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Genetic analysis of atherosclerosis identifies a major susceptibility locus in the major histocompatibility complex of mice

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Abstract

Background and aims: Recent genome-wide association studies (GWAS) have identified over 50 significant loci containing common variants associated with coronary artery disease. However, these variants explain only 26% of the genetic heritability of the disease, suggesting that many more variants remain to be discovered. Here, we examined the genetic basis underlying the marked difference between SM/J-*Apoe*^{-/-} and BALB/cJ-*Apoe*^{-/-} mice in atherosclerotic lesion formation.

Methods: 206 female F₂ mice generated from an intercross between the two *Apoe*^{-/-} strains were fed 12 weeks of western diet. Atherosclerotic lesion sizes in the aortic root were measured and 149 genetic markers genotyped across the entire genome.

Results: A significant locus, named *Ath49* (LOD score: 4.18), for atherosclerosis was mapped to the H2 complex [mouse major histocompatibility complex (MHC)] on chromosome 17. Bioinformatic analysis identified 12 probable candidate genes, including *Tnfrsf21*, *Adgrf1*, *Adgrf5*, *Mep1a*, and *Pla2g7*. Corresponding human genomic regions of *Ath49* showed significant association with coronary heart disease. Five suggestive loci on chromosomes 1, 4, 5, and 8 for atherosclerosis were also identified. Atherosclerotic lesion sizes were significantly correlated with HDL but not with non-HDL cholesterol, triglyceride or glucose levels in the F₂ cohort.

Conclusions: We have identified the MHC as a major genetic determinant of atherosclerosis, highlighting the importance of inflammation in atherogenesis.

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Conflict of interest

Authors declared no conflict of interest, including financial, personal or other relationships with other people or organizations, within the past three years that could have inappropriately influenced the work reported here.

Keywords

Atherosclerosis; Major histocompatibility complex; Quantitative trait locus; Mice

1. Introduction

Atherosclerosis, the primary cause of heart attack, ischemic stroke and peripheral arterial disease, is a complex disease resulting from interactions between environmental and genetic factors [1]. The important role of genetic factors in atherosclerosis has been clearly demonstrated in numerous studies, including prospective studies of twins, families and cohorts [2–4], and genome-wide association studies (GWAS) [5–7]. Apart from rare cases of Mendelian inheritance that are caused by missense or nonsense mutations with large effect, the vast majority of coronary heart disease is complex, probably involving many genes of small effect [8]. Recent meta-analysis of GWAS data have identified >50 loci harboring common variants significantly associated with coronary heart disease [5–7]. Together they only explain 26% of the genetic heritability of coronary heart disease [5], suggesting that many more loci have not been discovered. Furthermore, most of the loci identified have small effect sizes with odds ratios (OR) < 1.25 [5]; thus it's extremely challenging to establish causality between a genetic variant and disease in humans.

A complementary approach to finding genes and pathways involved in atherosclerosis is to study animal models. This allows for strict control over environmental influence and accurate phenotypic characterization of atherosclerotic lesions. Apolipoprotein E-null (*Apoe*^{−/−}) and LDL receptor-null (*Ldlr*^{−/−}) mouse models reproduce all phases of atherosclerotic lesions seen in humans [9,10]. Over a dozen intercrosses or backcrosses have been generated from atherosclerosis-susceptible and -resistant inbred strains with either *Apoe*^{−/−} or *Ldlr*^{−/−} background, leading to identification of 50 unique atherosclerosis susceptibility loci (<http://www.informatics.jax.org/allele>). Unfortunately, nearly all the crosses generated have chosen C57BL/6 (B6) mice as an atherosclerosis-susceptible strain; thus limiting their mapping power and coverage of allelic diversity. Creation of new genetic crosses using a different susceptible strain may discover new loci and also empower bioinformatics analysis for finding causative genes. When a QTL for the same trait has been mapped to the same chromosomal location with multiple crosses derived from different inbred strains, whole-genome sequences and SNP databases available for them can be utilized to prioritize candidate genes. We recently have shown that SM/J (SM) and SWR/J *Apoe*^{−/−} mice are susceptible to atherosclerosis compared to BALB/cJ (BALB) or C3H/HeJ *Apoe*^{−/−} mice [11]. In this study, we performed QTL analysis using a female F₂ cohort derived from an intercross between BALB/cJ (BALB)-*Apoe*^{−/−} and SM-*Apoe*^{−/−} mice to understand the genetic control of atherosclerosis susceptibility.

2. Materials and methods

2.1. Mice

BALB-*Apoe*^{−/−} and SM-*Apoe*^{−/−} mice were made in our laboratory using the congenic breeding method as previously reported [11]. The creation of a female F₂ cohort from the

two Apoe^{-/-} strains was recently described [12]. The animals were weaned at 3 weeks of age, and at 6 weeks of age switched onto a Western diet. After 12 weeks of Western diet, mice were euthanized for assessment of atherosclerotic lesion formation in the aorta.

2.2. Quantitation of aortic atherosclerosis

Atherosclerotic lesion areas in the aortic root of mice were measured as previously reported [13]. Lesion areas were measured on oil red O stained sections using Zeiss AxioVision 4.8 software. The eight largest lesion areas were added up for each mouse and this sum was used for statistical analysis.

2.3. Measurements of plasma glucose and lipid levels

Plasma total cholesterol, HDL cholesterol, triglyceride and glucose levels were measured using commercial kits as reported [14]. Non-HDL cholesterol was calculated as the difference between total and HDL cholesterol.

2.4. Genotyping

F₂ mice were genotyped using the Illumina LD linkage panel, as reported [12]. Microsatellite markers were typed by PCR for regions of chromosome 8 that were not covered by informative SNP markers. A total of 149 markers were included in QTL analysis.

2.5. Statistical analysis

QTL analysis was performed using J/qtl and MapManager QTX software, as reported [12]. LOD threshold values were determined from 1000 permutations of the observed data, which are provided in Data-in-Brief.

2.6. Human genetic association analysis

Human homologous genomic regions corresponding to mouse QTL intervals were examined for associations with coronary heart disease using the dataset from the CARDIoGRAMplusC4D Consortium that included 60,801 coronary artery disease cases and 123,504 controls [5]. Web-based software LocusZoom was used to determine associations of SNPs in regions of interest with coronary artery disease.

2.7. Prioritization of candidate genes

Bioinformatic tools were used to prioritize candidate genes for significant atherosclerosis QTL that was mapped in two or more crosses derived from different parental strains. Likely candidate genes were defined as those containing a non-synonymous SNP in a coding region or a SNP in upstream regulatory region, and this SNP was shared by the parental strains carrying the high allele but different from the one shared by the parental strains carrying the low allele of a QTL, as reported [15]. Analysis was performed using a combination of SNP sources, including the Sanger Mouse Genomes Project, Mouse Phenome Database, and Ensembl. Web-based software RaptorX was used to predict the potential impact of an amino acid substitution on the 3D structure of protein product (http://raptorx.uchicago.edu/Structure_Prediction/predict).

2.8. Gene expression analysis

mRNA expression levels of potential candidate genes in the thymus of BALB mice, which carry the H2^d haplotype, and C3H/HeJ mice, which have the H2^k haplotype, were determined by real-time quantitative PCR. RNA extraction, cDNA preparation and PCR amplification were performed as we previously reported [16]. The following forward and reverse primers were used for *Tnfrsf21*: (5'-TTGAAGCTTGTAGCAGCCCA-3'/5'-TATCCATTGGAGAAGGCCGC-3'; *Tdrd6*: 5'-CATCGAAAACCCTGGCTCCT-3'/5'-GAGATGGCTCGCTGCTTTTG-3'; *Mep1a*: 5'-TCTGGGCACGCCTTTTCTA-3'/5'-GAAGATCAAGCCAGCGATGC-3'; *Pla2g7*: 5'-CAGCTTGGAGCTGTCAGGAG-3'/5'-TGAGCATACAGCCTCCTCGT-3'; *Adgrf5*: 5'-GCAGCACTTACACGCTCAAG-3'/5'-CTCTGGCTCCATAGGCACTG-3'; *Adgrf1*: 5'-ACAGCCTCCAGGGTGACTAGA-3'/5'-GTGAAGAGAGGGACGAGCCA-3'; *Gapdh*: 5'-GAGGCCGGTGCTGAGTATGT-3'/5'-AAGGGTGGAGCCAAAAGGGTCATC-3'). PCR reactions were run in triplicate for each sample. The transcript level of each gene was analyzed using BioRad CFX Manager 3.1 software and expressed as a comparative cycle threshold (Ct) value relative to the level of Gapdh. PCR products amplified with fewer than 30 cycles were electrophoresed on agarose gel to evaluate PCR efficiency.

3. Results

3.1. QTL analysis of atherosclerotic lesions

Values of atherosclerotic lesion areas in 206 female F₂ mice were calculated by summing up the top eight sections for each mouse. These values display a normal distribution (Fig. 1). Genome-wide QTL analysis of these data revealed one significant QTL on chromosome 17 and five suggestive QTLs on chromosomes 1, 4, 5, and 8 for atherosclerosis (Fig. 2). Details of the QTLs detected, including locus name, LOD score, peak location, 95% confidence interval (CI), genome-wide P value, high allele, and mode of inheritance, are shown in Table 1. The QTL on chromosome 17 had a significant LOD score of 4.18 and peaked at 26.08 cM. It exerted effect in a dominant mode of inheritance with the BALB allele conferring susceptibility and the SM allele conferring resistance to atherosclerosis (Fig. 3A, Table 2). This QTL was overlapping in the confidence interval with Ath26, mapped in an AKR-*Apoe*^{-/-} × DBA-*Apoe*^{-/-} intercross [17]. Because this QTL was mapped in an intercross derived from distinct parental strains, it was named *Ath49* in accordance to the guideline provided by the International Committee on Standardized Genetic Nomenclature for Mice (<http://www.informatics.jax.org/mgihome/nomen/gene.shtml>). The QTL on chromosome 1 had a suggestive LOD score of 2.28 and peaked at 97.02 cM (Fig. 3B). This QTL replicated Ath1, mapped in several crosses [18–21]. The QTL on chromosome 4 peaked at 71.37 cM and had a LOD score of 2.52. It replicated Athsq1 mapped in a (MOLF/Ei × B6-*Ldlr*^{-/-}) × B6-*Ldlr*^{-/-} backcross [22]. Two suggestive QTLs were detected on chromosome 5, peaking at 34.19 and 69.4 cM, respectively (Figs. 2 and 3C). The distal QTL replicated Ath42, mapped in a B6-*Apoe*^{-/-} × BALB-*Apoe*^{-/-} intercross [19]. The proximal QTL replicated a suggestive one mapped in a B6-*Apoe*^{-/-} × C3H-*Apoe*^{-/-} intercross [20] and was named *Ath50*. The QTL on chromosome 8 peaked at 46.14 cM and had a suggestive LOD score of 2.18. It was partially overlapping with *Ath40* mapped in a B6 × BALB/cByJ *LDLR*^{-/-} intercross [23]. SM alleles were responsible for increased lesion sizes for the chromo-

1, 5 and 8 QTLs but decreased lesion sizes for *Ath49*. The chromosome 4 QTL affected lesion formation in a heterotic manner as F₂ mice with heterozygous alleles exhibited increased lesion size over those with homozygous alleles.

3.2. Candidate genes for *Ath49*

The QTL for atherosclerosis on chromosome 17 has also been mapped in an AKR-*ApoE*^{-/-} × DBA/2-*ApoE*^{-/-} intercross with the DBA/2 allele conferring susceptibility and the AKR allele conferring resistance to atherosclerosis [17]. The high allele strains BALB and DBA/2 share the same H2^d haplotype, which falls within the *Ath49* interval, whereas the low allele strains SM and AKR have either H2^v or H2^k haplotype. To find candidate genes for the QTL, we searched the Sanger SNP database for genes containing nonsynonymous SNP(s) or SNP(s) in upstream regulatory regions that are shared by the high allele strains (BALB, DBA/2) but are different from ones carried by the low allele strain (AKR) in the confidence interval. SM strain was not included because its genomic sequences for the region were incomplete. 12 candidate genes were identified. For these genes, we further searched other publicly available databases, including the Mouse Phenome Database (<http://phenome.jax.org/db/q?rtnsnp/home>) and Ensembl (<http://www.ensembl.org/index.html>), and compiled the results in Table 3. Of them, *Tnfrsf21*, *Adgrf1*, *Adgrf5*, *Mep1a*, and *Tdrd6* are located precisely underneath the linkage peak and contain one or more nonsynonymous SNPs leading to amino acid substitutions. SIFT (Sorting Intolerant From Tolerant) score, a parameter for estimating the impact of an amino acid substitution on protein function, suggest that the function of protein products encoded by *Adgrf1*, *Adgrf5*, and *Mep1a* is highly likely different between the high and low allele strains (Table 3). *Mep1a* contains over a dozen non-synonymous SNPs between the high and low allele strains. Three of them had extremely low SIFT scores, including substitutions at amino acid residues 247 F/Y (score: 0.01), 232 N/S (score: 0.02) and 201 H/Y (score: 0.06). We further used online software RaptorX to predict the impact of multiple amino substitutions on the 3D structure of *Mep1a* protein product (<http://raptorx.uchicago.edu/StructurePrediction/>). The predicted 3D structure of *Mep1a* protein was noticeably different between the high allele strain BALB and the low allele strain AKR (Fig. 4).

The expression of *Tnfrsf21*, *Adgrf1*, *Adgrf5*, *Mep1a*, *Pla2g7*, and *Tdrd6* in the thymus of BALB and C3H/HeJ mice were determined by real-time PCR. C3H/HeJ mice have the same H2^k haplotype as AKR mice. The transcript level of each gene was normalized to the level of reference gene *Gapdh*. *Adgrf1* and *Mep1a* expression levels were significantly lower in BALB than in C3H/HeJ mice ($p < 0.05$) (Fig. 5A). *Tdrd6* expression level was also lower in BALB mice although the difference was not significant ($p = 0.059$). Noticeable differences for these three genes were observed between the two strains on agarose gel electrophoreses of PCR products amplified with fewer than 30 cycles (Fig. 5B). No significant differences in expression levels were found for *Tnfrsf21*, *Adgrf5*, and *Pla2g7*.

3.3. Association with coronary artery disease in humans

The confidence interval of *Ath49* extended from 43 to 63 Mb, as denoted by the histogram of interval mapping graph (Fig. 3A). The corresponding human syntenic region spans approximately from 30 to 55 Mb on chromosome 6. We searched the human GWAS meta-

analysis dataset for variants in this region that were associated with coronary artery disease. Multiple SNPs, particularly those in the 39–40 Mb region, showed significant associations with the disease ($p < 3.3 \times 10^{-4}$) (Fig. 6). The lead SNP rs56336142 had a highly significant p value of 1.85×10^{-8} . Genes adjacent to this SNP include KCNK5, KCNK16, KCNK17, and SAYSD1. The mouse counterparts of these genes are located on mouse chromosome 14 and thus they are the causal genes of *Ath49*.

3.4. Correlations of atherosclerotic lesion sizes with plasma lipid and glucose levels

The associations of atherosclerotic lesion sizes with fasting plasma lipid and glucose levels were evaluated using the F₂ population (Fig. 7). A significantly inverse correlation with HDL cholesterol levels was observed ($r = -0.188$; $p = 0.0068$). Those F₂ mice with higher HDL cholesterol levels tended to develop smaller atherosclerotic lesions than those with lower levels. Nevertheless, HDL only accounted for 3.5% of the variance in atherosclerotic lesion sizes of the F₂ population, as denoted by the r^2 value, a measure of the correlation between the two variables. No significant correlations were found with plasma levels of non-HDL cholesterol ($r = 0.022$; $p = 0.757$), triglyceride ($R = -0.093$; $p = 0.184$), or glucose ($r = 0.00021$; $p = 0.998$).

4. Discussion

In this study, we performed QTL analysis using a female cohort derived from an intercross between BALB and SM *Apoe*^{-/-} mouse strains and identified one significant QTL (*Ath49*) on chromosome 17 and six suggestive QTLs on chromosomes 1, 4, 5, and 8 for atherosclerotic lesion sizes in the aorta. We then used bioinformatic tools to reduce the number of candidate genes for *Ath49* down to a dozen. The corresponding human homologous region of *Ath49* showed significant associations with coronary artery disease. Moreover, plasma HDL cholesterol was found to explain 3.5% of the variance in lesion sizes of the F₂ population.

Ath26 is an atherosclerosis susceptibility QTL on chromosome 17, initially mapped in an intercross between AKR-*Apoe*^{-/-} and DBA/2-*Apoe*^{-/-} mice [17]. In that cross, the QTL peaked at 36 or 47 Mb, depending on whether or not the lesion data were log-transferred. The confidence interval of *Ath26* overlaps with the H2 complex (mouse major histocompatibility complex). The present study also mapped an atherosclerosis susceptibility QTL to the same region, but because it was mapped with a cross derived from distinct parental strains, the QTL was given a new designation, *Ath49*, according to the guideline established by the International Committee on Standardized Genetic Nomenclature for Mice (<http://www.informatics.jax.org/mgihome/nomen/gene.shtml>). Direct evidence supporting an importance role for the H2 complex in regulation of atherosclerosis susceptibility comes from our study of C3.SW-*H2^bApoe*^{-/-} mice [24]. C3H/HeJ mice are extremely resistant to atherosclerosis even on the *Apoe* null background. However, after their *H2^k* allele was replaced with *H2^b* allele and bone marrow was reconstituted with syngeneic or allogenic marrow, these mice exhibited a 21-fold increase in atherosclerotic lesion size [24].

By examining genes containing SNPs that were shared among the high allele strains (BALB, DBA/2) but different from those carried by the low allele strain (AKR), we were able to

reduce the number of candidates down to 12 genes in the *Ath49* interval. This analysis was based on the observation that genetic variations between mouse strains are primarily inherited from their progenitors *M. m. musculus* and *M. m. domesticus* [25]. Mouse strains carrying an allele that results in a high value for a given trait often share the same ancestral allele harboring the underlying causal gene, while strains carrying an allele that produces a low value for the trait share a different ancestral allele [26]. A limitation of this analysis was that the causal gene would be missed if the QTL arose from a more recent mutation. Because QTL arises from changes in the function or the quantity of a gene product, we focused on genes that carry a nonsynonymous coding SNP or a SNP in the upstream regulatory region segregating between the high allele and low allele strains of the QTL crosses. *Tnfrsf21*, *Adgrf1*, *Adgrf5*, *Mep1a*, *Pla2g7* and *Tdrd6* are located underneath the linkage peak of *Ath49*, and contains one or more nonsynonymous SNPs or a SNP in the upstream regulatory region segregating between the high allele and low allele parental strains.

Genes in the H2 complex encode proteins that are directly involved in or indirectly associated with antigen presentation through interactions with T lymphocytes. The thymus is the primary lymphoid organ of the immune system where T lymphocytes mature; thus we compared the expression of above prioritized candidate genes in this organ of BALB and C3H/HeJ mice. C3H/HeJ mice carry the same H2^k haplotype as AKR mice, and BALB mice share the same H2^d haplotype with DBA/2 mice. The differential expression in an important immune issue between the high and low allele strains further support the probability for *Adgrf1* and *Mep1a* to be the underlying QTL genes. Moreover, both genes contain one or more nonsynonymous SNPs with deleterious SIFT scores that almost certainly affect protein function. *Mep1a* encodes the α subunit of meprin metalloproteinase, and the absence of this gene aggravates chronic inflammation in the colons of mice [27]. *Mep1a* probably also exerts an anti-inflammatory role in the arterial wall; thus its low expression or protein malfunction is expected to accelerate inflammatory responses and atherosclerosis in the high allele mouse strains.

Association of the MHC complex with coronary artery disease has been observed in recent human GWAS studies [5,27]. The present analysis of human GWAS meta-analysis dataset also revealed multiple variants in the MHC region associated with coronary artery disease. KCNK5 has been suggested to be an underlying causal gene contributing to coronary artery disease [5]. However, as KCNK5 is located on mouse chromosome 14 but not 17, obviously it is not the causal genes of *Ath49*. Furthermore, the chromosomal region harboring KCNK5 has only shown a suggestive linkage with atherosclerotic lesions [28].

In this study, we identified multiple suggestive QTLs for atherosclerotic lesion sizes, including the ones on chromosome 5. The LOD score plots for chromosome 5 revealed two distinct peaks, suggesting the existence of two QTLs for atherosclerosis. The distal QTL was overlapping with *Ath42* mapped in a B6 $\times$ BALB *Apoe*^{-/-} intercross [19]. The proximal QTL replicated a suggestive QTL mapped in a B6 $\times$ C3H *Apoe*^{-/-} intercross [20] and was named *Ath50*. Naming a suggestive locus is considered appropriate if it is repeatedly observed [29]. Promising candidate genes for this QTL include *Lrrc66*, *Pdgfra*, *Epha5*, *Cenpc1*, *Uba6*, and *Tmprss11g*. These genes are located underneath the linkage peak, and

contains one or more SNPs segregating between the high allele and low allele parental strains of the QTL crosses (data not shown). Moreover, the human syntenic region harboring these genes has been shown to be associated with coronary heart disease [5].

A slight but statistically significant correlation was observed between HDL cholesterol levels and aortic atherosclerotic lesion sizes in the F₂ cross. Similar findings have been observed with other mouse crosses [15,23,30]. These findings are consistent with the observation made from Mendelian randomization studies of HDL for role in human coronary heart disease [31,32]. Although Mendelian randomization studies of blood lipids have suggested a role for triglyceride and LDL cholesterol in human coronary heart disease, no significant correlations with atherosclerotic lesions were observed in the present cross or previous crosses [13,33].

In summary, we have identified a major QTL for atherosclerosis in the mouse MHC region using a segregating F₂ population, and further applied bioinformatic tools to define underlying candidate genes. As the MHC region harbors genes that play an important role in both innate and adaptive immunity, our findings highlight the significance of inflammation in atherogenesis and its potential for developing anti-atherosclerotic therapy.

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Fig. 1. Frequency distributions of atherosclerotic lesion sizes in 206 female F₂ mice derived from BALB-*Apoe*^{-/-} and SM-*Apoe*^{-/-} mice.

The lesion size of each mouse was the sum of lesion areas on 8 cross-sections with the largest readings. The graph was created using a plotting function of J/qtl software.

Fig. 2. A genome-wide scan to search for main effect QTLs influencing atherosclerotic lesion sizes in the F₂ cohort.

Chromosomes 1 through X are represented numerically on the X-axis. Each minor tick on the X axis represents one informative genetic marker. The Y-axis represents the LOD score. Two horizontal dashed lines denote genome-wide thresholds for suggestive ($P = 0.63$) and significant ($P = 0.05$) linkage.

Fig. 3. Interval mapping graphs for atherosclerotic lesion sizes plotted by MapManager QTX. A, chromosomes 17; B, chromosome 1; and C, chromosome 5. The black line represents LOD scores calculated at a 1-cM interval, the blue plot represents the effect of BALB allele, and the red plot represents the effect of SM allele. The histogram estimates the confidence interval of a QTL. Two green vertical lines represent genome-wide LOD thresholds for suggestive (left line) and significant linkage (right line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 4. Predicted 3D structure of Mep1a protein plotted by RaptorX software.

Left panel: AKR mouse, and right panel: BALB mouse. The predicted 3D structure is noticeably different between the two mouse strains, specifically for the region pointed by the arrow.

Fig. 5. Expression of candidate genes in the thymus of BALB and C3H/HeJ mice fed a chow diet. A, real-time PCR analysis of candidate gene expression. Results are expressed as comparative cycle threshold (Ct) values normalized by the reference gene *Gapdh*. Values are means $\pm$ SE of 3e4 biological replicates of each strain. * $P < 0.05$. B, semi-quantitative PCR analyses of gene expression in the thymus of BALB and C3H/HeJ mice. Each lane represents an individual mouse.

Fig. 6. Regional association plot for coronary heart disease using the dataset from the CARDIoGRAMplusC4D Consortium. Linkage disequilibrium (LD) was inferred based on the 1000 Genomes Phase 3 EUR reference panel.

Fig. 7.

Correlations of atherosclerotic lesion sizes with plasma levels of HDL, non-HDL cholesterol, triglyceride and glucose in the F_2 population. Each point represents values of an individual F_2 mouse. The correlation coefficient (r) and significance (p) are shown.

Table 1

QTLs identified for aortic lesion areas in female F₂ mice derived from BALB-*Apoe*^{-/-} and SM-*Apoe*^{-/-} mice.

Locus	Chr	LOD ^a	p-value ^b	Peak (cM)	Peak (Mb)	Peak marker	95% CI (cM) ^c	High allele	Mode of inheritance ^d
Ath1	1	2.28	0.499	97.02	177.27	rs13476259	5.52–97.02	SMJ	Recessive
Ath5ql	4	2.524	0.333	71.37	119.72	rs3688968	52.32–84.32	–	Heterosis
Ath50	5	2.664	0.263	34.19	61.51	rs6354160	29.4–45.4	SMJ	Recessive
Ath42	5	2.809	0.191	69.4	108.38	rs3726547	45.4–75.4	SMJ	Recessive
Ath40	8	2.176	0.585	46.14	89.35	D8Mit50	20.14–56.14	SM	Recessive
Ath49	17	4.184	0.01	26.08	43.09	rs4231494	16.08–35.34	BALB	Dominant

^a LOD score threshold for suggestive QTL: > 2.108; for significant: > 3.448 established by 1000 permutation tests.

^b p-values represent genome-wide significance at a locus.

^c 95% CI (confidence interval) was obtained from whole-genome scans with J/qtl.

^d Inheritance was determined based on the effect of each parental allele at the nearest genomic marker.

Effects of BALB and SM alleles on aortic lesion area at identified J/ql and PLINK QTLs in female F₂s derived from BALB-*Apoe*^{-/-} and SM-*Apoe*^{-/-} mice.

Table 2

Locus name	Chr	Peak marker	Peak (cM)	Peak marker (Mb)	BB	BS	SS	p-Value
Ath1	1	rs13476259	97.02	177.27	2.0 ± 0.9914 (55)	2.097 ± 0.9426 (95)	2.561 ± 0.975 (56)	0.00565
Athsq1	4	rs3688968	71.37	119.72	2.107 ± 0.8958 (51)	2.42e ± 0.1025 (103)	1.875 ± 0.892 (52)	0.00326
Ath50	5	rs6354160	34.19	61.51	2.065 ± 0.7986 (55)	2.046 ± 0.9883 (91)	2.578 ± 0.1057 (10)	0.414
Ath42	5	rs3726547	69.4	108.38	2.037 ± 0.8441 (53)	2.079 ± 0.9479 (89)	2.536 ± 0.108 (63)	0.00599
Ath40	8	D8Mit50	46.14	89.35	1.957 ± 9.706 (60)	2.106 ± 0.9422 (86)	2.525 ± 0.9261 (54)	0.00471
Ath49	17	rs4231494	26.08	43.09	2.259 ± 9.691 (55)	2.415 ± 0.9221 (96)	1.785 ± 0.9925 (55)	0.000577

Measurements for aortic lesion areas are expressed as means ± SD. The unit for these measurements is: mm² × 10⁶. BB, homozygous BALB allele; BS, heterozygous allele; SS, homozygous SMJ allele. Anova was performed on the data to determine the significance (p-value) of the differences among the BB, BS, and SS genotypes.

Table 3

Haplotype analysis for *Ath49* on chromosome 17 (43–66 Mb).

Chr	Position	Gene	dbSNP	Low allele		High allele		Consequence	Amino acid change	SIFT score
				AKR	BALB	DBA				
17	43040105	Tnfrsf21	rs48909028	A	C	C		missense_variant	387 A/T	0.41
17	43284853	Adgrf1	rs108340214	T	C	C		5_prime_utr_variant		
17	43305361	Adgrf1	rs47784933	G	T	T		missense_variant	297 S/F	0.01
17	43389663	Adgrf5	rs33570001	A	A	A		5_prime_utr_variant		
17	43441946	Adgrf5	rs51535437	T	A	A		missense_variant	593 D/V	0.02
17	43449807	Adgrf5	rs46753646	G	C	C		missense_variant	798 I/V	1
17	43486313	Mepla	rs50539426	T	G	G		missense_variant	288 V/I	1
17	43487139	Mepla	rs214680904	A	A	A		missense_variant	255 P/L	1
17	43487163	Mepla	rs215987489	T	C	C		missense_variant	247 F/Y	0.01
17	43487200	Mepla	rs252705192	T	T	T		missense_variant	235 E/K	0.18
17	43487208	Mepla	rs218712173	C	G	G		missense_variant	232 N/S	0.02
17	43487220	Mepla	rs229991911	T	G	G		missense_variant	228 P/Q	0.13
17	43487302	Mepla	rs250429860	A	A	A		missense_variant	201 H/Y	0.06
17	43487308	Mepla	rs221971200	G	T	T		missense_variant	199 y/h	0.44
17	43491606	Mepla	rs46011116	G	A	A		missense_variant	189 N/T	0.9
17	43491690	Mepla	rs50252056	G	C	C		missense_variant	161 F/S	0.21
17	43491700	Mepla	rs255741402	T	G	G		missense_variant	158 G/R	0.41
17	43491721	Mepla	rs48412763	A	G	G		stop_gained		
17	43491733	Mepla	rs107621975	C	C	C		missense_variant	147 Q/E	0.28
17	43491736	Mepla	rs108717471	T	C	C		missense_variant	146 D/N	0.77
17	43568147	Pla2g7	rs51948026	A	T	T		5_prime_utr_variant		
17	43585255	Pla2g7	rs230688248	G	A	A		5_prime_utr_variant		
17	43585259	Pla2g7	rs254851017	G	A	A		5_prime_utr_variant		
17	43585295	Pla2g7	rs227921889	G	A	A		5_prime_utr_variant		
17	43585297	Pla2g7	rs245576321	G	C	C		5_prime_utr_variant		
17	43624232	Tdrd6	rs29514745	C	T	T		missense_variant	1975 G/D	0.39
17	43624499	Tdrd6	rs221878489	T	C	C		missense_variant	1886 C/Y	0.36

Chr	Position	Gene	dbSNP	Low allele AKR	High allele BALB DBA	Consequence	Amino acid change	SIFT score
17	43624829	Tdrd6	rs240899227	T	T	missense_variant	1776 R/H	0.12
17	43625292	Tdrd6	rs220739854	C	G	missense_variant	1622 S/G	0.14
17	43625324	Tdrd6	rs260352790	A	C	missense_variant	1611 A/V	1
17	43625634	Tdrd6	rs51245534	A	T	missense_variant	1508 A/T	0.58
17	43626615	Tdrd6	rs49793949	A	G	missense_variant	1181 Y/H	0.19
17	43630049	Tdrd6	rs33049652	T	G	missense_variant	36 V/A	0.28
17	53676965	Sgol1	rs13465307	A	A	missense_variant	462 A/S	1
17	53679006	Sgol1	rs13465306	A	T	missense_variant	386 S/T	0.15
17	53683570	Sgol1	rs3671918	C	G	missense_variant	134 S/P	0.06
17	53687717	Sgol1	rs50806942	C	T	missense_variant	46 V/I	1
17	53687723	Sgol1	rs51504866	T	T	missense_variant	44 G/S	0.08
17	53688017	Sgol1	rs3704178	T	A	5_prime_utr_variant		
17	53688095	Sgol1	rs3704745	G	A	5_prime_utr_variant		
17	53689232	Sgol1	rs33531614	C	C	5_prime_utr_variant		
17	53689244	Sgol1	rs33700085	C	G	5_prime_utr_variant		
17	54297024	Slc5a7	rs51867594	C	T	missense_variant	38 R/H	0.1
17	54298695	Slc5a7	rs33594491	C	T	5_prime_utr_variant		
17	65382453	Tmem232	rs47821319	C	A	missense_variant	556 A/S	0.22
17	65382495	Tmem232	rs49652396	C	T	missense_variant	542 V/M	0.05
17	65402850	Tmem232	rs108289705	A	T	missense_variant	481 R/Q	0.5
17	65402904	Tmem232	rs45670940	G	T	missense_variant	463 M/K	0.08
17	65772739	Rab31	rs33048000	T	A	5_prime_utr_variant		
17	65829436	Ppp4r1	rs33569000	G	C	missense_variant	370 W/R	0.25
17	65829437	Ppp4r1	rs33596066	T	A ^a	stop_gained missense_variant	547 G/S; 370 W/ ^a	0.86
17	65829442	Ppp4r1	rs33439071	A	C	missense_variant	372 C/R	0.11
17	65850047	Ralbp1	rs48356103	C	G	missense_variant	617 I/T	0.74
17	65884698	Ralbp1	rs52562388	A	G	5_prime_utr_variant		
17	65885198	Ralbp1	rs33127414	T	C	5_prime_utr_variant		
17	65885592	Ralbp1	rs29523402	G	C	5_prime_utr_variant		

Analysis was performed using a combination of SNP sources, including the Mouse Genomes Project and Mouse Phenome Database.

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Functional candidate genes are denoted in bold.

SIFT (Sorting Intolerant From Tolerant): an algorithm for estimating the effect of a nonsynonymous variant on protein function. A smaller SIFT score denotes a higher likelihood of protein function change.

^aMost likely SNP.