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EDITORIAL COMMENT

Developing Medicines That Mimic the Natural Successes of the Human Genome

Lessons From *NPC1L1, HMGCR, PCSK9, APOC3*, and *CETP**

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Coronary heart disease (CHD) remains the leading cause of death in the industrialized world and will soon become so worldwide (1). Much research has focused on biological risk factors and developing medicines to modify them. However, remarkably few medicines (e.g., aspirin, statins, and antihypertensive agents) are proven to reduce CHD risk.

In several recent, large-scale, randomized-controlled trials, newly-developed medicines (e.g., dalcetrapib and darapladib) showed no benefit over placebo in reducing risk for coronary events (2–4). Often, these late-stage failures come after decades of research effort. Why were these results not anticipated? Two reasons stand out (5). First, the field has traditionally depended on in vitro or animal models; however, preclinical disease models often have limited ability to predict benefit in patients. For example, atherosclerosis in humans is the result of genetic makeup and decades of atherogenic stimuli; it is not surprising that this is difficult to recapitulate in cells or in nonhuman model organisms. Second, drugs that block a specific gene target are sometimes taken for many years; we often do not know the effect over such an extended period.

How can we more accurately anticipate whether a medicine will reduce risk for clinical CHD without adverse effects? In this issue of the *Journal*, Ference et al. (6) show that the human genome may be a valuable tool for prioritizing molecular targets in drug development. Medicines are typically designed to target a specific gene and its protein product. There is naturally occurring variation in nearly every gene, some of which resides in a specific drug's target gene. If this deoxyribonucleic acid (DNA) sequence variation modulates the gene's function or expression, then the phenotypic consequences of this variation in the human population could anticipate if a drug will safely reduce disease risk.

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This approach has now been applied to several drug-gene pairs. Some of the drugs are already in clinical use (ezetimibe targeting *NPC1L1*, statins targeting *HMGCR*) and some are in development (drugs targeting *PCSK9*, *APOC3*, and *CETP*). We will review each of these examples to understand the strengths and limitations of using genotype-phenotype correlations to anticipate a medicine's potential efficacy and safety.

Ezetimibe lowers plasma levels of low-density lipoprotein cholesterol (LDL-C) by blocking the Niemann-Pick C1-like 1 (NPC1L1) protein, a transporter allowing dietary cholesterol to enter the body from the intestinal lumen (7). Experiments in cells and mice confirmed that ezetimibe tightly binds to and blocks NPC1L1, and targeted deletion of *Npc1l1* in mice lowered plasma cholesterol and reduced atherosclerosis (8,9). In human volunteers, ezetimibe treatment blocked dietary cholesterol absorption by about 50% (10). However, addition of ezetimibe to background statin therapy failed to prevent atherosclerotic

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progression, as assessed by carotid intima-media thickness (11). These results raised uncertainty as to whether lowering LDL-C with ezetimibe would reduce risk for clinical CHD.

Ference et al. (6) mined the human genome to address this uncertainty. If ezetimibe successfully reduces risk for clinical CHD, DNA sequence variants that reduce NPC1L1 function (i.e., mimicking ezetimibe action) would be expected to lower LDL-C and protect against CHD risk. The results of several recently-published, large, genome-wide searches for DNA sequence variants affecting plasma LDL-C (12,13) were leveraged by Ference et al. (6) to pinpoint 5 independent variants at or near the *NPC1L1* gene and develop a genetic score using these variants. Those with an *NPC1L1* gene score below the median had a 2.4 mg/dl lower LDL-C level than those with scores above the median. After testing the *NPC1L1* gene score in 108,376 individuals, the investigators found that those with the lower gene scores also had reduced risk for CHD (4.8% lower).

Late last year, we published findings (14) consistent with those of Ference et al. (6). Through sequencing of the protein-coding regions of *NPC1L1*, we found that approximately 1 in 650 persons carried a large-effect mutation (nonsense, splice-site, or frameshift; "null" mutations) that completely inactivated 1 of the 2 copies of *NPC1L1* present in each genome. Ezetimibe mimics heterozygous null mutations, as both reduce protein function by about one-half. We found that *NPC1L1* null mutation carriers had a 12 mg/dl lower LDL-C level (an effect size similar to ezetimibe treatment) and 53% lower risk for CHD. Together, both studies provide a strong therapeutic hypothesis that ezetimibe, a small-molecular inhibitor of NPC1L1, will not only lower LDL-C, but will also reduce risk for CHD.

The IMPROVE-IT (Improved Reduction of Outcomes: Vytorin Efficacy International Trial; NCT00202878) has validated this hypothesis. To establish the clinical benefit and safety of ezetimibe, 18,144 high-risk individuals presenting with an acute coronary syndrome were randomized to simvastatin monotherapy versus simvastatin/ezetimibe combined therapy (15). The addition of ezetimibe significantly reduced the primary endpoint (cardiovascular death, myocardial infarction, unstable angina requiring hospitalization, coronary revascularization, or stroke), with a relative risk reduction of 6.4% and an absolute risk reduction of 2% over 7 years of treatment. This was the first trial to demonstrate that adding a nonstatin agent to a statin provides incremental clinical benefit. The average achieved LDL-C was 54 mg/dl with ezetimibe/simvastatin dual therapy versus 70 mg/dl with simvastatin monotherapy. These data suggest that, in the setting of secondary prevention, we should target LDL-C to levels even lower than conventional thresholds.

Beyond ezetimibe-*NPC1L1*, there are now at least 4 other examples of drug-gene pairs affecting cardiovascular therapeutics, suggesting that this approach may be generalizable (**Table 1** summarizes all 5 examples). Statins and their therapeutic target gene, 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGCR*), are another notable example. Numerous individual trials and comprehensive meta-analyses show that statins reduce the risk for either a first or recurrent CHD event (16). Although available for more than 2 decades, we only recently appreciated that statins lead to 2 important adverse events—modest weight gain and a small increase in the relative risk for type 2 diabetes (17,18). Could these adverse events have been anticipated using modern human genetic approaches?

We identified a naturally-occurring *HMGCR* gene variation associated with lower LDL-C that led to lower hepatic messenger ribonucleic acid expression and, thereby, a loss of gene function (19). Swerdlow et al. (18) demonstrated that those harboring an LDL-lowering allele at either of 2 *HMGCR* polymorphisms displayed higher body weight, increased fasting insulin, higher plasma glucose, and increased risk for type 2 diabetes. Clinical trial and genetic data are, thus, remarkably concordant in identifying both the efficacy and toxicity of statin therapy. Importantly, these data suggest that weight gain and glucose intolerance induced by statins is related to HMGCR inhibition, that is, an on-target class effect, rather than the characteristics of a single agent or off-target toxicity.

What might be the net effect on CHD of an intervention that lowers LDL-C while simultaneously increasing the risk of type 2 diabetes? From a meta-analysis of statin trials in the secondary prevention setting, it is clear that CHD risk is reduced with statin treatment (20). Importantly, human genetics suggests the same. In the study by Ference et al. (6), a genetic score of *HMGCR* polymorphisms was associated not only with lower LDL-C, but also with lower risk of CHD.

There are 3 examples of variation in target genes with therapies in development: *PCSK9*, *APOC3*, and *CETP*. About 1 in 50 black people carry a null mutation at the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene, causing lifelong inactivation of 1 copy of the gene (21). These individuals have 28% lower LDL-C and are protected from CHD (88% lower