Recent Advances in the Genetics of Atherothrombotic Disease and Its Determinants

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Over the past 2 years, the pace of scientific discovery in human genetics related to atherothrombotic disease and vascular biology has been rapid, with no shortage of innovative articles published in *ATVB*. Several studies have identified novel loci by genetic association approaches, whereas others focused on validating genome-wide association study (GWAS) data functionally. Strides were also made with micro-RNAs (miRNAs) and their use as biomarkers and therapeutic targets in disease. Furthermore, molecular and mechanistic bases of certain genetic conditions, including dyslipidemias, were characterized. Here, we review genetic-themed articles published in *ATVB* since 2015, which highlight rapid advances in the field.

Statistical Genetic Associations With Vascular and Related Traits

A popular type of study in human genetics is the association study, which classically is performed in 1 of 2 forms.[1] In the first, a quantitative phenotypic feature is measured in a population sample, genotypic strata are created from alleles of a common DNA variant, and differences between genotypic classes are tested statistically. In the second, cases with a discrete trait or disorder are matched with controls who are free of the trait. Both groups are genotyped for a common DNA variant, and differences in allele or genotype frequencies between cases and controls are evaluated statistically. Both forms of association studies can be performed millions of times with microarrays that genotype single nucleotide polymorphisms (SNPs) from across the human genome, with adjustments for multiple testing; the extreme case is GWAS. Often, when results are reported, there is no direct experimental testing of biological impact of the associated alleles. For instance, in 812 participants of the 15-year Bruneck study, a noncoding DNA microsatellite polymorphism in the promoter region of the *HMOX1* gene, encoding heme oxidase-1 was associated with increased carotid atherosclerosis and a trend to higher levels of oxidized phospholipids; however, the functional basis of this association was not characterized.[2] In another study of a single-gene locus, investigators genotyped 3 SNPs of the *IFNA1* gene, encoding type-I IFN (interferon)-α, creating a polygenic score that was strongly associated with induced production of IFN-α in leucocytes ex vivo but was not associated with other ex vivo stimuli or clinical coronary artery disease (CAD) events.[3] Following a similar method, 1747 markers that quantify ethnic admixture at the molecular level were evaluated on their possible impact on carotid intima media thickness, peripheral arterial disease, and calcification of coronary arteries and the abdominal aorta in participants from the Jackson Heart Study.[4] The investigators found that increasing levels of European admixture (determined molecularly) were associated with more favorable measures of subclinical atherosclerosis.[4]

Studies in human twins demonstrate another approach to further understand the genetic contributions toward various phenotypes. For example, Rao et al[5] performed a genome-wide linkage study in 386 monozygotic and dizygotic twins and showed that oxidized phospholipids on apo (apolipoprotein) B-containing lipoproteins were correlated with variation in Lp(a) (lipoprotein[a]) levels and with size variation at the *LPA* gene locus on chromosome 6.[5] In a subsequent study performed in 431 239 patients, Moriarty et al[6] demonstrated that *APOE* genotype was strongly associated with interindividual variation in circulating Lp(a) mass, with the highest levels in subjects with 1 or 2 *APOE* E4 alleles. Although not confirmed by functional studies, these results suggested alternate interactions or competition between Lp(a) and the different apoE isoforms—each isoform being essentially equivalent to *APOE* genotype.[6] In another study of *APOE* genotypes, the Heart Brain Connection Collaborative Research Group found that cerebral vasoreactivity was inversely associated with dementia, especially among carriers of the E4 allele.[7]

Expanding on simple associations, the Mendelian randomization study design allows investigators to determine whether genetic determinants found to be associated with certain traits or conditions, namely, risk factors or markers, have a direct or causal role in determining a clinical end point or phenotype.[8] If a causal relationship exists between the risk factor and end point, there will be an observable association between the 2; the absence of an association reflects a noncausal, biomarker relationship. Durda et al[9] showed that serum levels of sIL-2Rα (interleukin 2 receptor α) were associated with genetic variation on chromosome 10p15-14 although the actual gene and locus are unknown. These results suggested a role for sIL-2Rα in atherosclerosis and generated hypotheses for studies of multiple-associated SNPs near chromosome 10p15-14.[9] Liu et al[10] used Mendelian randomization to test whether plasma levels of fatty acid–binding protein 4, retinol-binding protein 4, and high molecular weight adiponectin were directly causal for cardiovascular mortality in 950 patients with type 2

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Nonstandard Abbreviations and Acronyms

AP	activator protein
apo	apolipoprotein
ARIC	Atherosclerosis Risk in Communities study
CAD	coronary artery disease
FH	familial hypercholesterolemia
GPER	G-protein estrogen receptor
GWAS	genome-wide association study
HDL-C	high-density lipoprotein cholesterol
IFN	interferon
LAL	lysosomal acid lipase
LDL-C	low-density lipoprotein cholesterol
Lp(a)	lipoprotein(a)
miRNAs	micro-RNAs
PAR	protease activated receptor
PCSK9	proprotein convertase subtilisin kexin type 9
sIL-2Rα	interleukin 2 receptor alpha
SNP	single nucleotide polymorphism
VLDL	very-low-density lipoprotein

diabetes mellitus and found no statistically significant associations. It is important to note that although this particular study did not show a significant association, the study design should be taken into consideration before permanently classifying something as causative or not. As is the case for all Mendelian randomization studies, depending on the risk factors and end points of interest, some studies may not be well powered enough to demonstrate a true causal association. Conversely, in a large study by Januzzi et al[11] on 3439 participants from the Framingham Heart Study, they found that higher concentrations of proneurotensin are associated with a greater risk of incident cardiovascular events but that the association did not vary according to the genotype of the sortilin receptor 1, the receptor for neurotensin.

Mendelian randomization performed by Kjaergaard et al[12] in 96 110 individuals from the Copenhagen cohorts definitively showed that although plasma levels of the inflammation-associated glycoprotein YKL-40 were associated with a 2-fold increased risk of venous thromboembolism, the association was not causal because the genotype that determined these plasma levels was itself not associated. In contrast, a Mendelian randomization experiment in 10 778 white and 3190 black participants in the ARIC study (Atherosclerosis Risk in Communities) found that variants in the *CELSR2-PSRC1-SORT1* region on chromosome 1 influenced circulating protein C levels, thus indicating a possible genetic link between lipoprotein metabolism and hemostasis.[13] Finally, a Mendelian randomization experiment by Coassin et al[14] found that genetic determinants of low HDL-C (high-density lipoprotein cholesterol) levels were not associated with worsened renal function (ie, estimated glomerular filtration rate), thus demonstrating that the relationship was not causal in nature.

Some genetic markers tested for association with cardiovascular disease are not sequence polymorphisms on autosomes. For instance, telomeres are regions at the ends of chromosomes that maintain chromosomal stability. Using an assay to measure leukocyte telomere length in 1525 postmenopausal women from the Women's Health Initiative, Carty et al[15] found that white women with shorter leukocyte telomere length had higher risks of mortality from cardiovascular disease, whereas shorter leukocyte telomere length was weakly associated with decreased mortality hazard in black women.

The Post-GWAS Era: Functional Validation of SNPs Associated With Atherothrombosis

Although the outcomes of GWASs have been fruitful in defining the genetic architecture of atherothrombotic disease, the disease-associated SNPs cannot explain the underlying mechanisms of disease. In the post-GWAS era, efforts have targeted this information gap by characterizing GWAS loci using bioinformatics analyses, wet-laboratory experiments, and gene editing.[1] One of the biggest hurdles in advancing GWAS information is the identification of causal genes and subsequent assessment of the functional impact of novel disease-associated alleles in the gene. These gaps in knowledge are being investigated using computational analyses, and both in vitro and in vivo experimental validation.

Many studies have fine-mapped GWAS signals and fine-tuned the list of candidate genes for atherosclerotic CAD. Rodríguez et al[16] analyzed a locus on chromosome 18 associated with high serum triglycerides in Mexicans. Using linkage disequilibrium analysis and a reporter assay, they found that the rs17259126-G allele alters expression of *TMEM241* in the METSIM Cohort (Metabolic Syndrome in Men) and that reduced *TMEM241* expression increases triglyceride levels.[16] Other studies further explored CAD GWAS data by working to annotate replicated CAD loci. In one such study, Ghosh et al[17] integrated large-scale GWA data with a large pathway database. Using network centrality analysis, they identified several novel gene candidates involved in CAD pathways. In a second CAD study, Brænne et al[18] performed a comprehensive bioinformatics analysis of sequence variation in known CAD loci to predict candidate causal genes. This led to a revised list of gene candidates for CAD and the expansion of the CAD genetic landscape.[18] Overall, these investigations revealed novel genes that impact plasma triglyceride levels and CAD phenotypes and represent examples of the initial successes of the post-GWAS era.

Other studies pursued in vitro and in vivo functional characterization of SNPs identified from GWASs. In a cohort of cardiac patients, Norman et al[19] performed a proof-of-principle study to show that rare *F2LR3* variants are involved in platelet reactivity and responsiveness to PAR (protease-activated receptor)-1 antagonist drugs. Studying the variant Y157C in a PAR4 expression construct, they found reduced PAR4 responses because of aberrant anterograde surface receptor trafficking, which validated the involvement of rare *F2RL3* variants in altering PAR4 reactivity after treatment with therapeutic PAR1 antagonists.[19] In another study, Hussain et al[20] considered the unknown link between estrogen deficiency and increased LDL-C (low-density lipoprotein cholesterol). They analyzed the role of GPER (G-protein estrogen receptor) in the regulation of both the LDL receptor and PCSK9 (proprotein convertase subtilisin kexin type 9). In whites, they found that

GPER downregulates PCSK9, leading to the upregulation of LDL receptor expression. They also identified an association between the P16L *GPER* variant and elevated LDL-C levels. Taken together, these results suggest a role of GPER in LDL metabolism.[20]

Additional studies pursued functional assessment of GWAS-identified SNPs, notably in relation to CAD. For instance, 1 group functionally investigated a coding variant, rs1051338, in *LIPA*, encoding lysosomal acid lipase and found that the risk allele leads to faster lysosomal acid lipase degradation, reduced protein levels, and decreased activity.[21] Confirmed in disease-relevant macrophages, these findings show the reduction of lysosomal acid lipase activity results from degradation. In another study by Wang et al,[22] 2-stage case–control studies in a Han Chinese population identified a SNP in the *SLC22A3-LPAL2-LPA* gene cluster, namely, rs3088442, that was significantly associated with both Lp(a) levels and CAD. A reporter gene analysis showed that the rs3088442-G allele may suppress the binding of miR-147a and, in turn, alter *SLC22A3-LPA* expression. Altogether, this suggests involvement of the *SLC22A3-LPAL2-LPA* gene cluster region in plasma Lp(a) levels and in turn association with CAD severity.

Other notable CAD-related functional studies include one by Turner et al,[23] where genetic and epigenetic fine-mapping were used to identify a causal SNP in intron 1 of *SMAD3*, rs17293632. Using chromatin immunoprecipitation and small interfering RNA–induced knockdowns, these investigators demonstrated that the rs17293632-T allele plays a protective role by disrupting a consensus AP (activator protein)-1 binding site in the *SMAD* enhancer region, reducing the activity of the enhancer and in turn, *SMAD3* expression. They demonstrated the involvement of the *SMAD3* SNP in arterial smooth muscle cell proliferation. A final example of exploring CAD GWAS data comes from Jiang et al,[24] who performed a case–control study in Chinese subjects and found that homozygotes for the -69C promoter polymorphism in the *NTRK2* gene encoding tyrosine kinase receptor B had significantly increased CAD risk. Together, these studies highlight the advances in understanding of CAD-related genetic loci.

The CRISPR-Cas9 gene-editing system has been at the forefront of molecular biology research in the post-GWAS era. CRISPR-Cas9 allows researchers to make specific, targeted changes to gene sequences in living cells and organisms and is a valuable tool for investigating GWAS-identified genes of interest. In a study using genetic fine-mapping and DNA resequencing, Beaudoin et al[25] identified a causal intronic variant in *PHACTR1*, rs9349379, where alternate alleles are differentially bound by myocyte enhancer factor-2. Using CRISPR-Cas9, they engineered a deletion of this transcription factor binding site and found altered expression levels of PHACTR1, suggesting a mechanism explaining the GWAS findings linking the *PHACTR1* gene to CAD risk. Zhu et al[26] also investigated the functional significance of rs1039084 in *STXBP5* using a CRISPR-Cas9 generated mouse model. Mice genetically engineered with this variant had decreased von Willebrand factor, thrombosis, and platelet secretion, supporting this SNP as a causal variant for a decreased thrombotic phenotype.

Additional studies used CRISPR-Cas9 to develop effective models for gene association research. In 1 study, CRISPR-Cas9 was used to target human *PCSK9* in hepatocytes and established humanized mice as a valuable model for preclinical assessment.[27] A second study used this gene-editing system to introduce frameshift mutations into the exonic sequence of *ABCA1*.[28] Gupta et al[28] were able to develop human pluripotent stem cell–derived macrophages to model reverse cholesterol transport, which can be used for future studies looking into molecular determinants affecting this process. These studies highlight the utility of CRISPR-Cas9 in the post-GWAS era of research, allowing manipulation and exploration of GWAS-identified loci, which could not otherwise be evaluated further.

Thus, the post-GWAS era has seen success in identifying causal genes through SNP associations, as well as developing effective and efficient model systems. However, as a word of caution, although multiple studies have excelled in developing antithrombotic models, some researchers have argued against the use of specific models originating from GWAS data. For example, Pasterkamp et al[29] reviewed atherosclerosis-causing genes in mice and compared these genes to their human orthologues. In their assessment, they could scarcely confirm a role for mouse genes contributing toward atherosclerotic lesion development genes in humans, suggesting uncertainty in murine atherosclerotic models for investigating atherosclerotic disease. This study emphasizes the need to evaluate model systems before use and to consider CRISPR-Cas9 to develop precise models for further study.

Micro-RNAs as Biomarkers and Therapeutic Targets in Atherosclerosis

MiRNAs have extensive involvement in atherosclerosis, including regulation of lipoprotein metabolism, vascular cell homeostasis, and inflammation.[30] Studies reported in *ATVB* emphasize the diversity of miRNA species that affect different pathways underlying atherogenesis and vascular biology. For instance, Ouimet et al[31] investigated whether miR-33 contributes to cholesterol homeostasis by targeting autophagy. They showed that miR-33 targets key autophagy regulators and effectors in macrophages to reduce lipid droplet catabolism, which is essential to generate free cholesterol for efflux. Furthermore, macrophages treated with anti–miR-33 showed increased efferocytosis, lysosomal biogenesis, and degradation of apoptotic material, collectively implicating miR-33 as a regulator of cholesterol homeostasis and atherosclerosis through multiple complementary biochemical and cellular mechanisms. The same group showed earlier that oxysterol-binding protein-like 6 is regulated by miRNAs and thus plays a key role in regulating cholesterol trafficking and efflux.[32] In another study that inhibited miR-33 in a mouse model of diet-induced obesity, Karunakaran et al[33] demonstrated conflicting results in which there were no changes in circulating lipid levels, body weight, or insulin resistance and the possibility of an increase in whole-body oxidative metabolism.

Another example in lipoprotein metabolism is miR-548p, which was bioinformatically predicted to interact with apoB mRNA.[34] Using site-directed mutagenesis and transfection, Zhou and Hussain[34] showed that miR-548p significantly reduced apoB secretion and also decreased lipid synthesis

in human hepatoma cells by reducing 3-hydroxy-3-methylglutaryl-coenzyme A reductase and acyl-CoA synthetase long-chain family member 4, both of which are involved in cholesterol and fatty acid synthesis. Thus, miR-548p is yet another miRNA target for treating hyperlipidemia and hepatosteatosis. Finally, the complex involvement of miRNAs in HDL metabolism was reviewed by Canfrán-Duque et al.[35]

Reports of miRNAs having a direct or indirect impact on various aspects of vascular biology have also been accruing at an accelerated rate. For instance, Zhu et al[36] showed in a murine model of hind-limb ischemia that miR-15b-5p was specifically expressed in vascular endothelial cells, targeted a protein kinase, and was remarkably downregulated after femoral artery ligation. Furthermore, in human patients, circulating miR-15b-5p discriminated between those with well-developed or poorly developed collateral vessels, supporting the idea that miR-15b-5p is a key regulator of angiogenesis.[36] Rajput et al[37] demonstrated that suppression by miR-150 of angiopoietin-2 was important for repair of vascular injury. In a related vein, Chen et al[38] observed in zebrafish that miR-126a direct lymphangiogenesis through interactions with chemokine and Flt4 signaling. MiR-126 has also been explored in angiogenesis therapeutics by Cao et al.[39] Their study revealed proangiogenic effects of miR-126-3p when coupled with the ultrasound-targeted microbubble destruction delivery method in rodents with chronic hind-limb ischemia.

Sun et al[40] reported that miR-182-3p modulates smooth muscle cell phenotype in the vascular wall, whereas Zhao et al[41] show that miR-22 plays a role in differentiation of smooth muscle cells. Sun et al[42] reported in transplant-associated arteriosclerosis that miR-155 directs the migration of smooth muscle progenitor cells by regulating monocyte chemoattractant protein 1. Another miRNA related to vascular smooth muscle cells, miR-590-3p, was shown to increase cell contractility in the presence of rs12731181-G and is supported functionally as a genetic risk factor for hypertension.[43] Reddy et al[44] showed that miR-504 also plays a role in vascular smooth muscle cell dysfunction in the context of diabetes mellitus, as does miR-34a.[45] Also in diabetes mellitus, miR-126 targeted tissue factor and reduced thrombogenicity.[46] Interestingly, Dangwal et al[47] demonstrated that diabetic individuals with peripheral artery disease and complications in wound healing have different circulating miRNA profiles, with miR-191 as the most differentially expressed; elevated levels of miR-191 reduce migration of fibroblasts and increase apoptosis of dermal fibroblasts, leading to reduced wound closure.

With respect to a role for miRNAs in vascular aging and degenerative changes, Deng et al[48] found that miR-146a targets polo-like kinase 2 expression to induce bone marrow cell apoptosis and senescence. Chao et al[49] showed in cell lines, animal models and human patients that miR-125b was associated with vascular calcification, and particularly uremia-associated calcification progression. In addition, in patients with acute ST-segment–elevation myocardial infarction, Templin et al[50] demonstrated significant upregulation of the angiomiRs, miR-378, and let-7b, in mobilized CD34$^+$ progenitor cells, suggesting that this unique miRNA expression pattern reflects an endogenous repair mechanism in response to acute myocardial infarction. Finally, Wei et al[51] noted effects from miR-155 on atherosclerotic lesion formation; however, this study unveiled an additional layer of complexity in that miR-155 had both pro- and antiatherogenic effects, depending on the stage of lesion formation.

The growing appreciation for the importance of miRNAs in atherosclerosis and thus potentially clinical end points has prompted a number of translational studies. For instance, in a study using integrative genomic analysis, up to 15 miRNAs were reported to be differentially expressed between human individuals with or without coronary heart disease,[52] suggesting a possible panel or profile of these markers for diagnosis and further suggesting a range of targets for possible therapeutic intervention. In addition, miRNA-mRNA coexpression analysis revealed that miR-1275, miR-365a-3p, and miR-150-5p may be causally linked to CAD given their predicted gene targets.[52]

Despite the importance of the above miRNAs in atherosclerosis, therapeutic modulation in a context-specific manner remains elusive. Gadde and Rayner[30] reviewed the emergence of miRNA-based therapies as potential therapies for atherosclerosis in coronary arteries and other vascular beds by targeting specific causal pathways. The review also introduced the concept of nanoparticle development as a platform to specifically target the vessel wall.[30]

Advances in Genetic Dyslipidemias

Advances in sequencing technologies have helped reveal new aspects of genetic dyslipidemias, of which familial hypercholesterolemia (FH) is perhaps the most clinically relevant.[53] For instance, in 313 patients with FH who were evaluated using a targeted next-generation sequencing panel and an assay to detect large-scale copy-number variation, Wang et al[54] found a genetic basis for extremely elevated LDL-C in ≈67% of patients. Of these individuals, 45% had a monogenic large-effect variant, 6% had a copy-number variation, and 17% had a strong polygenic component, as defined by accumulation of common GWAS-identified SNPs associated with LDL-C levels.[54] Thus, several types of genetic variants need to be considered when assessing patients with elevated LDL-C, including those contributing to both monogenic and polygenic risk.[55] Furthermore, in clinical assessment of patients with severe hypercholesterolemia, it is critical to rule out the contribution of secondary factors,[56] including drugs such as cyclosporine, which can raise LDL-C through both LDL receptor and non-receptor mechanisms.[57]

Early identification of patients with FH also enables earlier intervention.[58] For instance, Li et al[59] showed that among Chinese patients with extremely elevated LDL-C, those with molecular-sequencing and clinical diagnosis of definite or probable FH using Dutch Lipid Clinics Network criteria were more likely to have premature CAD with more severe angiographic involvement. They emphasized that genetic screening for FH should be undertaken as early as possible. National registries of FH patients might allow for assessment of diagnostic and therapeutic effectiveness.[60,61] For example, Perez de Isla et al[62] studied individuals from the SAFEHEART registry (Spanish Familial Hypercholesterolemia Cohort Study) and

showed that premature atherosclerotic cardiovascular disease, peripheral arterial disease, and CAD were significantly more common in individuals with FH than in those without FH, or to the general population. Interestingly, they saw no difference in cerebrovascular disease risk between these groups.[62] Observations in 227 human patients with FH by Ogura et al[63] indicated that decreased cholesterol efflux capacity was associated with a greater degree of corneal arcus and increased thickness of both Achilles tendons and carotid intima-media, suggesting that cholesterol efflux could be targeted therapeutically or used as a biomarker of atherosclerosis risk in patients with FH.

Although statins have shown efficacy in preventing complications in FH, these patients often need additional nonstatin agents to help further reduce LDL-C to target levels.[64] Among newer agents, inhibitors of PCSK9 show great promise.[64] Thedrez et al[65] assessed primary lymphocytes from 28 patients with a subtype of FH called autosomal recessive hypercholesterolemia who were treated with the PCSK9 inhibitor alirocumab and found that when coupled with a statin, there was a slight but significantly increased uptake of LDL suggesting some clinical utility in these patients. In a human genetic model of PCSK9 inhibition, namely, the loss-of-function *PCSK9* R46L variant, individuals from the EPIC (European Prospective Investigation of Cancer)-Norfolk study who were heterozygous for this variant had a broad range of antiatherosclerotic phenotypes, and in particular, reduced VLDL (very low-density lipoprotein) and LDL particle concentrations, lower Lp(a) levels, and lower secretory phospholipase A2 and lipoprotein-associated phospholipase A2 activity compared with noncarriers.[66]

Another promising, novel compound that lowers LDL-C, namely, bempedoic acid,[67] was studied by Samsoondar et al.[68] After treating *Ldlr*-null mice fed high-fat, high-cholesterol diets, bempedoic acid was found to reduce LDL-C and triglyceride levels and attenuate hyperinsulinemia, hyperglycemia, hepatic steatosis, obesity, and the development rate of atherosclerotic lesions.[69] Yet another relatively new agent that has been evaluated in patients with FH is mipomersen, an antisense oligonucleotide directed against apoB.[69,70] Santos et al[71] showed that in addition to lowering LDL-C, mipomersen also lowers Lp(a) in patients with hypercholesterolemia, which may confer additional benefits. In patients with FH, elevated Lp(a) can skew the LDL-C determination made in the clinical laboratory and also seems to amplify the risk of atherosclerosis end points, acting synergistically with raised LDL-C.[72,73] Population genetic analysis emphasizes the independent role of Lp(a) in driving risk of myocardial infarction and aortic valve disease and the potential benefits of Lp(a) reduction.[74] One established clinical approach for Lp(a) reduction is apheresis[75]; a 5-year observational study of lipoprotein apheresis performed in 170 patients with high Lp(a) levels confirmed sustained reductions in Lp(a) levels and in cardiovascular events.[76]

Epidemiological links between dyslipidemia and clinical end points emphasize the need to continuously clarify its underlying mechanistic basis, particularly in animal models. Roche et al[77] used a novel approach in generating a stable hyperlipidemic, atherosclerotic mouse model by injecting a gain-of-function *PCSK9* mutation into the liver using adeno-associated virus. Interestingly, parallel experiments in the severely hyperlipidemic *ApoE*-null mouse showed an even greater elevation of LDL-C levels and a more severe atherosclerotic phenotype, emphasizing synergism between these 2 mechanisms of raising atherogenic lipoproteins.[77] In different mouse models, deficiencies of certain gene products were shown to impact inflammation and atherosclerosis. For instance, Konaniah et al[78] knocked out *Lrp1* in adipocytes, increasing the proinflammatory state and atherosclerosis risk.

Probing a different mechanism, Babaev et al[79] introduced *Jnk1*-null hematopoietic cells into *Ldlr*-null mice and saw that apoptosis of macrophages was suppressed, which led to accelerated atherosclerosis. Very intriguingly, the dominating role of sex in atherosclerosis risk, through its effects on HDL, has been refined by studies using the 4 core genotypes mouse model: XX females, XX males, XY females, and XY males.[80] It seems that having 2 X chromosomes versus an X and Y chromosome complement drives sex differences in HDL-C and ultimately atherosclerosis.

Finally, there has been progress in understanding the genetic basis of hypertriglyceridemia, the complexity of which has recently come into focus.[81] The transcription factor CREBH is a determinant of triglycerides in mice and humans.[82] Rare loss-of-function mutations in the human *CREB3L3* gene are associated with hypertriglyceridemia.[83] Furthermore, knocking out this gene on an *Ldlr*-null background accelerates atherosclerosis.[84] A fascinating potential therapeutic intervention for the increased postprandial chylomicron excursion observed in CREBH-null mice was suggested when *Akkermansia muciniphila,* a mucin-degrading bacterium, was administered to these mice, resulting in enhanced LDL receptor expression and reduced hepatic endoplasmic reticulum stress and inflammation.[85]

Defining Atherosclerosis in Animal and Cellular Models

We continue to learn about molecular pathways and targets by studying genetic manipulations or other interventions in animal models of atherosclerosis, such as *ApoE*-null or *Ldlr*-null mice. For instance, compared with nonmanipulated apoE knockout mice, atherosclerosis was increased with concurrent endothelial glucocorticoid receptor knockout[86] but was decreased with knockout of the Fcgamma RIIb receptor,[87] knockout of the four-and-a-half LIM domain protein-2,[88] and transgenic overexpression of the sclerostin gene,[89] whereas it was unchanged with knockout of the V1 (VHS107.1.42) immunoglobulin heavy chain gene.[90] Deletion of methionine sulfoxide reductase A on the *ApoE*-null background did not affect atherothrombosis, but did promote neointimal hyperplasia and extracellular signal-regulated kinase 1/2 signaling.[91] In *ApoE*-null mice fed a Western diet, the DNA methyltransferase inhibitor 5-aza-2′-deoxycytidine significantly attenuated atherosclerotic lesions, because of abnormal methylation status of specific target genes and effects on vascular smooth muscle cell dedifferentiation and remodeling.[92] Finally, hypertensive *ApoE*-null mice developed fibrotic aortic valve stenosis.[93]

Furthermore, compared with nonmanipulated *Ldlr*-null mice, atherosclerosis was increased with concurrent deficiency of the sheddase, a disintegrin and metalloproteinase 17,[94] but was decreased (together with lower body weight and

reduced hepatosteatosis) in mice with reduced expression of the angiotensinogen gene,[95] decreased in mice with deficiency of IκB kinase,[96] and also decreased (together with decreased aortic aneurysm development) in mice with leukocyte-specific calpain 2 deficiency.[97]

Newer genetically manipulated mouse models have also contributed valuable pieces of information to the overall puzzle of atherosclerosis. For instance, high expression levels of telomerase reverse transcriptase in mice induce atherosclerosis-like smooth muscle cell morphology, whereas deletion of *TERT* decreases neointima formation through epigenetic regulation of proliferative gene expression.[98] Aortic valve interstitial cells from *Notch1* heterozygous deficient mice become fully activated myofibroblasts leading to enhanced dystrophic calcification.[99] Genetic ablation of CaV3.2 channels enhances the arterial myogenic response through modulation of the RyR–BKCa (ryodine receptors-large conductance Ca^{2+}-activated K^+ channels) axis.[100] Conditional knockout of the E-twenty six factor Ets variant 2 in endothelial cells resulted in impaired neovascularization in response to ischemic tissue injury.[101] Mice lacking neuropilin-1 in cardiomyocytes and vascular smooth muscle cells exhibited decreased survival, because of development of cardiomyopathy and aggravated ischemia-induced heart failure.[102] Finally, in a transgenic mouse expressing a peroxisome proliferator–activated receptor-γ mutant (E-V290M) selectively in endothelium, the time to occlusive thrombosis of the carotid artery was significantly shortened after either chemical or photochemical injury.[103]

A novel mechanism for disturbed vascular phenotype was examined in developing zebrafish, where mutations in 2 different aminoacyl-transfer RNA synthetases, namely, threonyl tRNA synthetase and isoleucyl tRNA synthetase, increased branching angiogenesis via the unfolded protein response pathway.[104] Another mechanism in vascular differentiation was explored in murine embryonic endothelial cells, where Jumonji C domain-containing protein 8 was upregulated during differentiation and was further found to regulate endothelial cell sprouting and metabolism by interacting with pyruvate kinase.[105] On a larger scale, transcriptome-wide analysis by RNA sequencing of primary human-derived macrophages showed a large number of alternative splicing events that defined different macrophage phenotypes and stages of development.[106]

Conclusion

Reports of genetic discoveries in *ATVB* continue apace since the last update.[107] A plethora of articles have used genetic tools and model systems to further detection, treatment, and underlying molecular basis of atherothrombotic disease and vascular biology. Recurring themes have included statistical genetic associations, functional validations, miRNA regulation and therapeutics, and genetic basis of human dyslipidemias. Emerging trends include combining genetic findings with other types of experiments and more rapid translation into the clinical realm. Individually and collectively, these studies provide stepping-stones on the path of future genetics research and progress in our field.

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