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

Sara Bretschger Seidelmann, Carl De Luca, Rudolph L. Leibel, Jan L. Breslow, Alan R. Tall, Carrie L. Welch

Objective—The purpose of this study was to examine genetic factors responsible for metabolic syndrome and atherosclerosis in a setting of low-density lipoprotein (LDL) receptor deficiency in a cross between C57BL/6J (B6) and PERA/Ei (PERA) inbred mouse strains.

Methods and Results—Comparison of metabolic phenotypes in B6 and PERA strains revealed the PERA genetic background to be dramatically more susceptible to hyperleptinemia, hyperglycemia, hypertriglyceridemia, elevated insulin levels, and body fat increase than the B6 background. To facilitate genetic analysis, metabolic syndrome–related traits and atherosclerotic lesion area were measured in 167 [(PERA×B6.129S7-Ldlrtm1Her/H11003)B6.129S7-Ldlrtm1Her/H11003]N2 male and female backcross mice that were homozygous for the Ldlr null allele. Quantitative trait locus analysis was performed using 153 polymorphic microsatellite markers spanning the genome. On chromosome 4, we identified a locus influencing plasma triglyceride, insulin, and leptin concentrations, body weight, and atherosclerosis. Several other genetic loci were identified with separate effects on plasma insulin, body weight, high-density lipoprotein cholesterol, and atherosclerosis.

Conclusions—The PERA strain is highly susceptible to the development of metabolic syndrome after feeding a Western-type diet. This susceptibility is due, in part, to a locus on murine chromosome 4 in which PERA alleles predispose to adiposity, increased insulin, and accelerated atherogenesis in the absence of marked hyperlipidemia.

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Key Words: quantitative trait loci ■ metabolic syndrome ■ atherosclerosis

The metabolic syndrome, also known as syndrome X, was reintroduced by Reaven in the late 1980s to describe the clustering of cardiovascular risk factors, including insulin resistance, impaired glucose tolerance, high triglyceride (TG) and low high-density lipoprotein cholesterol (HDL-C) concentrations, obesity, and hypertension.1 With increasing prevalence of obesity in the US, the metabolic syndrome poses a major public health threat now affecting 22% of the adult population.2 Atherosclerotic cardiovascular disease is a major clinical complication of the metabolic syndrome; patients with the syndrome have at least a 2-fold increase in risk for atherosclerosis compared with controls.3 However, the mechanism(s) for the association between metabolic risk factors and atherogenesis are poorly understood. Several studies have attempted to map and identify genetic factors contributing to metabolic syndrome in humans,4–7 but these have met with limited success. Because of the inherent difficulties in carrying out linkage analysis in humans, many geneticists have successfully turned to animal models.

Wild-type mice are resistant to atherosclerosis, developing only very small lesions when fed a diet high in fat and cholic acid.8 Genetically-engineered models, such as the low-density lipoprotein receptor knock-out (Ldlr−/−) and apolipoprotein E knock-out (apoE−/−) mouse strains, develop larger, more complex lesions with structural and histological features similar to those of humans.9–11 These knock-out mouse models have been successfully used in our laboratory, as well as others, in the mapping of atherosclerosis modifiers.12,13 Previously, we identified 2 atherosclerosis modifier loci in an interspecific cross between MOLF/Ei and...
B6.129S7-Ldlr<sup>mu</sup> (B6-Ldlr<sup>−/−</sup>) that did not affect traditional risk factors for atherogenesis. The goal of this study was to identify additional atherosclerosis modifier loci in a cross between 2 genetically distinct mouse strains: PERA/Ei (PERA) and B6-Ldlr<sup>−/−</sup>. The PERA strain was originally captured in 1961 in the Rimac Valley of Peru and is estimated to have diverged from the B6 strain 1 million years ago. In the process of carrying out this study, we discovered that PERA mice are extremely susceptible to diet-induced obesity and exhibit many features resembling metabolic syndrome in humans. Thus, we used quantitative trait locus (QTL) analysis to identify genetic loci influencing metabolic syndrome–related phenotypes in the PERA strain and to study their relationship to atherosclerosis.

**Methods**

**Mice**

PERA/Ei (PERA) and B6.129S7-Ldlr<sup>mu</sup> (B6-Ldlr<sup>−/−</sup>) mice were purchased from The Jackson Laboratory (Bar Harbor, Me). PERA females were mated with B6-Ldlr<sup>−/−</sup> males to produce F1 mice. Female F1s were backcrossed to B6-Ldlr<sup>−/−</sup> males to produce 167 N2 male and female mice homozygous for the Ldlr knockout allele. N2 mice were weaned onto standard laboratory chow (PicoLab Rodent 20, #5053) at 21 days of age and switched to a Western-type diet (WTD) at 8 to 12 weeks of age. The WTD contained 21% (wt/wt) butterfat and 0.15% (wt/wt) cholesterol and was ~4.53 kcal/g (42% of calories from fat, 15% from protein, and 43% from carbohydrate; Harlan Teklad Adjusted Calories TD 88137). Mice were bled after 2 weeks and 3 months of WTD feeding, and euthanized at the 3-month time point. The breeding colony was produced and maintained in a specific pathogen–free environment. All mice were given ad libitum access to food and water and maintained on a standard 12-hour light-dark cycle throughout the study. Food intake was measured 5x a week for parental strains, which were housed ≤2 per cage. All experimental protocols were approved by the Institutional Animal Care and Research Advisory Committee.

**Atherosclerotic Lesion Measurements**

Mice were anesthetized using Forane (Baxter) and killed by cervical dislocation. The hearts were perfused with 0.9% NaCl, and then the heart and aortic root were dissected and fixed in 10% formalin. The aortic root was sectioned and stained with oil red O, and lesion areas were quantified as described. Isolation of HDL-C by chemical precipitation (HDL reagent, Sigma), were performed according to the manufacturers’ instructions. Non–HDL-C was calculated by subtracting HDL-C from total cholesterol. To evaluate cholesterol concentrations within lipoprotein subfractions from mice grouped by genotype at D4Mit143, 100 μl of plasma was pooled from 10 males per group and separated by fast protein liquid chromatography (FPLC). Plasma was injected onto 2 Superose 6 columns and eluted at a constant flow rate of 0.5 mL/min with 0.1 mol/L Tris-HCl and 0.4% NaCl. Fractions of 0.5 mL were collected, and enzymatic measurements of cholesterol were assayed as described above. Insulin and leptin were measured by using a commercially available ELISA kit (Crystal Chem Inc).

**Plasma Lipoprotein, Insulin, and Leptin Measurements**

Mice were retro-orbitally bled, using Forane anesthesia in the middle of the light cycle after a 5- to 6-hour fast. Blood was collected directly into heparinized capillary tubes (Becton Dickson). Plasma was separated from cells by centrifugation and stored at −70°C.

Isolation of HDL-C by chemical precipitation (HDL reagent, Sigma), as well as enzymatic measurements of cholesterol and triglycerides (Wako Pure Chemical, Osaka), were performed according to the manufacturers’ instructions. Non–HDL-C was calculated by subtracting HDL-C from total cholesterol. To evaluate cholesterol concentrations within lipoprotein subfractions from mice grouped by genotype at D4Mit143, 100 μl of plasma was pooled from 10 males per group and separated by fast protein liquid chromatography (FPLC). Plasma was injected onto 2 Superose 6 columns and eluted at a constant flow rate of 0.5 mL/min with 0.1 mol/L Tris-HCl and 0.4% NaCl. Fractions of 0.5 mL were collected, and enzymatic measurements of cholesterol were assayed as described above. Insulin and leptin were measured by using a commercially available ELISA kit (Crystal Chem Inc).

**Glucose Measurement**

Blood samples for glucose analysis were taken from the cut tail and measured using a Blood Glucose Meter (Ascencia ELITE, Bayer Corp, NY).

**Body Fat Analysis**

Dual Energy X-Ray Absorptiometry measurements were used to determine body fat composition. Animals were anesthetized as previously described, placed in the prone position, and scanned on a Mouse Densitometer (PIXImus, GE Medical Systems). Measurements included total mass, total fat mass, total lean mass, and body fat percentage.

**DNA Extraction and Ldlr Genotyping**

DNA was extracted from tail tips by a quick alkaline lysis protocol as previously described. Ldlr genotyping was performed as previously described.

**DNA Genotyping and Genome Scan**

Initially, 350 fluorescently-labeled primers were tested to identify polymorphic markers between the B6-Ldlr<sup>−/−</sup> and PERA/Ei parental strains. Of those tested, 153 polymorphic markers spaced ~10 cm apart throughout the genome were selected for polymerase chain reaction (PCR), and the genome scan was performed using DNA samples from the N2 generation. Parental and F1 DNA served as controls for each marker. Markers were PCR amplified and products were analyzed by capillary electrophoresis using an Applied Biosystems 3700 DNA sequencer. PCR reactions and electrophoresis were performed by the Starr Center Genotyping Core Facility at The Rockefeller University using automated technology: Tecan, Genesis RSP 100, and Robbins Scientific (Mountain View, Calif) Hydra 384 robots. Genotypes were determined using Applied Biosystems GE-NOTYPE 3.6 NT software.

**Localization of QTLs**

Chromosomal linkage maps were constructed, and linkage analysis was performed to localize QTLs by using MAP MANAGER QTXb19 (http://www.mapmanager.org) for a backcross. All analyses were performed separately for males and females because of the strong effect of gender on atherosclerosis and lipoprotein phenotypes. Permutation analysis was performed to determine the threshold for “suggestive,” “significant,” and “highly significant” linkage using the genome-wide significance thresholds of P=0.05, and P=0.001, respectively. One thousand permutations were performed for each trait under investigation.

**Statistical Analysis**

ANOVA was performed using STATVIEW 5.0 (Abacus Concepts).

**Quantification of Lepr, Isoform B Gene Expression**

Hypothalami were dissected from each animal and placed in an RNA stabilization buffer (RNeasy Micro Kit, 74104; Qiagen). RNA was extracted using a guanidine thiocyanate method (RNeasy Mini Kit, 74104; Qiagen). RNA recovery was quantified by absorbance spectrophotometry (Ultrospec 2000 UV/Visible Spectrophotometer, Pharmacia Biotech). RNA was reverse transcribed using random hexamers and commercially available reverse transcriptase (Super-Script III First-Strand Synthesis System for RT-PCR, 18080-051; Invitrogen). Quantitative PCR was performed using DyNAmo Hot Start SYBR Green qPCR kit (Finzymes) with gene specific primers. Amplification and fluorescence detection were performed on an Opticon2 (MJ Research). The PCR conditions were 40 cycles of 95°C for 10 seconds, 55°C for 10 seconds, and 72°C for 10 seconds. The forward primer, reverse primer, and product size for each amplicon were as follows: Lepr-B, cagggctgtatgtcattg andtgcttggtaaaagatgct, 187 bp; Hprt, agcagtaagccaaaa and tttggcttttccagtttca, 195 bp. Melting curve analysis was performed to verify specificity of amplification. The data were corrected for amplification efficiency and normalized to Hprt mRNA content.
Although not significant, PERA mice also exhibited slightly increased HDL-C levels compared to B6 mice (Table 1). Phenotypes in parental and F1 mice are summarized in Table 1.

### Results

#### Characterization of Metabolic Syndrome–Related Phenotypes in Parental and F1 Mice

Plasma parameters and body weights of B6, PERA, and F1 strains fed a WTD for 2 weeks are summarized in Table 1. PERA mice displayed higher levels of plasma total cholesterol than B6 mice (P<0.05), reflecting differences in plasma non-HDL-C levels (P<0.05). Plasma glucose concentrations were increased in the PERA strain relative to the B6 strain (P<0.002). Although not significant, PERA mice also exhibited slightly higher plasma TG and insulin concentrations than the B6 strain.

To identify strain background effects on metabolic syndrome–related phenotypes, (PERA×B6-Ldlr<sup>−/−</sup>)F1 mice were generated and compared with (B6×B6-Ldlr<sup>−/−</sup>)F1 mice which served as controls (Table 1). After feeding a WTD, plasma TG and insulin concentrations were elevated in (PERA×B6-Ldlr<sup>−/−</sup>)F1 mice relative to (B6×B6-Ldlr<sup>−/−</sup>)F1 controls (P<0.001 and P<0.05, respectively). Because plasma concentrations of insulin and TG were also higher in the PERA, relative to the B6 parental strain, this indicated that dominant PERA alleles may contribute to these phenotypes. Further, insulin concentrations of (PERA×B6-Ldlr<sup>−/−</sup>)F1 mice were dramatically higher than either of the parental strains as well as (B6×B6-Ldlr<sup>−/−</sup>)F1 controls, indicating that this trait may be displaying overdominance. Strikingly, (PERA×B6-Ldlr<sup>−/−</sup>)F1 mice showed much higher body weights after feeding the WTD compared with controls (P<0.001).

### Table 1. Assessment of Body Weights and Plasma Parameters in C57BL/6J (B6), PERA/Ei (PERA), (B6×B6-Ldlr<sup>−/−</sup>)F1, and (PERA×B6-Ldlr<sup>−/−</sup>)F1 Male Mice Fed WTD (Two Weeks)

<table>
<thead>
<tr>
<th>Trait</th>
<th>B6</th>
<th>PERA</th>
<th>(B6×B6-Ldlr&lt;sup&gt;−/−&lt;/sup&gt;)F1</th>
<th>(PERA×B6-Ldlr&lt;sup&gt;−/−&lt;/sup&gt;)F1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>125±11</td>
<td>143±15*</td>
<td>238±37</td>
<td>215±23</td>
</tr>
<tr>
<td>HDL-C, mg/dl</td>
<td>47±19</td>
<td>49±16</td>
<td>97±25</td>
<td>88±20</td>
</tr>
<tr>
<td>Non-HDL-C, mg/dl</td>
<td>78±12</td>
<td>91±9</td>
<td>140±22</td>
<td>127±14</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>84±22</td>
<td>104±32</td>
<td>113±35</td>
<td>191±58†</td>
</tr>
<tr>
<td>BWT, g</td>
<td>31±2</td>
<td>31±3</td>
<td>39±7</td>
<td>51±6†</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>91±14</td>
<td>129±17</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>0.74±0.7</td>
<td>1.29±0.9</td>
<td>2.8±1.7</td>
<td>13±10†</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD. B6 and PERA mice were bled and weighed at ~3 months of age; F1 mice at approximately 6 months of age. Note that B6 and PERA strains are Ldlr<sup>B6−</sup>/B6− and Ldlr<sup>B6−</sup>/PERA−Ei respectively.

*P<0.05 vs B6; †P<0.002 vs B6; ‡P<0.001 vs (B6×B6-Ldlr<sup>−/−</sup>)F1; §P<0.05 vs (B6×B6-Ldlr<sup>−/−</sup>)F1.

#### Table 2. Quantitative Trait Locus Analysis of Metabolic Syndrome–Related Phenotypes and Atherosclerotic Lesion Area in [(PERA×B6-Ldlr<sup>−/−</sup>)F1×B6-Ldlr<sup>−/−</sup>]/N2 Mice

<table>
<thead>
<tr>
<th>Linked Marker</th>
<th>Chr Position (cM)</th>
<th>Trait</th>
<th>LOD (%VAR)†</th>
<th>LOD (%VAR)‡</th>
<th>LOD (%VAR)§</th>
<th>P&lt;sub&gt;emp&lt;/sub&gt;‡</th>
<th>Genotype Associated With Higher Value (% Increase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1Mit270</td>
<td>Chr 1 (92)</td>
<td>Insulin§</td>
<td>—</td>
<td>2.9 (16%)</td>
<td>—</td>
<td>≤0.02</td>
<td>BP (127%)</td>
</tr>
<tr>
<td>D2Mit405</td>
<td>Chr 2 (69)</td>
<td>Lesion area§</td>
<td>2.8 (16%)</td>
<td>1.1 (6%)</td>
<td>3.9 (11%)</td>
<td>≤0.04</td>
<td>BB (27%)‡</td>
</tr>
<tr>
<td>D4Mit143</td>
<td>Chr 4 (43)</td>
<td>TG**</td>
<td>3.6 (20%)</td>
<td>1.2 (6%)</td>
<td>4.8 (13%)</td>
<td>≤0.004</td>
<td>BP (52%)§</td>
</tr>
<tr>
<td>D7Mit253</td>
<td>Chr 7 (53)</td>
<td>HDL-C**</td>
<td>3.1 (18%)</td>
<td>—</td>
<td>—</td>
<td>≤0.04</td>
<td>BB (26%)‡</td>
</tr>
<tr>
<td>D8Mit291</td>
<td>Chr 8 (16)</td>
<td>BWT†</td>
<td>3.4 (19%)</td>
<td>—</td>
<td>—</td>
<td>≤0.02</td>
<td>BB (15%)‡</td>
</tr>
<tr>
<td>D13Mit134</td>
<td>Chr 13 (6)</td>
<td>BWT**</td>
<td>2.9 (16%)</td>
<td>—</td>
<td>—</td>
<td>≤0.04</td>
<td>BP (14%)‡</td>
</tr>
<tr>
<td>D13Mit139</td>
<td>Chr 13 (32)</td>
<td>HDL-C**</td>
<td>4.6 (26%)</td>
<td>—</td>
<td>—</td>
<td>&lt;0.001</td>
<td>BP (30%)‡</td>
</tr>
<tr>
<td>D13Mit144</td>
<td>Chr 13 (48)</td>
<td>Insulin**</td>
<td>1.1 (7%)</td>
<td>3.2 (15%)</td>
<td>4.3 (11%)</td>
<td>≤0.02</td>
<td>BP (53%)§</td>
</tr>
</tbody>
</table>

Only significant loci with genome-wide P<sub>emp</sub> ≤0.05 are presented. BB indicates homozygous for B6 alleles; BP, heterozygous for B6 and PERA/Ei alleles. BWT=body weight.

*Chromosome (Chr) Position indicates the distance of the peak marker from the centromere in centimorgans (cM) as listed in the Mouse Genome Database (http://informatics.jax.org).
†LOD indicates logarithm of odds ratio score; %VAR, percent variance explained by the locus.
‡P<sub>emp</sub> indicates empirically-determined probability for observing the corresponding LOD score by chance (calculated using the permutation test function of Map Manager QTXb19).
§Traits linked in both sexes; average percent increase for males and females is reported.
¶After feeding WTD for 1 months; mice were 20 weeks of age.
**After feeding WTD for 2 weeks; mice were 10 weeks of age.
††After feeding a laboratory chow diet; mice were 8 weeks of age.

#### Sequence Analysis

Direct bidirectional sequencing was performed on PCR-amplified cDNA using an ABI Genetic Analyzer 3100 (Applied Biosystems). Sequence was analyzed using Sequencer version 3.1, and compared with published sequence (Ensembl, ENSMUSG00000035212).
QTL Analysis of Phenotypes of Metabolic Syndrome and Atherosclerosis in Mice

A backcross design was chosen because of the suggestion of dominant alleles affecting metabolic syndrome–related phenotypes in the PERA genetic background and to facilitate Ldlr homozygosity, required for atherosclerotic lesion development. A cross was set up between (PERA×B6-Ldlr<sup>−/−</sup>)F1 female and B6-Ldlr<sup>−/−</sup> male mice to produce 167 [(PERA×B6-Ldlr<sup>−/−</sup>)F1×B6-Ldlr<sup>−/−</sup>]N2 mice homozygous for the Ldlr null allele. Animals heterozygous for the Ldlr null allele were not used in this study. To detect QTLs for metabolic syndrome phenotypes...
and aortic lesion area, we performed a genomic scan using 153 polymorphic microsatellite markers distributed throughout the genome, resulting in an average marker spacing of \(\approx 10\) cM. Significant QTLs, as determined by permutation analysis, are presented in Table 2 and Figure 1. QTLs influencing plasma insulin concentrations were detected on chromosomes 1 (females; logarithm of odds [LOD]=2.9) and 13 (females and males; LOD=4.3). Body weight QTLs were found on chromosomes 8 (males; LOD=3.4) and 13 (males; LOD=2.9). An atherosclerosis locus was detected on chromosome 2 (females and males; LOD=3.9), and 2 QTLs influencing HDL-C levels were detected on chromosomes 7 (males; LOD=3.1) and 13 (males; LOD=4.6).

Notably, a QTL on chromosome 4 was associated with multiple traits related to metabolic syndrome. The QTL displayed peak linkage to the microsatellite marker D4Mit143, located 43 cM from the centromere (22 to 50 cM, \(\pm 1\) LOD support interval) near the leptin receptor (Lepr; 46.7 cM). Primary linkage was to TG (females and males; LOD=4.8) and body weight (females and males; LOD=4.0). Additionally, ANOVA revealed significant genotypic effects on plasma insulin and leptin levels and atherosclerotic lesion area (Table 3). Plasma non–HDL-C and HDL-C levels were not altered in male mice, but non–HDL-C was significantly different in females (Table 3). FPLC confirmed that total and HDL-C levels were similar in males grouped by genotype at D4Mit143 (Figure 2), although very low–density lipoprotein levels were slightly elevated in mice carrying the PERA allele, as expected because plasma TG levels were elevated.

**Further Characterization of Metabolic Syndrome–Related Phenotypes in Parental Strains**

To further characterize the B6 and PERA parental strains, and to test Lepr as a candidate for the chromosome 4 metabolic syndrome locus, we studied the homeostatic response to a 2-week, high-fat WTD challenge in the 2 strains. PERA mice had \(>5\)-fold higher plasma concentrations of leptin relative to B6 mice (\(P<0.0001\), Figure 3A). Average daily food intake was also measured. PERA mice consumed \(\approx 16\%\) more calories per day than B6 mice (\(P<0.0001\), Figure 3B) and had an average weight gain of 7.1 g, whereas B6 mice gained an average of 2.5 g (\(P<0.0001\), Figure 3C). These differences reflected greater feed efficiency (gram weight gained per gram of food consumed) in PERA mice compared with B6 mice (0.154±0.057 and 0.061±0.024, respectively; \(P=0.0008\)). Dual-energy X-ray absorptiometry analysis showed that although the PERA strain had only slightly higher average total body mass compared with B6 mice, their lean body mass was lower (13.4 g versus 18.2 g) and fat mass was much higher (10.3 g versus 4.2 g) compared with the B6 strain (\(P<0.0002\) and \(P<0.0001\) respectively; Figure 3D). Overall, average percent body fat was 2.3-fold higher in PERA mice relative to B6 (\(P<0.0001\), Figure 3E).

To directly test Lepr as a positional candidate for the chromosome 4 metabolic syndrome locus, we sequenced the coding region of the PERA allele but found no sequence variants between the strains (data not shown). To eliminate the possibility of a promoter or enhancer variant altering expression between PERA and B6, Lepr mRNA abundance was measured in the hypothalamus of WTD-fed mice with primers specific for the B isoform, Lepr-B, the major signaling form. Relative levels of Lepr-B mRNA were not statistically different between the strains (Figure 3F), eliminating Lepr as a candidate for this locus.

**Discussion**

This study introduces the PERA strain as a mouse model to aid in the elucidation of genetic and molecular aspects of the
metabolic syndrome. Although the B6 mouse strain itself has been studied as an animal model of diet-induced obesity, insulin resistance, and the metabolic syndrome,18 our studies indicate that the PERA genetic background is strikingly more susceptible to these phenotypes than B6. The mapping population used in this study carried Ldlr null alleles. Although Ldlr deficiency has been suggested to contribute to murine obesity in at least one study,19 observation of the metabolic syndrome–related phenotypes in PERA mice carrying wild-type Ldlr alleles suggests that the loci mapped in this study likely represent unrelated genes present in the parental backgrounds.

The PERA mouse serves as a model of the human metabolic syndrome as it develops obesity, hyperleptinemia, hyperglycemia, hypertriglyceridemia, and elevated insulin levels on a high-fat WTD as compared with the B6 strain. Although PERA mice fed the high fat diet ingested more calories than B6 mice, they also demonstrated increased feed efficiency, suggesting that the extra weight gain in the PERA strain may not be a result of hyperphagia alone. However, strain differences in total energy expenditure, basal metabolic rate, and physical activity were not assessed in this study and warrant further investigation. Several mouse strains carrying genetic variants of the leptin receptor on permissive genetic backgrounds are known to develop obesity or diabetes disorders.20,21 Therefore, the leptin receptor was tested as a candidate for the metabolic disorder in PERA mice and eliminated based on sequence analysis and relative hypothalamic mRNA expression.

Although atherosclerosis is a major clinical result of the metabolic syndrome, the underlying mechanisms of increased atherosclerosis risk are poorly understood. In humans, part of the increased risk of atherosclerosis is derived from the low HDL-C and increased non–HDL-C levels common in metabolic syndrome patients. But altered lipoprotein levels do not explain all of the increased risk for atherosclerosis. In previous studies of atherosclerosis and metabolic syndrome in the mouse, such as the ob/ob Ldlr-deficient mouse, plasma cholesterol levels were so dramatically different that it is difficult to dissect out the other contributions to increased atherosclerosis susceptibility.22 Other studies have identified QTLs contributing to obesity, lipoprotein metabolism, and insulin levels but have not assessed effects on atherosclerosis.23–26 In this study, we have identified a locus on mouse chromosome 4 that predisposes to both metabolic syndrome phenotypes and atherosclerosis in the absence of marked hyperlipidemia. Because the PERA backcross males, which inherited a PERA allele at the chromosome 4 metabolic syndrome locus, showed increased levels of plasma insulin and also increased atherosclerosis, insulin resistance may be responsible for their increased atherosclerosis.

Insulin resistance is frequently present in individuals with metabolic syndrome, and some studies have suggested that it is an important cause of atherosclerosis. Many epidemiological studies have established an association between decreased insulin sensitivity and atherosclerosis susceptibility,27–29 which is not completely accounted for by traditional risk factors. It has been proposed that insulin resistance may elicit a proatherogenic response within the arterial wall. Decreased insulin signaling in vascular endothelial cells has been shown to lower levels of the atheroprotective molecule, endothelial nitric oxide synthase.30 In a separate study, defective insulin signaling in macrophages resulted in increased binding and uptake of oxidized LDL, which was partly mediated by elevated CD36 protein levels, predisposing to foam cell formation and presumably atherosclerosis.31 It is possible that insulin resistance at the arterial cellular level promotes atherosclerosis in mice carrying the PERA interval of the chromosome 4 locus, explaining why the more insulin resistant male mice develop more atherosclerosis, whereas the females do not.

Although environmental factors, common to a “Western” lifestyle, such as physical inactivity and a high fat diet, contribute to development of the metabolic syndrome, the syndrome also has a strong genetic component. Some genetic studies have focused on the individual components of the metabolic syndrome, establishing familial aggregation of plasma lipids,32–34 blood pressure,35–37 and non–insulin-dependent diabetes mellitus.38,39 But is the metabolic syndrome just a clustering of overlapping phenotypes brought about by environmental factors interacting with independent genetic proclivities, or is there a common major genetic factor? In a study of 2508 male twins, multivariate genetic modeling suggested the presence of a common factor responsible for the clustering of hypertension, diabetes, and obesity which was influenced by both genetic and environmental effects (59% genetic and 41% environmental).40 Evidence for a common genetic component of the syndrome was displayed by the probandwise concordance rate for the clustering of hypertension, diabetes, and obesity which was 5-fold higher in monozygotic versus dizygotic twins. A separate twin study suggested that triglycerides, insulin resistance, HDL-C, body mass index, and blood pressure are affected by a single genetic factor.41 Our study indicates the complexity of the genetics of metabolic syndrome. The linkage study revealed many genes contributing to the overall metabolic syndrome phenotype in PERA mice, with most of the loci affecting individual risk factors. However, the chromosome 4 locus may contain a single gene, having primary effects on body weight and TG but also affecting many features of metabolic syndrome.
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References
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