A Transcriptomics-Informed Genetic Association Study Identifies RHOA in Simvastatin-Induced Low-Density Lipoprotein Cholesterol Lowering

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Study Hypothesis
Inhibitors of HMGCR (or statins) are the most common and effective medications used for the treatment and prevention of cardiovascular morbidity and mortality. It is well known that statins mediate their effect primarily through the reduction of low-density lipoprotein cholesterol (LDLc) which is causally related to the development of coronary and other forms of atherosclerosis/thrombosis. Despite their effectiveness, there is significant variability in the LDLc-lowering effects of statins with few clinical predictors of the magnitude of statin-induced LDLc lowering. Recent genomewide studies of allelic variation of statin-induced LDLc have identified few genomic loci (APOE, ABCG2, LPA) in which genetic variation predicts the magnitude of LDLc lowering with statins. Although important, much of the observed variability in response remains unexplained. One potential explanation for the lack of association for the vast majority of genetic variants tested in genomewide association studies is the strict statistical criteria used to declare significance. As a consequence of these criteria, many potential variants with lesser levels of statistical evidence are often overlooked. Therefore, Medina et al sought to test the hypothesis that statin-induced changes in gene expression could be used as a filter to narrow genomic loci for subsequent genetic association studies of statin-induced LDLc.

How Was the Hypothesis Tested?
To test their hypothesis, the authors took advantage of a unique resource in pharmacogenomics research. From a previous study (Cholesterol and Pharmacogenetics [CAP] study) in which volunteers were exposed to 6 weeks of 40 mg/d simvastatin, the authors banked 480 lymphoblastoid cell lines (LCLs) from study participants. This resource allowed the authors to expose LCLs to simvastatin in vitro to assess drug-induced changes in genomewide gene expression. Using simvastatin-induced changes in HMGCR gene expression as a pharmacodynamic marker of statin response in LCL, the authors then identified novel transcripts that changed in response to simvastatin in a magnitude similar to that of HMGCR across all 480 LCLs. This critical, dimensionality reduction step filtered the large number of genes that are differentially expressed with in vitro simvastatin to those that are specifically coexpressed with HMGCR. The authors then focused on a novel candidate gene to emerge from this filter in subsequent functional studies in hepatoma cell lines. Furthermore, changes in gene expression in response to in vitro simvastatin exposure in LCLs were directly related to changes in vivo LDLc lowering by simvastatin from the CAP study. Finally, genetic variants in novel candidate genes were tested for association in statin-induced LDLc lowering in CAP and in another clinical trial, PRavastatin INflammation C-reactive protein Evaluation (PRINCE) (pravastatin 40 mg/d, 24 weeks, n=1306).

Principal Findings
Using their approach, the authors reduced the number of genes differentially expressed with simvastatin in LCLs (n=725) to a much more tractable number (n=45). As expected, many genes that were coexpressed with HMGCR with simvastatin treatment were related to cholesterol synthesis. However, 1 transcript, RHOA, was an attractive novel candidate for modulating the LDLc-lowering response to simvastatin. Through siRNA experiments in hepatoma cell lines, the authors demonstrate that knockdown of RHOA leads to an accumulation of cholesterol esters and an increased secretion of apolipoprotein B, the major protein of circulating LDL particles. Furthermore, changes in RHOA gene expression in LCLs with in vitro simvastatin correlated with in vivo reductions in LDLc from the CAP study. Finally, after constructing haplotypes from the RHOA locus, the authors identified the H3B haplotype, defined by rs11716445, as a predictor of lower statin-induced LDLc. Although the mechanism by which the H3B haplotype confers resistance to statin-induced LDLc was beyond the scope of the present study, the authors demonstrate that the minor allele of rs11716445 acts as a cis-acting splicing quantitative trait locus that promotes the inclusion of a novel, cryptic exon in RHOA.

Implications
The work by Medina et al demonstrates the power of an integrative genomics approach to identify novel pharmacogenomic loci implicated in drug responses. It is unlikely that genetic testing will be used in clinical care to predict statin-induced LDLc in patients at risk for cardiovascular disease. However, by identifying novel candidate loci implicated in the variable response to statins, this work provides insight into the potential pleotropic, or non–lipid-lowering, effects of statins and may explain why despite adequate LDLc lowering some statin-treated patients still go on to develop cardiovascular disease.

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Disclosures
None.

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