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REVIEW ARTICLE
Identifying Novel Genes for Atherosclerosis through Mouse-Human Comparative Genetics

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Susceptibility to atherosclerosis is determined by both environmental and genetic factors. Its genetic determinants have been studied by use of quantitative-trait–locus (QTL) analysis. So far, 21 atherosclerosis QTLs have been identified in the mouse: 7 in a high-fat–diet model only, 9 in a sensitized model (apolipoprotein E– or LDL [low-density lipoprotein] receptor–deficient mice) only, and 5 in both models, suggesting that different gene sets operate in each model and that a subset operates in both. Among the 27 human atherosclerosis QTLs reported, 17 (63%) are located in regions homologous (concordant) to mouse QTLs, suggesting that these mouse and human atherosclerosis QTLs have the same underlying genes. Therefore, genes regulating human atherosclerosis will be found most efficiently by first finding their orthologs in concordant mouse QTLs. Novel mouse QTL genes will be found most efficiently by using a combination of the following strategies: identifying QTLs in new crosses performed with previously unused parental strains; inducing mutations in large-scale, high-throughput mutagenesis screens; and using new genomic and bioinformatics tools. Once QTL genes are identified in mice, they can be tested in human association studies for their relevance in human atherosclerotic disease.

Introduction

Atherosclerosis is the pathological basis of ischemic coronary artery disease (CAD), the leading cause of death in industrialized nations. It is characterized by accumulation of plasma lipids, fibrous tissues, and cell components (mostly macrophages, smooth-muscle cells, and lymphocytes) in large arteries. Atherosclerosis is a complex disease caused by both genetic (with ~50% heritability) and environmental (mostly diet) factors. The underlying genes have been studied through genotype- and phenotype-driven approaches. In the genotype-driven approach, a gene suspected of contributing to atherosclerosis is tested for a pathological role either in atherogenesis, by use of transgenic/knockout animal models, or in rare Mendelian forms of human atherosclerosis. Studies with mouse models have identified >80 atherosclerosis-regulating genes, and some of their human orthologs have confirmed polymorphisms associated with human atherosclerotic diseases, including genes encoding the angiotensin-converting enzyme (*ACE* [MIM 106180]); adrenergic receptor, beta 2 (*ADRB2* [MIM 109690]); 5-lipoxygenase–activating protein (*ALOX5AP* [MIM 603700]); apolipoprotein E (*APOE* [MIM 107741]); coagulation factor VII (*F7* [MIM 227500]); fibrinogen, B beta polypeptide (*FGB* [MIM 134830]); glycoprotein 1b, alpha polypeptide (*GP1BA* [MIM 606672]); matrix metalloproteinase 3 (*MMP3* [MIM 185250]); matrix metalloproteinase 9 (*MMP9* [MIM 120361]); 5,10-methylenetetrahydrofolate reductase (*MTHFR* [MIM 607093]); nitric oxide synthase 3, endothelial cell (*NOS3* [MIM 163729]); serum paraoxonase (*PON1* [MIM 168820]); transforming growth factor, beta 1 (*TGFB1* [MIM 190180]); thrombospondin 2 (*THBS2* [MIM 188061]); thrombospondin 4 (*THBS4* [MIM 600715]); toll-like receptor 4 (*TLR4* [MIM 603030]) (Agah and Topol 2002; Humphries and Morgan 2004; Lusis et al. 2004; Manolio et al. 2004; McCarthy et al. 2004); and tumor necrosis factor (ligand) superfamily, member 4 (*TNFSF4* [MIM 603594]) (Wang et al. 2005*b*). In the phenotype-driven approach, no assumption is made as to which genes cause a disease phenotype, and atherosclerosis is linked to chromosome locations, QTLs, in a genomewide scan. This approach has the advantage of finding novel atherosclerosis-susceptibility genes. Two reviews summarize some of these QTLs (Allayee et al. 2003; Smith 2003).

Here, we present an updated review of atherosclerosis QTLs found in mice and humans, compare atherosclerosis QTLs found in the two most commonly used

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mouse models (diet-induced and sensitized), and comment on their concordance with human atherosclerosis QTLs. Finally, we propose future strategies for finding novel genes regulating human atherosclerosis.

QTLs for Atherosclerotic Diseases in Humans

So far, nine genomewide scans have been reported for human atherosclerotic diseases, including myocardial infarction (MI), CAD, acute coronary syndrome (ACS; acute MI and unstable angina), and carotid artery intimal-medial thickness (CIMT) (table 1). Thirty-one individual QTLs (those found in different populations, regardless of whether they are at the same chromosomal locations) have been identified. They are distributed on 27 chromosome locations, and four of them—on chromosomes 2, 3, 12, and 13—are found in two populations, so there are 27 unique QTLs (fig. 1). Six genes that have confirmed association with the risk of atherosclerotic diseases in at least two independent populations lie in 6 of the 27 QTLs. Analyses consistently indicate that *ALOX5AP* underlies the MI and stroke QTL on proximal chromosome 13 (Ben-Asher and Lancet 2004; Helgadottir et al. 2004; Lohmussaar et al. 2005). The R353Q polymorphism in *F7* is significantly associated with MI (McCarthy et al. 2004), so it is a candidate for the MI QTL on distal chromosome 13q. The C-1252T polymorphism in the promoter region of *MMP9* is associated with the size of complicated lesions and the stenosis of coronary arteries (Zhang et al. 1999; Pollanen et al. 2001), suggesting that it is the gene underlying the ACS QTL on chromosome 20q. The *C677T* SNP in *MTHFR* is significantly associated with CIMT in a Chinese population and a Japanese population (Humphries and Morgan 2004), making it a candidate for the top chromosome 1p QTL. More than two dozen studies indicate that the Q192R polymorphism of *PON1* underlies the CAD QTL on chromosome 7q (Durrington et al. 2001; Agah and Topol 2002), although a recent meta-analysis revealed only a weak relative risk of 1.12 (95% CI 1.07–1.16) (Wheeler et al. 2004). Association studies in two groups of patients with MI revealed that a polymorphism in the first intron of *TNFSF4* is associated with the risk of MI and CAD, suggesting that it underlies the CAD QTL on chromosome 1q (Wang et al. 2005*b*). In addition, it is highly likely that *MEF2A* (encoding myocyte enhancer factor 2A [MIM 600660]) is the gene underlying the chromosome 15q MI QTL, because there is a 7-aa deletion in MEF2A in all 13 of the patients with MI but not from the 8 normal individuals from a family (Wang et al. 2003*a*), and three novel mutations in exon 7 of *MEF2A* are present in 4 of the 207 study patients with CAD/MI but not in 191 control subjects (Bhagavatula et al. 2004).

Of the >80 genes shown to influence atherosclerosis in either gene-knockout or transgenic mouse models—and at least 17 genes having confirmed polymorphisms associated with human atherosclerotic diseases—only about a quarter of them fall within human atherosclerosis QTLs, as indicated in fig. 1. Half of the human atherosclerosis QTLs have no obvious candidate genes, which suggests that their genetic determinants are unknown.

QTLs for Atherosclerosis in Mice

Atherosclerosis QTLs in mice have been identified in two models: (1) a high-fat–diet model, in which a diet containing 15% fat, 1.25% cholesterol, and 0.5% cholic acid is used to induce atherosclerosis, and (2) the sensitized model, in which mice deficient in *Apoe*—or low-density lipoprotein receptor (*Ldlr*)—are used (Jawien et al. 2004; Meir and Leitersdorf 2004). *Apoe*-deficient mice develop atherosclerosis spontaneously when fed a chow diet; their lesions develop faster when they are fed a Western diet (containing 21% fat and 0.15% cholesterol) and more so when they are fed the high-fat diet described above. *Ldlr*-deficient mice also develop atherosclerotic lesions when fed the chow diet, but the lesions are very small and take considerable time to develop; feeding these mice a Western diet or the high-fat diet described above accelerates their lesion formation.

A total of 15 studies using mouse models of atherosclerosis detected 35 individual QTLs. Among them, there are 21 unique QTLs (QTLs identified in different crosses but at the same chromosomal location are counted as one unique QTL because they may have the same underlying gene [table 2 and fig. 2]). Of the 21 QTLs, 7 are revealed only in the high-fat–diet model, 9 only in the sensitized model, and 5 in both models (fig. 3). Of the 21 QTLs, 12 are replicated in more than one cross, which enables the use of haplotype analysis to narrow these QTLs (Wang and Paigen 2005*b*). The causal genes underlying 2 of the 21 mouse atherosclerosis QTLs have been identified: *Tnfsf4* (*Ox40l*) for *Ath1* (atherosclerosis susceptibility 1) on chromosome 1 (Wang et al. 2005*b*) and *Alox5* for *Artles* on chromosome 6 (Mehrabian et al. 2002). Additionally, *Angptl3* is probably one gene underlying a complex locus, *Ath8,* on chromosome 4, which may contain more than one QTL (Korstanje et al. 2004).

Concordance of Human and Mouse Atherosclerosis QTLs

The term "concordance" refers to the fact that QTLs for the same trait but from different species map to homologous genomic regions. It was first used to describe hypertension QTLs that map to homologous regions in rats, mice, and humans (Stoll et al. 2000; Sugiyama et

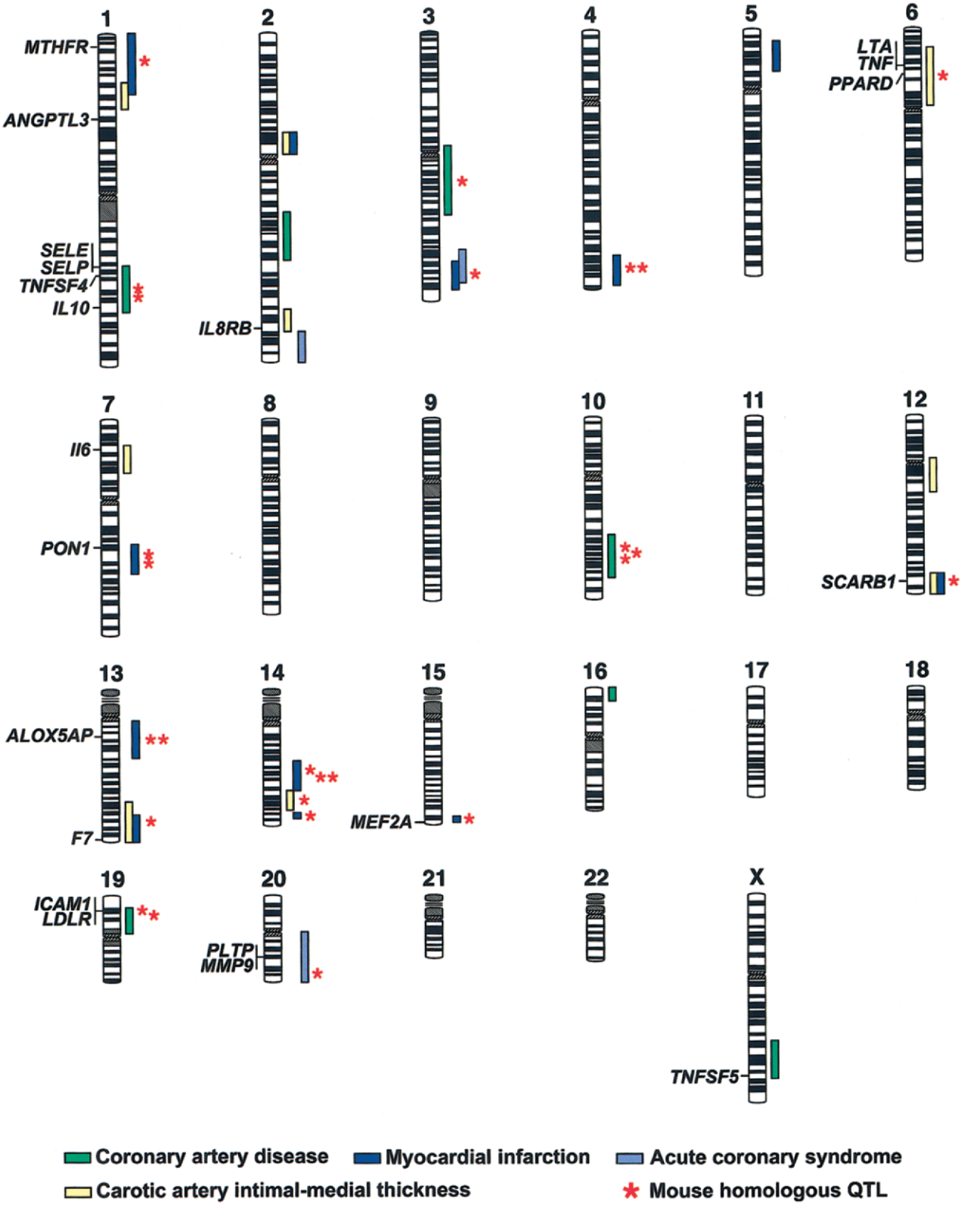

Figure 1 Chromosome map of human QTLs for atherosclerotic diseases. Chromosomes are drawn to scale, on the basis of the length of each from the Ensembl Human Genome Browser. Each bar to the right of a chromosome represents a human atherosclerosis QTL from one population (see table 1). The size of the QTL is determined either as a 1.5-LOD CI or as ±10 Mb on either side of the peak marker when the information on CI is unavailable. Candidate genes are listed to the left of the chromosomes. Human homologous regions of mouse QTLs that fall within the human QTLs are represented by red stars.

Table 1

QTLs for Atherosclerotic Diseases in Humans

Chromosome and Cytogenetic Band Peak[a] (95% CI[b])	Peak (cM)[c]	Peak, in Mb[d] (95% CI[b])	Nearest Marker	LOD	Mouse Homologous Regions[e] and Chromosome Peak, in cM (95% CI)	Disease	Population[f]	Reference
1:								
1p36.1 (p36-34)	47	21 (0–41)	*D1S3669*	11.7	4–66 (50–83)[g]	MI	U.S., 1,613/428	Wang et al. 2004*a*
1p33 (p34.3-32.2)	76	48	*D1S2134*	2.4	4–55 (49–61)	CIMT	U.S., 1,242/311	Fox et al. 2004
1q31.1 (q24.2-1q32.1)	202	184 (164–200)	*D1S518*	2.2	1–82 (69–85)[g]	CAD	International, 1,168/438	Hauser et al. 2004
2:								
2p11.2 (p12-11.2)	103	85	*D2S1790*	3.3	6–31	MI	U.S., 1,613/428	Wang et al. 2004*a*
2p11.2 (p12-11.2)	103	85	*D2S1790*	1.6	6–31	CIMT	U.S., 1,242/311	Fox et al. 2004
2q22.1 (q21.2-24.3)	150	142 (134–168)	*D2S129*	3.2	2–34 (30–38), 1–67	CAD	Finnish, 364/156	Pajukanta et al. 2000
2q34 (q33.3-35)	210	214 (205–221)	*D2S2944*	3.1	1–36 (30–44)	CIMT	Mexican Americans, 274/91	Wang et al. 2005*a*
2q36-37.3	250	…	…	2.6	1- (41–59)	ACS	61 Australian sib pairs	Harrap et al. 2002
3:								
3q13.31 (p12.1-3q22.1)	140	119 (86–134)	*D3S2460*	3.5	16–23 (21–44)[g], 6–38, 9–56	CAD	International, 1,168/438	Hauser et al. 2004
3q27.2 (q26.31-28)	199	187 (175–191)	*D3S1571-3686*	2.4	16–14 (14–22)[g], 3–13	MI	Indo-Mauritian, 535/99	Francke et al. 2001
3q26-27	…	…	…	1.8	16- (14–21)[g], 3- (11–15)	ACS	61 Australian sib pairs	Harrap et al. 2002
4:								
4q34.1 (q32.3-35.1)	176	175	*D4S2431*	4.4	8–31[g]	MI	U.S., 1,613/428	Wang et al. 2004*a*
5:								
5p14.3 (p15.2-13.3)	36	22	*D5S2845*	3.7	15–5	MI	U.S., 1,613/428	Wang et al. 2004*a*
6:								
6p22.1 (p22.3-12)	44	29 (19–65)	*D6S1022*	2.2	13–12 (9–28), 17- (11–30), 9–42[g]	CIMT	Mexican Americans, 274/91	Wang et al. 2005*a*
7:								
7p14.3 (p15.3-14.1)	50	31	*D7S817*	1.6	6–27 (23–29), 13–9	CIMT	U.S., 1,242/311	Fox et al. 2004
7q22.2 (q21.3-31.1)	114	104	*D7S1799*	3.6	5–78, 12–15[g]	MI	U.S., 1,613/428	Wang et al. 2004*a*
10:								
10q23.33 (q23.2-26.11)	116	95 (86–119)	*D10S185*	2.1	19–48 (35–55)[g], 14–16[g]	CAD	Indo-Mauritian, 535/99	Francke et al. 2001

12:								
12q12 (p11.21-q13.13)	56	42	*D12S1301*	1.7	15–55 (53–62)	CIMT	U.S., 1,242/311	Fox et al. 2004
12q24.33 (q24.23-24.33)	161	129	*D12S1045*	4.4	5–65, 10- (42–49)[g]	MI	U.S., 1,613/428	Wang et al. 2004*a*
12q24.33 (q24.23-24.33)	161	129	*D12S1045*	4.1	5–65, 10- (42–49)[g]	CIMT	U.S., 1,242/311	Fox et al. 2004
13:								
13q12.3 (q12.11-14.11)	24	30	*D13S289*	2.5	5–84 (82–88), 14-(20–30)[g], 3–29	MI	Iceland, 713/296	Helgadottir et al. 2004
13q33.3 (q31.1-34)	94	107 (80–109)	*D13S796*	1.3	8–2 (1–10)[g], 14- (51–68)	CIMT	Mexican Americans, 274/91	Wang et al. 2005*a*
13q33.3 (q32.1-34)	94	107	*D13S796*	3.6	8–5[g]	MI	U.S., 1,613/428	Wang et al. 2004*a*
14:								
14q23.1 (q22.1-24.2)	67	60	*D14S592*	4.2	12–31[g], 14–18[g]	MI	U.S., 1,613/428	Wang et al. 2004*a*
14q31.2 (q24.3-32.12)	92	83	*D14S606*	1.8	12–48 (37–57)[g]	CIMT	U.S., 1,242/311	Fox et al. 2004
14q32.2 (q32.2-32.31)	126	100 (98–101)	*D14S1426*	3.9	12–53 (52–57)[g]	MI	German, 1,406/513	Broeckel et al. 2002
15:								
15q26.3 (q26.2-26.3)	113	97 (96–99)	*D15S120*	4.2	7- (29–41)[g]	CAD/MI	U.S., 21/1	Wang et al. 2003*a*
16:								
16p13.3 (p13.3-13.2)	9	4 (0–8)	*D16S3027*	3.1	16–2 (1–7), 17–11 (8–16), 11–16	CAD	Indo-Mauritian, 535/99	Francke et al. 2001
19:								
19p13.12 (p13.2-12)	44	16	*D19S252*	1.7	8–34[g], 10–42[g], 17–20	CAD	International, 1,168/438	Hauser et al. 2004
20:								
20q11-13	…	…	…	1.6	2- (83–108)[g]	ACS	61 Australian sib pairs	Harrap et al. 2002
X:								
Xq25 (q23-26)	82	129 (109–137)	*DX1047*	3.5	X-16 (13–18), X-65	CAD	Finnish, 364/156	Pajukanta et al. 2000

[a] Peak cytogenetic bands were retrieved from the Ensembl database (release 26.35.1), according the positions of the peak markers.

[b] CIs 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 use of ±10 Mb around the peak marker in the absence of the above information.

[c] cM positions were retrieved from the genetic map at the Center for Medical Genetics Web site, by use of the markers named in the references.

[d] Mb are based on Ensembl release 26.35.1. CIs, in Mb, are shown only if CIs of cytogenetic bands were available from the references.

[e] Mouse homologous chromosome regions of the human cytogenetic bands were determined by use of the Mammalian Orthology Query Form (MGI). The ranges of the positions were derived either from CIs of cytogenetic bands if the data were available or from the mouse homologous regions of the peak cytogenetic bands if CIs of cytogenic bands were not available.

[f] Number of individuals/families.

[g] Mouse homologous regions of the human QTLs that fall within atherosclerosis QTLs (marker with peak LOD ± 10 cM) from mouse crosses shown in table 2.

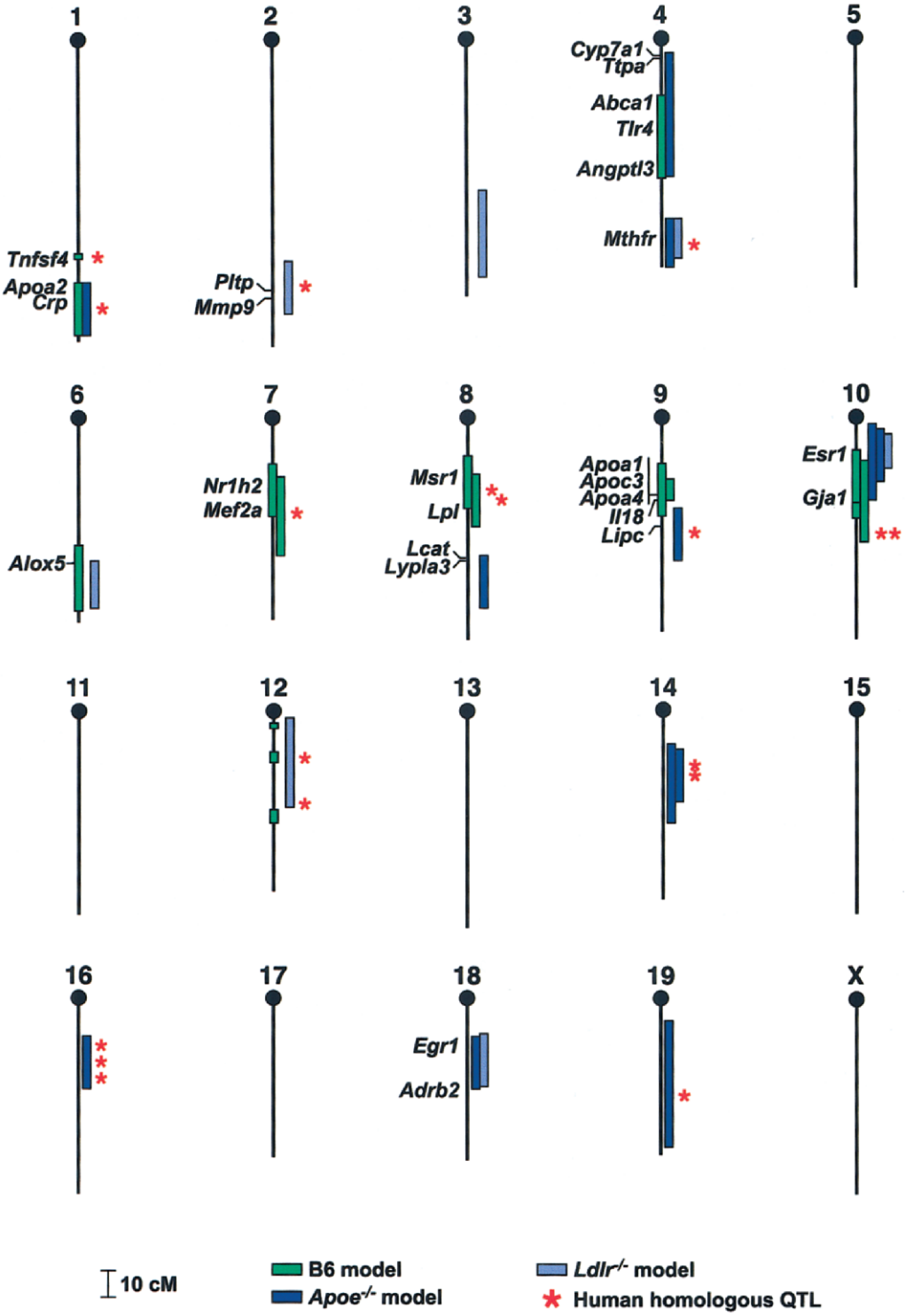

Figure 2 Chromosome map of mouse atherosclerosis QTLs. Each chromosome is represented by a vertical line (centromeres at the top). Genetic distances (in cM) from the centromere are drawn to scale (scale shown at lower left of the figure. Chromosomes are drawn to scale, on the basis of the estimated cM position of the most distally mapped locus on the Mouse Genome Informatics (MGI) Web site. Mouse atherosclerosis QTLs are represented by the bars to the right of each chromosome (interacting QTLs are not shown). Each bar represents a QTL from one cross (sharing the same parental strains, regardless of the breeding strategy) in the specified mouse model (see table 2). The size of the QTL is determined either as a 95% CI or as ±10 cM on either side of the LOD score peak when the information on CI is unavailable. Candidate genes are listed to the left of the chromosomes. Mouse homologous regions of human QTLs that fall within mouse QTLs are represented by red stars.

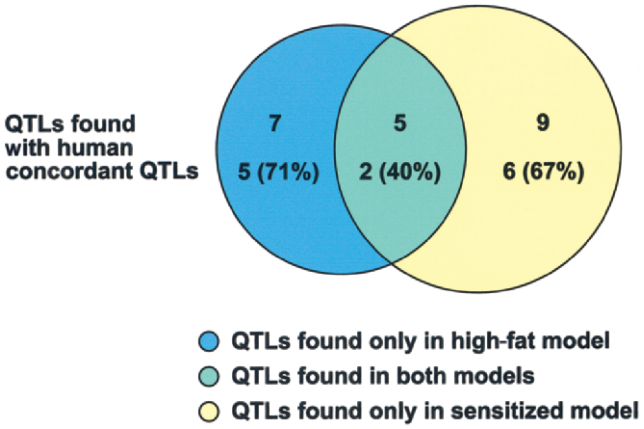

Figure 3 Comparison of mouse atherosclerosis QTLs found in high-fat and sensitized models (*Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice), shown in different colors. The number of QTLs concordant with human QTLs is also shown.

al. 2001). It has since been observed for kidney disease (Korstanje and DiPetrillo 2004) and plasma levels of high-density lipoprotein (HDL) (Wang and Paigen 2005*a*), low-density lipoprotein, and triglycerides (Wang and Paigen 2005*b*). For atherosclerosis, 13 (62%) of the 21 mouse QTLs are concordant with human QTLs, and 17 (63%) of the 27 human QTLs are concordant with mouse QTLs (figs. 1 and 2), suggesting that more than half of the mouse and human atherosclerosis QTLs have the same underlying genes. Thus, identifying them in one species should greatly facilitate identifying them in the other.

Concordant QTLs are found for five (71%) of the seven mouse atherosclerosis QTLs in the high-fat model, six (67%) of the nine in a sensitized model, and two (40%) of the five in both models (fig. 3). Altogether, more than half of the QTLs found in each model have concordant human QTLs, so it cannot be said that one model is more relevant to human atherosclerotic disease than the other. Three reasons may explain why some of the human QTLs do not have concordant mouse QTLs. First, not enough mouse crosses have been made. Second, and perhaps more important, C57BL/6 (B6) mice were used as the susceptible parental strain in 11 of the 13 crosses made so far to detect mouse atherosclerosis QTLs. Use of different susceptible strains may detect different QTLs that are concordant with human QTLs. Third, the size of atherosclerotic lesions in mice (used as the phenotype in all the QTL studies in mice so far) only partially corresponds to the incidence of human atherosclerotic diseases. In fact, the incidence of human ACS is determined not only by lesion size but also, more importantly, by plaque stability. Thus, future mouse QTL studies that include plaque stability as a phenotype may identify additional QTLs that are concordant with human atherosclerosis QTLs.

Strategies for Finding Novel Genes Regulating Human Atherosclerosis

Using the Mouse-to-Human Strategy

Finding QTL genes in mice is much more cost-effective, less time consuming, and less fraught with ethical issues than is finding them first in humans, especially now that many new genetic, genomic, and bioinformatics tools for the mouse are available (discussed in the "Integrating Traditional Genetic Approaches with New Genetic, Genomic, and Bioinformatic Resources and Tools" section). After a QTL gene is identified in the mouse, it can be tested in human association studies for its role in human diseases. The completion of the human HapMap project will greatly simplify such studies by facilitating the selection of representative SNPs of a haplotype block. In addition, dense SNPs throughout the human genome have made possible whole-genome association studies, whereby the candidacy of all the genes in human diseases are systematically studied (Ozaki et al. 2002; Carlson et al. 2004).

This mouse-to-human strategy has been successfully used to identify *POMC* (encoding pro-opiomelanocortin-alpha [MIM 176830]) as a gene influencing obesity (Krude et al. 1998; Hixson et al. 1999); *CTLA4* (encoding cytotoxic T-lymphocyte-associated protein 4 [MIM 123890]) as a gene contributing to autoimmune disorders, including Graves disease, autoimmune hypothyroidism, and type 1 diabetes (Ueda et al. 2003); and *EN2* (encoding engrailed 2 [MIM 131310]) as a gene for autism-spectrum disorder (Gharani et al. 2004). This strategy has also been used to identify an atherosclerosis-susceptibility gene in humans. *Ath1* is a QTL on chromosome 1 that makes B6 mice more susceptible to atherosclerosis than C3H mice (Paigen et al. 1987*a*, 1987*b*). By using congenic strains, we narrowed *Ath1* to a 0.66-cM region containing 11 genes (Phelan et al. 2002). Three of the 11 genes could be involved in atherosclerosis: *Prdx6* (peroxiredoxin 6 [also called "antioxidant protein 2"]) encodes an antioxidant (Steinberg and Witztum 2002), *Tnfsf4* encodes a membrane protein on lymphocytes and endothelial cells (Hansson et al. 2002), and *Fasl* (Fas ligand) is important for apoptosis (Geng 2001). After analyzing atherosclerosis susceptibility in mouse mutants for each of these genes, we found that only *Tnfsf4*-targeted mutants were significantly less susceptible to diet-induced atherosclerosis than were wild-type controls and that mice overexpressing *Tnfsf4* were more susceptible to atherosclerosis than were wild-type controls. We then tested SNPs in *TNFSF4* as well as in the nearby genes in human association studies, and we

Table 2

QTL for Atherosclerosis in Inbred Mouse Crosses

Chromosome, Locus, and Marker[a]	Peak cM (95% CI)[b]	LOD	Cross[c]	Sex[d]	High Allele (inheritance)[e]	Variance (%)	Human Ortholog Region[f]	Reference(s)
1:								
Ath1:								
D1Mit159	82	...	B × H and C × B RI lines	F	B6		1q25.1[g]	Paigen et al. 1987*b*; Phelan et al. 2002
1–169 Mb	100	3.4	(B6 × A) F_2	F	B6 (dom)		1q22-41[g]	N. Ishimori and B. Paigen, unpublished data
Ath9:								
D1Mit359	100	3.3	(B6.129-*Apoe*$^{-/-}$ × FVB.129-*Apoe*$^{-/-}$) F_2	F+M		7	1q22-41[g]	Dansky et al. 2002
2:								
D2Mit504	92	2.8	(PERA × B6.129-*Ldlr*$^{-/-}$) × B6.129-*Ldlr*$^{-/-}$)	M/	B6	16	20q11-13[g]	Seidelmann et al. 2005
3:								
D3Mit45	79 (55–88)	4.5	(B6.129-*Ldlr*$^{-/-}$ × FVB.129-*Ldlr*$^{-/-}$) F_2	F/	B6 (add)		1p13-31	Teupser and Breslow 2003
4:								
D4Mit111	22 (3–50)	3.0	(B6.129-*Apoe*$^{-/-}$ × C3H.129-*Apoe*$^{-/-}$) F_2	F	B6 (add)		9q22-33	Wang et al. 2003*b*
Ath8:								
D4Mit44	29 (18–51)	3.6	(SM × NZB) F_2	F	SM (rec)		9q22-33	Korstanje et al. 2004
4–135 Mb	76	4.6	(A × B6.129-*Apoe*$^{-/-}$) F_2	F	A (add)		1p35-36[g]	N. Ishimori and B. Paigen, unpublished data
Athsq1:								
D4Mit127	78 (66–81)	6.2	(MOLF × B6-*Ldlr*$^{-/-}$) × B6-*Ldlr*$^{-/-}$	F/	MOLF	32	1p35-36[g]	Welch et al. 2001
6:								
Artles:								
D6Mit256	61 (46–71)	6.7	(CAST × B6) F_2	F+M	B6 (rec)		12p13, 10q11, 3p25	Mehrabian et al. 2001, 2002
Athsq2:								
D6Mit110	64 (52–70)	6.7	(MOLF × B6-*Ldlr*$^{-/-}$) × B6-*Ldlr*$^{-/-}$	F, M	B6	16	12p13, 10q11, 3p25	Welch et al. 2001
7:								
D7Mit193	25	3.7	(B6 × DBA/2) F_2	F	DBA (add)	10	19q13, 11p15, 15q11, 15q26[g]	Colinayo et al. 2003
Ath3:								
Tyr	44 (20–50)	...	A × B and B × A RI lines	F+M	B6		11p15-q14, 15q24-26[g]	Stewart-Phillips et al. 1989
8:								
8–54 Mb	22	2.8	(B6 × A) F_2	F	A (add)		4q31-35[g], 8p22-23, 19p12-13[g], 13q34[g]	N. Ishimori and B. Paigen, unpublished data
D8Mit41	29	3.4	(B6 × DBA/2) F_2	F	B6 (add)	9	4q31-35[g], 8p22-23, 19p12-13[g], 16q12	Colinayo et al. 2003
8–109 Mb	60	2.0	(A × B6.129-*Apoe*$^{-/-}$) F_2	F	A (rec)		16q22-24, 1q42	N. Ishimori and B. Paigen, unpublished data
9:								
9–48 Mb	25	4.1	(B6 × A) F_2	F	B6 (add)		11q22-24, 15q21-24	N. Ishimori and B. Paigen, unpublished data
D9Mit330	26		SWR (SWR × SJL)	F	SJL		11q22-24, 15q21-24	Svenson and Paigen, unpublished data
9–64 Mb	42	3.7	(A × B6.129-*Apoe*$^{-/-}$) F_2	F	A (dom)		6p12-q13[g], 15q21-24	N. Ishimori and B. Paigen, unpublished data
10:								
Ath20[h]:								
D10Mit213	10 (5–40)	...	(B6 × 129) F_2	F	...	5	6q21-24, 10q21-22	Ishimori et al. 2004
Ath11:								
D10Mit213	11 (0–29)	5.1	(B6.129-*Apoe*$^{-/-}$ × FVB.129-*Apoe*$^{-/-}$) F_2	F, M	FVB (rec)	21	6q21-24, 10q21-22	Dansky et al. 2002

10–27 Mb	12	2.8	(A × B6.129-*Apoe*$^{-/-}$) F_2	F	A (dom)		6q21-24, 10q21-22	N. Ishimori and B. Paigen, unpublished data
D10Mit16	16 (4–17)	12.9	(B6.129-*Ldlr*$^{-/-}$ × FVB.129-*Ldlr*$^{-/-}$) F_2	F, M	FVB (rec)		6q21-24, 10q21-22	Teupser and Breslow 2003
10–27 Mb	20	3.9	(B6 × A) F_2	F	A (add)		6q21-24, 10q21-22	N. Ishimori and B. Paigen, unpublished data
Artles2:								
D10Mit42	30 (14–45)	4.5	(B6 × DBA/2) F_2	F	DBA (add)	11	6q21-22, 10q21-22, 12q24[g], 19p13[g], 21q22	Colinayo et al. 2003
Ath17:								
D10Mit31	34 (30–36)	6.6	(B6 × 129) F_2	F	129	10	6q21-22, 10q21-22, 12q24[g], 19p13[g], 21q22	Ishimori et al. 2004
11:								
Ath19[h]:								
D11Mit333	60 (55–70)	…	(B6 × 129) F_2	F	…	4	17q21-25	Ishimori et al. 2004
12:								
Ath6:								
D12Mit49	3 (2–4)	2.5	(B6-*db*/*db* × BKS) F_2	F	BKS		2p23-25	Mu et al. 1999; Purcell et al. 2001
Ath18:								
D12Mit243	16 (13–17)	3.7	(B6 × 129) F_2	F	B6 or 129	6	7q22-31[g], 2p25	Ishimori et al. 2004
D12Mit222	18 (0–34)	3.7	(B6.129-*Ldlr*$^{-/-}$ × FVB.129-*Ldlr*$^{-/-}$) F_2	F, M	B6 (add)		7q22-31[g], 2p23-25, 14q12-24[g]	Teupser and Breslow 2003
Ath7:								
D12Mit158	38 (35–40)		SWR × (SWR × SJL), SWR × SJL RI	F	SWR		14q23-31[g]	Svenson and Paigen, unpublished data
Ath21[h]:								
D12Mit7	50 (10–70)	…	(B6 × 129) F_2	F	…	5	14q23-32[g], 7q36	Ishimori et al. 2004
14:								
Ath13:								
D14Mit60	15 (10–40)	2.5	(B6.129-*Apoe*$^{-/-}$ × FVB.129-*Apoe*$^{-/-}$) F_2	M/	B6 (dom)	10	14q11-22[g], 10q21-23[g], 8p21, 13q12, 3p21	Dansky et al. 2002
14–52 Mb	22	2.6	(A × B6.129-*Apoe*$^{-/-}$) F_2	F	B6 (add)		14q11-22[g], 10q21-23[g], 8p21, 13q12, 3p21	N. Ishimori and B. Paigen, unpublished data
16:								
D16Mit103	22	2.5	(B6.129-*Apoe*$^{-/-}$ × FVB.129-*Apoe*$^{-/-}$) F_2	F+M		6	3q27-29[g], 3q13[g]	Dansky et al. 2002
18:								
D18Mit23	21	3.1	(B6.129-*Ldlr*$^{-/-}$ × FVB.129-*Ldlr*$^{-/-}$) F_2	F, M	B6		5q23-31, 18q12	Teupser and Breslow 2003
18–52 Mb	22	2.5	(A × B6.129-*Apoe*$^{-/-}$) F_2	F	B6 (add)		5q23-31, 18q12	N. Ishimori and B. Paigen, unpublished data
19:								
Ath16:								
D19Mit120	41 (6–54)	3.8	(B6.129-*Apoe*$^{-/-}$ × FVB.129-*Apoe*$^{-/-}$) F_2	M/	FVB (dom)	16	10q23-25[g]	Dansky et al. 2002
Unknown:								
Ath2:		…	A × B RI, B × A RI					Paigen 1995

[a] Peak marker. The non-MIT markers are all SNP markers with their positions (e.g., 1–169 Mb denotes a SNP on chromosome 1 at 169 Mb).

[b] Genetic distances were retrieved from MGI.

[c] RI strains; F2, intercross.

[d] F = only females were studied; M = only males were studied; F/ (M/) = mice of both sexes were studied, and QTLs were found in only in females (males); F, M = QTLs were detected in both female and male mice; F+M = data from both females and males were used in the same analysis.

[e] add = additive; dom = dominant; rec = recessive.

[f] Human homologous regions of the peak cM of mouse QTLs were retrieved from MGI Whole Genome Orthology Map.

[g] Falls within a QTL for either MI, CAD, ACS, or CIMT in humans (see table 1).

[h] Interacting QTL.

found that only a polymorphism of *TNFSF4* was significantly associated with the risk of MI and CAD in humans (Wang et al. 2005*b*). We therefore identified a human atherosclerosis gene from a mouse QTL.

Making More Mouse Crosses

Although 21 mouse atherosclerosis QTLs have been revealed, additional mouse crosses will expedite finding genes that regulate atherosclerosis, for the following reasons. First, among the 25 individual mouse atherosclerosis QTLs published since 2003, 14 are new, and only 11 are replicates of QTLs found before, suggesting that the mouse QTL map has not been saturated and that new QTLs will be identified in new crosses. Second, additional mouse crosses may confirm previously found QTLs (nearly half of the mouse QTLs are found in only one cross) and may provide more strains for haplotype analysis (Wang et al. 2004*b*; Wang and Paigen 2005*b*). Third, 11 of the 13 crosses use B6 as the susceptible parent. Using different susceptible strains—such as SM/J, SWR/J, and NON/LtJ—may reveal different QTLs. Fourth, there are 27 human and only 21 mouse QTLs, and 10 of the 27 human ones have no concordant mouse QTL. Additional mouse crosses may reveal QTLs concordant with these human QTLs and may expedite finding their underlying genes with the mouse-to-human strategy.

Including Plaque Stability and Coronary Atherosclerosis as Phenotypes

Effective treatment of human atherosclerosis should include stabilizing plaque as well as reducing the accumulation of plasma lipids, and consequently the formation of fibrolipid cores in the arterial wall. So far, the only atherosclerosis phenotype used in mouse genomewide-scan studies is the size of the lipid-staining lesions. Thus, some of the genes identified may regulate intimal lipid deposition but not plaque stability. The latter may be more relevant to clinical events of human atherosclerosis, because ACS is usually caused by plaque rupture and the subsequently formed thrombi that obstruct coronary arteries rather than increased plaque size. Thus, future genomewide scans should include phenotypes that reflect stability of atherosclerotic plaques, such as thickness and rupture of fibrous cap and formation of intraplaque hemorrhage, thrombus, and buried fibrous cap. Plaque rupture has been observed in B6 mice with deficiency of *Apoe* when mice are fed either chow (Rosenfeld et al. 2000) or a diet with high-cholesterol content (Johnson and Jackson 2001; Williams et al. 2002; Johnson et al. 2005). The most frequently observed site of rupture is the innominate (brachiocephalic) artery, as shown in the studies just cited, and genes controlling atherosclerosis in the innominate artery and aortic root may be different (Reardon et al. 2003; Teupser et al. 2004). The vessels most relevant to human atherosclerotic diseases are, of course, the coronary arteries. Advanced atherosclerotic lesions do occur in mouse coronary arteries when mice are deficient in either *Apoe, Ldlr,* both *Apoe* and *Ldlr,* or *Scarb1* (encoding scavenger receptor class B, member 1) and when they are fed either chow (Braun et al. 2002) or a diet with high-cholesterol content (Caligiuri et al. 1999; Calara et al. 2001). Thus, future genomewide scans in mice perhaps should include coronary atherosclerosis as a phenotype.

Finding Genes Regulating Human Atherosclerosis by Studying QTLs for Both Atherosclerosis and HDL

So far, 37 mouse and 30 human HDL-regulating QTLs have been identified (Wang and Paigen 2005*a*). Some of the genes underlying these QTLs may also regulate atherogenesis, given the compelling evidence that HDL protects against atherosclerosis (Brewer 2004; Gotto and Brinton 2004). Thus, human atherosclerosis-regulating genes can be identified by either (1) finding the gene underlying a mouse atherosclerosis QTL and then testing it for a role in human atherosclerosis or (2) finding the gene underlying a mouse HDL QTL and then testing whether it regulates HDL levels and atherosclerosis in humans.

Integrating Traditional Genetic Approaches with New Genetic, Genomic, and Bioinformatic Resources and Tools

A QTL interval is usually large and typically contains hundreds of genes. The first step toward finding a causal gene is to narrow the QTL. QTL-narrowing strategies that take advantage of recombinations in mouse crosses include one or more of the following: (1) interval-specific congenic strains, (2) advanced intercross lines, (3) heterogeneous stocks, (4) recombinant inbred (RI) segregation test, (5) recombinant progeny testing, (6) selective phenotyping, (7) yin-yang cross, and (8) collaborative cross.

Interval-specific congenic strains.—Mice that are recombinant in the QTL region are crossed for several generations with the background parental strain to eliminate alleles from the donor parental strain at all QTLs affecting the trait (other than the QTL being mapped). Animals are then intercrossed, and homozygotes for the recombinant haplotype are selected to establish one congenic strain. Several congenic strains with overlapping regions can differentiate which region contains the QTL gene (Darvasi 1998). Recently, two sets of mice with overlapping congenic regions, covering the whole genome, were generated; one set is B6.DBA/2, the other is B6.CAST. These sets will facilitate narrowing QTLs from B6 × DBA/2 and B6 × CAST crosses (Iakoubova et al. 2001).

Advanced intercross lines.—Mice from two inbred progenitor strains are crossed to yield F1 offspring, which are intercrossed to produce F2 offspring. Fifty F2 × F2 matings are made (avoiding brother-sister matings) to produce F3 offspring, and fifty F3 × F3 matings are made (avoiding brother-sister and cousin matings) to produce F4 offspring, and so on, until the desired advanced intercross (F5, F6, and so on) is attained. Thus, the many recombinations required to narrow a QTL accumulate in a relatively small final population (Darvasi 1998).

Heterogeneous stocks.—Heterogeneous stocks are derived from multiple inbred strains (eight strains in current stocks) through continued intercrossing. They are essentially advanced intercross lines made from multiple strains instead of two (Mott et al. 2000).

RI segregation test.—This strategy requires an existing set of RI strains and takes advantage of the high mapping resolution of RI strains and applies it to QTL mapping. An RI strain with a recombinant haplotype in the region of interest is crossed with both parental strains to produce two separate F1 populations. Intercrossing these F1 individuals then produces F2 mice. By analysis of the F2 populations, a QTL can be located relative to the recombination point, because the QTL previously mapped to this region will segregate in only one of the two F2 populations (Darvasi 1998).

Recombinant progeny testing.—Mice carrying a recombinant chromosome in the QTL region are crossed with one of the parental strains, and their progeny are phenotyped to determine the location of the QTL relative to the recombination point (Darvasi 1998).

Selective phenotyping.—In a large intercross or backcross population, only individuals recombinant at an interval previously found to contain a QTL are phenotyped, because only those individuals contribute to further mapping accuracy (Darvasi 1998).

Yin-yang cross.—Yin-yang cross is a generalization of the RI segregation test that uses inbred strains as if they are RI lines (Shifman and Darvasi 2005). This method is based on the observation that the many inbred laboratory mice are derived from a limited number of founder populations, so the large number of recombination events accumulated in inbred strains since their origin can be used to fine map a QTL to a very small region. The statistical analysis of a yin-yang cross depends on the knowledge of the SNPs of the strains used and is actually similar to haplotype analysis of multiple crosses (Hitzemann et al. 2002; Park et al. 2003; Manenti et al. 2004; Wang et al. 2004*b*).

Collaborative cross.—The collaborative cross is not an existing resource, but it has been recently proposed (Churchill et al. 2004). It is a panel of 1,000 RI strains derived from eight inbred strains. With an estimated 135,000 unique recombination events and known genotypes in the whole panel, QTLs can be mapped to a very small interval without making any crosses.

However, all these approaches require many recombinant animals, which makes the analyses slow, labor intensive, and sometimes difficult to generate because of the existence of recombination hotspots (Kelmenson et al. 2005). In addition to the above strategies, new genetic and genomic resources, especially mouse and human genome sequences, have led to new strategies in narrowing a QTL and even in identifying its underlying gene by analysis of combined cross data, comparison of mouse and human homologous QTLs, analysis of SNPs and haplotypes in multiple strains, and detection of *in silico* QTLs, as discussed by Wang and Paigen (2005*b*). It should be cautioned that successfully identifying a QTL gene from haplotype analysis and *in silico* QTL mapping depends in large part on detailed genome sequence/SNP information in many strains, which is only partly available now. A complete sequence of more mouse strains is likely to fully empower haplotype analysis and *in silico* QTL mapping, as reviewed by Flint et al. (2005). Once a QTL is narrowed with the above approaches, candidate genes can be tested for sequence and expression difference between parental strains (Wang and Paigen 2005*b*). To study the genetics of gene expression, Jansen and Nap proposed a genomewide scan using gene-expression data as phenotypes that can be obtained from microarray analysis (Jansen and Nap 2001); chromosome segments thus obtained are called "expression QTLs" (eQTLs). Because the vast majority of eQTL genes are *cis*-regulated (Bystrykh et al. 2005; Chesler et al. 2005; Hubner et al. 2005), candidate genes of a physiological QTL (pQTL) (with use of clinical traits as phenotype) can be identified by overlapping such a pQTL with eQTLs: differentially expressed genes in a pQTL region could be good candidates (Schadt et al. 2003; Chesler et al. 2005; Hubner et al. 2005).

Using Mutagenesis as a Complementary Approach

Although the QTL approach takes advantage of spontaneous mutations that had accumulated in the past and had been fixed in the various inbred strains of laboratory mice (Lander and Botstein 1989), a mutagenesis approach is capable of generating, in a short time, large numbers of mutations across the genome (Balling 2001). Very efficient at low toxicity, ENU is an alkylating mutagen that induces ~1×10^{-3} random point mutations per locus. It affects germ cells, particularly spermatogonial stem cells. Once adult mice are mutagenized, they are bred, and offspring abnormalities are detected in high-throughput phenotyping screens. Deviants are tested for heritability (Balling 2001). The large-scale and high-throughput features of mutagenesis can potentially generate mutations in every gene. Some of these mutations will help

us understand a gene's functions. The HLB Mutagenesis Program at The Jackson Laboratory is using ENU mutagenesis to identify genes regulating a wide range of cardiovascular phenotypes (Svenson et al. 2003). Atherosclerosis in mutagenized mice cannot be screened with either cross-sectional (Paigen et al. 1987*c*) or *en face* analysis (Palinski et al. 1994), because deviant mice need to be kept for breeding. Instead, noninvasive methods should be used. Magnetic resonance imaging (MRI) provides high-resolution images on not only lesion size (Fayad et al. 1998; Choudhury et al. 2002; Hockings et al. 2002) but also its components (lipid core and cap) (McAteer et al. 2004; Schneider et al. 2004). MRI could be used for an atherosclerosis screen in mutagenized mice if the throughput could be increased.

Summary

The most efficient way to find atherosclerosis-regulating genes in humans is to first find orthologs underlying concordant QTLs, preferably replicate ones, in the mouse. Such QTLs will increase in numbers as new mouse crosses are performed, and they can be rapidly narrowed by analysis of combined cross data, *in silico* QTL analyses, and haplotype analyses. Because replicated QTLs are likely caused by conserved ancestral alleles, causal genes within the narrowed region should exhibit sequence and/or expression differences consistent with their allele effects in all parental strains. These candidate genes, if associated with atherosclerotic diseases in humans, can then be tested, positively identified, and their functions studied in transgenic and/or knockout mice.

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Web Resources

The URLs for data presented herein are as follows:

Center for Medical Genetics, http://research.marshfieldclinic.org/genetics/

Ensembl Human Genome Browser, http://www.ensembl.org/Homo_sapiens/

MGI, http://www.informatics.jax.org/

MGI Whole Genome Orthology Map, http://www.informatics.jax.org/reports/homologymap/mouse_human.shtml

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for *ACE, ADRB2, ALOX5AP, APOE, F7, FGB, GP1BA, MMP3, MMP9, MTHFR, NOS3, PON1, TGFB1, THBS2, THBS4, TLR4, TNFSF4, MEF2A, POMC, CTLA4,* and *EN2*)

References

Agah R, Topol EJ (2002) Genetic testing for coronary heart disease: the approaching frontier. Expert Rev Mol Diagn 2: 448–460

Allayee H, Ghazalpour A, Lusis AJ (2003) Using mice to dissect genetic factors in atherosclerosis. Arterioscler Thromb Vasc Biol 23:1501–1509

Balling R (2001) ENU mutagenesis: analyzing gene function in mice. Annu Rev Genomics Hum Genet 2:463–492

Ben-Asher E, Lancet D (2004) 5-Lipoxygenase activating protein (ALOX5AP): association with cardiovascular infarction and stroke. Isr Med Assoc J 6:318–319

Bhagavatula MR, Fan C, Shen GQ, Cassano J, Plow EF, Topol EJ, Wang Q (2004) Transcription factor MEF2A mutations in patients with coronary artery disease. Hum Mol Genet 13: 3181–3188

Braun A, Trigatti BL, Post MJ, Sato K, Simons M, Edelberg JM, Rosenberg RD, Schrenzel M, Krieger M (2002) Loss of SR-BI expression leads to the early onset of occlusive atherosclerotic coronary artery disease, spontaneous myocardial infarctions, severe cardiac dysfunction, and premature death in apolipoprotein E-deficient mice. Circ Res 90:270–276

Brewer HB Jr (2004) High-density lipoproteins: a new potential therapeutic target for the prevention of cardiovascular disease. Arterioscler Thromb Vasc Biol 24:387–391

Broeckel U, Hengstenberg C, Mayer B, Holmer S, Martin LJ, Comuzzie AG, Blangero J, Nurnberg P, Reis A, Riegger GA, Jacob HJ, Schunkert H (2002) A comprehensive linkage analysis for myocardial infarction and its related risk factors. Nat Genet 30:210–214

Bystrykh L, Weersing E, Dontje B, Sutton S, Pletcher MT, Wiltshire T, Su AI, Vellenga E, Wang J, Manly KF, Lu L, Chesler EJ, Alberts R, Jansen RC, Williams RW, Cooke MP, de Haan G (2005) Uncovering regulatory pathways that affect hematopoietic stem cell function using "genetical genomics." Nat Genet 37:225–232

Calara F, Silvestre M, Casanada F, Yuan N, Napoli C, Palinski W (2001) Spontaneous plaque rupture and secondary thrombosis in apolipoprotein E-deficient and LDL receptor-deficient mice. J Pathol 195:257–263

Caligiuri G, Levy B, Pernow J, Thoren P, Hansson GK (1999) Myocardial infarction mediated by endothelin receptor signaling in hypercholesterolemic mice. Proc Natl Acad Sci USA 96:6920–6924

Carlson CS, Eberle MA, Kruglyak L, Nickerson DA (2004) Mapping complex disease loci in whole-genome association studies. Nature 429:446–452

Chesler EJ, Lu L, Shou S, Qu Y, Gu J, Wang J, Hsu HC, Mountz JD, Baldwin NE, Langston MA, Threadgill DW, Manly KF, Williams RW (2005) Complex trait analysis of gene expression uncovers polygenic and pleiotropic networks that modulate nervous system function. Nat Genet 37:233–242

Choudhury RP, Aguinaldo JG, Rong JX, Kulak JL, Kulak AR, Reis ED, Fallon JT, Fuster V, Fisher EA, Fayad ZA (2002) Atherosclerotic lesions in genetically modified mice quanti-

fied in vivo by non-invasive high-resolution magnetic resonance microscopy. Atherosclerosis 162:315–321

Churchill GA, Airey DC, Allayee H, Angel JM, Attie AD, Beatty J, Beavis WD, et al (2004) The Collaborative Cross, a community resource for the genetic analysis of complex traits. Nat Genet 36:1133–1137

Colinayo VV, Qiao JH, Wang X, Krass KL, Schadt E, Lusis AJ, Drake TA (2003) Genetic loci for diet-induced atherosclerosis lesions and plasma lipids in mice. Mamm Genome 14:464–471

Dansky HM, Shu P, Donavan M, Montagno J, Nagle DL, Smutko JS, Roy N, Whiteing S, Barrios J, McBride TJ, Smith JD, Duyk G, Breslow JL, Moore KJ (2002) A phenotype-sensitizing Apoe-deficient genetic background reveals novel atherosclerosis predisposition loci in the mouse. Genetics 160: 1599–1608

Darvasi A (1998) Experimental strategies for the genetic dissection of complex traits in animal models. Nat Genet 18: 19–24

Durrington PN, Mackness B, Mackness MI (2001) Paraoxonase and atherosclerosis. Arterioscler Thromb Vasc Biol 21: 473–480

Fayad ZA, Fallon JT, Shinnar M, Wehrli S, Dansky HM, Poon M, Badimon JJ, Charlton SA, Fisher EA, Breslow JL, Fuster V (1998) Noninvasive in vivo high-resolution magnetic resonance imaging of atherosclerotic lesions in genetically engineered mice. Circulation 98:1541–1547

Flint J, Valdar W, Shifman S, Mott R (2005) Strategies for mapping and cloning quantitative trait genes in rodents. Nat Rev Genet 6:271–286

Fox CS, Cupples LA, Chazaro I, Polak JF, Wolf PA, D'Agostino RB, Ordovas JM, O'Donnell CJ (2004) Genomewide linkage analysis for internal carotid artery intimal medial thickness: evidence for linkage to chromosome 12. Am J Hum Genet 74:253–261

Francke S, Manraj M, Lacquemant C, Lecoeur C, Lepretre F, Passa P, Hebe A, Corset L, Yan SL, Lahmidi S, Jankee S, Gunness TK, Ramjuttun US, Balgobin V, Dina C, Froguel P (2001) A genome-wide scan for coronary heart disease suggests in Indo-Mauritians a susceptibility locus on chromosome 16p13 and replicates linkage with the metabolic syndrome on 3q27. Hum Mol Genet 10:2751–2765

Geng YJ (2001) Biologic effect and molecular regulation of vascular apoptosis in atherosclerosis. Curr Atheroscler Rep 3:234–242

Gharani N, Benayed R, Mancuso V, Brzustowicz LM, Millonig JH (2004) Association of the homeobox transcription factor, ENGRAILED 2, 3, with autism spectrum disorder. Mol Psychiatry 9:474–484

Gotto AM Jr, Brinton EA (2004) Assessing low levels of high-density lipoprotein cholesterol as a risk factor in coronary heart disease: a working group report and update. J Am Coll Cardiol 43:717–724

Hansson GK, Libby P, Schonbeck U, Yan ZQ (2002) Innate and adaptive immunity in the pathogenesis of atherosclerosis. Circ Res 91:281–291

Harrap SB, Zammit KS, Wong ZY, Williams FM, Bahlo M, Tonkin AM, Anderson ST (2002) Genome-wide linkage analysis of the acute coronary syndrome suggests a locus on chromosome 2. Arterioscler Thromb Vasc Biol 22:874–878

Hauser ER, Crossman DC, Granger CB, Haines JL, Jones CJH, Mooser V, McAdam B, et al (2004) A genomewide scan for early-onset coronary artery disease in 438 families: the GENECARD Study. Am J Hum Genet 75:436–447

Helgadottir A, Manolescu A, Thorleifsson G, Gretarsdottir S, Jonsdottir H, Thorsteinsdottir U, Samani NJ, et al (2004) The gene encoding 5-lipoxygenase activating protein confers risk of myocardial infarction and stroke. Nat Genet 36:233–239

Hitzemann R, Malmanger B, Cooper S, Coulombe S, Reed C, Demarest K, Koyner J, Cipp L, Flint J, Talbot C, Rademacher B, Buck K, McCaughran J Jr (2002) Multiple cross mapping (MCM) markedly improves the localization of a QTL for ethanol-induced activation. Genes Brain Behav 1: 214–222

Hixson JE, Almasy L, Cole S, Birnbaum S, Mitchell BD, Mahaney MC, Stern MP, MacCluer JW, Blangero J, Comuzzie AG (1999) Normal variation in leptin levels in associated with polymorphisms in the proopiomelanocortin gene, *POMC*. J Clin Endocrinol Metab 84:3187–3191

Hockings PD, Roberts T, Galloway GJ, Reid DG, Harris DA, Vidgeon-Hart M, Groot PH, Suckling KE, Benson GM (2002) Repeated three-dimensional magnetic resonance imaging of atherosclerosis development in innominate arteries of low-density lipoprotein receptor-knockout mice. Circulation 106: 1716–1721

Hubner N, Wallace CA, Zimdahl H, Petretto E, Schulz H, Maciver F, Mueller M, Hummel O, Monti J, Zidek V, Musilova A, Kren V, Causton H, Game L, Born G, Schmidt S, Muller A, Cook SA, Kurtz TW, Whittaker J, Pravenec M, Aitman TJ (2005) Integrated transcriptional profiling and linkage analysis for identification of genes underlying disease. Nat Genet 37:243–253

Humphries SE, Morgan L (2004) Genetic risk factors for stroke and carotid atherosclerosis: insights into pathophysiology from candidate gene approaches. Lancet Neurol 3:227–235

Iakoubova OA, Olsson CL, Dains KM, Ross DA, Andalibi A, Lau K, Choi J, Kalcheva I, Cunanan M, Louie J, Nimon V, Machrus M, Bentley LG, Beauheim C, Silvey S, Cavalcoli J, Lusis AJ, West DB (2001) Genome-tagged mice (GTM): two sets of genome-wide congenic strains. Genomics 74:89–104

Ishimori N, Li R, Kelmenson PM, Korstanje R, Walsh KA, Churchill GA, Forsman-Semb K, Paigen B (2004) Quantitative trait loci analysis for plasma HDL-cholesterol concentrations and atherosclerosis susceptibility between inbred mouse strains C57BL/6J and 129S1/SvImJ. Arterioscler Thromb Vasc Biol 24:161–166

Jansen RC, Nap JP (2001) Genetical genomics: the added value from segregation. Trends Genet 17:388–391

Jawien J, Nastalek P, Korbut R (2004) Mouse models of experimental atherosclerosis. J Physiol Pharmacol 55:503–517

Johnson J, Carson K, Williams H, Karanam S, Newby A, Angelini G, George S, Jackson C (2005) Plaque rupture after short periods of fat feeding in the apolipoprotein E-knockout mouse: model characterization and effects of pravastatin treatment. Circulation 111:1422–1430

Johnson JL, Jackson CL (2001) Atherosclerotic plaque rupture in the apolipoprotein E knockout mouse. Atherosclerosis 154: 399–406

Kelmenson PM, Petkov P, Wang X, Higgins DC, Paigen BJ, Paigen K (2005) A torrid zone on mouse chromosome 1 containing a cluster of recombinational hotspots. Genetics 169: 833–841

Korstanje R, DiPetrillo K (2004) Unraveling the genetics of chronic kidney disease using animal models. Am J Physiol Renal Physiol 287:F347–F352

Korstanje R, Eriksson P, Samnegard A, Olsson PG, Forsman-Semb K, Sen S, Churchill GA, Rollins J, Harris S, Hamsten A, Paigen B (2004) Locating *Ath8,* a locus for murine atherosclerosis susceptibility and testing several of its candidate genes in mice and humans. Atherosclerosis 177:443–450

Krude H, Biebermann H, Luck W, Horn R, Brabant G, Gruters A (1998) Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by *POMC* mutations in humans. Nat Genet 19:155–157

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Lohmussaar E, Gschwendtner A, Mueller JC, Org T, Wichmann E, Hamann G, Meitinger T, Dichgans M (2005) *ALOX5AP* gene and the *PDE4D* gene in a central European population of stroke patients. Stroke 36:731–736

Lusis AJ, Mar R, Pajukanta P (2004) Genetics of atherosclerosis. Annu Rev Genomics Hum Genet 5:189–218

Manenti G, Galbiati F, Gianni-Barrera R, Pettinicchio A, Acevedo A, Dragani TA (2004) Haplotype sharing suggests that a genomic segment containing six genes accounts for the pulmonary adenoma susceptibility 1 (*Pas1*) locus activity in mice. Oncogene 23:4495–4504

Manolio TA, Boerwinkle E, O'Donnell CJ, Wilson AF (2004) Genetics of ultrasonographic carotid atherosclerosis. Arterioscler Thromb Vasc Biol 24:1567–1577

McAteer MA, Schneider JE, Clarke K, Neubauer S, Channon KM, Choudhury RP (2004) Quantification and 3D reconstruction of atherosclerotic plaque components in apolipoprotein E knockout mice using ex vivo high-resolution MRI. Arterioscler Thromb Vasc Biol 24:2384–2390

McCarthy JJ, Parker A, Salem R, Moliterno DJ, Wang Q, Plow EF, Rao S, Shen G, Rogers WJ, Newby LK, Cannata R, Glatt K, Topol EJ (2004) Large scale association analysis for identification of genes underlying premature coronary heart disease: cumulative perspective from analysis of 111 candidate genes. J Med Genet 41:334–341

Mehrabian M, Allayee H, Wong J, Shi W, Wang XP, Shaposhnik Z, Funk CD, Lusis AJ, Shih W (2002) Identification of 5-lipoxygenase as a major gene contributing to atherosclerosis susceptibility in mice. Circ Res 91:120–126

Mehrabian M, Wong J, Wang X, Jiang Z, Shi W, Fogelman AM, Lusis AJ (2001) Genetic locus in mice that blocks development of atherosclerosis despite extreme hyperlipidemia. Circ Res 89:125–130

Meir KS, Leitersdorf E (2004) Atherosclerosis in the apolipoprotein-E-deficient mouse: a decade of progress. Arterioscler Thromb Vasc Biol 24:1006–1014

Mott R, Talbot CJ, Turri MG, Collins AC, Flint J (2000) A new method for fine mapping quantitative trait loci in outbred animal stocks. Proc Natl Acad Sci USA 97:12649–12654

Mu JL, Naggert JK, Svenson KL, Collin GB, Kim JH, McFarland C, Nishina PM, Levine DM, Williams KJ, Paigen B (1999) Quantitative trait loci analysis for the differences in susceptibility to atherosclerosis and diabetes between inbred mouse strains C57BL/6J and C57BLKS/J. J Lipid Res 40: 1328–1335

Ozaki K, Ohnishi Y, Iida A, Sekine A, Yamada R, Tsunoda T, Sato H, Sato H, Hori M, Nakamura Y, Tanaka T (2002) Functional SNPs in the lymphotoxin-α gene that are associated with susceptibility to myocardial infarction. Nat Genet 32:650–654

Paigen B (1995) Genetics of responsiveness to high-fat and high-cholesterol diets in the mouse. Am J Clin Nutr 62:458S–462S

Paigen B, Albee D, Holmes PA, Mitchell D (1987*a*) Genetic analysis of murine strains C57BL/6J and C3H/HeJ to confirm the map position of *Ath-1,* a gene determining atherosclerosis susceptibility. Biochem Genet 25:501–511

Paigen B, Mitchell D, Reue K, Morrow A, Lusis AJ, LeBoeuf RC (1987*b*) *Ath-1,* a gene determining atherosclerosis susceptibility and high density lipoprotein levels in mice. Proc Natl Acad Sci USA 84:3763–3767

Paigen B, Morrow A, Holmes PA, Mitchell D, Williams RA (1987*c*) Quantitative assessment of atherosclerotic lesions in mice. Atherosclerosis 68:231–240

Pajukanta P, Cargill M, Viitanen L, Nuotio I, Kareinen A, Perola M, Terwilliger JD, Kempas E, Daly M, Lilja H, Rioux JD, Brettin T, Viikari JSA, Rönnemaa T, Laakso M, Lander ES, Peltonen L (2000) Two loci on chromosomes 2 and X for premature coronary heart disease identified in early- and late-settlement populations of Finland. Am J Hum Genet 67:1481–1493

Palinski W, Ord VA, Plump AS, Breslow JL, Steinberg D, Witztum JL (1994) ApoE-deficient mice are a model of lipoprotein oxidation in atherogenesis: demonstration of oxidation-specific epitopes in lesions and high titers of autoantibodies to malondialdehyde-lysine in serum. Arterioscler Thromb 14: 605–616

Park YG, Clifford R, Buetow KH, Hunter KW (2003) Multiple cross and inbred strain haplotype mapping of complex-trait candidate genes. Genome Res 13:118–121

Phelan SA, Beier DR, Higgins DC, Paigen B (2002) Confirmation and high resolution mapping of an atherosclerosis susceptibility gene in mice on Chromosome 1. Mamm Genome 13:548–553

Pollanen PJ, Karhunen PJ, Mikkelsson J, Laippala P, Perola M, Penttila A, Mattila KM, Koivula T, Lehtimaki T (2001) Coronary artery complicated lesion area is related to functional polymorphism of matrix metalloproteinase 9 gene: an autopsy study. Arterioscler Thromb Vasc Biol 21:1446–1450

Purcell MK, Mu JL, Higgins DC, Elango R, Whitmore H, Harris S, Paigen B (2001) Fine mapping of *Ath6,* a quantitative trait locus for atherosclerosis in mice. Mamm Genome 12:495–500

Reardon CA, Blachowicz L, Lukens J, Nissenbaum M, Getz GS (2003) Genetic background selectively influences innominate artery atherosclerosis: immune system deficiency as a probe. Arterioscler Thromb Vasc Biol 23:1449–1454

Rosenfeld ME, Polinsky P, Virmani R, Kauser K, Rubanyi G, Schwartz SM (2000) Advanced atherosclerotic lesions in the innominate artery of the ApoE knockout mouse. Arterioscler Thromb Vasc Biol 20:2587–2592

Schadt EE, Monks SA, Drake TA, Lusis AJ, Che N, Colinayo V, Ruff TG, Milligan SB, Lamb JR, Cavet G, Linsley PS, Mao M, Stoughton RB, Friend SH (2003) Genetics of gene expression surveyed in maize, mouse and man. Nature 422: 297–302

Schneider JE, McAteer MA, Tyler DJ, Clarke K, Channon KM, Choudhury RP, Neubauer S (2004) High-resolution, multicontrast three-dimensional-MRI characterizes atherosclerotic plaque composition in ApoE$^{-/-}$ mice ex vivo. J Magn Reson Imaging 20:981–989

Seidelmann SB, De Luca C, Leibel RL, Breslow JL, Tall AR, Welch CL (2005) Quantitative trait locus mapping of genetic modifiers of metabolic syndrome and atherosclerosis in low-density lipoprotein receptor-deficient mice: identification of a locus for metabolic syndrome and increased atherosclerosis on chromosome 4. Arterioscler Thromb Vasc Biol 25: 204–210

Shifman S, Darvasi A (2005) Mouse inbred strain sequence information and yin-yang crosses for quantitative trait locus fine mapping. Genetics 169:849–854

Smith J (2003) Quantitative trait locus mapping for atherosclerosis susceptibility. Curr Opin Lipidol 14:499–504

Steinberg D, Witztum JL (2002) Is the oxidative modification hypothesis relevant to human atherosclerosis? Do the antioxidant trials conducted to date refute the hypothesis? Circulation 105:2107–2111

Stewart-Phillips JL, Lough J, Skamene E (1989) ATH-3, a new gene for atherosclerosis in the mouse. Clin Invest Med 12: 121–126

Stoll M, Kwitek-Black AE, Cowley AW Jr, Harris EL, Harrap SB, Krieger JE, Printz MP, Provoost AP, Sassard J, Jacob HJ (2000) New target regions for human hypertension via comparative genomics. Genome Res 10:473–482

Sugiyama F, Churchill GA, Higgins DC, Johns C, Makaritsis KP, Gavras H, Paigen B (2001) Concordance of murine quantitative trait loci for salt-induced hypertension with rat and human loci. Genomics 71:70–77

Svenson KL, Bogue MA, Peters LL (2003) Invited review: identifying new mouse models of cardiovascular disease: a review of high-throughput screens of mutagenized and inbred strains. J Appl Physiol 94:1650–1659

Teupser D, Breslow JL (2003) Quantitative trait locus mapping of atherosclerotic lesions in a cross between B6.129-*Ldlr*$^{-/-}$ and FVB.129-*Ldlr*$^{-/-}$ mice. Paper presented at the 5th Annual Conference on Arteriosclerosis, Thrombosis and Vascular Biology, San Francisco, May 6–8, 2004

Teupser D, Pavlides S, Tan M, Gutierrez-Ramos JC, Kolbeck R, Breslow JL (2004) Major reduction of atherosclerosis in fractalkine (CX3CL1)-deficient mice is at the brachiocephalic artery, not the aortic root. Proc Natl Acad Sci USA 101: 17795–17800

Ueda H, Howson JM, Esposito L, Heward J, Snook H, Chamberlain G, Rainbow DB, et al (2003) Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. Nature 423:506–511

Wang D, Yang H, Quinones MJ, Bulnes-Enriquez I, Jimenez X, De La Rosa R, Modilevsky T, Yu K, Li Y, Taylor KD, Hsueh WA, Hodis HN, Rotter JI (2005*a*) A genome-wide scan for carotid artery intima-media thickness: the Mexican-American Coronary Artery Disease family study. Stroke 36: 540–545

Wang L, Fan C, Topol SE, Topol EJ, Wang Q (2003*a*) Mutation of MEF2A in an inherited disorder with features of coronary artery disease. Science 302:1578–1581

Wang Q, Rao S, Shen G-Q, Li L, Moliterno DJ, Newby LK, Rogers WJ, Cannata R, Zirzow E, Elston RC, Topol EJ (2004*a*) Premature myocardial infarction novel susceptibility locus on chromosome 1p34-36 identified by genomewide linkage analysis. Am J Hum Genet 74:262–271

Wang S, Shi W, Wang X, Lusis AJ (2003*b*) Identification of a locus in mice that influences aortic aneurysm formation. Circulation 108:IV–177

Wang X, Korstanje R, Higgins D, Paigen B (2004*b*) Haplotype analysis in multiple crosses to identify a QTL gene. Genome Res 14:1767–1772

Wang X, Paigen B (2005*a*) Genetics of variation in HDL cholesterol in humans and mice. Circ Res 96:27–42

——— (2005*b*) Genome-wide search for new genes controlling plasma lipid concentrations in mice and humans. Curr Opin Lipidol 16:127–137

Wang X, Ria M, Kelmenson PM, Eriksson P, Higgins DC, Samnegard A, Petros C, Rollins J, Bennet AM, Wiman B, de Faire U, Wennberg C, Olsson PG, Ishii N, Sugamura K, Hamsten A, Forsman-Semb K, Lagercrantz J, Paigen B (2005*b*) Positional identification of *TNFSF4,* encoding OX40 ligand, as a gene that influences atherosclerosis susceptibility. Nat Genet 37:365–372

Welch CL, Bretschger S, Latib N, Bezouevski M, Guo Y, Pleskac N, Liang CP, Barlow C, Dansky H, Breslow JL, Tall AR (2001) Localization of atherosclerosis susceptibility loci to chromosomes 4 and 6 using the Ldlr knockout mouse model. Proc Natl Acad Sci USA 98:7946–7951

Wheeler JG, Keavney BD, Watkins H, Collins R, Danesh J (2004) Four paraoxonase gene polymorphisms in 11 212 cases of coronary heart disease and 12 786 controls: meta-analysis of 43 studies. Lancet 363:689–695

Williams H, Johnson JL, Carson KG, Jackson CL (2002) Characteristics of intact and ruptured atherosclerotic plaques in brachiocephalic arteries of apolipoprotein E knockout mice. Arterioscler Thromb Vasc Biol 22:788–792

Zhang B, Ye S, Herrmann SM, Eriksson P, de Maat M, Evans A, Arveiler D, Luc G, Cambien F, Hamsten A, Watkins H, Henney AM (1999) Functional polymorphism in the regulatory region of gelatinase B gene in relation to severity of coronary atherosclerosis. Circulation 99:1788–1794