Purpose of review
Since 2007, genome-wide association studies (GWAS) have led to the identification of numerous loci of atherosclerotic cardiovascular disease. The majority of these loci harbor genes previously not known to be involved in atherogenesis. In this review, we summarize the recent progress in understanding the pathophysiology of genetic variants in atherosclerosis.

Recent findings
Fifty-eight loci with \( P < 10^{-7} \) have been identified in GWAS for coronary heart disease and myocardial infarction. Of these, 23 loci (40%) overlap with GWAS loci of classical risk factors such as lipids, blood pressure, and diabetes mellitus, suggesting a potential causal relation. The vast majority of the remaining 35 loci (60%) are at genomic regions where the mechanism in atherogenesis is unclear. Loci most frequently found in independent GWAS were at Chr9p21.3 (ANRIL/CDKN2B-AS1), Chr6p24.1 (PHACTR1), and Chr1p13.3 (CELSR2, PSRC1, MYBPHL, SORT1). Recent work suggests that Chr9p21.3 exerts its effects through epigenetic regulation of target genes, whereas mechanisms at Chr6p24.1 remain obscure, and Chr1p13.3 affects plasma LDL cholesterol.

Summary
Novel GWAS loci indicate that our understanding of atherosclerosis is limited and implicate a role of hitherto unknown mechanisms, such as epigenetic gene regulation in atherogenesis.

Keywords
1p13.3, 6p24.1, 9p21.3, atherosclerosis, GWAS

INTRODUCTION
Findings from genome-wide association studies (GWAS) are a treasure trove for our understanding of the pathophysiology of atherosclerosis. The first GWAS in 2007 identified a locus on chromosome 9p21.3 (Chr9p21.3), which is the strongest genetic factor of atherosclerosis known today [1–4]. Since then, additional loci have been constantly added, resulting in over 50 loci. The majority is completely novel and the current challenge in the ‘post GWAS era’ is to identify the responsible genes and integrate them into our understanding of the pathophysiology of this frequent disease.

Here, we focus on the most robust loci identified by GWAS and review some of the approaches recently used to tease out their complex pathophysiology. These approaches include expression quantitative trait loci (eQTL) and functional studies in tissues from patients with defined genotypes, which are essential to single-out the culprit gene at loci usually containing multiple transcripts. Moreover, overlap with GWAS hits of cardiovascular risk factors and seemingly unrelated phenotypes gives hints to potentially causal relations. Finally, cell culture studies and mouse models using knockout and overexpression strategies are essential, in particular at loci involving completely novel pathophysiology. Understanding the mechanisms of these loci in atherogenesis is a prerequisite for later therapeutic targeting.
Forty percent overlap with genomic loci for classical risk factors, whereas mechanisms at the remaining 60% are unclear.

The three most frequently found loci are at Chr9p21.3, Chr6p24.1, and Chr1p13.3.

Recent work suggests a role of epigenetic gene regulation by a noncoding RNA as a novel mechanism of atherogenesis at Chr9p21.3.

Mechanisms at Chr6p24.1 are unclear, and Chr1p13.3 likely works through affecting plasma LDL-cholesterol.

Atherosclerosis is a disease affecting arterial blood vessels, leading to different disease phenotypes depending on the anatomical location and stage of the disease process. Most GWAS have been performed for the phenotype of coronary heart disease (CHD), which includes a broad spectrum of patients with stable and unstable coronary disease, myocardial infarction (MI) survivors and patients undergoing coronary angiography (Table 1) [3–8,9,10*,11–13,16,17,18**,19]. A smaller number of GWAS has specifically dealt with the phenotype MI, which overlaps with CHD because CHD almost always precedes MI. However, MI clearly involves additional mechanisms, such as thrombosis. In this review, we are not covering stroke, which requires differentiation into several subtypes of ischemic and hemorrhagic stroke with different underlying pathophysiology [22]. We are also not explicitly covering peripheral atherosclerosis and its surrogate marker ankle brachial index, where until now GWAS have only revealed the Chr9p21.3 locus with genome-wide significance in a study of more than 40,000 individuals [23].

GWA S LOCI OF CORONARY HEART DISEASE AND MYOCARDIAL INFARCTION

Searching the GWAS catalogue (www.genome.gov/gwastudies; accessed May 2013; [21]) with a stringent cutoff (P < 10⁻⁷), we have assembled 58 loci from 18 publications for the phenotypes of CHD and MI (Table 1) reporting the best P values (including combined analyses with replication) [1,3–8,9*,10**,11–17,18**,19,20]. A predominant number of these variants has been identified by the CARDIoGRAMplusC4D consortium [18**], which also tested for overlap with genetic variants for established risk factors (Table 1). As summarized in Fig. 1, we found that GWAS loci for CHD and MI overlap with 14 loci for lipids (24% of all risk loci), six loci for blood pressure/hypertension (10%), one locus for diabetes mellitus (2%), and two loci with at least two risk factors (4%). Thirty-five (60%) loci did not co-segregate with loci of classical risk factors but out of these, six overlapped with loci from seemingly unrelated GWAS (Table 1, Supplementary material).

Another approach to get insights into function is to investigate the effects of the genotype on mRNA expression of genes at GWAS loci and to map eQTLs. This might be particularly helpful to identify the culprit gene(s) at loci harboring many
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### Table 1. Summary of 58 GWAS loci for CHD and MI with \( P < 1 \times 10^{-7} \) as of May 2013

| Region | Genes | SNP | FRA | \( p \)-value | OR | Reference |
|--------|-------|-----|-----|-------------|----|-----------|
| 10p11.23 | HYD1B | rs2954029 | A | 0.55 | 5 x 10^{-5} | A |
| 10p11.23 | NFATC4 | rs2123536 | T | 0.39 | 7 x 10^{-5} | A |
| 10q25.1 | CYP17A1 | rs1842896 | T | 0.76 | 1 x 10^{-5} | A |
| 11q23.3 | SMG6, SRR | rs4252120 | T | 0.73 | 5 x 10^{-5} | A |
| 11q22.3 | HDAC4, HDAC5 | rs12190287 | C | 0.62 | 1 x 10^{-5} | A |
| 11p13.3 | ADAMTS7 | rs12413409 | G | 0.89 | 1 x 10^{-5} | A |
| 11q22 | CDKN2B-AS1 | rs9982601 | T | 0.15 | 4 x 10^{-5} | A |
| 11q22 | COL4A1, COL4A2 | rs94652 | T | 0.47 | 1 x 10^{-5} | A |
| 11q22 | FURIN-FES | rs264 | G | 0.86 | 3 x 10^{-5} | A |
| 11q22 | APOL2-B | rs11066280 | A | 0.17 | 2 x 10^{-5} | A |
| 11q22 | SMG6, SRR | rs1746048 | C | 0.87 | 3 x 10^{-5} | A |
| 11q22 | ZEB2 | rs2252641 | G | 0.36 | 1 x 10^{-5} | A |
| 11q22 | HLA-C | rs1561198 | C | 0.51 | 1 x 10^{-5} | A |
| 11q22 | APAH2, APAH1 | rs46522 | T | 0.53 | 4 x 10^{-5} | A |

Loci are from the GWAS catalogue (www.genome.gov/gwastudies; accessed May 2013, [21]). Overlap with loci for risk factors of atherosclerosis was added: Black – loci for CHD from GWAS catalogue, dark grey – loci for MI from GWAS catalogue, grey – loci for risk factors of atherosclerosis (lipids, blood pressure, hypertension, diabetes) and other phenotypes from GWAS catalogue, light blue – loci for risk factors and other phenotypes reported in [18**], dark blue – loci for risk factors and other phenotypes reported both in GWAS catalogue and in [18**]. Notes A–O with information of co-segregating GWAS hits (\( P < 10^{-7} \)) can be found in the Supplementary material. C, Chinese cohort; CHD, coronary heart disease; E, European cohort; EA, European and South-Asian cohorts; FRA, frequency of risk allele; GWAS, genome-wide association studies; J, Japanese; K, Korean cohort; M, Middle Eastern cohort; MI, myocardial infarction; NR, not reported. Loci with two-SNP haplotypes (TSH [12], TSH1 – rs11924705, rs6789378 [risk alleles C,A]; TSH2 – rs7697839, rs7673097 [risk alleles G,G]; TSH3 – rs1165568, rs1165669 [risk alleles G,C]); loci for MI [19], Chr1p13.3 – rs6467761, FRA 0.81, \( P \) value 8 x 10^{-12}, OR 1.19, Chr1p32.3 – FRA 0.81, \( P \) value 1 x 10^{-8}, OR 1.15, Chr1q41 – FRA 0.72, \( P \) value 1 x 10^{-9}, OR 1.14, Chr9p21.3 – FRA 0.56, \( P \) value 3 x 10^{-4}, OR 1.29, Chr10q11.21 – FRA 0.84, \( P \) value 7 x 10^{-9}, OR 1.17, Chr19p13.2 – FRA 0.75, \( P \) value 2 x 10^{-6}, OR 1.15, Chr21q21.11 – FRA 0.13, \( P \) value 6 x 10^{-11}, OR 1.2.
Overlap between atherosclerosis loci and loci from genome-wide association studies (GWAS) hits for other nonrisk factor-associated traits (26% of all loci).

Genes. Testing cis-regulation of a genetic variant at a genome-wide level requires large cohorts where transcriptome-wide mRNA expression has been assayed in each individual and where genome-wide SNP data are also available. Folkersen et al. [28] have systematically tested lead SNPs from GWAS of CHD and MI and found evidence for cis-regulation at five loci in different vascular tissues and liver samples (Chr1p13.3: SORT1, PSRC1, CELSR2; Chr2q33.2: NBEAL1; Chr3q22.3: MRAS; Chr6q25.1: MTHFD1L; Chr21q22.11: SLCSA3). Wild et al. [11] performed a comparable analysis using mRNA expression data from monocytes of 1494 individuals from a population-based study [29] and found three eQTLs (Chr1p13.3: PSRC1; Chr2q33.2: WDR12; Chr1q23.31: LIPA). Results at Chr2q33.2 are particularly interesting since expression analysis in different tissues apparently led to different findings. A similar approach was taken by the C4D consortium, which systematically tested for eQTLs at newly identified loci [9*]. A current limitation of this very promising approach is the limited availability of large cohorts with tissue collections for transcriptome-wide expression analysis.

**Chr9p21.3 (ANRIL): Role of a Long Noncoding RNA (ncRNA) in Atherogenesis**

Chr9p21.3 is the most replicated locus of human atherosclerosis (reviewed in [30,31]). The locus lacks associations with common cardiovascular risk

![FIGURE 1. Overlap between atherosclerosis loci and loci for common risk factors. Out of 58 loci for coronary heart disease (CHD) and myocardial infarction (MI), 24% overlapped with lipid loci (LDL cholesterol, HDL cholesterol, total cholesterol, triglycerides), 10% with blood pressure, 2% with diabetes-related traits, 2% with lipids and diabetes-related traits, and 2% with all three risk factors. Sixty percent (n = 35) of CHD and MI loci did not overlap with loci for common risk factors suggesting novel pathophysiology. The inner circle shows additional overlap with genome-wide association studies hits for other nonrisk factor-associated traits (26% of all loci).](image)

![FIGURE 2. Haplotype analysis (HapMap CEU) and annotated genes at the three most frequently identified loci for coronary heart disease (CHD) and myocardial infarction (MI). Single-nucleotide polymorphisms with strongest signals of the respective phenotype and corresponding references are given. (a) Chr9p21.3 CHD and MI locus and adjacent hits for cancer, diabetes, and other traits. (b) Chr6p24.1 CHD and MI locus overlapping with migraine. Significance of pulse pressure and femoral neck width loci is unclear. (c) Chr1p13.3 CHD and MI locus co-segregating with genome-wide association studies (GWAS) hits for lipids.](image)
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ANRIL [63] proposed that apoptosis et al. express in modulating atherosclerosis susceptibility. Knock-down led to decreased ANRIL as major CDKN2A belongs to the group of gene networks. Expression is tightly function at ANRIL may act as a scaffold, guiding regressive complexes 1 and 2 (PRC1, PRC2) and potentially others to chromatin. Transcription of ANRIL is complex and more than 20 linear and several circular isofoms are known today [55,57,59]. As a mechanism for differential expression, Harismendy et al. [63] proposed that ANRIL expression in Chr9p21.3 risk allele carriers was induced by disruption of an inhibitory STAT1-binding site. Functional studies in mammalian cells revealed that ANRIL knock-down led to decreased proliferation [64–67]. Recent work has expanded these findings, showing that ANRIL overexpression not only led to accelerated proliferation but also increased adhesion and decreased apoptosis [59]. These are key mechanisms of atherogenesis and the direction of effects would be in line with the proatherogenic role of ANRIL suggested from expression studies (Fig. 3) [59].

But how does ANRIL exert these effects at the molecular level? ANRIL belongs to the group of large noncoding RNAs which have been shown to regulate gene expression through RNA–RNA, RNA–DNA, or RNA–protein interactions [68–70]. For ANRIL, binding to epigenetic silencer Polycomb repressive complexes 1 and 2 (PRC1 and PRC2) [59,66,67] and to PRC-associated activating proteins RYBP and YY1 [71,72] has been demonstrated (Fig. 3). We have recently shown that trans-regulation was dependent on an Alu-DEIN motif [74,75], which marked the promoters of ANRIL target genes and was mirrored in ANRIL RNA transcripts (Fig. 3). The functional relevance of Alu motifs in ANRIL was confirmed by deletion and mutagenesis, reversing trans-regulation and restoring normal cellular functions [59]. Recent work by Jeck et al. has also demonstrated that Alu motifs are preferably incorporated in noncoding RNA lariats, which might represent inactive isofoms and were also shown to exist for ANRIL [55,76]. Whether integration of Alu motifs in ncRNA lariats leads to silencing of the effector sequences remains to be determined.

FIGURE 3. Model of ANRIL/CDKN2B-AS1 function at Chr9p21 according to [59]. The atherosclerosis risk allele leads to up-regulation of the long ncRNA ANRIL. Increased ANRIL expression modulates networks of genes in-trans, leading to proatherogenic cell properties [increased cell adhesion, increased proliferation, decreased apoptosis]. On the molecular level, ANRIL may act as a scaffold, guiding epigenetic modifier proteins of Polycomb repressive complexes 1 and 2 (PRC1, PRC2) and potentially others to chromatin. These functions depend on Alu motifs, which mark the promoters of ANRIL target genes and are mirrored in ANRIL RNA, suggesting an Alu-mediated RNA–DNA interaction as effector mechanism.
In summary, the robust association of ANRIL with the risk genotype, its correlation with atherosclerosis severity, and functional data strongly support ANRIL as Chr9p21.3 effector gene. Recent work has not only broadened our understanding of ANRIL's function but also suggested a novel molecular mechanism for long ncRNA-mediated trans-regulation.

**Chr6p24.1 (PHACTR1): Frequently Replicated But Poorly Understood**

Chr6p24.1 is the second most often identified GWAS hit for CHD and MI. The locus was found in European, Asian, and Middle Eastern populations and therefore appears to be relevant across ethnicities [9*,10**,15,16,18**,19]. Chr6p24.1 is also associated with coronary calcification [40]. Until now, virtually nothing is known about the mechanism of Chr6p24.1 in atherogenesis. The region contains a single gene, protein phosphatase and actin regulator 1 (PHACTR1), spanning a very large genomic distance of ∼500 kb, and extending over three haplotype blocks (Fig. 2b). Lead SNPs for CHD and MI are in the proximal haplotype block and the same SNPs were independently identified in a GWAS for migraine [42]. Intriguingly, alleles conferring migraine susceptibility were also associated with risk for CHD suggesting a common pathophysiology. The distal haplotype block of PHACTR1 also contains hits in the GWAS catalogue (www.genome.gov/gwastudies; accessed May 2013; [21]), originating from a 100k GWAS for femoral neck width in females of the Framingham Heart Study [43] and a linkage study for pulse pressure in 63 Chinese sib-pairs [44] (Fig. 2b). However, these findings have not been firmly replicated and their significance is still unclear. In addition, these SNPs are ∼300-kb apart and seemingly unrelated to the lead atherosclerosis SNPs, speaking against a causal relation.

PHACTR1 is highest expressed in human heart and brain [77] and is a member of a family of proteins that bind actin and interact with protein phosphatase 1 (PP1) [78]. PP1 is an ubiquitous enzyme, regulating essential cellular processes such as cell cycle progression, protein synthesis, muscle contraction, carbohydrate metabolism, transcription, and neuronal signaling (reviewed in [79]). For PHACTR1, a role in cell migration, motility and invasiveness of breast cancer, and melanoma tumor cells was described [80,81]. Moreover, PHACTR1 is expressed in endothelial cells and involved in regulation of endothelial tubulogenesis and apoptosis [82,83]. In summary, even though PHACTR1 is an obvious candidate gene at Chr6p24.1, current data on its function is scarce and its mechanism in atherogenesis is still unclear.

**Chr1p13.3 (PSRC1/CELSR2/MYBPHL/SORT1): Lipids and Coronary Heart Disease**

The Chr1p13.3 locus has been discovered in the first surge of GWAS for CHD even before it was also identified as one of the top GWAS hits for plasma LDL cholesterol concentrations [84–86]. Genetic variation at the locus is associated with reduced plasma LDL-cholesterol and reduced risk of coronary artery disease [10**,25,45] suggesting that Chr1p13.3 exerts its effect on atherosclerosis by modulating LDL-cholesterol levels. The lead SNPs of CHD and LDL-cholesterol are located in a haplotype block encoding three genes, cadherin EGF LAG seven-pass G-type receptor 2 (CELSR2), proline/serine-rich coiled-coil 1 (PSRC1), and myosin binding protein H-like (MYBPHL) (Fig. 2c). Wild et al. found differential expression of PSRC1 in monocytes at the locus [11]. The majority of functional work, however, has focused on sortilin 1 (SORT1), which is located in a haplotype block distal of PSRC1, CELSR2, and MYBPHL (Fig. 2c) containing GWAS hits for major depressive disorder [46] and chronic kidney disease [47]. Schadt et al. [87] and Folkers et al. [28] found that mRNA expression of CELSR2, PSRC1, and SORT1 were all strongly associated with Chr1p13.3 in liver. Although SORT1 was highly expressed in many tissues, genotype-dependent differential regulation was only seen in liver [28]. Musunuru et al. [26] identified a SNP in linkage disequilibrium with the lead SNP, creating a C/EBP transcription factor binding site in the 3' UTR of CELSR2 and altering expression of SORT1. These data suggested that SORT1 expression might be affected by cis-regulation through the neighboring haplotype block [26].

SORT1 is a member of the VSP10p receptor family of sorting receptors, which have been intensively studied in neuroscience and direct proteins through secretory and endocytic pathways of the cell (for review see [88,89]). In 2010, three independent groups published first mechanistic work on the role of SORT1 in LDL-metabolism with in part paradoxical results: The first study overexpressed SORT1 in HEK293 cells, resulting in increased uptake of LDL and LDL-receptor-related protein [25]. A second article used viral overexpression in mouse liver, demonstrating that increased SORT1 decreased plasma LDL-cholesterol and VLDL levels by reducing hepatic VLDL secretion [26]. Inverse results were seen after SORT1 knock-down [26]. Both studies were well in line with the observation that increased expression of SORT1 mRNA in human liver was correlated with decreased LDL-cholesterol [26], even though the proposed mechanisms would be either through increased LDL uptake [25] or reduced...
VLDDL secretion [26]. Results of a third article, published virtually at the same time, were seemingly at odds with the two previous articles. Using mice on the Ldlr−/− background, these authors demonstrated that complete Sort1 deficiency ameliorated hypercholesterolemia and atherosclerosis [24]. Additional studies on the subcellular level indicated that Sort1 interacts with apoB100 in the Golgi apparatus, thereby facilitating formation and hepatic export of apolipoprotein B containing lipoproteins [24].

Recent work [27] has reconciled the divergent hypotheses on the function of SORT1 in lipoprotein metabolism. These authors proposed a model in which hepatic SORT1 binds intracellular apoB100 containing particles in the Golgi as well as extracellular LDL at the plasma membrane and traffics them to lysosomal degradation. They suggested a hyperbolic relationship in which complete lack as well as increased SORT1 would both lead to a reduction in apoB and VLDL secretion, whereas intermediate SORT1 expression would increase secretion [27]. Although common variants in SORT1 have subtle effects on LDL-cholesterol, a recent publication provided data speaking against a role of SORT1 missense mutations in autosomal dominant hypercholesterolemia [90].

Until now, the majority of work on the molecular mechanism at Chr1p13.3 has clearly focused on SORT1. Very little is known about the functions of PSRC1, CELSR2, and MYBPHL, which are closer to the lead Chr1p13.3 SNPs. More work is clearly warranted to establish or firmly exclude a role of these genes in lipid metabolism and atherogenesis.

**CONCLUSION**

Current GWAS have added additional loci to the ‘genomic landscape’ of CHD and MI bringing the total count to 58 at a significance cutoff of \(P < 10^{-7}\). Recent advances in functional characterization of some loci promise the discovery of hitherto unknown pathways influencing atherosclerosis risk. One such example is the most replicated locus on Chr9p21.3, which might influence atherogenesis through epigenetic chromatin modification by the long ncRNA ANRIL. Nevertheless, our current understanding of potential causal variants and mechanisms at most GWAS loci of atherosclerotic cardiovascular disease is very limited. Although some of these loci co-segregate with known risk factors suggesting a potential causal relation, the majority is still ‘terra incognita’. This is exemplified by the second most frequently found locus on Chr6p24.1, where virtually nothing is known about its function in atherogenesis. Owing to their small effect size, the utility of genetic variants for diagnostic purposes is limited. The major promise of identified GWAS loci therefore lies in understanding their function in atherogenesis as a prerequisite for later therapeutic targeting.

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**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES AND RECOMMENDED READING**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 456).

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