for the major allele the mean reduction in total cholesterol was 42.0 mg/dL (absolute difference, 9.2 mg/dl, 95% CI 3.8-14.6 mg/dL; p=0.001). For rs17238540, individuals homozygous for the major allele (T) had a 22.3% lower reduction of total cholesterol compared to heterozygotes with an absolute difference of 9.3 mg/dL (95% CI, 3.8-14.7 mg/dL, p<0.001). Whether titrated dose adjustment of statins based on inheritance will improve cholesterol level control and thereby further impact cardiovascular outcomes needs to be elucidated in prospective trials.

HMGCR typically undergoes alternative splicing of exon 13 which encodes a portion of the statin binding domain [14]. Previous studies have identified certain SNPs and haplotypes that are associated with a reduced statin efficacy [15]. In an attempt to understand the underlying mechanism of this observation, Medina et al studied alternative splicing variation using a sample of simvastatin-incubated immortalized lymphocyte cell lines derived from participants in the Cholesterol and Pharmacogenetics (CAP) study. They postulated that the intronic SNPs rs17244841, rs17238540 and rs384662 which form a haplotype (H7) may impact mRNA splicing. They demonstrated that the H7 haplotype was associated with an alternatively spliced *HMGCR* mRNA lacking exon 13, which is important for proper statin binding. The fact that statins do not efficiently bind to *HMGCR* lacking exon 13 may explain inter-individual variation in the effect of statins on blood lipid levels. There is evidence that alternative splicing may also be important in other genes in the cholesterol synthesis pathway such as HMG-CoA synthase [16] and mevalonate kinase [17]. A better understanding of the mechanisms of how genetic variation contributes to drug response could help direct future clinical use of statins in reducing cardiovascular disease outcomes.

While the above mentioned genes are directly involved in the cholesterol synthesis pathway, genes that regulate other compensatory mechanisms of LDL regulation may be involved in modulating statin efficacy in lipid lowering. Paraprotein convertase subtilisin/kexin type 9 (*PCSK9*) is one such gene involved in regulating LDL receptor expression on the surface of hepatocytes. PCSK9 acts as a 'chaperone' for the intracellular uptake of LDL receptor and its degradation and recycling. Thus, down regulation of *PCSK9* leads to an increased LDL receptor expression on surface hepatocytes and lower LDL levels resulting from increased clearance of LDL particles [18, 19]. It might be expected therefore, that blocking PCSK9 might enhance statin efficacy, and thereby increase clearance of atherogenic lipoproteins. Conversely, the use of statins and the subsequent lower intracellular LDL concentration may be associated with a compensatory up regulation of *PCSK9* with a resultant lowering of efficacy of statins on prolonged use [20].

In a nested case-control study in the TNT (Treating to New Targets) Trial, baseline circulating PCSK9 levels predicted the outcome of

cardiovascular disease only in patients randomized to low dose atorvastatin group (10 mg) and not in participants who were randomized to the high dose atorvastatin group (80 mg), after an initial run in period of low dose atorvastatin. However, the PCSK9 levels measured at baseline and 1 year after randomization did not change significantly among the low and high dose participants therefore making a causal inference difficult [21].

In the JUPITER trial (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin), various polymorphisms in PCSK9 were found to influence the patient response to rosuvastatin in a GWAS study of 6989 participants who were randomly allocated to rosuvastatin or placebo [22]. In a case control analysis of 668 cases and 1217 controls with hypercholesterolemia selected from the Utrecht Cardiovascular Pharmacogenetics (UCP) studies, two SNPs in *PCSK9* (rs10888896 and rs505151 (E670G)) were found to modify the efficacy of statins in preventing MI. Participants with the variant allele E670G did not benefit from statin use (OR 0.63, 95% C.I. 0.30-1.32) whereas homozygous wild type carriers benefited from statin treatment (OR 0.36, 95% C.I. 0.28-0.45) [23]. On the other hand, there is contrary literature as well with no association seen with the E670G polymorphism in the PROSPER trial [24] and PROVE IT_TIMI trial [25]. In addition, another GWAS study conducted by Vrablik et al also did not demonstrate any influence of PCSK9 polymorphisms on statin efficacy in modulating lipid levels [26].

Association of statin usage with CVD phenotypes

There have been multiple reports on the effect of statins in reducing the risk of cardiovascular events and mortality. These studies have included heterogeneous participant groups including individuals with and without known occlusive vascular disease [27]. A meta-analysis of 26 randomized clinical trials of statins versus placebo reported a reduction in all-cause mortality associated with statins of 10% per 1 mmol/L reduction in LDL cholesterol (RR 0.90, 95% CI 0.87-0.93; p<0.0001) [28]. In another meta-analysis of 18 randomized trials analyzing the effect of statins in the primary prevention of cardiovascular events, statins were found to reduce both all-cause mortality (OR 0.86, 95% CI 0.79 to 0.94) and the rate of cardiovascular events (RR 0.75, 95% CI 0.70-0.81) [29]. In contrast, another meta-analysis of 8 randomized trials evaluated the risk of cardiovascular events in a primary prevention setting, although 8.6% of the participants in the meta-analysis had baseline vascular occlusive disease. In this analysis, total mortality was not affected by randomization to statins (RR 0.95, 95% CI 0.89-1.01), however there was a reduction in the rate of cardiovascular events associated with statins (0.82, 0.77-0.87). This latter benefit however was due to a significant benefit among men aged 30-69, while no benefit was seen among women (0.98, 0.85-1.12) or for participants older than 69 years (0.94, 0.77-1.15) [30]. However, a more recent meta-analysis of 18 randomized clinical trials of statins with sex specific outcomes showed a protective effect of statins on both primary and secondary cardiovascular event rates in women (OR 0.81, 95% C.I. 0.75-0.89) as well as men (OR 0.77, 95% C.I. 0.71-0.83) irrespective of baseline risk [31]. The all-cause mortality was significantly lower in women when primary prevention trials were examined (OR 0.87, 95% C.I. 0.78-0.97) while the effect was not significant for all-cause mortality in secondary prevention trials (OR 1.03, 95% C.I. 0.84-1.25). Conversely, the all-cause mortality was lower in men when secondary prevention trials were considered (OR 0.76, 95% C.I. 0.66-0.87) and not significantly lower for primary prevention trials (OR 0.92, 95% C.I. 0.84-1.01).

Overall, statins have proven to be beneficial in primary and secondary reduction of cardiovascular events and mortality. The observed variations in the results of studies evaluating these relationships may be linked to a number of variables including environmental factors and genetic risk. A better understanding of the effect of genetic variation in the effect of statins on cardiovascular disease outcomes may be helpful in identifying sub-populations that are more likely to benefit from statin intervention.

Association of lipid metabolism genes with CVD phenotypes

To explore the effect of polymorphisms in genes that affect lipid metabolism and cardiovascular outcomes Kathiresan and colleagues used samples from the cardiovascular cohort of the

Malmo Diet and Cancer study (MDCS) [9]. In this analysis, a "genotype score" was developed which was based on the number of unfavorable alleles in 9 SNPs in genes associated with high LDL or low HDL. The results of this study demonstrated an increase in the crude rate of cardiovascular events from 3.1/1,000 to 11/1,000 person years for individuals with a genotype score of ≤6 vs. ≥13. After multivariable adjustment, genotype score was significantly associated with an increased risk of a cardiovascular event (HR 1.15 per copy of an unfavorable allele, C.I 1.07-1.24). While there was a significant relationship between genotype score and time to first cardiovascular event, the genotype score did not have any additional value in predicting cardiovascular events at 10 years of follow-up. In addition, the authors could not discriminate the effect of specific SNPs on cardiovascular outcomes.

Other studies have looked at variation in another gene involved with statin metabolism, the kinesis-like protein 6 gene (KIF6), that encodes for kinesin a protein involved in transport of proteins, membrane organelles and messenger ribonucleic acid (mRNA) along microtubules [32]. In a study of genotype data from the Cholesterol and Recurrent Events (CARE) and West of Scotland Coronary Prevention Study (WOSCOPS) investigators found that the Trp719Arg allele polymorphism (rs20455) in the KIF6 gene was associated with a 50% increased risk of coronary events compared to non-carriers in a dominant model (HR 1.5, 95 C.I., 1.05 to 2.15) [33]. Iakoubova and colleagues also studied the effect of polymorphisms in the KIF-6 gene on the relationship between statins and cardiovascular outcomes among participants in the Pravastatin or Atorvastatin Evaluation and Infection Therapy: Thrombolysis in Myocardial Infarction 22 (PROVE IT-TIMI 22) trial [33]. They found that 59% of study participants were carriers of the rs20455 polymorphism in KIF-6 and that carriers of this polymorphism had a significantly greater benefit from intensive statin therapy with lower rates of coronary events (HR 0.59, 95% CI 0.45-0.77), while no benefit was observed among non-carriers 0.94 (95% CI 0.70-1.27). While carriers of rs20455 were found to be at increased risk of fatal or nonfatal coronary events in other studies [34, 35], in the Iakoubova study, there was no increased risk of cardiovascular events for rs20455 carriers that were treated with statins. The authors proposed that the increased risk associated with rs20455 may be offset by intensive statin therapy suggesting that rs20455 may be both a prognostic and a predictive polymorphism. Further evidence of genetic vulnerability of KIF6 polymorphism and LDL cholesterol comes from a meta-analysis of 37 studies including case control, prospective cohort and randomized clinical trials by Ference et al. In their analysis, the KIF6 719 Arg allele was not associated with risk of CVD, but participants with one or more copies of the KIF 719 Arg allele had a 15% greater increase in CVD per mmol/L increase in LDL and at the same time experienced a 13% greater reduction in CVD risk per mmol/L decrease in LDL cholesterol in response to statins compared to non-carrier status [36]. However, the exact mechanism by which KIF6 gene modulates statin efficacy is not known.

Association of statin usage with cancer phenotypes

As indicated above, statins were primarily developed for the purpose of lowering serum cholesterol; however there is evidence that statins may also have chemo preventive and therapeutic effects for a number of different cancers. In-vitro data has shown anti-proliferative, apoptotic and anti-invasive properties of statins in cancer cell line studies [37-43]. In vitro studies of breast and lung cancer cell lines have shown statin induced decrease in expression of anti-apoptotic Bcl2 and increase in pro-apoptotic Bax protein [44]. It was postulated that a change in the balance of pro-apoptotic and anti-apoptotic signals toward the former may enhance the apoptotic activity of chemotherapy.

Another possible chemo preventive activity of statins may be related to statin induced inhibition of the mevalonate pathway. This inhibition leads to lower levels of downstream products including farnesyl diphosphate (FPP), geranylgeranyl diphosphate (GGPP) and dolichol which are involved in post translational modification of many different proteins [45-47]. In particular, the Ras molecule requires farnesylation, mediated by FPP, to attach onto the cellular membrane which in turn helps transmit downstream signaling from surface receptors [48]. Ras

increases gene transcription and proliferation by acting through the MEK and PI3K/Akt pathways [37, 38] which are up regulated in many cancers.

GGPP is involved in geranylgeranylation of Rho proteins including RhoGTPases [39] which in turn maintains function of Rho kinases that are involved in various cellular functions including gene expression, actin cytoskeleton migration, adhesion and contractility of cells [49]. By inhibiting the production of GGPP, statins may reduce cell migration and have anti-proliferative and anti-invasive properties. In vitro studies of cerivastatin have shown dose dependent reduction in nuclear factor kappa B (NFkB) along with Rho A inhibition, resulting in a decrease in matrix metalloproteinase 9 (MMP9) and urokinase levels which are important in cell migration [50] and may lead to reduced metastatic potential in cancer cells. Statins are also associated with increase in p21 and p27 [41, 51-53] which are two cyclin mediated kinases whose degradation is mediated by RhoGTPases [54]. P27 acts as an inhibitor in cell cycle progression and thereby reduces cell proliferation. Thus the implication of statins in G1-S arrest mediated by the increase in p27 [41, 43] may explain the anti-proliferative effects of statins.

Epidemiologic studies of the association between statins and cancer risk have demonstrated mixed results with some studies showing a protective effect for some cancers and other studies showing an increased risk or no association [42, 55-57]. Two meta-analyses evaluating the effect of statins on breast cancer risk, one which included 7 randomized trials and 9 observational studies [58], and the other 7 randomized trials [59] showed no significant association. A recent prospective cohort analysis showed no significant relationship between statins and colon, lung, pancreatic, breast or bladder cancer however there was a reduction in risk of melanoma, endometrial cancer and NHL [60]. Another recent nested case control study revealed an increased risk of bladder, colon and lung cancer although a reduced risk of hematological malignancies including myeloma, leukemia and lymphoma [61] and in a meta-analysis of twenty case control studies, there was a protective effect for colon cancer but no effect on breast, lung and prostate cancer risk [62]. Other studies which have demonstrated a protective effect of statins include a US veteran's study which demonstrated a reduced risk of lung cancer [63] and three recently completed meta-analyses showed a reduced risk of colon cancer [64-66]. As indicated in the review, the results in the epidemiological studies on statins and cancer risk are heterogeneous and point towards possible inter-individual variation in the protective effect of statins with regards to cancer risk.

Association of lipid metabolism genes and cancer phenotypes

A number of investigators have looked at the relationship between inter-individual variation in genes associated with lipid metabolism pathways and the risk of cancer [2, 67, 68]. In a population based case-control study in Shanghai, China, Andreotti et al. examined the association of 12 SNPs in 5 genes in the lipid metabolism pathway with risk of biliary tract cancers and stones [2]. Results from this showed that individuals with the G allele in rs440446 SNP (MAF=0.375) of the *APOE* gene had a 1.8 fold increased risk of gallbladder cancer (95% CI 1.1-3.3), a 3.7 fold increased risk of bile duct cancer (95% CI 2-7) and a 4 fold increased risk of ampullary cancer (95% CI 1.4-12). Individuals who had a T allele for the SNP rs520354 in the *APOB* gene had a 1.6 fold increased risk of bile duct cancer (CI 95% 1.1-2.3). The increased cancer risk was independent of the occurrence of gallstones and may hint towards other pathways that are affected by variations in SNPs that modify the risk of biliary tract cancer. However, this study did not explore the interaction of statin therapy, lipid levels and risk of biliary tract cancer.

Lipkin et al. examined how genetic variation in the *HMGCR* gene modifies the chemo preventive activity of statins for colorectal cancer among participants in the population-based Molecular Epidemiology of Colon Cancer (MECC) study in Northern Israel [67]. Individuals with the AA genotype for SNP rs12654264 in the *HMCGR* gene who were also statin users, had a greater reduction in the risk of colorectal cancer compared to individuals who had the TT genotype. In a case only analysis, the odds of colorectal cancer among statin users vs. non-users with the AA genotype was 0.3 (95% C.I. 0.18-0.51), whereas the odds of colorectal cancer for users vs. non-users associated with the TT genotype was 0.66 (95% C.I. 0.41-1.06) and

the AT genotype had an intermediate odds ratio (*p* value for interaction 0.0012). In addition, the AA genotype was associated with lower serum levels of LDL among all cases and controls compared to AT or TT genotype [67].

Lipkin et al further evaluated the potential mechanism involving alternative splicing by cell culture studies and *HMGCR* mRNA isoform quantification. The rs12654264 variant, which was also discussed earlier, was found to be a noncoding SNP located in intron 12 of *HMGCR*. As previously noted, Medina et al. had described an alternative spliced form of *HMGCR* (HMGCR v1) that does not include exon 13, which encodes a critical portion of the statin binding domain and that this truncated transcript is less effective at binding statins [15]. The rs12654264 A allele was found to be associated with full length mRNA transcripts, whereas the T allele was associated with the truncated HMGCR v1 transcript. Thus the molecular mechanism by which the rs12654264 transcript modifies cholesterol synthesis in colorectal cancer cell lines, was found to be related to the concept of alternative splicing. Genotype alone was not associated with colorectal cancer risk after adjustment for multiple comparisons. Furthermore, in this study rs12654264 was not associated with altered overall *HMGCR* mRNA expression levels when tested in non-statin users, suggesting that genetic variation in *HMGCR* modifies colorectal cancer risk only in the presence of statins. Other downstream targets impacted by increased cholesterol synthesis in colon cancer cells may be involved in why individuals homozygous for rs12654264 have increased protection from colorectal cancer.

Discussion

In summary, variation in genes involved in the cholesterol synthesis pathway influence lipid levels and response to statins. These polymorphisms also modify the efficacy of statins in prevention of cancer and may allow us to categorize individuals that may have a greater or lesser benefit from statins in regards to cardiovascular disease risk reduction and prevention of cancer. SNPs that predict lesser response are equally important in targeting individuals that may need more than standard intervention to result in similar cardiovascular outcomes as compared to individuals carrying SNPs that predict better response. While this is important in moving towards personalized medicine, it is a daunting task to identify all important polymorphisms with their respective variations. Some identified polymorphisms such as the PCSK9 with a well-described mechanism of action, do not always modulate the effects of statins as expected in experimental studies. Furthermore, there may be racial or ethnic differences in genetic effects, as exemplified by a study by Krauss et al. that included 326 African-Americans and 596 whites and evaluated possible racial differences in LDL lowering efficacy of statins. African-Americans with H7 or H2 haplotype had lower baseline LDL levels and also attenuated LDL reduction response to simvastatin treatment (*p* value 0.02) compared with African-Americans who did not carry this haplotype, whereas, there was no effect of the haplotype on baseline LDL or LDL reduction response in whites [69]. These ethnic variations in SNPs allele frequencies should be incorporated into plans to use these polymorphisms for screening purposes. Moreover, genetic variations in *HMGCR* may modify the chemo preventive activity of statins for colorectal cancer [67]. SNPs in the lipid metabolism pathway have also been linked to the risk of biliary tract cancer, [68] independent of the occurrence of gall stones, which is an independent risk factor for the occurrence of gall bladder cancer. With further elucidation of associations between genotype and statin response in cardiovascular disease and cancer prevention and treatment, statin therapy may be tailored based on genotype profile.

Disclosure of conflict of interest

None.

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