Linkage analysis of neointimal hyperplasia and vascular wall transformation after balloon angioplasty

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NIH is triggered by vascular endothelial cell (VEC) disruption and injury that leads to smooth muscle cell (SMC) migration to subendothelial injury sites, followed by SMC proliferation and apoptosis. Significant neointimal thickening and decreased lumen areas are seen within 2 wk after vascular injury (16, 32). However, vascular occlusion can continue after 2 wk as SMC size increases (16, 32) and proteoglycan matrix deposits continue to thicken the neointima (8, 28, 36, 37, 45). Many factors are involved in, and possibly responsible for, genetically determined variations in the NIH injury response between different strains of animals and among individual patients (1). We postulate that heritable variations in the genes for these vascular injury response elements are possible candidates for the quantitative trait loci (QTL) governing the differences in postinjury NIH among different individuals and animal strains.

We utilized a rat model for NIH formation after vascular injury to genetically dissect this complex biological process (1). In this model, we create a vascular injury by denuding the VECs of the vessel wall, similar to injury occurring in human vessels after cardiovascular interventions. The objective of the present work was to identify chromosomal regions containing the gene(s) responsible for the differences in NIH formation after vascular injury, previously observed between inbred spontaneously hypertensive rat (SHR) and Brown Norway (BN) rat strains (1). We performed a genome scan on a segregating population of 301 F2 (SHR × BN) rats that identified multiple QTL for postinjury NIH and other vascular wall phenotypes.

MATERIALS AND METHODS

Animals. Inbred SHR and BN rats were obtained from Harlan Sprague-Dawley (Indianapolis, IN) and were maintained in the Division of Laboratory Animal Medicine at the Medical University of Ohio, where the genetic crosses were bred. Our previous experiments suggested that the alleles responsible for NIH growth were inherited in a codominant manner (1), and thus we selected an F2 (SHR × BN) intercross population for the genome scan. Female SHR and male BN rats were intercrossed to produce F1 (SHR × BN) progeny that were then used to breed an F2 (SHR × BN) population of 301 males. Reciprocal crosses were not used to breed the initial F1 population because of difficulties in BN rat husbandry. Therefore, the Y chromosome is inherited from the BN strain whereas the X chromosome is equally inherited from both strains. Male SHR and BN rats were also concomitantly raised for comparison. Rats were weaned at 28 days and fed a standard rat chow (Ralston Purina, Diet 5001) with ad libitum access to water.

Vascular injury. Surgery was performed on male rats at 11–12 wk of age (250–300 g) as previously described (1). Briefly, rats were anesthetized intraperitoneally with 80 mg/kg ketamine and 12 mg/kg xylazine, and the inner left thigh was shaved and disinfected. The skin over the femoral artery was opened, and the femoral artery was isolated by blunt dissection, dilated with lidocaine, and cannulated with a 2-Fr balloon catheter (1, 4). The 2-Fr balloon catheter was advanced to the distal aorta, inflated with 0.1 ml of normal saline, retracted, and deflated until slight resistance was encountered while...
being withdrawn through the iliac artery. This process was repeated
three times to ensure complete endothelial denudation. Finally, the
left femoral artery was ligated, and the tissue layers and skin were
approximated with an absorbable suture. This procedure produced the
“standard vascular injury.” This animal study was approved by the
Medical University of Ohio Institutional Animal Care and Use Com-
mittee and complied with the Guide for the Care and Use of Labo-
ratory Animals (National Institutes of Health Pub. No. 86-23, revised
1985).

Perfusion-fixation and preparation of vessel sections. Rats were
anesthetized as above, and the body weight of each rat was recorded
at the appropriate time intervals after surgery. A standard perfusion-
fixation technique was performed as previously described (1). Primary
agarose embedding was used before tissue processing and paraffin
embedding to ensure an exact perpendicular orientation of the fixed
vessels to the microtome blade. Each harvested vessel was “pinned out” on a straight axis in small, agarose-filled chambers. Once the
anterior or the posterior, the embedded vessels were cut at the midpoint of the
artery and submitted to standard paraffin processing. The “true”
perpendicular axes of the midiliac arteries were then placed in blocks
for sectioning. Midiliac artery sections were then stained with Ver-
hoeff-van Gieson stain for elastin.

Vessel measurements. Image analysis measurements of the media
area (MA), neointimal area (NIH area), and internal elastic lamina
(IEL) length were made on perpendicular cross sections of control and
injured arteries. These parameters were used to calculate the percent-
age of total arterial wall area due to neointimal growth (designated as
%NIH), MA, media width (MW), circular area (CA), and lumen size
(LS) of each vessel (1). Body weight-adjusted heart weight (BWadj-
HW) was calculated by adjusting heart weight for differences in body
weight, using the regression of heart weight on body weight for each
animal (14, 21). Because lumens of sectioned vessels were sometimes
partially collapsed, the CA was calculated with the length of the IEL
directly measured by image analysis. CAs were calculated by
the formula CA = (IEL2)/4π. To correct for asymmetric neointimal
growth in the injured vessels, injured vessel lumen size (inj-LS) was
calculated by subtracting the neointimal area of each vessel from its
CA: inj-LS = CA − neointimal area. As control vessels did not have
a neointimal layer, their CA and LS were identical. The MA of each
vessel was directly measured and, in conjunction with IEL length,
allowed calculation of MW as follows:

\[ MW = r_2^2 - r_1^2 \]

where \( r_2 \) is the radius of the entire vessel and \( r_1 \) is the radius of its CA.

area of entire vessel = \( πr_2^2 \)

CA + MA or, substituting, \( \frac{IEL^2}{4π} + MA \)

Therefore,

\[ r_2 = \sqrt{\frac{IEL^2 + MA}{\pi}} \]

Because \( r_1 = IEL/2π \),

\[ MW = \sqrt{\frac{IEL^2}{4π} + MA - \frac{IEL}{2π}} \]

In addition to BWadj-HW, 10 vascular parameters were measured:
parameters related to vascular injury include injured vessel CA,
injured vessel MA, injured vessel MW, injured vessel NIH area,
injured vessel MA + NIH area, injured vessel LS, and injured vessel
%NIH. We also measured parameters in the control vessels that were
not related to the vascular injury, including control vessel CA, control
vessel MA, and control vessel MW.

Statistical analysis. Distributions of each phenotype in the F2
(SHR × BN) population were assessed for normality with the SPSS
computer program (SPSS, Chicago, IL) by skewness, kurtosis, and the
Kolmogorov-Smirnov test. Of the phenotypes measured, two required
transformation for normality as follows: %NIH values were trans-
formed by taking the square root and BWadj-HW values were norma-
ized by removing outliers from Box Plot analysis and then taking
1 divided by the value (1/X). Both of the transformed phenotypic
values (%NIH and BWadj-HW) were found to have a normal distri-
bution by the Kolmogorov-Smirnov test. Many of the distributions
were nearly (98.8%) normal and only required removal of outliers as
determined by Box Plot analysis. Furthermore, statistical analyses of
differences and/or associations between these parameters measured in
the parental and F2 (SHR × BN) rats were also analyzed with one-way ANOVA, \( χ^2 \) and a nonparametric statistical method to test
for possible associations between these parameters without assump-
tions about their underlying distribution.

Genotyping with PCR. Microsatellite loci were genotyped by PCR
amplification. Genomic DNA was prepared from the unfixed livers of
BN, SHR, and F2 (SHR × BN) rats isolated with a kit (Dnasey
96-well kit, Qiagen, Valencia, CA). PCR amplification was performed
with a PTC-100-96Ag thermocycler (MJ Research, Waltham, MA) as
previously described (53). PCR products were electrophoretically
size-fractionated on 4% 3:1 (Amresco, Solon, OH) or 4% Metaphor
(Cambrex Bio Science, Walkersville, MD) agarose gels and visual-
ized with ethidium bromide staining.

Identification of putative QTL by interval mapping. Putative QTL
were first identified by selecting genotypes of a portion of the F2
(SHR × BN) population. Specifically, the highest (\( n = 45 \)) and lowest
(\( n = 45 \)) 15% of the population with regard to NIH formation were
genotyped with polymorphic microsatellite markers, spaced on average
13–25 cM apart (see Supplemental Table S1, which is available at the
Physiological Genomics web site).1 The Map Manager QTX
computer program (35) was used to order genotypic data into linkage
groups, perform interval mapping, and identify putative QTL (29, 44).
Potential errors in typing, i.e., loci involved in double-recombination
events, were retested to confirm or correct the results. Threshold
values for suggestive linkage of a locus to a QTL were as defined by
Lander and coworkers (29, 30), corresponding to the expectation of
one false positive per genome scan. Each rat chromosome (RNO) that
contained at least one marker suggesting linkage in this %NIH-
directed genome scan (RNO2–RNO7, RNO15, and RNO20) was
used to order genotypic data into linkage regions. Map Manager QTX
was used to organize interval maps of the chromosomal regions contain-
ing NIH QTL.

RESULTS

Genome scan. %NIH was measured in the injured vessels of
301 F2 (SHR × BN) rats 8 wk after a standard vascular injury.
Initially, 158 markers were used to selectively genotype micro-
satellite polymorphisms covering rat chromosomes (1–20,
X) in the F2 (SHR × BN) population, with the highest (top
15%, \( n = 45 \)) and lowest (bottom 15%, \( n = 45 \)) %NIH selected
from 301 male F2 rats after the standard vascular injury.
Chromosomal regions with logarithm of odds (LOD) scores
suggestive of linkage (\( ≥ 2.0 \)) to the measured phenotype for
both control and injured vessel phenotypes, %NIH, MA, NIH

1 The Supplemental Material for this article (Supplemental Table S1)
is available online at http://physiolgenomics.physiology.org/cgi/content/full/
00135.2005/DC1.
area, MA + NIH area, CA, LS, MW, and BWadj-HW, were then selected for genotyping in the remaining 211 rats of the F2 (SHR × BN) population. The average spacing of microsatellite markers was 13.6 cM, with a mean maximum distance of 25.1 cM between markers on a chromosome, resulting in 99.4% of the genome being within 15 cM of a microsatellite marker (see Supplemental Table S1). The resulting map length using the Kosambi correction of all rat chromosomes was 1,780.3 cM, similar to previous estimates (5, 7, 15). Figure 1 shows a LOD plot of the F2 (SHR × BN) genome scan for the %NIH phenotype based on the top and bottom 15% of postinjury NIH formation (n = 90 rats). This LOD plot for the %NIH genome scan used a free model of inheritance without any assumptions regarding inheritance. Three chromosomal regions showed suggestive linkage to %NIH in this initial screen: 1) RNO2, with a peak LOD score near D2Rat20; 2) RNO3, with a peak LOD score near D3Arb12; and 3) RNO15, with a peak LOD score near D15Rat50 (Fig. 1). QTL for vascular wall measurements of control and injured vessels, as well as for BWadj-HW (RNO2, near marker D2Rat111) were also found in this initial, %NIH-directed genome scan; these include QTL for control vessel measurements unrelated to NIH formation for MA, CA, LS, and MW on RNO2 near marker D2Rat3, MA, CA, and LS on RNO4 near marker D4Rat198, and MA and MW on RNO6 near marker D6Rat44.

Furthermore, vascular wall measurements of injured vessel QTL related to NIH growth were found for MA on RNO4 near marker D4Rat198, CA and LS on RNO5 near marker D5Rat20, as well as MA on RNO7 and RNO20 near markers D7Rat20 and D20Rat1, respectively. Complete genotyping of these QTL by chromosome was then performed for the remaining 211 F2 (SHR × BN) rats for RNO2–RNO7, RNO15, and RNO20.

Table 1 summarizes the characteristics of the QTL confirmed in the complete F2 (SHR × BN) population of 301 rats, including the locus nearest the LOD plot peak for each phenotype and its mean value for the three genotypes. The mode of inheritance resulting in the highest significant LOD score for each QTL is also given (30). Fourteen QTL were found, of which only ten (injured vessel QTL) were directly related to postinjury NIH production (Table 1). LOD plots for the QTL identified on RNO3, RNO4, RNO6, and RNO15 are shown in Fig. 2, A–D, respectively. Two significant QTL for injured vessel CA (LOD 4.6) and inj-LS (LOD 4.2) were found on RNO3 located near marker D3Arb12 (Fig. 2A). We also found

<table>
<thead>
<tr>
<th>RNO</th>
<th>Parameter</th>
<th>Vessel</th>
<th>Locus</th>
<th>Genotype</th>
<th>LOD Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>MA, mm²</td>
<td>Injured</td>
<td>D3Rat227</td>
<td>BN/BN</td>
<td>0.092 ± 0.02, 0.095 ± 0.02, 0.103 ± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>CA, mm²</td>
<td>Injured</td>
<td>D3Rat12</td>
<td>BN/SHR</td>
<td>0.416 ± 0.08, 0.436 ± 0.08, 0.471 ± 0.11</td>
</tr>
<tr>
<td>3</td>
<td>LS, mm²</td>
<td>Injured</td>
<td>D3Rat13</td>
<td>SHR/SHR</td>
<td>0.392 ± 0.08, 0.415 ± 0.08, 0.450 ± 0.10</td>
</tr>
<tr>
<td>3</td>
<td>%NIH</td>
<td>Injured</td>
<td>D3Arb12</td>
<td></td>
<td>0.409 ± 0.07, 0.448 ± 0.11, 0.456 ± 0.09</td>
</tr>
<tr>
<td>3</td>
<td>LS, mm²</td>
<td>Injured</td>
<td>D3Arb12</td>
<td></td>
<td>0.182 ± 0.12, 0.188 ± 0.13, 0.125 ± 0.09</td>
</tr>
<tr>
<td>3</td>
<td>NIH area, mm²</td>
<td>Injured</td>
<td>D3Arb12</td>
<td></td>
<td>0.387 ± 0.07, 0.431 ± 0.09, 0.432 ± 0.11</td>
</tr>
<tr>
<td>4</td>
<td>MA + NIH area, mm²</td>
<td>Injured</td>
<td>D4Rat79</td>
<td></td>
<td>0.023 ± 0.02, 0.025 ± 0.02, 0.016 ± 0.01</td>
</tr>
<tr>
<td>6</td>
<td>%NIH</td>
<td>Injured</td>
<td>D6Rat44</td>
<td></td>
<td>0.119 ± 0.03, 0.114 ± 0.03, 0.128 ± 0.03</td>
</tr>
<tr>
<td>6</td>
<td>MA, mm²</td>
<td>Control</td>
<td>D6Rat44</td>
<td></td>
<td>0.198 ± 0.13, 0.157 ± 0.11, 0.169 ± 0.12</td>
</tr>
<tr>
<td>6</td>
<td>MW, mm²</td>
<td>Control</td>
<td>D6Rat44</td>
<td></td>
<td>0.106 ± 0.02, 0.098 ± 0.02, 0.096 ± 0.02</td>
</tr>
<tr>
<td>6</td>
<td>MA, mm²</td>
<td>Control</td>
<td>D6Rat150</td>
<td></td>
<td>0.105 ± 0.02, 0.096 ± 0.02, 0.098 ± 0.02</td>
</tr>
<tr>
<td>6</td>
<td>MW, mm²</td>
<td>Control</td>
<td>D6Rat150</td>
<td></td>
<td>0.042 ± 0.01, 0.040 ± 0.01, 0.044 ± 0.03</td>
</tr>
<tr>
<td>15</td>
<td>MA, mm²</td>
<td>Injured</td>
<td>D15Rat95</td>
<td></td>
<td>0.093 ± 0.02, 0.095 ± 0.02, 0.104 ± 0.03</td>
</tr>
</tbody>
</table>

Data are means ± SD; all 301 F2 [spontaneously hypertensive rat (SHR) × Brown Norway (BN)] animals were genotyped in quantitative trait loci (QTL) regions. RNO, rat chromosome; MA, media area; CA, circular area; LS, lumen size; NIH, neointimal hyperplasia; %NIH, % of total arterial wall area due to neointimal growth; MW, media width; Locus, locus nearest QTL peak; LOD, logarithm of odds ratio; Model, mode of inheritance resulting in LOD score with highest significance.
several suggestive QTL related to injured vessel MA, CA, LS, NIH area, and %NIH, primarily residing in the same area of the distal portion of the q arm of RNO3 (Fig. 2A). The RNO3 QTL associated with injured vessel MA (blue) was located near D3Rat227, with a LOD score of 2.0. The RNO3 QTL associated with injured vessel CA (red) were located near D3Rat12 and D3Arb12, with LOD scores of 2.9 and 4.6. Also in the q terminus of RNO3 located near D3Arb12 are QTL for %NIH, injured vessel LS, and NIH area. The %NIH (transformed data, black) and NIH area (nontransformed data, orange) QTL have similar LOD plots peaking near D3Arb12 and LOD scores of 2.5 and 3.1, respectively. Finally, QTL peaks were found near both D3Rat12 (LOD = 2.8) and D3Arb12 (LOD = 4.2) for injured vessel LS (yellow).

Suggestive QTL were also identified on RNO4, RNO6, and RNO15 (Fig. 2, B–D, respectively): a QTL for MA + NIH area was found on RNO4 with a LOD score of 2.4 located near D15Rat95 (Fig. 2D).

**BWadj-HW and control vessels.** Table 2 shows the mean ± CV of BWadj-HW and the CA, MA, and MW of BN, SHR, and F2 (SHR × BN) control vessels harvested 8 wk after the vascular injury. The mean BWadj-HW of the hypertensive SHR rats was significantly higher than that of normotensive BN rats (Table 2), with the mean BWadj-HW F2 (SHR × BN) values intermediate to those of the parental strains (P < 0.001, Table 2). Also, SHR control vessels had smaller CA and larger MA and MW than BN control vessels (Table 2). No associations were found between BWadj-HW and control vessel CA of the individual BN and F2 (SHR × BN) rats [P = not significant (NS)]. Furthermore, the F2 (SHR × BN) control vessels had larger CA than SHR control vessels (P < 0.001, Table 2).

**Injured and control vessels.** In a preliminary time after injury study, BN and SHR vessels were harvested at 0, 2, 4, 7, 18, 23, and 28 days in addition to 8 wk after injury. There was considerable scatter in the measurements of the vessels taken at these earlier time periods. The only significant difference found was a relative increase in the BN vessel NIH area and %NIH at 8 wk after injury (P < 0.05; data not shown). However, injury-induced neointima was observed in both

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**Fig. 2.** Individual LOD plots for phenotypes measured in the %NIH-driven genome scan. Linkage for postinjury NIH production and other vessel-wall phenotypes were found on rat chromosomes RNO3 (A), RNO4 (B), RNO6 (C), and RNO15 (D) for the F2 (SHR × BN) population (n = 301). Thresholds differ for the individual chromosomes because of the use of different models of inheritance (30). CA, circular area; LS, lumen size; NIH, neointimal hyperplasia; MA, media area; MW, media width.
strains only 2 days after the vascular injury, arguing against differences in intimal denudations from the initial injury being responsible for the 8-wk strain differences in neointima production. The IEL in sections of these vessels from the early postinjury time points were also graded in a coded study for the “number of IEL breaks” and “waviness or corrugation” (graded 0–6), a morphology pattern associated with reactive vasospasm (3, 16, 33). Interestingly, the sections of the vessels taken 0–18 days after injury (30 vessels from each strain) revealed that the SHR IEL had more breaks (mean = 14.3, range 2 to >40) than the BN IEL (mean = 8.7, range 0–32, P < 0.025). Surprisingly, the average “wave grade” of the injured BN vessels was 2 (range 0–6), compared with only 0.6 (range 0–3) for the injured SHR vessels (P < 0.001). Together, these results are consistent with the thinner BN vessels (see below, Tables 2 and 3), which are also more fragile than the SHR vessels (11, 23), being more easily distended than the more rigid SHR vessels. Thus the BN vessels had fewer injury-induced IEL breaks. This difference in injury pattern between the two strains, i.e., the numbers of injury-induced IEL breaks, may have led to differences in injury-induced vasospasm (waves) and possibly contributed to the strain variations in neointimal growth.

Injured BN vessels did have higher NIH area and %NIH than injured SHR vessels (Table 3; Ref. 1). In contrast to our earlier results (1), this increased neointima formation was not associated with a smaller LS for the injured BN vessels, which had a 10% increase in CA compared with the noninjured control vessels, compensating for the occlusive effects of the NIH ingrowth (Table 4). However, the NIH ingrowth in the injured F2 (SHR × BN) vessels did lead to a significant decrease in LS compared with control vessels (Table 4).

The F2 (SHR × BN) control vessels were similar to the BN control vessels and showed strong associations between individual vessel CA and MA (P < 0.001) but also resembled the SHR control vessels, with strong associations found between individual MW and MA (P < 0.001). In summary, the control vessels of BN and SHR rats showed strain-specific associations of the CA, MA, and MW, with the control vessels of the F2 (BN × SHR) rats having variables of both parental strains. Most importantly, for both of the parental BN and SHR rat strains, no associations were found between the individual control vessel CA, MA, MW, and BWadj-HW measurements and any of the parameters measured in the injured vessels of the same rats (P = NS). In contrast, control vessel MW of individual F2 (SHR × BN) rats were significantly related to the injured vessel MA (P < 0.001) and MW (P < 0.001) of the same F2 rats. Furthermore, values of the individual F2 (SHR × BN) injured vessel MA, MW, CA, MA + NIH area, and %NIH were all positively associated (P < 0.001). Finally, the BWadj-HW of individual F2 (SHR × BN) rats also showed a positive association with the injured vessel CA, MA + NIH area, and %NIH (P < 0.001).

DISCUSSION

Ultimately, we would like to facilitate the discovery of effective therapies to prevent vascular restenosis by taking advantage of the 2.5-fold difference in NIH formation found between the inbred BN and SHR rat strains (1). The genome scan of the F2 (SHR × BN) intercross population identified regions controlling the formation of NIH on RNO3 and RNO6. Surprisingly, the %NIH-directed genome scan led to the discovery of QTL for vascular wall parameters in both control and injured vessels that were not directly related to NIH formation. The injured vessel vasculature changes were associated with QTL for MA, CA, and LS on RNO3 (Fig. 2A), MA + NIH area on RNO4 (Fig. 2B), and MA on RNO15 (Fig. 2D). The control vessel vasculature changes were particularly interesting, with QTL for both MA and MW found in two distinct peaks on RNO6 (Fig. 2C).

Table 3. Vascular parameters measured for control BN, SHR, and F2 (SHR × BN) vessels

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BN</th>
<th>SHR</th>
<th>F2 (SHR × BN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA, mm²</td>
<td>0.500 ± 0.70 (0.290–1.790)</td>
<td>0.347 ± 0.22 (0.273–0.591)†</td>
<td>0.442 ± 0.21 (0.092–0.858)‡</td>
</tr>
<tr>
<td>LS, mm²</td>
<td>0.471 ± 0.73 (0.261–1.742)</td>
<td>0.335 ± 0.24 (0.217–0.580)§</td>
<td>0.420 ± 0.21 (0.091–0.846)§</td>
</tr>
<tr>
<td>MA, mm²</td>
<td>0.072 ± 0.13 (0.039–0.148)†</td>
<td>0.088 ± 0.26 (0.046–0.126)</td>
<td>0.097 ± 0.23 (0.029–0.174)‡</td>
</tr>
<tr>
<td>MW, mm²</td>
<td>0.028 ± 0.20 (0.020–0.040)‡</td>
<td>0.040 ± 0.21 (0.024–0.053)§</td>
<td>0.039 ± 0.18 (0.021–0.062)‡</td>
</tr>
<tr>
<td>NIH area, mm²</td>
<td>0.029 ± 0.58 (0.005–0.058)</td>
<td>0.012 ± 0.118 (0.001–0.058)</td>
<td>0.022 ± 0.85 (0.000–0.104)§</td>
</tr>
<tr>
<td>MA + NIH area, mm²</td>
<td>0.101 ± 0.37 (0.048–0.197)</td>
<td>0.100 ± 0.25 (0.062–0.148)</td>
<td>0.118 ± 0.25 (0.030–0.224)‡</td>
</tr>
<tr>
<td>%NIH</td>
<td>0.271 ± 0.41 (0.070–0.442)†</td>
<td>0.116 ± 0.97 (0.004–0.391)</td>
<td>0.171 ± 0.70 (0.001–0.613)§</td>
</tr>
</tbody>
</table>

Data are means ± CV, with range given in parenthesis, for 16 BN, 20 SHR, and 301 F2 (SHR × BN) vessels harvested 8 wk after the standard vascular injury. *P < 0.001 vs. F2 (SHR × BN) by 1-way ANOVA; †P < 0.001 vs. SHR by 1-way ANOVA; ‡P < 0.001 vs. BN by 1-way ANOVA.
Several NIH-related and injured vessel QTTL were found on the distal arm of RNO3 (Fig. 2A): a QTTL-containing region was identified on RNO3 near marker D3Arb12 for %NIH, NIH area, CA, and LS. In addition, QTTL were found on RNO3 for injured vessel MA near marker D3Rat227 as well as LS and CA near marker D3Rat12. We have examined these regions of the rat genome for known genes and are targeting several candidates for further study that may be controlling the NIH injury response.

Initially, vascular injury triggers platelet aggregation and release of PDGF (1, 39), a chemoattractant for SMCs in the media and other inflammatory cells in the circulation and adjacent tissues (39). SMCs migrate through the ECM of the media, pass through the injured IEL, and proliferate and undergo apoptosis at the subendothelial injury site. SMC migration and proliferation are both triggered and modulated by PDGF (32, 48, 54), TGFβ (32, 36, 40, 48), endothelial cell-produced heparin (32, 45), estradiol (19), progesterone (31), IGF-I and IGF-II (48), EGF (48), basic fibroblastic growth factor (bFGF) (48), somatomedin C (32), epidermal growth factor (48), IL-1 (48), and enzymes, some possibly related to the renin-angiotensin-aldosterone system (13, 45). ECM macromolecules such as collagen, elastin, proteoglycans, and adhesive glycoproteins are also involved in maintaining vascular wall integrity after injury (17, 28, 36, 37), and signaling cascades induced by changes in these ECM macromolecules can regulate the migration (6, 45, 51), apoptosis (6, 18, 41), proliferation (16, 32, 45), and differentiation (34) of vascular SMCs, VECs, and fibroblasts. Intriguingly, the QTTL interval on RNO3 includes matrix metalloproteinase-9 (Mmp9) and cadherin 22 (Cdh22).

The MMP family consists of 17 known enzymes classified as collagenases, gelatinases, stromelysins, and metalloelastases. Mmp9, of the gelatinase subfamily, is located within the NIH QTTL region on RNO3 near locus D3Arb12 and is involved in remodeling the vascular ECM (20). MMP enzymatic activation is controlled by inducible nitric oxide synthase (iNOS) signaling. The endothelial nitric oxide synthase (eNOS) isoforms are constitutively active and produce NO in small quantities. eNOS protects the uninjured vasculature by inducing vasorelaxation in response to the shear stress of increased blood pressure (2, 10, 42), inhibits the transvascular migration and proliferation of SMCs and leukocytes (2, 9, 12, 52), blocks the adherence and aggregation of platelets to the vessel walls (2, 9, 12, 38, 52), and promotes endothelial cell growth and proliferation (2). iNOS produces NO in large quantities, is stimulated by vascular damage, and abrogates the vascular protective effects of eNOS-produced NO. NO produced by iNOS thereby promotes injury-induced vasospasm, adherence and aggregation of platelets to the injury site, transvessel migration of SMCs and inflammatory cells (2, 9, 12, 26, 38, 52), and upregulation of MMP expression (26). MMP expression leads to the enzymatic degradation of the ECM and facilitates SMC migration through the media and IEL of the injured vessel.

Another candidate gene in the RNO3 QTTL-containing region is a cadherin, Cdh22. Cadherins are adhesion molecules involved in calcium-dependent cell-cell adhesions and may aid the migration of SMCs through the ECM. Cdh22 also participates in a signal transduction pathway regulating expression levels and localization of β-catenin, which also controls cell proliferation, migration, and apoptosis by the Wnt growth factor pathway (22). We also scanned genes present in the RNO6 QTTL-containing region. Although several genes could potentially be involved in the process of NIH formation, none was as obvious a candidate as Mmp9 and Cdh22 for governing the NIH injury response.

We previously found (1) slight decreases in the CA (P = NS) and LS of injured BN vessels compared with noninjured controls. It was possible, however, that this finding was in part due to the noninjured BN vessels being more flexible and difficult to properly orient in the tissue blocks for perpendicular sections than the injured vessels, i.e., the noninjured vessels lacked the rigidity of the injured vessels imparted by the neointima itself and the postinjury inflammatory response. If this was the case, such bias in section perpendicularity would artificially elevate the control vessel CA values. In the present study, we used primary agarose embedding before processing and paraffin embedding to ensure an exact perpendicular orientation of the fixed vessels to the microtome blade (see MATERIALS AND METHODS). A different result from that previously was found for sections prepared in this manner, with a 10% increase of CA of the injured BN vessels occurring, which more than compensated for any occlusive effects of the neointima on the LS of the injured BN vessels (P = NS, Table 1).

BWad)-HW and vessel-wall parameters such as CA, MW, and %NIH are complex phenotypes, presumably controlled by the interplay of multiple genetic and environmental factors. Phenotypes influenced by the same environmental or genetic factors are expected to have expression levels that are positively or negatively linked. Phenotypes without common underlying causal factors would not be expected to show a coordinated pattern of expression. Also, the degree of expression of certain phenotypes before injury might influence the

### Table 4. Comparison of parameters measured for control and injured BN, SHR, and F2 (SHR × BN) vessels

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>CA, mm²</th>
<th>MA, mm²</th>
<th>MW, mm²</th>
<th>MA + NIH area, mm²</th>
<th>LS, mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>BN control</td>
<td>16</td>
<td>0.454±46</td>
<td>0.087±25</td>
<td>0.035±13</td>
<td>0.087±25</td>
<td>0.454±46</td>
</tr>
<tr>
<td>BN injured</td>
<td>16</td>
<td>0.500±70</td>
<td>0.072±37</td>
<td>0.028±20</td>
<td>0.101±37</td>
<td>0.471±73</td>
</tr>
<tr>
<td>P, 1-way ANOVA</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>SHR control</td>
<td>20</td>
<td>0.342±13</td>
<td>0.094±22</td>
<td>0.042±18</td>
<td>0.094±22</td>
<td>0.342±13</td>
</tr>
<tr>
<td>SHR injured</td>
<td>20</td>
<td>0.347±22</td>
<td>0.088±26</td>
<td>0.040±21</td>
<td>0.100±25</td>
<td>0.335±24</td>
</tr>
<tr>
<td>P, 1-way ANOVA</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>F2 control</td>
<td>301</td>
<td>0.439±25</td>
<td>0.100±21</td>
<td>0.040±16</td>
<td>0.100±21</td>
<td>0.439±25</td>
</tr>
<tr>
<td>F2 injured</td>
<td>301</td>
<td>0.442±21</td>
<td>0.097±23</td>
<td>0.039±18</td>
<td>0.118±25</td>
<td>0.420±21</td>
</tr>
<tr>
<td>P, 1-way ANOVA</td>
<td>NS</td>
<td>NS</td>
<td>0.002</td>
<td>&lt;0.0001</td>
<td>0.019</td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± %CV. NS, not significant.
extent, and thereby the subsequent repair, of the standard vascular injury, i.e., the standard vascular injury might produce different degrees of damage to thick- and thin-walled vessels. If this were the case, then the initial MA or MW of a given F2 (SHR × BN) vessel might have a direct bearing on the amount of injury transmitted to that vessel and, in turn, influence its postinjury NIH production. In support of this, the differing numbers of postinjury IEL “breaks” and “waves” between the BN and SHR parental strains also suggest that a genetic difference in vessel fragility and distensibility (11, 23) may directly influence the postinjury responses of the vasculature. We are currently trying to determine whether or not these differences in postinjury vascular changes between the BN and SHR strains can be influenced by endothelial vasodilators.

As the SHR rat is perhaps the best-studied genetic model of hypertension and F2 (SHR × BN) populations are extensively used to identify QTL for blood pressure and related traits (46, 49, 50), elevated blood pressures may have influenced the response to the standard vessel injury in our population of F2 (SHR × BN) rats. Each of the F2 (SHR × BN) rats in our population was phenotyped for BWadj-HW, which shows a strong correlation with blood pressure in rat models (43, 55), including segregating populations of rats (reviewed in Ref. 47). Here we used BWadj-HW as an indirect (and imperfect) measure of the blood pressures observed in the population. As expected, the mean BWadj-HW of F2 (SHR × BN) rats was intermediate to, and significantly different from, the higher values of SHR rats and the lower values of BN rats (Table 2). The lack of association between BWadj-HW and control vessel CA of the individual BN and F2 (SHR × BN) rats and the increased CA observed in F2 (SHR × BN) control vessels, compared with those of SHR control vessels (Table 2), suggest that the mechanism(s) producing small, thick-walled vessels in the SHR rats is not present in the F2 (SHR × BN) rats.

The finding of QTL for non-NIH-related phenotypes, especially those for control vessel MA and MW (Table 1), in the %NIH-driven genome scan supports the possibility of causal connections between certain control vessel parameters and postinjury NIH production. Specifically, the initial genome scan was performed on rats at the extremes of the F2 (SHR × BN) %NIH phenotypic distribution, followed by genotyping of the remaining F2 rats in the chromosome regions where QTL were identified. The complete %NIH-driven genome scan revealed 14 QTL, of which only 10 (injured vessel parameters) were directly related to NIH (Table 1). The non-NIH-related QTL (control vessel parameters) can only be explained by chance or causal connections between postinjury NIH and these phenotypes.

If future studies with congenic strains confirm these associations between the control vessel parameters and postinjury neointima production, these relationships may allow the identification of vessels or individuals with an increased susceptibility to NIH after vascular procedures. Also, development of congenic strains that delimit the chromosomal intervals in which causative gene(s) for %NIH-related QTL reside should facilitate the future identification of the genes responsible for the strain differences in postinjury NIH formation observed between BN and SHR rats. This discovery may, in turn, facilitate the identification of orthologous, NIH-related genes in humans, as disease-specific QTL found in rodent studies have often been shown to be located within orthologous human chromosomal regions containing QTL for the same (or related) phenotypes (25, 56). Ultimately, the detection of NIH QTL may lead to the identification of the gene(s) controlling vascular stenosis and the development of new therapies to prevent this complication.

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GRANTS

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REFERENCES


