Genetic Risk and Altering Lipids With Lifestyle Changes and Metformin
Is Fate Modifiable?

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It is not in the stars to hold our destiny but in ourselves
—William Shakespeare

The widespread availability and lower costs of genotyping and sequencing have resulted in the performance of a large number of genotype–phenotype association studies in cardiovascular medicine. The stringent requirements for correction for multiple testing and replication have particularly favored genotype–phenotype studies examining the association between genetic variation and quantitative traits that allows for greater statistical power. Multiple genome-wide association studies and a subsequent meta-analysis have identified >180 common and rare genetic variants associated with lipid traits.1–2 However, the effect size of these individual genetic variants is small, explaining only a small fraction of phenotypic variation prompting investigators to use genetic risk scores (GRS) that represent an aggregate of genetic risk to demonstrate clinical utility. Single-nucleotide polymorphisms (SNPs) and GRS have been used to predict cardiovascular disease such as coronary artery disease (CAD) and hypertension, surrogate markers of disease such as coronary calcium, cardiovascular outcomes such as myocardial infarction, and intermediate traits such as blood pressure and lipids.3

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Genetic Loci Associated with Lipid Levels

The Global Lipids Genetics Consortium has performed the largest genetic association study of lipid levels in 188,577 individuals from a total of 60 studies.1–2 There were 157 genetic loci that were identified, 95 were described previously and 62 were novel. The lipid level variance explained by the novel loci in this study ranged from 1.6% for high-density lipoprotein cholesterol (HDL-C) levels to 2.6% for total cholesterol levels. The lipid level variance explained by the previously described loci was 10% to 12%. The population studied was predominantly of European ancestry, and subjects on lipid-lowering therapy were excluded. The association of these genetic loci with lipid levels and a change in lipid levels with intervention in a prediabetic population has not been studied until recently.

In a study published in this issue of Circulation: Cardiovascular Genetics, Varga et al3 performed a genotype–phenotype association study to study the association of known lipid-associated genetic loci with multiple lipid traits and the effect of interventions such as lifestyle and metformin on these interactions in 2993 participants who were at a high risk for diabetes mellitus from the Diabetes Prevention Program (DPP). Participants in the intensive lifestyle intervention received extensive education taught by case managers on a one-to-one basis to achieve a weight reduction of at least 7% of initial body weight and were expected to engage in physical activity of moderate intensity for at least 150 minutes/wk. Lifestyle intervention and metformin reduced the incidence of diabetes mellitus by 58% and 31%, respectively.

SNPs were selected from the Global Lipids Genetics Consortium meta-analysis2 that were associated with major lipid traits (150 SNPs) such as total cholesterol, triglycerides, HDL-C, and low-density lipoprotein cholesterol (LDL-C) and various lipid subfractions (91 SNPs). Lipid trait-specific GRS were also created. The phenotypes assessed were baseline, 1 year, and change in lipid and subfraction levels at 1 year. The authors after performing a total of 1110 genotype–phenotype association tests using multiple different combinations of SNPs with baseline lipid and lipid subfraction traits determined 20.5% to have met significance thresholds at a critical \( \alpha = 0.05 \) and 2.5% replicated at a Bonferroni-adjusted level of \( P < 4.5 \times 10^{-5} \). \( P \) value of <0.05 was considered significant for replicating SNPs that had previously met genome-wide level of significance for a particular lipid or lipid subfraction trait; however, Bonferroni correction was applied to determine significance of association of SNPs to those subfraction traits that were significantly correlated with a major lipid trait. There were 673 (61% of the total) such correlated subfraction trait association tests performed in this study.

Interpreting the Effects of Lipid-Associated Genetic Loci

The lack of a more robust genotype–lipid trait association in this study is not surprising, considering the small effect size of the individual genetic variants and the relatively small sample size of the population studied. The results of this study that did not adjust for the use of lipid-lowering therapy could also have been affected by the confounding use of statins in 11% to
15% of participants. It is also important to note that the lipid-associated genetic loci that were identified by Global Lipids Genetics Consortium used in this multiethnic study (only 55% of participants in the DPP were white) were largely derived from a population of European Ancestry. There clearly maybe ethnic-specific SNPs that are most significantly associated with lipid traits that may have not been included in this analysis. The frequency of the SNPs that were included in this analysis may differ in the various ethnic groups thus affecting results. However, when weighted GRS were used, 28 of the total 34 association tests performed were significantly associated with their respective baseline lipid or subfraction traits. The weighted GRS accounted for 6% of the variance in overall lipid and subfraction baseline levels and trait-specific weighted GRS explained an average of 2.4% of the phenotypic variation similar to findings presented by the Global Lipids Genetics Consortium.

Clinical Implications of Lipid-Associated Genetic Loci
Identifying genetic loci associated with lipid levels may not only have value in discovering new lipid biology and therapeutic targets but could also help predict associated cardiovascular traits and the relative importance of that association. The Global Lipids Genetics Consortium demonstrated a significant association of LDL-C, triglyceride, and total cholesterol trait-increasing alleles, HDL-C trait-decreasing alleles, and CAD risk (P=2×10−12, 2×10−16, 0.006, and 0.02, respectively). After accounting for the pleiotropic effects of lipid-associated SNPs on the various lipid levels, LDL-C and triglyceride but not HDL-C–associated genetic loci were significantly associated with CAD risk. These findings were extended by the analysis of rare and low-frequency variants using whole-genome sequence data in 119,146 Icelanders that demonstrated that a non-HDL cholesterol (total cholesterol minus HDL-C) GRS and no other lipid trait GRS was significantly associated with CAD (P=2.7×10−20). Obese individuals with a higher triglyceride-associated GRS not only have higher fasting triglyceride levels than obese individuals with lower GRS but also have higher triglyceride levels than overweight and normal body mass index individuals with similar high GRS. Whether lipid-associated genetic susceptibility plays a role in the variable penetrance of CAD in obese individuals remains to be explored. Genetic variation in lipid-associated loci can identify high-risk populations; however, to demonstrate clinical utility, the important questions that need to be answered are whether genetic risk influences treatment outcomes and whether treatment or intervention based on genetic information alters outcomes.

Genetic Risk Influencing Treatment Outcomes
The strength of the study by Varga et al9 is the examination of the influence of lipid-associated genetic loci on lipid levels with treatment or intervention. The association of lipid-associated genetic variation and change in lipids was assessed in the 2 intervention arms of the DPP, intensive lifestyle modification versus placebo and metformin use versus placebo, by interaction analyses. No SNP associations were significant using inverse normalized lipid trait values when assessed for 1-year follow-up lipids after correcting for lifestyle or metformin treatment interventions. Lifestyle intervention significantly modified the effect of the unweighted GRS for large HDL-C particle numbers at 1 year that is participants with a high GRS had lower baseline-adjusted large HDL-C particle numbers at 1 year than those with a low GRS. However, this interaction was not significant using a wGRS and was not observed in the metformin or placebo groups. Importantly, participants with a higher GRS had improvement in 17 of 20 lipid traits with lifestyle changes and in 14 of 20 traits with the use of metformin suggestive that genetic predisposition to lipid traits may not necessarily modify the favorable effect of these interventions on overall lipid profiles.

This observation has held to be true for genetic risk for CAD in a recent study involving 55,685 participants from 3 prospective longitudinal cohorts.10 This study showed that despite a 91% increased risk of incident CAD in those with the highest genetic risk, a favorable lifestyle was associated with a 46% lower risk of coronary events. Favorable lifestyle was defined as 3 of 4 lifestyle factors that included no current smoking, no obesity, regular physical activity, and a healthy diet. The relative risk reduction for coronary events when compared with unfavorable lifestyle observed in the intermediate and low genetic risk groups with a favorable lifestyle was similar to the high genetic risk group at 47% and 45%, respectively. The major limitation of this observational study similar to the study by Varga et al9 is that treatment or intervention was not prospectively randomized based on genetic risk.

The lack of association of lipid traits or CAD-associated genetic loci with treatment outcomes may not be surprising as these genetic variants were not primarily identified to be associated with treatment response. For example, the entire field of pharmacogenomics is devoted to the role of inheritance in drug pharmacokinetics and pharmacodynamics, and the identification of these genetic variants is based on its association with drug efficacy or adverse effects.11 This concept is exemplified by the SLCO1B1 gene, variation in which was found to be genome-wide significant in statin efficacy and toxicity but was not associated with baseline lipid levels.12 In addition, the efficacy of interventions such as statin therapy may be related to mechanisms other than LDL-C lowering such as a reduction in inflammation, the genetic determinants of which are not related to LDL-C reduction.13 The favorable changes observed in the lipid subfractions (including large HDL-C numbers noted by Varga et al) with lifestyle intervention in the DPP were related to changes in insulin resistance, BMI and adiponectin.14 The genetic variation in these and other pathways related to oxidative stress and inflammation or agnostic genome-wide association studies perhaps should be considered when evaluating the variable response to regular exercise and diet modifications on lipid traits.

Treatment Based on Genetic Testing Altering Outcomes
To demonstrate clinical utility of genetic testing, the Evaluation of Genomic Applications in Practice and Prevention Working Group recommends the demonstration of improvement in clinical outcomes using genetic testing compared with
routine care. There are few prospective cardiovascular clinical trials that have tested the utility of genetic testing to deliver individualized care. The largest ongoing cardiovascular genotype-based randomized clinical trial is the National Heart Lung Blood Institute sponsored TAILOR PCI trial (Tailored Antiplatelet Initiation to Lessen Outcomes Due to Decreased Clopidogrel Response After Percutaneous Coronary Intervention; https://www.clinicaltrials.gov, NCT01742117) that is examining the role of prospective genetic testing to guide antiplatelet therapy to reduce major adverse cardiac events. A recent prospective randomized clinical trial, MI-GENES trial (Myocardial Infarction Genes), tested the hypothesis that disclosing genetic risk for CAD by a genetics counselor in addition to a conventional risk score to participants when compared with a conventional risk score alone would lower LDL-C levels. The GRS group had lower LDL-C levels (96.5 versus 105.9 mg/dL; \( P = 0.04 \)) and greater statin use (39% versus 22%, \( P < 0.01 \)) than the conventional risk score group, indicating that knowledge of genetic risk could be helpful in improving clinical care and possibly clinical outcomes.

**Conclusion**

Varga et al demonstrated significant association of known lipid-associated genetic loci in prediabetic participants of the DPP with baseline lipid profiles and that intensive lifestyle changes result in improvement of most lipid traits despite genetic risk. Whether lipid-associated genetic loci are associated with CAD or attenuate clinical outcomes remains to be proven. Recently completed and ongoing genotype-based RCTs using CAD risk scores or pharmacogenetic markers may fulfill the promise of precision medicine by targeting high genetic risk patients.

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