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

Andrew T. Grainger [a, 1], Michael B. Jones [b, 1], Jing Li [b], Mei-Hua Chen [b], Ani Manichaikul [c], Weibin Shi [a, b, *]

[a] Department of Biochemistry & Molecular Genetics, University of Virginia, Charlottesville, VA, USA
[b] Department of Radiology and Medical Imaging, University of Virginia, Charlottesville, VA, USA
[c] Department of Public Health Sciences, University of Virginia, Charlottesville, VA, USA

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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 F_2 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 F_2 cohort.

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

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 (*Lldlr*$^{-/-}$) 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

* Corresponding author. University of Virginia, Snyder Bldg Rm 266, 480 Ray C. Hunt Dr, P.O. Box 801339, Fontaine Research Park, Charlottesville, VA, 22908, USA.
E-mail address: ws4v@virginia.edu (W. Shi).
[1] Authors contributed equally.

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strains with either *Apoe*$^{-/-}$ or *Lldlr*$^{-/-}$ 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 F_2 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 F_2 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

F_2 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′-TCTGGGCACGCCTTTTTCTA-3′/5′-GAAGATCAAGCCAGCGATGC-3′; *Pla2g7*: 5′-CAGCTTGGAGCTGTCAGGAG-3′/5′-TGAGCATACAGCCTCCTCGT-3′; *Adgrf5*: 5′-GCAGCACTTACACGCTCAAG-3′/5′-CTCTGGCTCCATAGGCACTG-3′; *Adgrf1*: 5′-ACAGCCTC-CAGGGTGACTAGA-3′/5′-GTGAAGAGAGGGACGAGCCA-3′; *Gapdh*: 5′-GAGGCCGGTGCTGAGTATGT-3′/5′-AAGGGTGGAGCCAAAAGGGT-CATC-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 (ΔΔCt) 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 F_2 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