Coding variation in ANGPTL4, LPL, and SVEP1 and the risk of coronary disease

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CROSS REFERENCE

\*The full names, degrees, and affiliations of the Myocardial Infarction Genetics and CARDIoGRAM Exome Consortium Investigators are listed in the Appendix.

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Although genomewide association studies have identified more than 56 loci associated with the risk of coronary artery disease, the disease-associated variants are typically common (minor-allele frequency >5%) and located in noncoding sequences; this has made it difficult to pinpoint causal genes and affected pathways. This lack of a causal mechanism has in part hindered the immediate translation of the findings of genomewide association studies into new therapeutic targets. However, the discovery of rare or low-frequency coding-sequence variants that affect the risk of coronary artery disease has facilitated advances in the prevention and treatment of disease. The most recent example of such advances is the development of a new class of therapeutic agents that is based on the discovery of the gene encoding proprotein convertase subtilisin/kexin type 9 (PCSK9) as a regulator of low-density lipoprotein (LDL) cholesterol and the discovery that low-frequency and loss-of-function variants in this gene protect against coronary artery disease.

Recently, low-frequency coding variation across the genome was systematically tabulated with the use of next-generation exome and whole-genome sequencing data from more than 12,000 persons of various ancestries (including a major contribution from the National Heart, Lung, and Blood Institute Exome Sequencing Project). Protein-altering variants (i.e., nonsynonymous, splice-site, and nonsense single-nucleotide substitutions) that were observed at least twice among these 12,000 persons were included in a genotyping array (hereafter referred to as the exome array). In addition, the exome array contains previously described variants from genomewide association studies, a sparse genomewide grid of common markers, markers that are informative with regard to ancestry (i.e., African American, Native American, and European), and some additional content. Additional information on the design of the exome array is provided at http://genome.sph.umich.edu/wiki/Exome_Chip_Design. In this study, we focused on the 220,231 autosomal variants that were present on the array and were expected to alter protein sequence (i.e., missense, nonsense, splice-site, and frameshift variants) and used these to test the contribution of low-frequency coding variation to the risk of coronary artery disease.

**METHODS**

**STUDY DESIGN AND PARTICIPANTS**

We performed a discovery study involving 42,335 patients with coronary artery disease and 78,240 controls from 20 individual studies (hereafter referred to as the discovery cohort). For variants with suggestive associations, we sought replication of our findings in an independent study of 30,533 patients and 42,530 controls assembled from 8 individual studies (hereafter referred to as the replication cohort). The names of the individual studies and information on the numbers of participants and phenotypic definitions of patients and controls in the discovery cohort and the replication cohort are provided in Tables S1 and S2, respectively, in the Supplementary Appendix, available with the full text of this article at NEJM.org. The 5755 participants from the Bangladesh Risk of Acute Vascular Events (BRAVE) study and the 22,072 participants from the Pakistan Risk of Myocardial Infarction Study (PROMIS) were of South Asian ancestry; all other participants were of European ancestry.

**GENOTYPING AND QUALITY CONTROL**

Samples were genotyped on the Illumina HumanExome BeadChip array, version 1.0 or 1.1, or the Illumina OmniExome array (which includes markers from the HumanExome BeadChip) in accordance with the manufacturer’s recommended protocol. Our genotyping methods, as well as the quality-control procedures that were used to remove low-quality samples and variants, are described in the Supplementary Appendix.

**FOLLOW-UP ANGPTL4 SEQUENCING**

The exon sequences of ANGPTL4 were obtained from the exome sequences of 6924 persons who had early-onset myocardial infarction and those of 6834 persons who were free from coronary artery disease (see the Methods section in the Supplementary Appendix for details). The names of the individual studies and information on the numbers of participants and phenotypic definitions of patients and controls for ANGPTL4 se-
quencing are provided in Table S3 in the Supplementary Appendix.

**Statistical Analysis**

Of the 220,231 variants on the exome array, 54,003 (covering 13,715 genes) were present in our study at sufficient frequency (minor-allele frequency >0.01%) to allow for individual-variant association testing as described in the Supplementary Appendix. In the discovery phase, we defined a suggestive novel association between a variant and the risk of coronary artery disease as one with a meta-analysis P value of $1 \times 10^{-4}$ or lower. For variants with suggestive association, we performed association analysis in the replication studies as described in the Supplementary Appendix. We defined significant novel associations as those that were nominally significant ($P<0.05$) in the replication cohort and that had an overall P value of less than $7.7 \times 10^{-8}$ in the discovery and replication cohorts combined (a Bonferroni-corrected threshold that accounted for 54,003 markers with minor-allele frequencies >0.01% being tested initially and 12 markers being tested in the replication cohort).

To test for association between variants and risk factors for coronary artery disease, we examined the relationship between low-frequency variants that were significantly associated with disease in the combined (i.e., discovery and replication) analysis and plasma lipid levels in 10,088 samples from the discovery cohort; in order to minimize any effect of ascertainment bias, we limited the analysis to persons without coronary artery disease who had lipid measurements available. We also examined the relationships between the significantly associated low-frequency variants and blood pressure in 146,562 persons from the Cohorts for Heart and Aging Research in Genomic Epidemiology Plus (CHARGE+) consortium. Tables S4 and S5 in the Supplementary Appendix list the individual studies and numbers of participants that contributed to the analyses of plasma lipid levels and blood pressure, respectively. Finally, we queried publicly available exome-array data to explore the relationship between significantly associated low-frequency variants and type 2 diabetes (data from the Type 2 Diabetes Genetic Exploration by Next-Generation Sequencing in Multi-Ethnic Samples [T2D-GENES] Consortium, Genetics of Type 2 Diabetes [GoT2D] Consortium, and the Diabetes Genetics Replication and Meta-Analysis [DIAGRAM] Consortium; accessed in November 2015 at www.type2diabetesgenetics.org/). Additional details of the risk-factor association analyses are provided in the Methods section in the Supplementary Appendix.

From the sequencing data, we used linear regression to test the association between ANGPTL4 loss-of-function alleles and plasma lipid levels in 8085 persons for whom lipid measurements were available, using models described in the Supplementary Appendix. We calculated the significance of the association between ANGPTL4 loss-of-function alleles and the risk of coronary artery disease with the use of 100,000 study-stratified permutations of case–control phenotypes.

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**Results**

**Low-Frequency Coding Variants Associated with Coronary Artery Disease**

The discovery cohort comprised 120,575 persons (42,335 patients and 78,240 controls) (Table S1 in the Supplementary Appendix). In the discovery cohort, we found significant associations between low-frequency coding variants in the LPA and PCSK9 genes and coronary artery disease (Table 1). Both gene loci also harbor common noncoding variants associated with coronary artery disease that had previously been discovered through genomewide association studies. These variants were also present on the exome array and had significant associations with coronary artery disease in our study (Table 1). In a conditional analysis, the associations between coronary artery disease and the low-frequency coding variants in both LPA and PCSK9 were found to be independent of the associations between coronary artery disease and the more common variants (Table 1).

In addition to the variants in LPA and PCSK9, 12 additional suggestive associations ($P<1 \times 10^{-4}$) between low-frequency coding variants and coronary artery disease were identified in the discovery study (Table S6 in the Supplementary Appendix). We tested for associations between these variants and coronary artery disease among the 73,063 persons (30,533 patients and 42,530 controls) in the replication cohort (see Tables S2...
ANGPTL4, LPL, and SVEP1 Variation and Coronary Disease

Variants in two genes showed association in the replication set and reached the genome-wide level of significance (P < 7.7 x 10^{-8}) in the combined data set (Table 2). A missense variant (encoding p.D2702G; minor-allele frequency, 3.60%) in the gene SVEP1 (which encodes sushi, von Willebrand factor type A, EGF and pentraxin domain containing 1) was associated with an increased risk of coronary artery disease, and a low-frequency missense variant (encoding p.E40K; minor-allele frequency, 2.01%) in ANGPTL4, which encodes angiopoietin-like 4, was associated with protection against coronary artery disease (Table 2).

ASSOCIATION BETWEEN VARIANTS AND RISK FACTORS FOR CORONARY ARTERY DISEASE

We next examined the associations of the SVEP1 p.D2702G and ANGPTL4 p.E40K variants with plasma lipid levels, blood pressure, and type 2 diabetes to determine whether their associations with coronary artery disease could potentially be mediated through an effect on these known risk factors. To minimize any potential effect of case–control ascertainment in testing the association with plasma lipid levels, we restricted our analysis to 10,088 controls who were free from coronary artery disease and 120,770 controls free from the disease.

Table 1. Low-Frequency Coding Variations Previously Associated with Coronary Artery Disease.

<table>
<thead>
<tr>
<th>Locus and SNP</th>
<th>Chromosome and Nucleotide Position</th>
<th>Allele 1/Allele 2</th>
<th>Frequency of Allele 1</th>
<th>Functional Effect</th>
<th>Odds Ratio</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPA rs3798220</td>
<td>6: 160961137 C/T</td>
<td>1.99</td>
<td>p.I4399M</td>
<td>1.54</td>
<td>9.7 x 10^{-24}</td>
<td>1.9 x 10^{-18}</td>
</tr>
<tr>
<td>LPA rs2048327</td>
<td>6: 160863532 C/T</td>
<td>37.36</td>
<td>NA</td>
<td>1.09</td>
<td>8.1 x 10^{-21}</td>
<td>1.8 x 10^{-15}</td>
</tr>
<tr>
<td>PCSK9 rs11591147</td>
<td>1: 55505647 T/G</td>
<td>1.52</td>
<td>p.R46L</td>
<td>0.78</td>
<td>8.0 x 10^{-10}</td>
<td>1.9 x 10^{-7}</td>
</tr>
<tr>
<td>PCSK9 rs11206510</td>
<td>1: 55496039 C/T</td>
<td>18.23</td>
<td>NA</td>
<td>0.93</td>
<td>1.7 x 10^{-8}</td>
<td>4.6 x 10^{-6}</td>
</tr>
</tbody>
</table>

* NA denotes not applicable, and SNP single-nucleotide polymorphism.
† Chromosome numbers and positions refer to genome build GRCh37.
‡ Odds ratios are for the development of disease in carriers of allele 1.
§ The conditional P value is the P value from an association test conditioning on the other marker listed in the locus.

Table 2. Novel Low-Frequency Coding Variations Showing Significant Association with Coronary Artery Disease.

<table>
<thead>
<tr>
<th>Locus and SNP</th>
<th>Chromosome and Nucleotide Position</th>
<th>Allele 1/Allele 2</th>
<th>Frequency of Allele 1</th>
<th>Functional Effect</th>
<th>Stage</th>
<th>Odds Ratio</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SVEP1 rs111245230</td>
<td>9: 113169775 C/T</td>
<td>3.60</td>
<td>p.D2702G</td>
<td>Discovery</td>
<td>1.14</td>
<td>1.1 x 10^{-7}</td>
<td></td>
</tr>
<tr>
<td>SVEP1 rs111245230</td>
<td>9: 113169775 C/T</td>
<td>3.60</td>
<td>p.D2702G</td>
<td>Replication</td>
<td>1.13</td>
<td>1.0 x 10^{-3}</td>
<td></td>
</tr>
<tr>
<td>SVEP1 rs111245230</td>
<td>9: 113169775 C/T</td>
<td>3.60</td>
<td>p.D2702G</td>
<td>Combined‡</td>
<td>1.14</td>
<td>4.2 x 10^{-10}</td>
<td></td>
</tr>
<tr>
<td>ANGPTL4 rs116843064</td>
<td>19: 8429323 A/G</td>
<td>2.01</td>
<td>p.E40K</td>
<td>Discovery</td>
<td>0.87</td>
<td>3.0 x 10^{-5}</td>
<td></td>
</tr>
<tr>
<td>ANGPTL4 rs116843064</td>
<td>19: 8429323 A/G</td>
<td>2.01</td>
<td>p.E40K</td>
<td>Replication</td>
<td>0.86</td>
<td>3.4 x 10^{-4}</td>
<td></td>
</tr>
<tr>
<td>ANGPTL4 rs116843064</td>
<td>19: 8429323 A/G</td>
<td>2.01</td>
<td>p.E40K</td>
<td>Combined‡</td>
<td>0.86</td>
<td>4.0 x 10^{-8}</td>
<td></td>
</tr>
</tbody>
</table>

* Chromosome numbers and positions refer to genome build GRCh37.
† Odds ratios are for the development of disease in carriers of allele 1. P values for testing differences between the effects observed in the discovery and replication results were 0.73 for rs111245230 and 0.11 for rs116843064 (Cochran’s Q test for heterogeneity).
‡ The combined stage refers to a meta-analysis that included 72,868 patients with coronary artery disease and 120,770 controls free from the disease.
blood pressure were performed on a set of 146,562 samples from the CHARGE+ consortium, and the results for tests of association with type 2 diabetes were extracted from publicly available databases. Additional details of the sources and samples for these analyses of risk factors are provided in Tables S4 and S5 in the Supplementary Appendix. We did not observe any significant association between 𝐴𝑁𝐺𝑃𝑇𝐿4 𝑝.𝐸40𝐾 and LDL cholesterol level (Table 3). Both 𝑆𝑉𝐸𝑃1 𝑝.𝐷2702G and 𝐴𝑁𝐺𝑃𝑇𝐿4 𝑝.𝐸40 𝐾 were nominally associated with type 2 diabetes in a direction concordant with the associated risk of coronary artery disease.

**AN GMT L4 LOSS-OF-FUNCTION MUTATIONS, PLASMA LIPID LEVELS, AND CORONARY ARTERY DISEASE**

The finding that a missense allele in 𝐴𝑁𝐺𝑃𝑇𝐿4 reduced the risk of coronary artery disease, potentially by reducing triglyceride levels, raised the possibility that complete loss-of-function variants in 𝐴𝑁𝐺𝑃𝑇𝐿4 may have an even more dramatic effect on triglyceride concentrations and the risk of coronary artery disease. We therefore examined sequence data for the seven protein-coding exons of 𝐴𝑁𝐺𝑃𝑇𝐿4 in 6924 patients with early-onset myocardial infarction and 6834 controls free from coronary artery disease (details of the patients and controls are provided in Table S3 in the Supplementary Appendix). We discovered a total of 10 variants that were predicted to lead to loss of gene function (Fig. 1A, and Table S9 in the Supplementary Appendix), carried by 28 heterozygous persons; no homozygous or compound heterozygous persons were discovered. Carriers of loss-of-function alleles had significantly lower levels of triglycerides than did noncarriers (a mean of 35% lower among carriers, 𝑃=0.003) (Fig. 1B, and Table S10 in the Supplementary Appendix), with no significant difference in LDL or HDL cholesterol levels. Moreover, we found a lower risk of coronary artery disease among carriers of loss-of-function alleles (9 carriers among 6924 patients vs. 19 carriers among 6834 controls; odds ratio for disease, 0.47; 𝑃=0.04) (Table S11 in the Supplementary Appendix). A similar investigation was performed for the 48 protein-coding exons of 𝑆𝑉𝐸𝑃1; however, only 3 loss-of-function allele carriers were

<table>
<thead>
<tr>
<th>Variant and Risk Factor</th>
<th>Effect (95% CI)†</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SV EP1 rs111245230 C allele</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.011 (−0.066 to 0.088)</td>
<td>0.78</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.023 (−0.054 to 0.099)</td>
<td>0.56</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.050 (−0.025 to 0.125)</td>
<td>0.19</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.94 (0.58 to 1.3)</td>
<td>3.0×10⁻⁷</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.57 (0.35 to 0.79)</td>
<td>4.4×10⁻⁷</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>1.13 (1.10 to 1.16)</td>
<td>0.0062</td>
</tr>
<tr>
<td><strong>AN TPL4 rs116843064 A allele</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>−0.064 (−0.153 to 0.025)</td>
<td>0.16</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.295 (0.206 to 0.384)</td>
<td>8.2×10⁻¹¹</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>−0.335 (−0.424 to −0.246)</td>
<td>1.6×10⁻¹³</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>−0.18 (−0.66 to 0.30)</td>
<td>0.46</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>−0.13 (−0.43 to 0.16)</td>
<td>0.38</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>0.90 (0.87 to 0.92)</td>
<td>0.028</td>
</tr>
</tbody>
</table>

† Shown in the table are low-frequency variants associated with coronary artery disease that are outside of known loci that were previously identified in genomewide association studies. CI denotes confidence interval, HDL high-density lipoprotein, and LDL low-density lipoprotein.

The effect is the difference per each copy of the allele in units of standard deviation change (in LDL cholesterol level, HDL cholesterol level, and the natural logarithm of triglyceride level), millimeters of mercury (for systolic and diastolic blood pressure), or the odds ratio for disease (for type 2 diabetes) for carriers as compared with noncarriers.
discovered (2 carriers among 6924 patients vs. 1 carrier among 6834 controls).

**Coding Variation in LPL and the Risk of Coronary Artery Disease**

On the basis of the fact that a loss of ANGPTL4 function was associated with reduced risk of coronary artery disease and that ANGPTL4 inhibits lipoprotein lipase (LPL), one would expect a gain of LPL function to also be associated with a lower risk of coronary artery disease, whereas a loss of LPL function would be expected to be associated with a higher risk. In observations consistent with these expectations, we found a low-frequency missense variant in LPL on the exome array that was associated with an increased risk of coronary artery disease (p.D36N; minor-allele frequency, 1.9%; odds ratio, 1.13; P=2.0×10^−4) (Table S12 in the Supplementary Appendix); previous studies have shown that this allele (also known as p.D9N) is associated with LPL activity that is 20% lower in allele carriers than in noncarriers. We also identified a nonsense mutation in LPL on the exome array that was significantly associated with a reduced risk of coronary artery disease (p.S447*; minor-allele frequency, 9.9%; odds ratio, 0.94; P=2.5×10^−7) (Table S12 in the Supplementary Appendix); previous studies have shown that this allele (also known as p.D9N) is associated with LPL activity that is 20% lower in allele carriers than in noncarriers. We also identified a nonsense mutation in LPL on the exome array that was significantly associated with a reduced risk of coronary artery disease (p.S447*; minor-allele frequency, 9.9%; odds ratio, 0.94; P=2.5×10^−7) (Table S12 in the Supplementary Appendix). Contrary to most instances in which the premature introduction of a stop codon leads to loss of gene function, this nonsense mutation, which occurs in the penultimate codon of the gene, paradoxically induces a gain of LPL function.

**Discussion**

Through large-scale exomewide screening, we identified a low-frequency coding variant in ANGPTL4 that was associated with protection against coronary artery disease and a low-frequency coding variant in SVEPI that was associated with an increased risk of the disease. Moreover, our results highlight LPL as a significant contributor to the risk of coronary artery disease and support the hypothesis that a gain of LPL function or loss of ANGPTL4 inhibition protects against the disease.

ANGPTL4 has previously been found to be involved in cancer pathogenesis and wound healing. Previous functional studies also revealed that ANGPTL4 regulates plasma triglyceride concentration by inhibiting LPL. The minor allele at p.E40K has previously been associated with lower levels of triglycerides and higher levels of HDL cholesterol. We now provide independent confirmation of these lipid effects. In vitro and in vivo experimental evidence suggests that the lysine allele at p.E40K results in destabilization of ANGPTL4 after its secretion from the cell in which it was synthesized. It may be that the p.E40K variant leads to increases in the enzymatic activity of LPL because of this destabilization.

To provide confirmatory orthogonal evidence that a loss of ANGPTL4 function is associated

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**Figure 1. Loss-of-Function Alleles in ANGPTL4 and Plasma Lipid Levels.**

Panel A shows the loss-of-function mutations discovered by sequencing the seven exons of ANGPTL4 in 9731 persons of European ancestry. The locations of the individual mutations are depicted along the length of the ANGPTL4 gene, starting at the 5′ end (left), along with the predicted functional effect. An asterisk indicates the introduction of a premature stop codon. Panel B shows the mean plasma lipid levels according to loss-of-function allele carrier status. T bars indicate standard deviations. P values were calculated from a linear regression (with the use of a logarithm transformation in the case of triglycerides) with covariates of age, sex, and study. HDL denotes high-density lipoprotein, and LDL low-density lipoprotein.
with a decreased risk of coronary artery disease, we searched for loss-of-function mutations in this gene. We found that ANGPTL4 loss-of-function mutations were associated with substantially lower triglyceride levels (35% lower than in persons who were not carriers of a loss-of-function mutation), and we also found that these loss-of-function alleles were associated with a 53% lower risk of coronary artery disease. The identification by Dewey et al. of additional ANGPTL4 inactivating mutation carriers, now reported in the Journal, provides further evidence of the association between a loss of ANGPTL4 function and lower triglyceride levels and a reduced risk of coronary artery disease.

Because ANGPTL4 inhibits LPL, we examined coding variants in LPL that were present on the exome array. A gain-of-function mutation in LPL was found to be associated with protection against coronary artery disease, whereas a partial loss-of-function mutation was associated with increased risk. These data corroborate evidence that has connected the risk of myocardial infarction to coding-sequence mutations in two other genes encoding proteins that modulate LPL activity: apolipoprotein A5 and apolipoprotein C3. Thus, the available human genetic evidence, including our current findings, is coalescing around the conclusion that, in addition to elevated levels of LDL cholesterol, disordered metabolism of triglyceride-rich lipoproteins through the LPL pathway contributes substantively to coronary artery disease (Fig. 2). These data also support the hypothesis that therapeutic modulation of this pathway — either by direct enhancement of LPL activity or by blocking of the effects of natural LPL inhibitors, such as ANGPTL4 (Fig. 2B) — should reduce both triglyceride levels and the risk of coronary artery disease. Studies involving inhibitors of APOC3, or of other inhibitory regulators in the LPL pathway will be needed to address this possibility.

The identification of a disease-associated missense variant in SVEP1 points to a potentially novel genetic mechanism leading to atherosclerosis. SVEP1 is a cell-adhesion molecule that acts as a ligand for integrin α9β1; knockdown of SVEP1 alters inflammatory signaling in a cellular model of sepsis. Although we observed that the low-frequency variant that was associated with an increased risk of coronary artery disease was also associated with significantly higher blood pressure, the latter effect was modest as compared with that found in a previous study, in which a genetically mediated 1.6–mm Hg increase in systolic blood pressure was associated with a 10% increased risk of coronary artery disease (in our study, SVEP1 p.D2702G was associated with an approximately 1-mm Hg increase in systolic blood pressure but a 14% increased risk of coronary artery disease). Thus, the exact mechanism by which SVEP1 contributes to coronary artery disease and the extent to which hypertension and diabetes mediate this risk remain to be clarified.

Several limitations of our study deserve consideration. In our samples, approximately three quarters of the variants on the array were either monomorphic (i.e., only the human reference allele was observed among the persons in the discovery cohort) or very rare (frequency below 0.1%); therefore, we cannot make any estimates of their contribution to the risk of coronary artery disease. Moreover, we estimate that the Illumina HumanExome BeadChip, version 1.0, covers only approximately 80% of coding variants in persons of European ancestry that have an allele frequency of 0.1% or higher (Fig. S1 in the Supplementary Appendix). Furthermore, despite a combined sample size of more than 120,000 persons in the discovery cohort, we had only 80% power to detect an odds ratio of approximately 1.5 for variants with a minor-allele frequency of 0.5% (or an odds ratio of approximately 2.0 for variants with a minor-allele frequency of 0.1%) at the required stringent level of significance (Fig. S2 in the Supplementary Appendix). Hence, analyses of even larger data sets and more comprehensive coverage of exonic variants may reveal further coding variants associated with the risk of coronary artery disease.

In summary, through large-scale exomewide screening, we identified several low-frequency coding variants that are associated with either an increased risk of or protection against coronary artery disease. Our results highlight the LPL pathway as a significant contributor to the risk of coronary artery disease and support speculation that therapeutic modulation of this pathway might be protective against coronary artery disease.
Figure 2. Genetic Variants Affecting the Lipoprotein Lipase Pathway and the Risk of Coronary Artery Disease.

Panel A shows normal physiological function of lipoprotein lipase (LPL) and regulation of LPL by gene products of ANGPTL4, APOC3, and APOA5. LPL, which is both transported across and anchored to capillary endothelial cells by the protein GPIHBP1, normally hydrolyzes the triglycerides that are present in circulating lipoproteins and reduces the plasma triglyceride level. Its activity is reduced by ANGPTL4 and APOC3 and increased by APOA5. Not shown here are other important regulators of LPL activity, including APOC2 and ANGPTL3. Green arrows indicate enhancers, and red blocked arrows indicate inhibitors. IDL denotes intermediate-density lipoprotein, and VLDL very-low-density lipoprotein. Panel B shows altered function of LPL in mutation carriers. Mutations affecting LPL and proteins interacting with LPL are shown, along with expected effect on LPL activity, plasma triglyceride levels, and risk of coronary artery disease. LPL loss of function refers to p.D36N, and gain of function refers to p.S477* (see Table S12 in the Supplementary Appendix). ANGPTL4 loss of function refers to both p.E40K and loss-of-function mutations (Tables S9, S10, and S11 in the Supplementary Appendix). APOC3<sup>7,18</sup> and APOA5<sup>7</sup> loss of function refers to multiple loss-of-function mutations.
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APPENDIX

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Sharing Data in a Public Health Emergency

The case for sharing data, and the consequences of not doing so, have been brought into stark relief by the Ebola and Zika outbreaks. In response, the New England Journal of Medicine has become a journal signatory to the following statement.

“In the context of a public health emergency of international concern, it is imperative that all parties make available any information that might have value in combatting the crisis. As research funders and journals, we are committed to working in partnership to ensure that the global response to public health emergencies is informed by the best available research evidence and data. Journal signatories will make all content concerning the Zika virus free access. Any data or preprint deposited for unrestricted dissemination ahead of submission of any paper will not preempt later publication in these journals. Funder signatories will require researchers undertaking work relevant to public health emergencies to establish mechanisms to share quality-assured interim and final data as rapidly and widely as possible, including with public health researchers and communities and the World Health Organization.

We urge other journals and research funders to make the same commitments.”

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