Identification of two susceptibility loci for vascular fragility in the Brown Norway rat

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Harris, Eugenie L., Monika Stoll, Gregory T. Jones, Mary A. Granados, William K. Porteous, Andre M. van Rij, and Howard J. Jacob. Identification of two susceptibility loci for vascular fragility in the Brown Norway rat. Physiol Genomics 6: 183–189, 2001.—A trait of vascular fragility, characterized by the formation of abrupt defects within the elastic laminae of the abdominal aorta, has been identified in Brown Norway (BN) rats. These lesions are greatly exacerbated in F1 rats from a BN × New Zealand genetically hypertensive (GH) intercross, implying that the genetic background provided by the GH rat influences lesion severity. The F2 progeny of the BN × GH intercross were used to identify susceptibility loci for the lesions as well as exacerbating loci. Two major quantitative trait loci (QTLs) for number of internal elastic lamina lesions were identified on rat chromosomes 5 and 10, with the maximum “log of the odds ratio” (LOD) scores at D5Rat119 (LOD 5.0) and at D10Mit2 (LOD 4.5), respectively, together contributing 33.5% to the genetic variance. Further analysis revealed that the chromosome 10 locus exhibits a dominant mode of inheritance, with BN alleles being associated with increased lesion number ($P < 0.0002$) compared with GH homozygotes. This locus was in epistasis to a modifier locus on rat chromosome 2 at D2Mit14 (LOD score 2.12). A second major locus was identified on chromosome 5, exhibiting a semidominant mode of inheritance, again with the BN allele being significantly associated with increased lesion number ($P < 0.0001$). Furthermore, a locus influencing lesion severity was identified on chromosome 3 wherein GH alleles associated with increased severity. This is the first study to identify susceptibility loci for vascular elastic tissue fragility.

abdominal aorta; atherosclerosis; hypertension; internal elastic lamina; quantitative trait loci

FORMATION OF ATHEROSCLEROTIC plaques and aneurysms are common vascular diseases, with potential lethal outcomes, that are caused by a complex array of both genetic and environmental factors. These disorders share a number of features, including breaks in the internal elastic lamina (IEL) and inflammatory cell infiltration into the vascular wall. Features of advanced atherosclerotic disease, typically consisting of an intimal lipid accumulation beneath a fibromuscular cap, are well characterized. The earlier initiating features are, however, far less defined. Two typical features are the presence of fatty streak, widely proposed as the first detectable form of atherosclerosis, and the migration of medial smooth muscle cells through the IEL into the intima, where they proliferate and form an intimal thickening. Importantly, studies show that fragmentation of the IEL in human neonates are sites prone to the development of atherosclerosis in adult life (14, 27). Correspondingly, elastin degradation and fragmentation constitute the sine qua non of conditions such as abdominal aortic aneurysm (AAA) (26). Elastin fragmentation in AAA is generally viewed as part of the advanced disease process. However, occasional abrupt defects in the IEL are present in young human aortas before either atherosclerosis or AAA develops (27).

Morphologically similar IEL defects develop spontaneously within the caudal artery of most rat strains (5, 29, 30). In contrast to other strains, the normotensive Brown Norway (BN) rat also develops numerous abrupt elastic tissue defects within the distal abdominal aorta (2, 20). These abdominal aortic IEL lesions constitute the visible manifestation of a condition of vascular fragility that extends throughout the arterial vasculature of the BN rat (2). The defects are mostly transversely orientated and range in size from small oval tears to breaks that run over half the circumference of the abdominal aorta. Other rat strains, including hypertensive strains, are relatively free of such defects (2, 19, 20). Recently, we have shown that the New Zealand genetically hypertensive (GH) rat has genetic components that appear to interact with the severity of the elastic lesion phenotype (19).

An intercross, conducted by our group, between BN and GH rats allowed further characterization of the BN rat abdominal aortic IEL lesion phenotype and suggested an autosomal dominant mode of inheritance (19). The GH rat was chosen as the contrast strain because it is not prone to IEL lesions despite manifesting full-blown hypertension; yet, considering the increase in lesion severity in F1 rats of our BN × GH cross (19), there must be at least one locus in the GH genome capable of exacerbating lesion severity. Thus

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in crossing BN to GH, we endeavored to simultaneously map both the lesion locus (lesion number quantitative trait loci (lesion number QTLs)) and loci impacting on lesion severity (severity QTLs). In the current study, the male F2 progeny of this cross were used to perform a genome-wide scan to locate susceptibility loci for the IEL lesion trait and simultaneously search for modifier loci that may influence the phenotypic outcome of this trait.

**METHODS**

**Animals.** Four male GH/Omr rats were mated with 4 female BN/Pit rats. F1 offspring were brother-sister mated to produce 180 F2 rats of both sexes. The GH progenitors had been inbred for over 90 generations. The BN rats were at generation 35 of inbreeding when received from the University of Pennsylvania in 1986 and have been continuously inbred thereafter at the University of Otago.

**F2 phenotyping.** At age 18 ± 1 wk the peak systolic tail-cuff blood pressure and pulse rate were determined at least three times on three separate occasions on conscious, restrained rats that had been acclimated to the procedure. The data were recorded using a microphonic manometer machine (ITTC), and the average of these measurements provided the blood pressure and heart rate phenotypic determinations. Rats were killed by CO2 asphyxiation, and the aortas were carefully removed and examined en face (Fig. 1). Total aortic elastic tissue lesion number was counted, and lesion severity was graded on a semiquantitative scale ranging from 0 (no lesions) to 4 (over half the vessel circumference in length, involving multiple medial elastic laminae and with associated adherent thrombus), as previously described (19). Additional phenotypes including left ventricular mass (LVM), body weight, left kidney mass (n = 120), total length, and tail length were also measured.

**Total genome scan.** A multi-phased genotyping strategy was used, based on the initial report by Lander and Botstein (22) that demonstrated the power of focusing genotyping efforts on progeny representing the extremes of the phenotypic distribution. In brief, for a specific quantitative trait, ~81% of the linkage information available in an entire study population is contained in 33% of the progeny whose phenotypes deviate from the mean by > 1 SD. This “targeting” strategy has more power than randomly selecting 33% of the progeny. Using a modification of this strategy, we selected the 46 male F2 animals most informative for lesion number, lesion severity, blood pressure, heart rate, body weight, LVM, and LVM index (LVMi; LVM/body wt) to initiate the genome scan.

DNA was extracted from liver using a modified cationic detergent method (10, 12). A genome-wide scan was performed on the 46 male animals, using 248 polymorphic simple sequence-length polymorphism markers with an average spacing of 10 centimorgans (cM), as described previously (1, 15, 37). To calculate genetic distance, genetic maps were constructed using MAPMAKER/EXP (Version 3.0; see Ref. 1) and the Kosambi algorithm. The genetic distance of the autosomal genome was 1,673.9 cM, which is in accordance with the previously published maps for the rat (37). Genetic markers flanking regions of the genome that exceed a predicted “log of the odds ratio” (LOD) score or the threshold for suggestive/significant linkage were genotyped in the full male population (93 rats) to verify positive linkage.

**Linkage analyses.** Prior to linkage analysis, phenotypic distribution was tested using the Kolmogorov-Smirnov test to assure normal distribution of the trait within the F2 population, as required for parametric linkage analysis. Traits failing the requirements of normality in the Kolmogorov-Smirnov test were transformed using either a logarithmic or square root transformation and retested for normalcy.
Parametric linkage analysis. Traits with a normal distribution were analyzed using the parametric genome scan function of the MAPMAKER/QTL computer package (23). Primary scans were performed under the assumption of free genetics and, if indicated by the scan, followed by genome scans modeling the different modes of inheritance (dominant/recessive and additive). Linkage thresholds were assigned in accordance with the guidelines set by Lander and Kruglyak (24). The thresholds for positive and suggestive linkage in this study were LOD scores of 4.3 and 2.8, respectively. The 95% confidence interval for each QTL was determined by calculating the genetic distance based on the drop of 1.0 LOD unit from the peak.

Nonparametric linkage analysis. A uniform testing procedure, nonparametric linkage analysis, available in MAPMAKER/QTL (v. 1.9b; see Ref. 25), was used for all traits, both those which could be normalized using either the natural logarithm or square root transformation, as well as those that did not pass our rigorous tests for normalcy. This was necessary since traditional QTL methods cannot be directly applied in nonnormalized cases due to the risk of low statistical power or unacceptably high false-positive rates (24). Nonparametric linkage analysis (25) was performed assuming all possible models, and the best fit was chosen. Based on the given maps under the assumption of an even spacing of markers, the threshold of significance for our cross structure was calculated and determined to be a Z score \( Z > 3.9 \).

Statistics. Genotype-phenotype correlation was performed at genetic markers closest to the QTL peak using one-way ANOVA followed by the appropriate post-hoc tests (Student’s t-test). Phenotype-phenotype correlation was performed by linear regression analysis.

RESULTS

The phenotypic characteristics of this BN × GH intercross have been described previously (19). Whereas spontaneous IEL defects are only rarely observed in GH rats (Fig. 1A), BN rats, of both sexes, develop spontaneous IEL defects (Figs. 1B and 2A) from 4 wk of age. At the F1 generation all rats had IEL defects in numbers similar to that of the BN parental strain but with increased severity (Fig. 2B). These data suggest an autosomal dominant mode of inheritance.

Using our total genome scan approach and both parametric and nonparametric linkage analysis, we identified two major QTLs for number of aortic lesions in the male F2 population. Since lesion numbers were not normally distributed within the F2 population, a transformation was performed to normalize the distribution prior to parametric linkage analysis. Before transformation, one QTL was located at the telomeric end of chromosome 5 with a LOD score of 5.0 and a 95% confidence interval extending from D5Rat59 to D5Rat118 (Fig. 3). The log-transformed trait mapped to the identical genomic region with a LOD score of 4.3. In addition, nonparametric analysis was performed, confirming the location of a QTL for IEL lesion number with a peak Z score of 4.4 in the dominant-additive model. All three statistical analyses verify that this region of chromosome 5 harbors at least one gene responsible for lesion number. Subsequent analyses addressing the mode of inheritance for this trait revealed an additive mode of inheritance wherein the BN allele associated with a significantly increased lesion number (\( P < 0.0001 \)) in our male progeny (Table 1). The second major QTL for lesion number was identified on chromosome 10, extending from D10Mgh10 to D10Mit1 with a peak LOD score of 4.5 at D10Mit2, spanning a genetic distance of \( \sim 25 \) cM (Fig. 4). Nonparametric analysis confirmed the location of this sec-

![Fig. 2. Longitudinally orientated histological sections of abdominal aorta in a male BN (A) and BN × GH F1 rat (B). Notice that in the BN rat the elastic tissue (stained black) lesion is primarily associated with the IEL. In the F1 animal, two lesion sites are present. The left lesion is particularly severe, involving multiple medial elastic laminae and containing microthombi (arrowhead). We used Verhoeff’s elastic tissue stain and Curtis’ modified van Gieson stain. Scale bars represent 100 \( \mu \)m in both A and B.](https://www.physiolgenomics.org/content/6/6/185)
increased lesion number (recessive model, the BN allele again associating with vascular fragility phenotype in the F2 male population created a genetic background that influenced the vascular fragility. Three loci is presented in Table 1. The influence of the respective alleles on the phenotype that the RNO2 locus is a genuine observation. RNO10 LOD score to 5.5, strengthening the likelihood that the RNO2 locus is a genuine observation. The influence of the respective alleles on the phenotypic outcome of elastic tissue lesion numbers at all three loci is presented in Table 1.

Crossing the BN rat to the hypertensive GH rat created a genetic background that influenced the vascular fragility phenotype in the F2 male population, allowing detection of QTLs for lesion severity as well as QTLs associated with lesion number. Although it was not possible to identify any major severity QTLs with LOD scores above 4.3, the significance threshold in our cross structure, a modest QTL for lesion severity was identified on RNO3, with a maximum LOD score of 2.4 near D3Rat91, contributing 11.2% of the genetic variance to the trait. As a correlation exists between lesion severity and lesion number (19), the interaction between D10Mit2 and D5Rat118 with the severity locus on chromosome 3 was tested. For this analysis, the variance components were individually removed for both D5Rat118 and D10Mit2, and D3Rat91 was retested. The D5Rat118 locus, but not the D10Mit2 locus, results in the reappearance of D3Rat91 with a LOD score of 2.8 for lesion severity. Interestingly, the lesion severity is attributable to the GH allele (P < 0.02; Table 2).

In addition to traits associated with vascular fragility, other cardiovascular parameters such as LVM, LVMi, and systolic tail blood pressure were measured and included in the linkage analysis. No significant QTLs for blood pressure were detected. The lack of any blood pressure QTL coinciding with a lesion number QTL and the absence of correlation between blood pressure and lesion number or severity in the F2 subgroups (19) indicate that the IEL lesions in this model are not the result of an increase in blood pressure.

DISCUSSION

Spontaneous defects of the elastic laminae have been observed in numerous vessels prone to the development of atherosclerosis. In humans, specific vessels known to develop elastic lamina defects include the abdominal aorta (27) and the coronary (27,28), cerebral (3,6,38), carotid (27), coeliac (17,36), iliac (27), and popliteal (32) arteries. This structural degeneration typically begins with IEL failure and progressively involves the underlying medial elastic laminae. As well as the iliac, femoral, and carotid bifurcations, the distal abdominal aorta appears particularly vulnerable to IEL defect formation. Although there is controversy as to the nature of elastic tissue damage in atherosclerosis and AAA (40), there is clear evidence that biophysical fatigue is at least one factor (39). Disruption of the IEL is by no means unique to human and rat arteries and has been reported within the arteries of numerous vertebrates, including reptiles (9). Morphologically, IEL disruptions in the BN rat, humans, and other animals share many common features regardless of the species involved. These include abrupt “tear” edges, luminal recoil of the break edge, predominantly trans-

Table 1. Genotype/phenotype correlations for aortic lesion number

<table>
<thead>
<tr>
<th>Marker</th>
<th>BB</th>
<th>BG</th>
<th>GG</th>
<th>P Value</th>
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<tbody>
<tr>
<td>D5Rat119</td>
<td>37.9 ± 13.6</td>
<td>27.2 ± 14.1</td>
<td>17.4 ± 12.9</td>
<td>0.0001</td>
</tr>
<tr>
<td>D10Mit2</td>
<td>33.3 ± 15.7</td>
<td>31.0 ± 14.8</td>
<td>17.4 ± 11.2</td>
<td>0.0002</td>
</tr>
<tr>
<td>D2Mit14</td>
<td>39.7 ± 12.8</td>
<td>28.8 ± 16.8</td>
<td>21.5 ± 14.8</td>
<td>0.0359</td>
</tr>
</tbody>
</table>

Values are means ± SD. Peak markers at D5Rat119 (LOD 5.0), D10Mit2 (LOD 4.5), and D2Mit14 (LOD 2.3) were associated with increased number of aortic lesions in rats that carried the Brown Norway (B) alleles. G, New Zealand genetically hypertensive. Significant differences shown are derived from one-way ANOVA tests.
verse orientation and associated reduplicated elastic tissue formation in the overlying intima.

Atherosclerosis and AAA incur high mortality and morbidity, particularly in aging populations. Although it is well known that concomitant hypertension provides an exacerbating factor in both these diseases, the presence of IEL breaks at sites where atherosclerosis develops is not as well recognized. In contrast, although IEL fragmentation is well recognized in AAA, it is not known whether this is an initiating factor or part of the advanced disease progression. We have recently reported on the spontaneous development of elastic tissue lesions in the abdominal aorta of the BN rat (19, 20), a finding first reported by Osborne-Pellegrin and coworkers (2). Our present study design, using a genome-wide scan in the F₂ progeny of a BN × GH cross, allowed not only identification of elastic lamina lesion susceptibility loci but also investigation of the role the genetic background of the GH rat might play in exacerbating lesion severity.

As is often the case with other traits of cardiovascular pathology in both rats and humans, a strong sexual dimorphism was observed in our BN × GH cross with respect to both lesion number and lesion severity, with the effect being greater in males than females (19). Because of the significantly different phenotypic distributions in male and female F₂ rats, we were not able to combine the data to perform a genome-wide scan in a gender-mixed population. Here, we report the results obtained in our male progeny. Two significant QTLs for IEL lesion number were identified on RNO5 and RNO10 with LOD scores of 5.0 and 4.5, respectively. At both loci, increased lesion number associated with BN alleles. The identification of a QTL on rat chromosome 10 exhibiting a dominant mode of inheritance confirmed the preliminary results obtained in our previous study in the F₁ progeny (19). Furthermore, the absence of any blood pressure QTLs coinciding with either of these loci confirmed a genetic basis for this trait independent of blood pressure. In addition to the RNO5 and RNO10 lesion number QTLs, a suggestive QTL for lesion severity was identified on RNO3, peaking at D3Rat91 with a LOD score of 2.8 after fixing for D5Rat118. In this QTL, GH alleles associated with the increase in elastic lamina lesion severity, suggesting the genetic background provided by the GH strain can indeed exacerbate lesion severity and confirming our previous observation of more severe lesions in the F₁ progeny compared with F₀ BN (19).

Although there are numerous reports of QTLs on RNO10 affecting the vasculature (11, 13, 16, 21, 31), the present study provides the first report of such a locus at the telocentric end of RNO5. The density of genes on the telomeric end of RNO5 is sparse, with the only known gene being the Moloney murine sarcoma virus gene (Mos). The homology between the extreme telomeric regions of RNO5 and MMU4 (mouse chromosome 4) indicated Lyn, the Yamaguchi sarcoma viral (v-yes-1)-related oncogene homolog, was likely to lie within the lesion gene QTL. To anchor this region between rat and mouse, we developed a marker to map at the telomeric end of D3Mit2. The 95% CI spans a genetic distance of ~24 cM. Note: the chromosome 10 locus exhibits a dominant mode of inheritance, with the BN allele “carrying” the IEL lesion phenotype.

![Graph showing LOD scores](image-url)

**Table 2. Genotype/phenotype correlations for lesion severity**

<table>
<thead>
<tr>
<th>Marker</th>
<th>BB</th>
<th>BG</th>
<th>GG</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>D5Rat119</td>
<td>2.62 ± 0.86</td>
<td>2.00 ± 0.83</td>
<td>1.98 ± 0.91</td>
<td>0.0164</td>
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<tr>
<td>D5Rat91</td>
<td>1.59 ± 0.83</td>
<td>2.25 ± 0.84</td>
<td>2.27 ± 0.93</td>
<td>0.0183</td>
</tr>
</tbody>
</table>

Values are means ± SD. Peak markers were identified at D3Rat91 and D5Rat119. Increased severity associated with BN alleles at the D3Rat91 (LOD 2.1) locus and appears to be consistent with the previously identified strong correlation between lesion number and severity (19). Interestingly, GH (G) alleles associated with increased severity associated at the D3Rat91 (LOD 2.8) locus, indicating that both strains contributed genes influencing lesion severity. Significant differences shown are derived from one-way ANOVA tests.
tified, genes within the interval of the QTL also need to be considered in future studies.

In contrast to the proximal telomeric region of RNO5, the genomic region on RNO10 is well covered with gene-based markers at a high density due to the huge interest this region attracted after the discovery of a blood pressure locus in the vicinity of Ace in an SHRSP × WKY intercross (13, 16). Our RNO10 lesion number QTL maps proximal to the Ace gene, and indeed Ace is outside the 99% confidence level of the QTL. The location of the peak LOD scores for our RNO10 lesion number QTL points toward genes located upstream of Ace such as Nos2 (the inducible nitric oxide synthase gene), Il4 (interleukin-4), Myh3 (and other skeletal myosin heavy chain genes), and Sparc (secreted acidic cysteine-rich glycoprotein or osteonectin). Sparc is known to interact with growth factors and extracellular matrix proteins (41) and could be considered a candidate gene by position and function.

Osborne-Pellegrin and coworkers (33) found a relationship between aortic elastin and the IEL ruptures in the BN rat. Recently, an association was sought between elastin expression and an RNO8 marker for the elastin gene (34). However, the study found the elastin locus only accounted for a minor portion of aortic elastin content, which is not surprising, given the complexity of elastin gene expression and the chance that a single marker will coincide with the genomic region containing a gene potentially responsible for a selected trait. Overall, the lack of association between the elastin gene marker and elastin expression was in accord with our results showing no lesion gene QTLs on RNO8.

In summary, this study identifies, for the first time, susceptibility loci containing genes that cause vascular fragility in the IEL of the BN rat. The existence of this susceptibility loci containing genes that cause vascular diseases such as atherosclerosis and AAA in humans.

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