Beyond Genome-Wide Association Studies

The Usefulness of Mouse Genetics in Understanding the Complex Etiology of Atherosclerosis

Carrie L. Welch

Abstract—The development of population-based genome-wide association studies has led to the rapid identification of large numbers of genetic variants associated with coronary artery disease (CAD) and related traits. Together with large-scale gene-centric studies, at least 35 loci associated with CAD per se have been identified with replication. The majority of these associations are with common single-nucleotide polymorphisms exhibiting modest effects on relative risk. The modest nature of the effects, coupled with ethical/practical constraints associated with human sampling, makes it difficult to answer important questions beyond gene/locus localization and allele frequency via human genetic studies. Questions related to gene function, disease-causing mechanism(s), and effective interventions will likely require studies in model organisms. The use of the mouse model for further detailed studies of CAD-associated loci identified by genome-wide association studies is highlighted herein. (Arterioscler Thromb Vasc Biol. 2012;32:207-215.)

Key Words: atherosclerosis ■ coronary artery disease ■ genome-wide association study ■ mouse genetics

The development of population-based genome-wide association studies has led to the rapid identification of large numbers of genetic variants associated with coronary artery disease (CAD)/myocardial infarction (MI) and related traits, such as plasma low-density lipoprotein (LDL) cholesterol, high-density lipoprotein cholesterol, triglycerides, obesity, and hypertension (reviewed in 1-7). The majority of these associations are with common single-nucleotide polymorphisms (SNPs) exhibiting modest effects on relative risk. The use of combined analyses, or metaanalyses, increases the power to detect modest associations.3,8,9 Currently, at least 35 loci associated with CAD per se have been identified and, importantly, replicated in at least 1 independent study (Table). Although some of the loci are associated with traditional risk factors, many of the loci likely affect atherogenesis via nontraditional mechanisms.

Notably, the CAD loci identified by genome-wide association studies thus far have been estimated to explain only ≈10% of the additive genetic variance of human CAD.8 Several human genetic approaches toward detecting loci representing the unexplained variance have been discussed. These include approaches for detecting rare SNPs10,11 or copy number variants12 associated with disease. However, as the list of new disease loci grows, it will be important to establish the clinical or public health importance of the identified loci.

Model organisms provide useful tools for obtaining data related to clinical relevance. Because of the modest size effects of SNP variants and ethical/practical constraints associated with human sampling, questions regarding gene function and disease-causing mechanism(s) can be assessed more definitively in model organisms. Furthermore, effective interventions and potential modifiers of SNP-disease associations can be tested in model organisms before the design of clinical trials in humans. The mouse has become the model of choice because of small size, breeding efficiency, availability of genetic manipulation technologies, and high degree of genome similarity. Human-mouse genomic homologies have been identified for most SNP association loci identified to date (Table). Although some CAD-associated loci have been found in gene-poor regions or regions of unknown biological relevance, the candidate causal variants in these cases may function by regulating expression of neighboring genes. Thus, genetic studies of mice exhibiting altered expression or function of CAD SNP-residing or neighboring genes may be relevant. Mutant models—including transgenic models, knockouts derived by gene-targeting or gene trap technologies, chemical- or radiation-induced mutagenesis, subchromosomal locus deletion, and spontaneous mutation—are available for many of the human CAD-associated loci identified to date (Table). Furthermore, more than 9000 conditional targeted alleles in mouse embryonic stem cells have recently become available.13 Lastly, random genetic variation...
Table. Human-Mouse Genomic Homologies for Coronary Artery Disease–Associated Loci: Availability of Mutant Mouse Models

<table>
<thead>
<tr>
<th>Human Chr</th>
<th>SNP</th>
<th>Gene(s) in Region</th>
<th>Related Phenotype (Human)</th>
<th>Murine Homolog(s)</th>
<th>Mouse Chr</th>
<th>Mutant Models*</th>
<th>Lesion Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rs11206510</td>
<td>PCSK9</td>
<td>LDL, TC, LDL, TC</td>
<td>Pcsk9</td>
<td>4</td>
<td>KO, Tg (liver)</td>
<td>LDL, TC</td>
</tr>
<tr>
<td>1</td>
<td>rs17114036</td>
<td>PPAP2B</td>
<td>LDL, TC</td>
<td>Ppap2b</td>
<td>4</td>
<td>KO (embryonic lethal)</td>
<td>LDL, TC</td>
</tr>
<tr>
<td>1</td>
<td>rs599839</td>
<td>SORT1</td>
<td>LDL, TC</td>
<td>Sort1</td>
<td>3</td>
<td>KO, Tg</td>
<td>LDL, TC</td>
</tr>
<tr>
<td>1</td>
<td>rs17465637</td>
<td>MIA3</td>
<td>LDL, TC</td>
<td>Mia3</td>
<td>1</td>
<td>KO (perinatal lethal)</td>
<td>LDL, TC</td>
</tr>
<tr>
<td>2</td>
<td>rs4299376</td>
<td>ABCG8</td>
<td>LDL, TC, sitosterolemia</td>
<td>Abcg8</td>
<td>17</td>
<td>KO, Tg</td>
<td>LDL, TC, sitosterolemia</td>
</tr>
<tr>
<td>2</td>
<td>rs6725887</td>
<td>WDR12</td>
<td>Wdr12</td>
<td>Wdr12</td>
<td>1</td>
<td>KO (ES cell)</td>
<td>LDL, TC</td>
</tr>
<tr>
<td>3</td>
<td>rs2306374</td>
<td>MRAS</td>
<td>Mras</td>
<td>Mras</td>
<td>9</td>
<td>KO</td>
<td>LDL, TC</td>
</tr>
<tr>
<td>5</td>
<td>rs2706399</td>
<td>IL5</td>
<td>Il5</td>
<td>Il5</td>
<td>11</td>
<td>KO, Tg</td>
<td>LDL, TC</td>
</tr>
<tr>
<td>6</td>
<td>rs6903956</td>
<td>Cllor105</td>
<td>953000BL14Rik</td>
<td></td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>rs12526453</td>
<td>PHACTR1</td>
<td>Phactr1</td>
<td>Phactr1</td>
<td>13</td>
<td>KO (ES cell)</td>
<td>LDL, TC</td>
</tr>
<tr>
<td>6</td>
<td>rs1760949</td>
<td>ANKS1A</td>
<td>Anks1</td>
<td>Anks1</td>
<td>17</td>
<td>KO (perinatal lethal)</td>
<td>LDL, TC</td>
</tr>
<tr>
<td>6</td>
<td>rs1219028</td>
<td>TCF21</td>
<td>Tcf21</td>
<td></td>
<td>10</td>
<td>KO</td>
<td>LDL, TC</td>
</tr>
<tr>
<td>6</td>
<td>rs3798220</td>
<td>LPA</td>
<td>LDL, TC; Lp(a)</td>
<td></td>
<td></td>
<td></td>
<td>LDL, TC, Lp(a)</td>
</tr>
<tr>
<td>7</td>
<td>rs1095341</td>
<td>BCAP29</td>
<td>Bcap29</td>
<td>Bcap29</td>
<td>12</td>
<td>KO (ES cell)</td>
<td>LDL, TC</td>
</tr>
<tr>
<td>7</td>
<td>rs1155692</td>
<td>ZC3HC1</td>
<td>Zc3hc1</td>
<td></td>
<td>6</td>
<td>KO</td>
<td>LDL, TC</td>
</tr>
<tr>
<td>8</td>
<td>rs17321515</td>
<td>TRIB1</td>
<td>Trib1</td>
<td></td>
<td>15</td>
<td>KO</td>
<td>LDL, TC</td>
</tr>
<tr>
<td>9</td>
<td>rs4977574</td>
<td>ANRIL</td>
<td>No contiguous segment</td>
<td></td>
<td>4</td>
<td>Locus deletion</td>
<td>129Sw locus deletion, HFD</td>
</tr>
<tr>
<td>9</td>
<td>rs579459</td>
<td>ABO</td>
<td>LDL, TC</td>
<td>Abo</td>
<td>2</td>
<td>KO (ES cell)</td>
<td>LDL, TC</td>
</tr>
</tbody>
</table>

(Continued)
<table>
<thead>
<tr>
<th>Human Chr</th>
<th>SNP</th>
<th>Gene(s) in Region</th>
<th>Related Phenotype (Human)</th>
<th>Murine Homolog(s)</th>
<th>Mouse Chr</th>
<th>Mutant Models*</th>
<th>Lesion Phenotype</th>
<th>Related Phenotype (Mouse)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>rs2505083</td>
<td>KIAA1462</td>
<td></td>
<td>9430020K01Rik</td>
<td>18</td>
<td>KO (ES cell)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>rs1746048</td>
<td>CXCL12</td>
<td></td>
<td>Cxcl12</td>
<td>6</td>
<td>KO (embryonic lethal)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>rs2246942</td>
<td>LIPA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>rs1241340</td>
<td>CYP17A1</td>
<td>Blood pressure</td>
<td></td>
<td>19</td>
<td>KO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>rs974819</td>
<td>NTRC2</td>
<td></td>
<td></td>
<td></td>
<td>KO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>rs9641844</td>
<td>APOA1/C3/A4/A5</td>
<td>TG, HDL</td>
<td></td>
<td>9</td>
<td>KO, Tg (single and compound alleles)</td>
<td>B6-huAPOA1 Tg, HFD</td>
<td>HDL, LDL, TC, TG</td>
</tr>
<tr>
<td>12</td>
<td>rs3184504</td>
<td>SH2B3</td>
<td>Blood pressure</td>
<td></td>
<td>5</td>
<td>KO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>rs4773144</td>
<td>COL4A1/A2</td>
<td></td>
<td></td>
<td>8</td>
<td>KO, chem/rad-induced</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>rs2895811</td>
<td>HHIPL1</td>
<td></td>
<td></td>
<td>12</td>
<td>KO (ES cell)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>rs3825807</td>
<td>ADAMTS7</td>
<td></td>
<td></td>
<td>9</td>
<td>KO (ES cell)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>rs2161726</td>
<td>SMG6</td>
<td></td>
<td></td>
<td>11</td>
<td>KO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>rs1293658</td>
<td>PEMT</td>
<td></td>
<td></td>
<td>11</td>
<td>KO, spont mutation</td>
<td>B6-Ldlr-/-, Pemt-/-, HFD</td>
<td>TG, LDL, VLDL, TC</td>
</tr>
<tr>
<td>17</td>
<td>rs46522</td>
<td>GIP</td>
<td></td>
<td></td>
<td>11</td>
<td>KO (ES cell)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>rs1122608</td>
<td>MRPS6</td>
<td></td>
<td></td>
<td>16</td>
<td>KO (cell line)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Diets were as follows: WTD, Western-type diet (0.15% cholesterol, 21% fat); HFD, high-fat diet (1.25% cholesterol/15% fat/0.5% sodium cholate); HFDb, high-fat diet (10.8% total fat, 0.75% cholesterol without sodium cholate); HF/HC, high-fat/high cholesterol diet (1.25% cholesterol, 20% olive oil). Chr indicates chromosome; SNP, lead disease–associated single nucleotide polymorphism; LDL, low-density lipoprotein; KO, knockout allele(s); Tg, transgenic allele(s); HDL, high-density lipoprotein; EGFP, enhanced green fluorescent protein; chem-induced, chemically induced mutant allele(s); rad-induced, radiation-induced mutant allele(s); spont mutation, spontaneous mutant allele(s); VLDL, very-low-density lipoprotein; FH, familial hypercholesterolemia; Tc, total cholesterol; ES, embryonic stem; Lp(a), lipoprotein (a); TG, triglycerides.

among different inbred strains of mice can lead to the identification of novel genes underlying atherosclerosis.

**Mendelian Disease Genes Exhibiting Common Associations**

Some of the CAD loci underlying common susceptibility to disease were previously identified in relationship to rare Mendelian forms of hypercholesterolemia/premature CAD. These include LDLR, APOE, PCSK9, ABCG8, and LIPA. Importantly, gene-specific mutant mouse models displayed effects on atherogenesis and related traits (ie, plasma cholesterol levels, plant sterol levels, xanthomatosis, lipase deficiency) similar to those observed in humans (Table). These provide “positive controls” indicating that the use of mutant mouse models can be relevant to the study of human CAD loci.

The disease-causing mechanism for Mendelian disease genes is at least partly established. Although clearance of circulating lipoproteins is a common mechanism underlying disease-causing mechanisms for LDLR, APOE, and PCSK9, new studies in mouse models are revealing additional roles in inflammation (Apoe),14,15 and apolipoprotein B secretion (Pcsk9).16 Common variants of ABCG8 are associated with both plasma phytosterol and LDL levels.17 Detailed studies in mice have outlined the role of Abcg8 in dietary cholesterol absorption18 and intestinal cholesterol excretion,19 but the role of plant sterols in atherogenesis remains unresolved. LIPA encodes a lysosomal acid lipase involved in the breakdown of cholesterol esters and triglycerides. The LIPA risk allele is associated with increased lipase expression but not altered lipid levels, suggesting a novel pathogenic mechanism. Thus, even the Mendelian-associated genes have the potential to reveal new pathogenic mechanisms.

**New Genes/Loci Associated With Traditional Risk Factors**

A few of the newly identified loci are associated with known risk factors for CAD, suggesting disease-causing mechanisms. For example, SORT1 and TRIB1 are associated with lipoprotein levels in human association studies. Functional validation of these associations was obtained via gene-specific over- and underexpression of these genes in genetically uniform mouse models of atherosclerosis.20,21 Furthermore, Sort1 and Trib1 were shown to modulate hepatic very-low-density lipoprotein secretion and production, respectively, from primary hepatocytes in mice.20,21 These data suggest that modulation of 2 novel regulatory pathways for lipoprotein metabolism may alter susceptibility to CAD/MI in humans. The ABO gene is associated with multiple CAD-related traits: LDL levels, thrombosis, inflammatory gene expression, and plant sterol levels.6,22,23 Additional studies are required to delineate the relative role of each pathway in the pathogenesis of CAD.

**New Genes/Loci Underlying Novel Pathogenic Mechanisms for CAD/MI**

The majority of the loci listed in the Table have some degree of known protein function but no known role in CAD/MI pathogenesis. For these loci, basic knowledge of directional effects and tissue relevance can be sorted out in mouse models. Directional effects (ie, for regulatory variants, whether increased or decreased gene expression is associated with disease) can be confirmed/established using general knockout or transgenic models crossed onto an Apoe \(^{-/-} \) or Ldlr \( ^{-/-} \) proatherosclerotic background. In some cases, existing congenic,24 spontaneous, chemically induced, or radiation-induced mutants may be queried (Table). Tissue relevance (ie, the specific tissue type affecting disease pathogenesis) can be assessed using bone marrow transplantation or tissue-specific knockouts. Reciprocal bone marrow experiments using a chromosome 4 congenic model harboring the 9p21 region of homology and exhibiting decreased expression of macrophage Cdkn2a indicated that bone marrow–derived cells, but not resident vascular cells, were sufficient to confer the proatherosclerotic phenotype of the congenic mouse.24,25 Direct testing of the candidate gene showed that bone marrow–derived cells from Cdkn2a \( ^{-/-} \) mice were sufficient to confer accelerated atherogenesis in the Ldlr \( ^{-/-} \) background.24 Of note, tissue macrophages and mixed monocyte/macrophage populations, but not circulating monocytes, were implicated in the study.24 This study suggests that macrophage deficiency of Cdkn2a may partly explain the association of 9p21 with CAD/MI in humans. The data are consistent with human studies reporting a lack of association of the 9p21 risk allele with Cdkn2a expression in circulating monocytes26 or resident vascular cells27 but significant association with decreased levels in T lymphocytes.28 Studies in human macrophages have not been reported.

Genetic variants of Anril have been implicated at the 9p21 human CAD/MI locus. ANRIL is a noncoding RNA implicated in both long-range cis-acting and trans-acting transcriptional control of the syntenic tumor suppressor genes CDKN2A (encoding p16 \( ^{INK4a} \) , p14 \( ^{ARF} \)) and CDKN2B (encoding p15 \( ^{INK4b} \)). Multiple ANRIL splice variants are present in human tissues, complicating genetic association studies of the structural gene.29 A murine deletion mutant covering the homologous region exhibited decreased expression of the neighboring tumor suppressor genes, supporting the hypothesis of an ANRIL regulatory variant underlying the 9p21 locus.30 A potential effect of the variant on atherosclerosis was not observed in the highly atheroresistant model tested. However, testing of the deletion in a more atherosusceptible model has not been carried out as yet.

Two of the CAD-associated loci listed in the Table were identified as open reading frames or cDNA clones via annotation efforts of the Human Genome Project (c6orf105) or the RIKEN Genome Science Laboratory (KIAA1462) but have no known biological function. C6orf105 exhibits ethnic-specific CAD association among Chinese Han populations,31 but KIAA1462 exhibits association in both European and Chinese populations, with similar allele frequencies and size effects.32 Both loci have homologous DNA sequences in the mouse genome, and thus, targeted deletion or transcriptional disruption may shed light on the biological functions of novel proteins.

Additional mechanistic data, including stage of lesion development, genetic background effects, potential effects on lesion regression, and overlapping roles of CAD loci in...
multiple diseases, can be assessed using unique strains and experimental designs. Stage of lesion development can be tested in dietary time course studies of gene-specific knock-out or transgenic mice bred onto standard mouse models of atherosclerosis or with conditional knockouts induced before or after lesion development. Genetic background effects can be tested using different inbred strains of mice (exhibiting differences in susceptibility to atherosclerosis), different engineered models of atherosclerosis, or mutant mice carrying mutations in more than 1 CAD locus. The Reversa mouse32,33 is a model of atherosclerosis regression that may be useful for differentiating hyperlipidemia from other genetic effects on lesion regression. Finally, introduction of CAD-associated mutations into mouse models of diabetes, hypertension, obesity, and metabolic syndrome may shed light on shared points of regulation among multiple disease phenotypes.

**New Discovery of Atherosclerosis Susceptibility Genes**

As mentioned above, the CAD loci identified by human genome-wide association studies thus far have been estimated to explain only a fraction of the genetic variance of human CAD.8 Mouse genetic/mapping approaches provide a means of identifying new genes, perhaps intractable by human genetic approaches because of their modest effect. Although mouse linkage studies pinpoint disease susceptibility loci to relatively large genomic intervals containing large numbers of genes, several techniques have been applied to narrow the list of disease causal candidate genes. Refined mapping of loci can be obtained through the generation of interval-specific congenic strains. A cross between B6-Apoε-/- and the more atheroresistant strain FVB-Apoε-/- revealed 2 intervals contributing to atherosclerosis susceptibility; 1 locus was narrowed to 7 genes, the other to 21 genes.34 Similarly, the congenic mapping efforts in a cross between B6-Ldlr-/- and a wild-derived MOLF strain revealed 2 atherosclerosis loci on chromosome 4.24 Cdkn2a was identified as a disease-causing gene in 1 of the intervals, but mapping of the distal locus is still under way. Combining a congenic mapping approach with gene expression profiling, Gargarovic et al identified Znrx235 as a novel regulator of plasma lipid metabolism.35 Copy number variants can also be applied to mouse mapping studies. In a cross between B6 and C3H, gene expression levels and several metabolic traits mapped to 3 unique copy number variants, suggesting novel loci involved in regulation of plasma lipoprotein levels, glucose, and body weight.36 Recently, a hybrid mouse panel was developed for high-resolution association studies in mice. This approach aims to provide refined mapping and increased sensitivity compared with linkage studies. Together, these studies have the potential to reveal new genes and pathways underlying atherosclerosis susceptibility.

**Limitations of the Mouse Model**

Many of the loci listed in the Table were discovered through case-control studies of MI. Although atherogenesis precedes MI, not all cases of atherosclerosis lead to acute complications. This suggests that different pathologies underlie these clinical phenotypes. Furthermore, the ABO gene was specifically associated with MI in the presence of coronary atherosclerosis.22 Currently available mouse models are susceptible to atherosclerosis but resistant to acute complications. Thus, studies of genes affecting plaque rupture may be limited in the mouse. ABO is associated with thrombosis,22 and at least 1 spontaneous mutation in mice leads to atherothrombosis.37 In addition, murine plaques exhibit features of human vulnerable plaques, a precursor to plaque rupture and infarction. These can be assessed by qualitative changes in plaque morphology.38,39 Thus, although plaque rupture/MI may not be amenable to study per se in murine models, pathogenic mechanisms leading to clinical consequences may be queried.

The identification of phenotypically causal variants underlying CAD susceptibility is important for the delineation of biological genotype-phenotype relationships, as well as discovering potential predictors of disease. Lead association SNPs may represent causal variants or may be associated by circumstance alone (ie, exhibiting strong linkage disequilibrium with the lead SNP). In most cases, disease-causing variants will not be the same in human and mouse. In particular, regulatory variants in noncoding regions may not be conserved. For example, the putative causal variant at SORT1 is human specific. The causal allele creates a binding site for the CEBP family of transcription factors that does not exist in the mouse.20 However, data supporting the role of a regulatory variant can be gained from studies in mice. For example, the lead SNP identified for TRIB1, a triglyceride-, LDL-, and CAD-associated locus, is located downstream of the coding sequence and suggested a regulatory effect on gene expression.3,17,40 Subsequent studies in Trib1-overexpressing and -deficient mice showed decreased and increased plasma triglyceride levels, respectively.21 Demonstration of regulatory effects stemming from an allele-specific mutant construct will be necessary to solidify the genotype-phenotype relationship.

Most disease-associated SNPs exhibit modest effects on relative risk. Thus, the relevance of complete gene knockout and highly expressing transgenic mice comes into question. Several genetic methods exist for testing modest effects. Mice carrying heterozygous deficiency of a particular gene will likely demonstrate differences in gene expression more closely mimicking the situation in humans.34 Second, bacterial artificial chromosome transgenic mice generally express only 1 to 3 copies of a transgene. Third, spontaneous, radiation-induced, and chemically induced mutants usually harbor point mutations. Some of these models are available for the human CAD-associated loci (Table) and can be tested for differences in atherosclerosis susceptibility or plaque morphology.

**Conclusions**

Although recent human genetic studies have met with remarkable success in terms of identifying CAD/MI-associated loci, many details regarding the underlying genes/mechanisms remain unanswered. The high degree of genomic similarity between humans and mice, along with the wide array of genetic tools available, indicate that much can be learned from parallel studies of mice and human.
Sources of Funding
This work was supported by National Institutes of Health Research Grant R01-HL102206.

Disclosures
None.

References


57. Welcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature.* 2007;447:661–678.


Beyond Genome-Wide Association Studies: The Usefulness of Mouse Genetics in Understanding the Complex Etiology of Atherosclerosis
Carrie L. Welch

doi: 10.1161/ATVBAHA.111.232694

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/32/2/207

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org/subscriptions/