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Toward understanding the molecular basis of atherosclerosis with genetics and genomics

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Summary

Atherosclerosis is a very complex disease involving both genetic and environmental risk factors, and their interactions. In the general population, genetic polymorphisms of many genes in the pathways of lipid metabolism, inflammation, and thrombogenesis are likely responsible for the wide range of susceptibilities to myocardial infarction, the most deadly consequence of atherosclerosis. To identify these polymorphisms, genetic linkage studies have been carried out in both humans and mouse models. Approximately 40 quantitative trait loci for atherosclerotic disease have been found in humans, and approximately 30 in mice. Recently, genome-wide association studies have been used to identify atherosclerosis-susceptibility polymorphisms. Although finding new atherosclerosis genes through these approaches remains challenging, the pace of finding these polymorphisms is accelerating due to the rapidly improving bioinformatics resources and biotechnologies. The results from these efforts will not only reveal the molecular basis of, but will facilitate finding drug targets and individualized medicine for, atherosclerotic disease.

Introduction

Atherosclerosis is characterized by lipid accumulation, inflammatory response, cell death, and fibrosis in the arterial wall. Our understanding of the pathogenesis of this process has evolved in the last few decades, guiding our efforts to find treatments. Because low-density lipoprotein cholesterol (LDL) is pro-atherogenic, statin drugs were developed to lower plasma LDL cholesterol (LDL-C) levels and reduce the risk of cardiovascular events. Recent studies suggest that reducing LDL-C levels to below current guideline targets further slows down atherogenesis and reduces coronary events (Todd and Farmer, 2006). Statin drugs have reduced new cardiovascular events by one third; although this is significant, it is clear that additional therapies are needed. Based on a plethora of evidence suggesting that increasing plasma high-density lipoprotein cholesterol (HDL-C) levels inhibits atherogenesis, HDL-raising agents are expected to retard atherogenesis. Three classes of HDL-raising agents exist: niacin, fibrate, and statin. Promising new ones include apoA-I-phospholipid complexes, human apoA-I and apoA-I mimetic peptides, and inhibitors of cholesterol ester transfer protein (CETP; questions arise with the failure of Pfizer's Torcetrapib, though), (Brousseau, 2005). New HDL-raising targets are being sought with genetics and genomics approaches (Rollins et al., 2006; Wang and Paigen, 2005a). The relatively recent appreciation that inflammatory response plays a key role in atherogenesis implies that inhibiting inflammation may provide new anti-

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atherosclerosis therapy. However, immune response can both promote and retard atherosclerosis (Hansson and Libby, 2006). The challenge will be to inhibit atherosclerosis-specific inflammation without compromising global immune responses.

To better understand the molecular mechanism of atherogenesis, two major approaches have been undertaken. The first is the candidate gene approach, in which genes in known atherogenesis pathways are tested for their role in atherosclerosis *in vitro*, *in vivo* and in association studies. This approach has been fruitful and role of many genes in atherogenesis became known as a result (Arnett et al., 2007; Hansson and Libby, 2006; Lusis et al., 2004). The second approach is conducting genome-wide linkage studies to find atherogenesis-regulating quantitative trait loci (QTL). This is an unbiased method that has the potential to find new atherosclerosis genes. Recently, the availability of whole genome sequences in humans and mice, especially the abundant SNP and haplotype information, has made it possible to perform genome-wide association studies, another unbiased approach, to identify disease genes relatively quickly as compared with traditional genetic methods. In deed, the pace of finding disease genes using such unbiased methods has been greatly accelerated in recent years because of the ever improving SNP/haplotype information, experimental design, statistical methods, and the decreasing cost and the high throughput of sequencing. In this review, we focus on finding novel atherosclerosis-regulating genes using genome-wide linkage and genome-wide association studies, summarize successful examples of using these approaches, and present detailed human-mouse comparative genomics maps of all known atherosclerosis-regulating QTL. These maps should be helpful for finding new atherosclerosis-regulating genes.

Genetic linkage studies of atherosclerotic diseases in humans

Atherosclerotic disease linkage studies have been conducted in 19 human populations (Table 1). Four of these studies were for linkage to coronary artery disease (CAD), six for linkage to myocardial infarction (MI), one for linkage to both CAD and MI, one for linkage to acute coronary syndrome (ACS), two for linkage to carotid intimal-medial thickness (CIMT), two for linkage to coronary artery calcification (CAC), two for linkage to stroke, and one for linkage to peripheral artery occlusive disease (PAOD). Forty atherosclerotic disease-regulating QTL have been identified: nine for CAD, twelve for MI, one for CAD/MI, three for ACS, nine for CIMT, four for CAC, one for stroke, and one for PAOD. Two meta-analysis of these genome-wide linkage analysis have been reported: in the meta-analysis of four studies, two linkage regions (2q and 3q) in the original studies were confirmed (Chiodini and Lewis, 2003); in another meta-analysis of 5 studies, 4 linkage regions in the original studies (1p, 5p, 12q, 13q) were confirmed, while four new linkage regions that were not found in the original studies (6p, 2 on 8q, 14p) were revealed (Zintzaras and Kitsios, 2006). It is noteworthy that among the 40 human atherosclerosis QTL, only three are replicated in more than one population for the same disease: one CAD QTL on chr 2, one stroke QTL on chr 5, and one MI QTL on chr 17. However, when all the seven disease types in were treated together as “atherosclerotic disease”, then there are 33 QTL regions, and 11 of them are replicates (Fig. 1). This relatively low replicate rate is possibly due to the small sample size and population difference in many studies. By using methods discussed previously (Rollins et al., 2006), we found that 31 of the 40 human QTL are concordant with mouse QTL (Fig. 1), suggesting that identifying the genes underlying the mouse QTL will facilitate identifying the genes underlying the human QTL.

Putative disease genes have been found from only three QTL for atherosclerotic disease, but the pace of identifying these genes is expected to be accelerating (Farrall et al., 2006; Hakonarson et al., 2005). The DeCode Genetics group in Iceland mapped a QTL for both MI and stroke to chr 13q12-13, and subsequently identified 5-lipoxygenase activating protein (*ALOX5AP*, also called *FLAP*) as the underlying gene (Helgadottir et al., 2004). ALOX5AP

contributes to the conversion of ALOX5 in the leukotriene pathway, yielding to production of leukotriene B_4, one of the most potent leukocyte pro-inflammatory mediators. This finding is consistent with the discovery that 5-lipoxygenase (*Alox5*) is the underlying gene for an atherosclerosis QTL on mouse Chr 6, and that ALOX5/ALOX5AP pathway influences the development of atherosclerosis (discussed below). In another study, Wang *et al*. studied a large family with 13 patients who displayed an autosomal dominant pattern of CAD (nine of whom developed acute MI). They found a linkage of CAD/MI to a region on 15q26, which consists of 93 genes. One of the genes, myocyte-enhancing factor 2A (*MEF2A*), is expressed in endothelial cells of coronary arteries. A 21-base pair deletion was identified in exon 11 in all ten living members with CAD in the family, and not in family members and an additional 119 individuals with normal angiograms, strongly suggesting that the deletion is responsible for CAD and MI in this large family (Wang *et al*., 2003a). MEF2A mutations may be a rare cause of CAD and MI because they are present in less than 2% of a U.S. population of 207 CAD/MI patients (Bhagavatula et al., 2004). Later, in another two studies, the 21-base pair deletion was found in a total of six subjects (3 in each study) older than 60 years of age who had no history nor symptoms of CAD (Kajimoto et al., 2005b; Weng et al., 2005), which seems to argue against the causality of the 21-base pair deletion in CAD/MI (Altshuler and Hirschhorn, 2005). However, in these later two studies, the CAD status in control subjects was not confirmed with angiography, as Wang et al did in the original publication (Wang *et al*., 2003a), making the conclusion not definitive. A recent study found that a P279L variant in MEF2A is associated with the risk of MI (11 of 483, or 2.3%, in MI patients, and 9 of 1189, or 0.8%, in controls; OR=3.06) (Gonzalez et al., 2006). In this study, the CAD status in controls was not confirmed with angiography either; however, this might add to the strength of the association because some of the nine “controls”, who were all under 50 years of age, may develop MI at older ages. More recently, association studies were done on candidate genes in the 1-LOD supporting interval of the 3q13 QTL for CAD they identified (Hauser et al., 2004), and authors found that four closely located genes on Chr 3 were associated with the risk for CAD: *CDGAP* at 120.5 Mb, *MYLK* at 124.8 Mb, and *KALRN* at 125.3 Mb in one study, (Wang et al., 2007), and GATA2 at 129.7 Mb in another study (Connelly et al., 2006). The first three are all in the GTPase-signaling pathway. How these genes affect CAD risk is not clear, although KALRN inhibit inducible nitric oxide synthase, and GATA1 may affect endothelial functions.

Genetic linkage studies of atherosclerosis in mice

The three most often used mouse models of atherosclerosis are the following: 1) a high fat model in which inbred mice are fed a high fat and cholesterol diet (15% fat, 1.25% cholesterol, and 0.5% cholic acid), 2) *Apoe*-deficient mice fed either chow, a Western diet (21% fat and 0.15% cholesterol), or a high fat and cholesterol diet without cholic acid, and 3) *Ldlr*-deficient mice fed either a Western diet or a high fat and cholesterol diet. The latter two models are also called sensitized models (Dansky *et al.*, 2002). Some early QTL studies took advantages of the existing recombinant inbred lines, such as BxH and CxB (Paigen *et al.*, 1987a; Paigen *et al.*, 1987b; Paigen *et al.*, 1987c). Since then, fourteen different F2 or N2 crosses have been generated to find atherosclerosis-regulating QTL: seven crosses involved normal inbred mice, five involved inbred mice with *Apoe*-deficiency, and two involved inbred mice with *Ldlr*-deficiency (Table 2). Thirty different mouse atherosclerosis QTL have been found: fifteen in one cross, eleven in two different crosses, and four in three or more crosses. Among the thirty atherosclerosis-regulating QTL, seven were found only in the high fat model, twelve only in the *Apoe*-deficient model, four only in the *Ldlr*-deficient model, two in both the *Apoe*-deficient and the *Ldlr*-deficient models, and five in both the high fat and the sensitized models. Among the 30 QTL, 13 are replicates (the same QTL region found in different studies). A comparative map (Fig. 2) of mouse and human atherosclerosis-regulating QTLs indicates that most of the mouse QTL found in both the high fat model (86%) and the sensitized models (61%) are

concordant with the human QTL. In all, twenty of the thirty mouse QTLs are concordant with the human QTL. This comparative QTL map will be helpful in two ways. First, it will help researchers focus on the concordant QTL because finding the underlying genes in the mouse might facilitate finding them in humans. Second, by overlapping the concordant QTL and excluding the non-overlapping regions, based on the assumption that the same orthologous genes underlie the QTL in both species, we might be able to significantly narrow many of the mouse QTL. Of course, this assumption may not always be true. Mouse QTL can also be narrowed with other bioinformatics methods, such as combined cross analysis, haplotype analysis, and whole-genome association studies (DiPetrillo et al., 2005). Candidate genes can be analyzed for sequence and expression patterns, and a shortened list of candidates can be verified in animal models and in human association studies (Wang *et al.*, 2005b).

Two of the genes underlying mouse atherosclerosis QTL have been identified: *Alox5* for the QTL *Artles* on Chr 6, and *Tnfsf4* (*Ox40l*) for the QTL *Ath1* (atherosclerosis susceptibility 1) on Chr 1 (Table 2). *Artles* was identified in a cross between CAST and C57BL/6 (Mehrabian *et al.*, 2001). By using *Alox5* targeted mutant mice with or without *Ldlr*-deficiency, a positional candidate, 5-lipoxygenase (*Alox5*), was shown to increase atherosclerosis (Ghazalpour *et al.*, 2006;Mehrabian *et al.*, 2002). Other evidence that the ALOX5/ALOX5AP pathway is involved in atherogenesis seems compelling: genetic linkage and association studies in humans have revealed that *ALOX5AP* confers risk of MI and/or stroke (Helgadottir et al., 2005;Helgadottir et al., 2004;Kajimoto et al., 2005a;Kaushal et al., 2007;Lohmussaar et al., 2005); promoter variants of *ALOX5* are associated with CIMT in humans (Dwyer *et al.*, 2004); expression levels of ALOX5 are elevated in symptomatic plaques and are associated with acute ischemic syndromes (Cipollone et al., 2003;Qiu et al., 2006); ALOX5AP inhibitor reduces atherosclerosis in mice (Jawien et al., 2006). Because ALOX5 and ALOX5AP are involved in leukotriene formation, leukotriene receptors and leukotriene-forming enzymes are studied for their role in atherosclerosis. It was found that CP-105,696, a leukotriene B_4 (LTB4) receptor antagonist, decreases atherosclerosis in both $Apoe^{-/-}$ and $Ldlr^{-/-}$ mouse models, and the effect was dependent on the presence of CCL2 (MCP1) because the drug has no effect in $Ccl2^{-/-}$ mice (Aiello *et al.*, 2002). In addition, targeted mutation of LTB4 receptor 1 (*Ltb4r1*) leads to reduced early atherosclerosis (Heller et al., 2005;Subbarao et al., 2004). Furthermore, a polymorphism of leukotriene A4 hydrolase (*LTA4H*), whose gene product hydrolyzes LTA4 into the leukocyte chemoattractant LTB4, is associated with the risk of MI in an Icelandic cohort (Helgadottir et al., 2004). The above observations strongly suggest that ALOX5/ALOX5AP pathway influences the progress of atherosclerotic disease.

However, it is noteworthy that conflicting data do exist. In both $Apoe^{-/-}$ and $Ldlr^{-/-}$ mouse models, ALOX5 deficiency does not significantly affect lesion development in a study by Zhao et al (Zhao *et al.*, 2004), in contrast to the observation by Mehrabian et al (Ghazalpour et al., 2006; Mehrabian et al., 2002). Apart from the differences in study design (age of the mice, length of diet, methods for measuring the lesions), the *Alox5* knockout mice used by these two different groups likely had different lengths of 129 flanking regions, which may exert additional effects on atherogenesis other than those by *Alox5* itself. In fact, the *Alox5* region contains at least two, and may be as many as four, genes that affect atherosclerosis (Ghazalpour et al., 2006). In addition, the association of *ALOX5AP* polymorphisms with MI and/or ischemic stroke has not been confirmed in other studies (Koch et al., 2007; Meschia et al., 2005; Zee et al., 2006). This is most likely due to the population/ethnic differences, as the associations are more significant in Icelandic, less or not so in non-Icelandic populations. Therefore, ALOX5/ALOX5AP pathway may confer ethnicity-specific risk for MI and stroke. Nonetheless, the use of the relatively isolated Icelandic population reveals that ALOX5/ALOX5AP pathway influences atherosclerotic disease. One possible mechanism is that macrophage ALOX5 cascade generates leukotrienes, which then act on neighboring endothelial cells (and possibly also T cells and macrophages themselves), thereby activating the release of CC and CXC

chemokines, resulting in the recruitment of inflammatory cells into the artery (Zhao *et al.*, 2004). Therefore ALOX5 and ALOX5AP may be targeted for treating human atherosclerotic disease.

The second atherosclerosis-regulating QTL gene found in the mouse was *Tnfsf4* (OX40 ligand). OX40 ligand and its receptor OX40 belong to the TNF/TNFR superfamily. OX40 is mainly expressed on activated T cells, and OX40L is expressed on antigen-presenting cells (B cells, dendritic cells, macrophages, and endothelial cells) and T cells. The function of the OX40-OX40L costimulatory pathway has been studied in *Ox40*- (Kopf et al., 1999) and *Ox40l*-deficient mice (Chen *et al.*, 1999; Murata *et al.*, 2000). These studies demonstrated that OX40-OX40L interactions are crucial for optimum T-cell function and the generation of memory T cells by promoting the survival of effector T cells after antigen priming (Sugamura *et al.*, 2004). Because *Ox40l* is a candidate gene for *Ath1* (Paigen *et al.*, 1987c; Phelan *et al.*, 2002), we studied the role of the OX40/OX40L pathway in atherosclerosis. We found that OX40L is expressed on endothelial cells, macrophages, and lymphocytes in mouse atherosclerotic lesions. OX40L is pro-atherogenic in mice: *Ox40l*-deficient mice have smaller lesions, and transgenic mice over-expressing *Ox40l* have larger lesions than do controls (Wang *et al.*, 2005c). More recently, van Wanrooij et al found that treating $Ldlr^{-/-}$ mice with antibody again OX40 reduces atherosclerosis by 53% (van Wanrooij et al., 2007), supporting the pro-atherogenic role of OX40L. Based on the mouse studies, we performed candidate gene association studies and found that OX40L may also influence atherosclerosis in humans: in two independent populations, the less common allele of SNP rs3850641 in *OX40L* is significantly more frequent ($P \leq 0.05$) in individuals with myocardial infarction than it is in controls (Wang *et al.*, 2005c). More recently, polymorphisms of *OX40* were found to be associated with the risk of MI (Ria et al., 2006), confirming that OX40L/OX40 pathway influences the risk of MI. However, the underlying mechanism is unclear. The OX40L expressed on vascular ECs might bind to OX40 on lymphocytes, facilitating their activation and recruitment into the arterial wall. Or, by reverse signaling, ligation of OX40L on vascular ECs, macrophages, and lymphocytes may induce the production of chemoattractants, drawing and directing leukocytes from the blood into atherosclerotic plaques (Wang, 2006). This possibility is supported by the observation that interaction of recombinant OX40 with human umbilical vein endothelial cells (HUVECs) induces the endothelial cells to produce the CC chemokine RANTES (CCL5) (Kotani *et al.*, 2002), a chemoattractant for monocyte and T cells. RANTES may promote atherosclerosis: intraperitoneal injection of RANTES receptor antagonist Met-RANTES reduces the progression of atherosclerosis in $Ldlr^{-/-}$ mice, accompanied by decreased leukocyte infiltration into lesions (Veillard *et al.*, 2004). It is also possible that the engagement of OX40L and OX40 may induce the production of inflammatory cytokines, as shown in a recent study indicating that the engagement of OX40L on human airway SMCs with recombinant OX40 releases IL-6, a pro-inflammatory cytokine, from these SMCs (Burgess *et al.*, 2004).

There is evidence that angiopoietin-like 3 (*Angptl3*) underlies *Ath8*, an atherosclerosis-regulating QTL on mouse Chr 4 (Korstanje et al., 2004): *Angptl3* has three non-synonymous polymorphisms between NZB and SM, the parental strains of the cross revealing *Ath8*; ANGPTL3 inhibits lipoprotein lipase activity and increases plasma total cholesterol and triglycerides levels (Koster et al., 2005); ANGPTL3 binds to integrin alpha(v)beta(3), suggesting it plays a role in angiogenesis and atherogenesis (Camenisch et al., 2002); polymorphisms of *ANGPTL3* are associated with the size of coronary atherosclerotic plaques in one human population (Korstanje et al., 2004).

Genome-wide association studies

Testing the association of a gene with atherosclerosis disease is usually motivated by existing pieces of evidence that the gene might be involved in certain atherogenic pathways. However, single gene association studies are notorious for high incidence of false positive findings and low level of reproducibility. Recently, the advent of dense SNPs, detailed haplotype information and low cost and high throughput genotyping has made it possible to carry out unbiased whole genome association studies to find disease genes, including those for atherosclerosis. In a pioneering, large-scale, case-control association study of a Japanese population, Ozaki *et al* used 92,788 gene-based single-nucleotide polymorphism (SNP) markers across the genome and identified the association of *LTA* (lymphotoxin-alpha) polymorphisms with susceptibility to MI (Ozaki *et al.*, 2002). This association with CAD/MI has been replicated in some (Laxton et al., 2005; Mizuno et al., 2006; PROCARDIS-Consortium, 2004) but not other studied populations (Clarke et al., 2006), suggesting a population-dependent effect. Following the results of their initial study, Ozaki *et al.* used an *E. coli* two-hybrid system and phage display to show that the LTA protein binds to LGALS2 (lectin, galactose-binding, soluble 2). They then found that an SNP in *LGALS2* is significantly associated with susceptibility to MI in a Japanese population, and that smooth muscle cells and macrophages in the atherosclerotic lesions express both LGALS2 and LTA (Ozaki et al., 2004). However, the association of *LGALS2* polymorphisms with MI was not confirmed in a Caucasian population (Mangino et al., 2006), which may be due to population heterogeneity. More studies are needed to address this inconsistency. More recently, prompted by the finding that binding of LTA to its receptor strongly activates NF-κB, an important regulator of pro- and anti-inflammatory genes and cell survival in atherosclerotic lesions (de Winther et al., 2005; Monaco and Paleolog, 2004), by proteasomal degradation of its inhibitory partner, IκB19, Ozaki et al tested the association of a variation in the genes encoding proteasomal proteins and the risk of MI (Mizuno et al., 2006). The study revealed that a common SNP (rs1048990) in the proteasome subunit alpha type 6 gene (*PSMA6*) confers risk of MI. The association was confirmed in a second cohort of 867 MI and 1,104 control subjects. They further found that rs1048990 affects the transcription activity of *PSMA6*, and suppression of *PSMA6* expression using siRNA reduces the activation of NF-κB. These results demonstrate that large-scale association studies can quickly identify novel disease-causing genes (such as *LTA*), and, in combination with functional genomics studies, additional disease genes can be identified (for example *PSMA6*). Later, in a large-scale association studies using 11,053 SNPs, Shiffman et al found four gene variants associated MI (*PALLD*, *KOS1*, *TAS2R50*, *OR13G1*), and the association was confirmed in two additional populations (Shiffman et al., 2005). In another large scale association study using 11,647 SNPs, these authors found that *VAMP8* and *HNRPUL1* were associated with the risk of MI, and the association was confirmed in two additional populations (Shiffman et al., 2006). The possible functions of these two genes in MI are not clear; VAMP7 may activate platelet thereby affecting MI. more recently, three different studies, in which 100K- to 500K-SNP sets and 12 different populations were used, identified SNPs at the same region (9p21.3, ≈22.1 Mb) that are associated with CAD and MI (Helgadottir et al., 2007; McPherson et al., 2007; Wellcome Trust Case Control Consortium, 2007). These SNPs are near CDKN2A and CDKN2B, two tumor suppressor genes.

Genome-wide association studies have also been used in mice to find genes affecting plasma HDL cholesterol levels (Pletcher *et al.*, 2004). The availability of dense SNP maps in more than 50 inbred mouse strains and a better understanding of mouse haplotype structures have greatly improved the feasibility and reliability of such studies.

Genome-wide/large-scale association studies can help find new atherosclerosis-regulating genes in three ways. First, the few genes in a usually very narrow associated region can be confirmed and their functions determined in separate populations. Second, an associated region

can be used to pin-point candidate genes in a coincident QTL and thereby facilitate identifying its underlying gene. Third, some QTL regions can be selected for large-scale gene association studies by testing dense SNPs in the region.

Using linkage and association studies to find atherosclerosis genes in mice and humans: expectations, limitations and future directions

Traditional models of atherosclerosis have been very useful in shedding light on this very complex disease, especially on the early stages of atherogenesis. However, we need good models for the clinical outcomes of plaque rupture, which cause thrombotic occlusions and myocardial infarction. Additionally, *Apoe*- and *Ldlr*-deficient mutants with genetic backgrounds other than C57BL/6 should be produced: the use of such strains along with newly available bioinformatics tools will likely reveal novel atherosclerosis QTL and facilitate identifying their underlying genes (Wang *et al.,* 2005b).

It is noteworthy that finding an atherosclerosis QTL gene may subsequently reveal a new pathway for atherogenesis. Even if the human ortholog of an atherosclerosis-regulating mouse gene is not a good target, other genes in the new pathways identified from genetics and genomics may be.

According to our current knowledge, two major disturbances affect the progression of atherosclerosis: dyslipidemia and inflammatory response. Identifying their genetic determinants may provide new therapies. The use of mouse models to identify genes regulating plasma lipid levels and atherosclerotic lesions has been reviewed recently (Rollins et al., 2006; Wang et al., 2005b; Wang and Paigen, 2005a; Wang and Paigen, 2005b). Two genes identified in human linkage studies of MI (*ALOX5AP* and *MEF2A*), the two in mouse linkage studies of atherosclerosis (*Alox5* and *Tnfsf4* in mice), and the two genes in human genome-wide association studies of MI (*LTA* and *PSMA6*) are all involved in immune and inflammatory response, not directly in lipid metabolism. This may not be a coincidence, because when atherosclerosis or MI is used as an end point, the genes that are historically under selective pressure are easier to find. It is conceivable that genes involved in defense against infection (rather than those involved in influencing plasma lipid and blood pressure levels) have been under greater selective pressure for human beings to reproduce and survive, therefore, their polymorphisms are more tightly associated with inflammatory diseases (such as atherosclerosis) and the associations are easier to find. This observation (that all the six genes found are involved in inflammation) also suggests that genetic determinants of inflammatory pathways account for significant part of population variance of atherosclerotic disease incidence.

Genetic linkage, candidate gene association, and genome-wide associations studies all have their advantages and limitations. Genetic linkage studies have been very successful in finding genes for diseases with simple Mendelian inheritance. However, although there are more and more successfully examples in the last few years (discussed above), finding human atherosclerosis genes from linkage studies remains difficult, and testing the usually 100–200 genes in a QTL remains a daunting task. The fact that only 3 out of the 40 QTL for the same atherosclerosis disease have been replicated, and most of the variants identified from genome-wide association studies are not in any previously found QTL, reflects the nature and complexity of atherosclerotic disease: etiological heterogeneity of the disease, many influencing genes each with very small effect, and strong influence of environmental factors (life-style, diet, social-economical status, etc). Adding to the complexity is the fact that genes affecting a trait tend to be clustered in chromosomes and they may interact with each other to cause a change in the phenotype, making positional cloning difficult. As recently shown in an elegant study by Galzalpour *et al*., a gene identified by positional cloning may not be the only

one that affects the phenotype of the QTL (Ghazalpour et al., 2006). By breaking the original Chr 6 CAST.B6 "congenic" region into 16 subregions, the authors found that, in addition to *Alox5*, two to four other genes in the original Chr 6 QTL region affect atherosclerosis. This suggests that the original congenic strain is not coisogenic, and the time-consuming process of generating subcongenic lines is actually powerful for finding the real and multiple QTL genes. "Peakwide mapping" (Wang et al., 2007), ie. doing association studies for genes in the QTL peak, as discussed above, should be useful for identifying multiple causal genes for a QTL. Using mouse-human comparative genomics approach should also increase the chance of finding human atherosclerosis genes, as demonstrated in the case of *OX40L* (Wang et al., 2005c). Candidate gene association studies are more powerful and successful in testing whether the gene of interest confers risk to atherosclerotic disease. However, only genes suspected of affecting atherosclerosis will be tested (therefore this approach is a biased), and significant association from one studies needs to be confirmed in additional studies to exclude false positives, which is often seen in candidate gene association studies. Another limitation of this approach is that the association itself does not tell whether and how the polymorphisms affect the functions of the genes to be tested, such as in the case of *ALOX5*, *ALOX5AP* and *OX40L*. Ultimately, functional studies in vitro and in vivo are needed to find the causal polymorphisms and to evaluate the gene as new therapeutic target. As another unbiased approach, the more recent genome-wide association studies have been very successful in finding unexpected candidate disease genes, including those for atherosclerotic disease. The availability of dense SNP sets will likely increase the power of this approach further. However, at this time the cost of doing this study is still high (millions of USD). More recently, the technological advances, most notably the rapid and low-cost microarray and genotyping methods, have made it possible to treat gene expression data as phenotypes and locate QTL for them; these are so-call expression QTL (eQTL). This allows quantifying the expression of tens of thousands of genes simultaneously in an unbiased way. Combining eQTL and traditional clinical QTL (cQTL; for example atherosclerosis and HDL QTL), candidate genes can be found for further testing. For example, if a cis-eQTL is found within the interval of a cQTL, the cis-eQTL gene would be good candidates for further test (Hubner et al., 2005; Yaguchi et al., 2005). Using this approach, *Abcc6* was found as a causal gene for dystrophic cardiac calcification in mice (Meng et al., 2007). We can expect that new atherosclerosis-related genes will be found with this approach in the coming years.

Linkage, candidate gene association, genome-wide association studies and gene expression analysis all have their advantages and limitations. By combining them together, we will have a better chance of defining the complex network underlying atherosclerotic disease.

Translating genome information into medication of atherosclerosis: future perspectives

Researchers are very hopeful that the sequencing of human and model organism genomes, along with the many other technological advances in biomedical research tools, will lead to dramatic improvements in cardiovascular disease therapies. Although two people may have the same MI symptoms, those symptoms may be caused by different combinations of genetic and environmental factors, and may call for different therapies. As such, finding genetic determinants for atherosclerotic disease is crucial for individualized medication. Finding these genes and knowing their functions will greatly facilitate drug target development. Some of the genes and their products may also be used as biomarkers for diagnosis, and for stratifying patients according to genetic risk factors in order to take early preventive measures.

Recently, the DeCode investigators performed a randomized, prospective, placebo-controlled, crossover phase II trial in 191 MI patients with at-risk variants of either the *ALOX5AP* or the leukotriene A_4 hydrolase gene to determine the therapeutic effects of DG-031, an ALOX5AP

inhibitor (Hakonarson et al., 2005). In patients with specific at-risk variants of the two genes in the leukotriene pathway, DG-031 effectively reduces leukotriene B_4 production by activated neutrophils and increases urinary leukotriene E_4 levels. DG-031 also suppresses three biomarkers of increased MI risk (C-reactive protein, intercellular adhesion molecule, myeloperoxidase). This trial was the first to use a gene-specific and individualized approach to treat atherosclerosis, and these preliminary results illustrate the potential to translate genomics information into novel diagnostics and treatments.

Obviously, atherosclerotic disease is very complex, and a large amount of research is required before genetics can be translated into clinical practice (Arnett et al., 2007). The genetic approaches discussed in this review will greatly accelerate the pace with which we acquire that knowledge. Together with clinical data, this knowledge will help us better understand cardiovascular pharmacogenetics and pharmacogenomics, and facilitate the development of individualized diagnosis, preventatives and treatments for atherosclerosis (Arnett et al., 2007).

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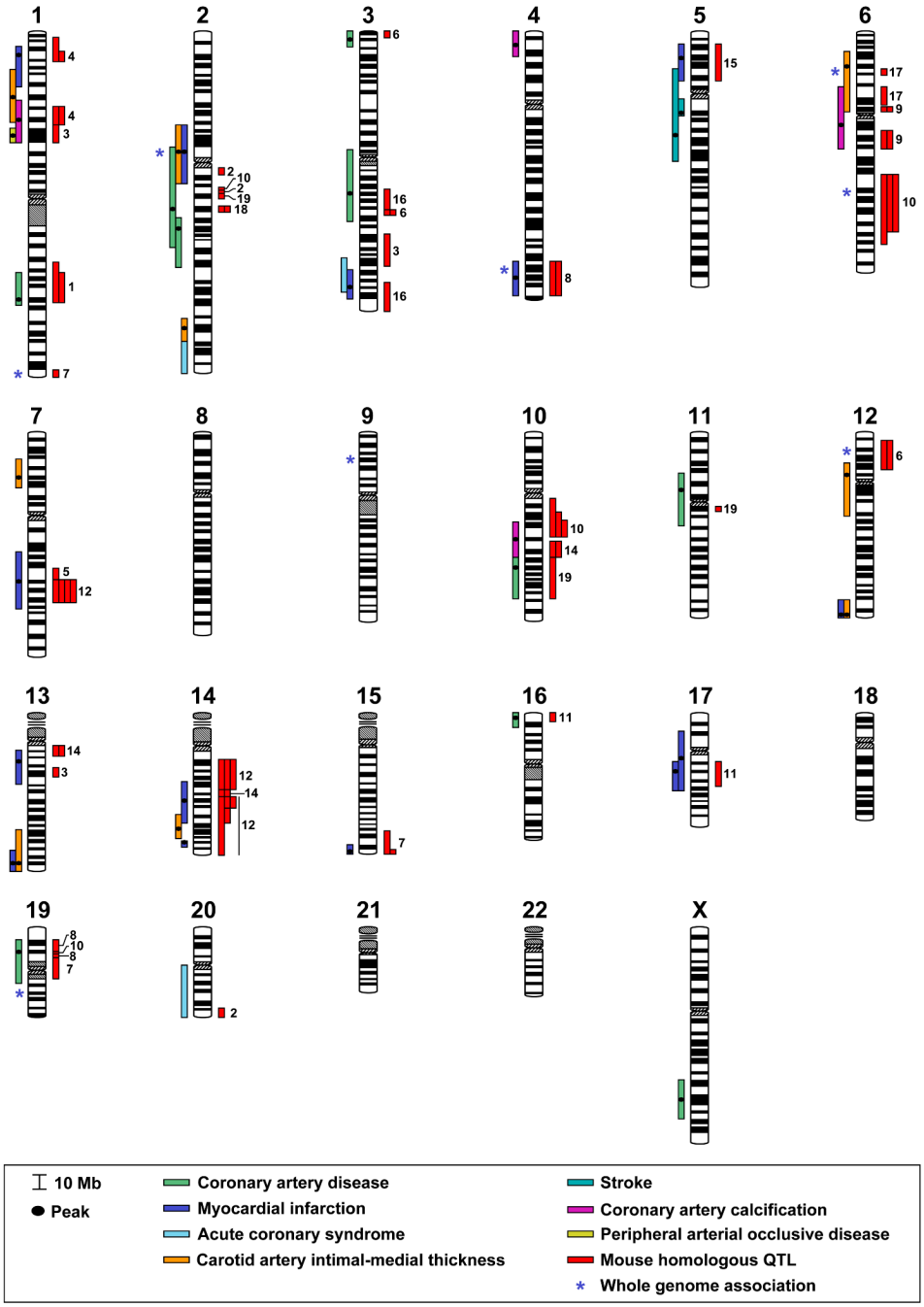

Figure 1.

Chromosome map of human and mouse concordant QTLs for atherosclerotic diseases. Human HDL-C QTLs are represented by bars to the left of each chromosome. Each bar represents a QTL from one population as shown in Table 1. QTL sizes are given either as 1.5-LOD drop intervals (if LOD score figures are available), or ± 15 cM centered around the LOD score peak (when LOD score figures are unavailable). Human homologues of mouse HDL-C QTLs are represented by red bars to the right of each chromosome, and the chromosome numbers of these mouse QTLs are to their right.

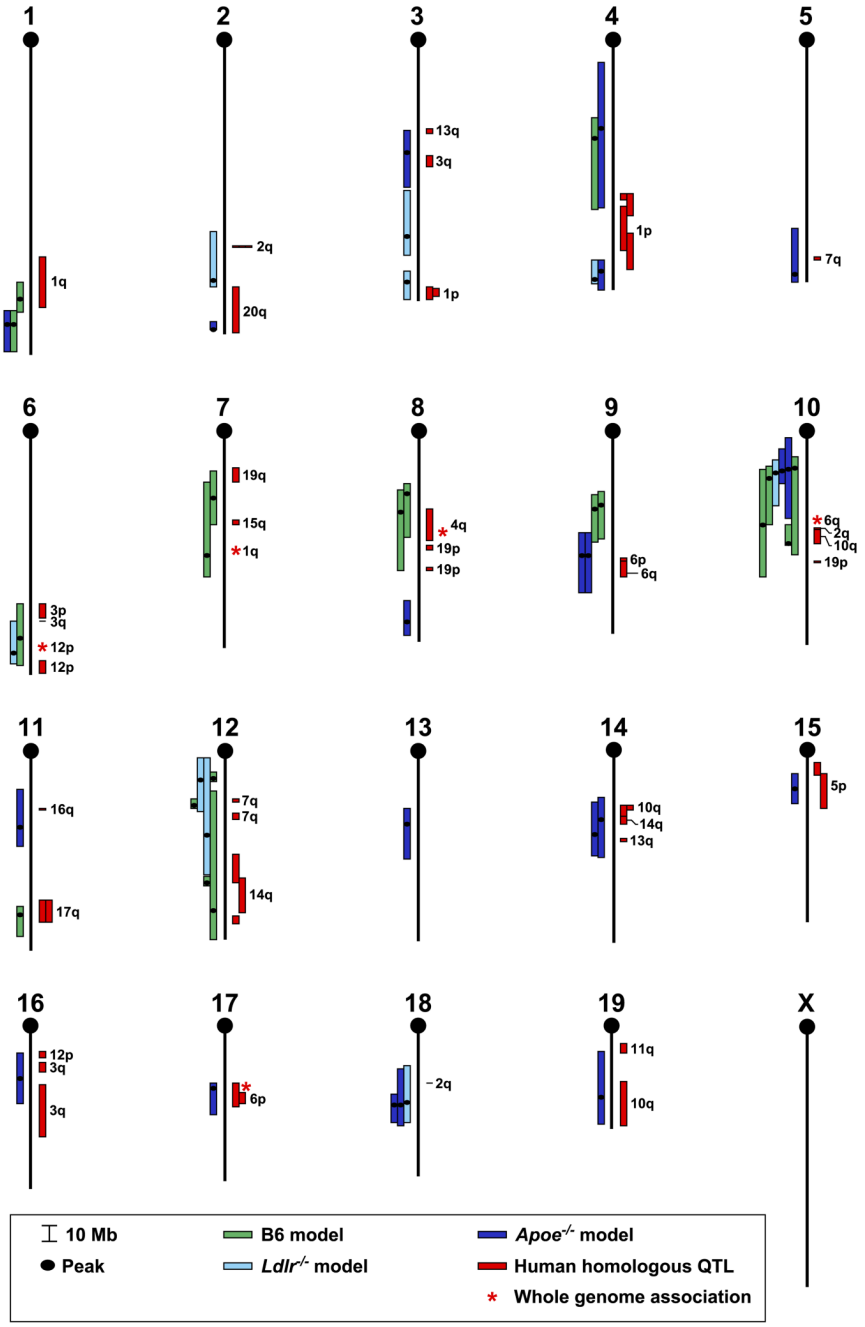

Figure 2.

Chromosome map of mouse and human concordant atherosclerosis QTLs. A vertical black line represents each chromosome, with the centromere at the top. Genetic distances in Mb from the centromere are shown by the scale at the lower left of the figure. Mouse atherosclerosis QTLs are represented by bars to the left of each chromosome. Each bar represents a QTL from one cross as shown in Table 2. QTL sizes are given either as 95% confidence intervals (CIs), 1.5-LOD drop intervals (if 95% CIs are not available but LOD score figures are available), or ± 10 cM centered around the LOD score peak (when neither CIs nor LOD score figures are available). To change cM into Mb, we found MIT markers at the cM positions in MGI (informatics.jax.org) and retrieved their Mb positions in Ensembl Mouse Genome Server NCBI

m36. Mouse homologues of human HDL-C QTLs are represented by red bars to the right of each chromosome, and the chromosome numbers of these human QTLs are to their right.

Table 1

QTLs for atherosclerotic diseases in humans

Chr	Cytogenetic band Peak (CI)[a]	Peak cM	Peak (CI) Mb[b]	Nearest Marker	LOD	Disease	Population[d]	Reference
1	1p36.13 (p36.21-34.2)	47	21 (13–41)	*D1S3669*	11.7	MI	U.S., 1613/428	(Wang et al., 2004)
	1p33 (p35.2-31.3)	76	48 (31–61)	*D1S2134*	2.4	CIMT	U.S., 1242/311	(Fox et al., 2004)
	1p31.3 (p32.3-31.1)	92	61 (52–80)	…	2.1	CAC	U.S. whites, 948/393 sibships	(Turner et al., 2005)
	1p31.1 (p31.1-31.1)	102	71 (70–76)	*D1S2895*	3.9	PAOD	Icelanders, 116/272	(Gudmundsson et al., 2002)
	1q31.1 (q24.2-32.1)	202	184 (164–200)	*D1S518*	2.2	CAD	International, 1168/438	(Hauser et al., 2004)
	1q44[f]	287	244	*OR13G1*	OR=1.2	MI	US whites, 3 case-control studies	(Shiffman et al., 2005)
2	2p11.2 (p13.3-q12.2)	103	85 (70–106)	*D2S1790*	3.3	MI	U.S., 1613/428	(Wang et al., 2004)
	2p11.2 (p13.3-q12.2)	103	85 (70–106)	*D2S1790*	1.6	CIMT	U.S., 1242/311	(Fox et al., 2004)
	2p11.2[f]	103	86	*rs1010*	OR=1.8	MI	U.S., 3 case-control studies	(Shiffman et al., 2006)
	2q14.3 (p11.2-q23.3)	134	128 (85–152)	*D2S2271*	2.7	CAD	U.K.,/1698	(Samani et al., 2005)
	2q22.1 (q21.2-24.3)	150	142 (134–168)	*D2S129*	3.2	CAD	Finnish, 364/156	(Pajukanta et al., 2000)
	2q34 (q33.3-35)	210	214 (205–221)	*D2S2944*	3.1	CIMT	Mexican Americans, 274/91	(Wang et al., 2005a)
	2q36-37.3	250	(221–243)[e]	…	2.6	ACS	61 Australian sib pairs	(Harrap et al., 2002)
3	3p26.1 (p26.3-25.3)	19	6 (0–9)	…	1.5	CAD	Europeans, 1464 sibpairs	(Farrall et al., 2006)
	3q13.31 (p12.1-q22.1)	140	119 (86–134)	*D3S2460*	3.5	CAD	International, 1168/438	(Hauser et al., 2004)
	3q27.2 (q26.31-28)	199	187 (175–191)	*D3S1571-3686*	2.4	MI	Indo-Mauritians, 535/99	(Francke et al., 2001)
	3q26-27	…	(162–190)[e]	…	1.8	ACS	61 Australian sib pairs	(Harrap et al., 2002)
4	1p16.1 (p16.3-15.33)	13	7 (0–15)	…	2.0	CAC	U.S. whites, 948/393 sibships	(Turner et al., 2005)
	4q32.3[f]	170	170	*PALLADIN*	OR=1.3	MI	US whites, 3 case-control studies	(Shiffman et al., 2005)
	4q34.1 (q32.2-35.1)	176	175 (162–185)	*D4S2431*	4.4	MI	U.S., 1613/428	(Wang et al., 2004)
5	5p14.3 (p15.2-13.2)	36	22 (10–34)	*D5S2845*	3.7	MI	U.S., 1613/428	(Wang et al., 2004)
	5q12.1 (q11.2-12.1)	69	59 (57–61)	*D5S2080*	4.4	Stroke	Icelanders, 914/179	(Gretarsdottir et al., 2002)
	5q13.3 (p13.3-q14.3)	82	76 (33–83)	*D5S424*	2.0	Stroke	Northern Swedish, 117/56	(Nilsson-Ardnor et al., 2005)
6	6p22.3-21.1[g]		(15–46)			CAD/MI		(Zintzaras and Kitsios, 2006)
	6p22.1 (p22.3-12)	44	29 (19–65)	*D6S1022*	2.2	CIMT	Mexican Americans, 274/91	(Wang et al., 2005a)
	6p21.33[f]	45	32	*LTA*	OR=1.8	MI	Japanese, 1133 MI, 1006 controls	(Ozaki et al., 2002)
	6q12 (p21.1-q14.3)	83	72 (42–86)	*D6S1053*	2.2	CAC	U.S. whites, 94/29 sibships	(Lange et al., 2002)
	6q22.1[f]	169	118	*ROS1*	OR=1.2	MI	US whites, 3 case-control studies	(Shiffman et al., 2005)
7	7p14.3 (p15.3-14.1)	50	31 (22–43)	*D7S817*	1.6	CIMT	U.S., 1242/311	(Fox et al., 2004)
	7q22.2 (q21.12-31.33)	114	104 (87–124)	*D7S1799*	3.6	MI	U.S., 1613/428	(Wang et al., 2004)
8	8q13.2-22.2[g]		(68–99)			CAD/MI		(Zintzaras and Kitsios, 2006)
	8q24.21-24.3[g]		(127–140)			CAD/MI		(Zintzaras and Kitsios, 2006)
9				*Rs10757274*	OR=1.2–1.9	CAD/MI	10 different populations	(Helgadottir et al., 2007; McPherson et al., 2007; Wellcome Trust Case Control Consortium, 2007)
	9p21.3	38	22					
10	10q22.2 (q21.3-23.2)	95	77 (64–88)	*D10S1432*	3.2	CAC	U.S. whites, 94/29 sibships	(Lange et al., 2002)

Chr	Cytogenetic band Peak (CI)[a]	Peak cM	Peak (CI) Mb[b]	Nearest Marker	LOD	Disease	Population[d]	Reference
	10q23.33 (q23.2-26.11)	116	95 (86–119)	*D10S185*	2.1	CAD	Indo-Mauritians, 535/99	(Francke et al., 2001)
11	11p11.2 (p13-q13.1)	66	43 (35–63)	...	1.7	CAD	Europeans, 1464 sibpairs	(Farrall et al., 2006)
12	12p13.2[f]	22	11	*TAS2R50*	OR=1.3	MI	US whites, 3 case-control studies	(Shiffman et al., 2005)
	12q12 (p12.1-q13.2)	56	42 (21–55)	*D12S1301*	1.7	CIMT	U.S., 1242/311	(Fox et al., 2004)
	12q24.33 (q24.31-24.33)	161	129 (124–132)	*D12S1045*	4.4	MI	U.S., 1613/428	(Wang et al., 2004)
	12q24.33 (q24.31-24.33)	161	129 (124–132)	*D12S1045*	4.1	CIMT	U.S., 1242/311	(Fox et al., 2004)
13	13q12.3 (q12.12-14.11)	24	30 (23–42)	*D13S289*	2.5	MI	Icelanders, 713/296	(Helgadottir et al., 2004)
	13q33.3 (q31.1-34)	94	107 (80–109)	*D13S796*	1.3	CIMT	Mexican Americans, 274/91	(Wang et al., 2005a)
	13q33.3 (q32.3-34)	94	107 (98–110)	*D13S796*	3.6	MI	U.S., 1613/428	(Wang et al., 2004)
14	14p13-q13.1[g]		(0–32)			CAD/MI		(Zintzaras and Kitsios, 2006)
	14q23.1 (q21.3-24.3)	67	60 (49–73)	*D14S592*	4.2	MI	U.S., 1613/428	(Wang et al., 2004)
	14q31.2 (q24.2-32.12)	92	83 (70–92)	*D14S606*	1.8	CIMT	U.S., 1242/311	(Fox et al., 2004)
	14q32.2 (q32.2-32.31)	126	100 (98–101)	*D14S1426*	3.9	MI	Germans, 1406/513	(Broeckel et al., 2002)
15	15q26.3 (q26.2-26.3)	113	97 (96–99)	*D15S120*	4.2	CAD/MI	U.S., 21/1	(Wang et al., 2003a)
16	16p13.3 (p13.3-13.2)	9	4 (0–8)	*D16S3027*	3.1	CAD	Indo-Mauritians, 535/99	(Francke et al., 2001)
17	17q11.2 (p12-q22)	51	23 (14–50)	...	2.9	MI	Europeans, 739 sibpairs	(Farrall et al., 2006)
	17q21.2 (q12-22)	67	36 (32–50)	...	1.6	MI	Europeans, 344 sibpairs	(Farrall et al., 2006)
19	19p13.12 (p13.2-q13.11)	44	16 (9–40)	*D19S252*	1.7	CAD	International, 1168/438	(Hauser et al., 2004)
	19q13.2[f]	65	47	*Rs11881940*	OR=1.9	MI	U.S., 3 case-control studies	(Shiffman et al., 2006)
20	20q11-13	...	(28–62)[e]	...	1.6	ACS	61 Australian sib pairs	(Harrap et al., 2002)
X	Xq25 (q23-26)	82	129 (109–137)	*DX1047*	3.5	CAD	Finnish, 364/156	(Pajukanta et al., 2000)

[a] Peak cytogenetic bands were retrieved from Ensembl Human NCBI 35, version 37.35j (accessed 3-1-06), according to the positions of the peak markers. Confidence intervals were determined either by the 1.5-LOD drop method when LOD score curves and markers were available, found in LOD score tables, or by using ± 15 cM around the peak marker in the absence of the above information.

[b] Mb: based on Ensembl Human NCBI 35, version 37.35j (accessed 3-1-06).

[c] Mouse homologous chromosome regions of the human QTLs were determined as described in the text. Peak position is listed first, followed by regions arranged by chromosome number.

[d] Number of individuals/families.

[e] Intervals estimated according to positions of cytogenetic bands.

[f] Detected in genome-wide association studies.

[g] Results from Meta-analysis

ACS, acute coronary syndrome (MI+unstable angina); CAC, coronary artery calcification; CAD, coronary artery disease; CIMT, carotid artery intimal-medial thickness; MI, myocardial infarction; PAOD, peripheral arterial occlusive disease.

Table 2

Quantitative trait loci for atherosclerosis in inbred mouse crosses

Chr	Cross[a]	marker[b]	cM (CI)[c]	Mb (CI)[d]	LOD[e]	locus	Reference
1	B × H and C × B RI lines	*D1Mit159*	82	162 (151–170)	…	Ath1	(Paigen et al., 1987c; Phelan et al., 2002)
	(B6 × A) F2	1–169 Mb	100	178 (169–195)	3.4		Ishimori et al, unpublished
	(B6.129-*Apoe*$^{-/-}$ × FVB.129-*Apoe*$^{-/-}$) F2	*D1Mit359*	100	178(169–195)	3.3	Ath9	(Dansky et al., 2002)
2	(PERA × B6.129-*Ldlr*$^{-/-}$) × B6.129-*Ldlr*$^{-/-}$	*D2Mit405*	69	148 (117–152)	2.8	Athla1	(Bretschger Seidelmann et al., 2005)
	(AKR.129-*Apoe*$^{-/-}$ × DBA/2.129-*Apoe*$^{-/-}$) RpF2	*rs13476938*	107	179 (174–179)	3.3	Ath28	(Smith et al., 2006)
3	(AKR.129-*Apoe*$^{-/-}$ × DBA/2.129-*Apoe*$^{-/-}$) RpF2	*rs13477166*	33	67 (53–89)	2.7	Ath23	(Smith et al., 2006)
	(B6.129-*Ldlr*$^{-/-}$ × FVB.129-*Ldlr* $^{-/-}$) F2	*D3Mit57*	55	119 (90–131)	4.1	Ascla4	(Teupser et al., 2006)
	(FVB.129-*Ldlr*$^{-/-}$ × B6.129-*Ldlr*$^{-/-}$) F2	*D3Mit45*	79	148 (141–160)	3.3	Ascla3	(Teupser et al., 2006)
4	(B6.129-*Apoe*$^{-/-}$ × C3H.129-*Apoe*$^{-/-}$) F2	*D4Mit111*	22 (3–50)	52 (10–102)	3.0		(Wang et al., 2003b)
	(SM × NZB) F2	*D4Mit44*	29 (18–51)	59 (46–104)	3.6	Ath8	(Korstanje et al., 2004)
	(A × B6.129-*Apoe*$^{-/-}$) F2	4–135 Mb	76	143 (136–154)	4.6		Ishimori et al, unpublished
	(MOLF × B6-*Ldlr*$^{-/-}$) × B6-*Ldlr*$^{-/-}$	*D4Mit127*	78 (66–81)	149 (136–151)	6.2	Athsq1	(Welch et al., 2001)
5	(AKR.129-*Apoe*$^{-/-}$ × DBA/2.129-*Apoe*$^{-/-}$) RpF2	*rs13478585*	84	147 (130–139)	3.4	Ath24	(Smith et al., 2006)
6	(CAST × B6) F2	*D6Mit256*	61 (46–71)	127 (105–144)	6.7	Artles	(Mehrabian et al., 2001)
	(MOLF × B6-*Ldlr*$^{-/-}$) × B6-*Ldlr*$^{-/-}$	*D6Mit110*	64 (52–70)	136 (115–143)	6.7	Athsq2	(Welch et al., 2001)
7	(B6 × DBA/2) F2	*D7Mit193*	25	51 (33–68)	3.7	Aorls2	(Colinayo et al., 2003)
	A × B and B × A RI lines	*Tyr*	44 (20–50)	87 (41–100)	…	Ath3	(Stewart-Phillips et al., 1989)
8	(B6 × A) F2	8–54 Mb	22	36 (31–67)	2.8		Ishimori et al, unpublished
	(B6 × DBA/2) F2	*D8Mit41*	29	49 (39–88)	3.4		(Colinayo et al., 2003)
	(A × B6.129-*Apoe*$^{-/-}$) F2	8–109 Mb	60	120 (106–129)	2.0		Ishimori et al, unpublished
9	(B6 × A) F2	9–48 Mb	25	43 (34–63)	4.1		Ishimori et al, unpublished
	SWR (SWR × SJL)	*D9Mit330*	26	45 (36–66)			Svenson et al, unpublished
	(A × B6.129-*Apoe*$^{-/-}$) F2	9–64 Mb	42	74 (60–98)	3.7		Ishimori et al, unpublished
	(B6.129-*Apoe*$^{-/-}$ × C3H.129-*Apoe*$^{-/-}$) F2	*D9Mit156*	42	74 (60–98)	4.1	Ath22	(Su et al., 2006)
10	(B6 × 129) F2	*D10Mit213*	10 (5–40)	19 (12–74)	…	Ath20[g]	(Ishimori et al., 2004)
	(B6.129-*Apoe*$^{-/-}$ × FVB.129-*Apoe*$^{-/-}$) F2	*D10Mit213*	11 (0–29)	20 (0–51)	5.1	Ath11	(Dansky et al., 2002)
	(A × B6.129-*Apoe*$^{-/-}$) F2	10–27 Mb	12	22 (7–29)	2.8		Ishimori et al, unpublished
	(B6.129-*Ldlr* $^{-/-}$ × FVB.129-*Ldlr* $^{-/-}$) RpF2	*D10Mit16*	16	21 (14–43)	13.1	Ascla1	(Teupser et al., 2006)
	(B6 × A) F2	10–27 Mb	20	26 (18–55)	3.9		Ishimori et al, unpublished
	(B6 × DBA/2) F2	*D10Mit42*	30 (14–45)	55 (20–88)	4.5	Aorls1	(Colinayo et al., 2003)
	(B6 × 129) F2	*D10Mit31*	34 (30–36)	67 (55–68)	6.6	Ath17	(Ishimori et al., 2004)
11	(B6.129-*Apoe* $^{-/-}$ × C3H.129-*Apoe* $^{-/-}$) F2	*D11Mit236*	20	44 (20–56)	2.4		(Su et al., 2006)
	(B6 × 129) F2	*D11Mit333*	60 (55–70)	99 (94–113)	…	Ath19[g]	(Ishimori et al., 2004)
12	(B6-*db*/*db* × BKS) F2	*D12Mit49*	3 (2–4)	13 (9–15)	2.5	Ath6	(Mu et al., 1999)
	(B6.129-*Ldlr*$^{-/-}$ × FVB.129-*Ldlr*$^{-/-}$) RpF2	*D12Mit82*	3	13 (0–39)	3.9	Ascla5	(Teupser et al., 2006)
	(B6 × 129) F2	*D12Mit243*	16 (13–17)	36 (32–38)	3.7	Ath18	(Ishimori et al., 2004)
	(B6.129-*Ldlr*$^{-/-}$ × FVB.129-*Ldlr*$^{-/-}$) RpF2	*D12Mit189*	24 (0–34)	55 (0–79)	4.8	Ascla6	(Teupser et al., 2006)
	SWR × (SWR × SJL), SWR × SJL RI	*D12Mit158*	38 (35–40)	85 (81–86)		Ath7	Svenson et al, unpublished
	(B6 × 129) F2	*D12Mit7*	50 (10–70)	103 (27–119)	…	Ath21[g]	(Ishimori et al., 2004)

Chr	Cross[a]	marker[b]	cM (CI)[c]	Mb (CI)[d]	LOD[e]	locus	Reference
13	(AKR.129-*Apoe*$^{-/-}$ × DBA/2.129-*Apoe*$^{-/-}$) RpF2	*rs13481782*	26	43 (33–67)	2.8	Ath25	(Smith et al., 2006)
14	(B6.129-*Apoe*$^{-/-}$ × FVB.129-*Apoe*$^{-/-}$) F2	*D14Mit60*	15 (10–40)	45 (28–70)	2.5	Ath13	(Dansky et al., 2002)
	(A × B6.129-*Apoe*$^{-/-}$) F2	14–52 Mb	22	55 (31–69)	2.6		Ishimori et al, unpublished
15	(AKR.129-*Apoe*$^{-/-}$ × DBA/2.129-*Apoe*$^{-/-}$) RpF2	rs13482467	14	20 (10–29)	3.3	Ath22	(Smith et al., 2006)
16	(B6.129-*Apoe*$^{-/-}$ × FVB.129-*Apoe*$^{-/-}$) F2	*D16Mit103*	22	29 (13–45)	2.5		(Dansky et al., 2002)
17	(AKR.129-*Apoe*$^{-/-}$ × DBA/2.129-*Apoe*$^{-/-}$) RpF2	*rs13482966*	20	36 (33–54)	4.3	Ath26	(Smith et al., 2006)
18	(B6.129-*Ldlr*$^{-/-}$ × FVB.129-*Ldlr*$^{-/-}$) F2	*D18Mit23*	21	44 (21–57)	3.1		(Teupser and Breslow, 2004)
	(A × B6.129-*Apoe*$^{-/-}$) F2	18–52 Mb	22	46 (23–59)	2.5		Ishimori et al, unpublished
	(AKR.129-*Apoe*$^{-/-}$ × DBA/2.129-*Apoe*$^{-/-}$) RpF2	rs13483316	22	41 (39–57)	3.6	Ath27	(Smith et al., 2006)
19	(B6.129-*Apoe*$^{-/-}$ × FVB.129-*Apoe*$^{-/-}$) F2	*D19Mit120*	41 (6–54)	42 (12–58)	3.8	Ath16	(Dansky et al., 2002)
?	A × B RI, B × A RI				…	Ath2	(Paigen, 1995)

[a] RI, recombinant inbred strains; F2, intercross; RpF2, reciprocal F2—both strains were used as female and male parental strains, and the F2s were combined for analysis.

[b] Peak marker. The non-MIT markers are all SNP markers with their positions (for example, 1–169 Mb means a SNP on Chr 1, at 169 Mb).

[c] cM, peak centimorgan. Genetic distances were retrieved from MGI at http://www.informatics.jax.org. CI: 95% confidence interval.

[d] Mb, megabase, derived from the physical positions (Ensembl NCBI m36, Gene build April 2006) of the markers at the corresponding cM position. In the absence of information on CI in cM, peak ± 10 cM is used instead.

[e] LOD, logarithm of the odds ratio