Quantitative genetic basis of arterial phenotypes in the Brown Norway rat

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The Brown Norway (BN) rat presents several genetically determined arterial phenotypes of interest, i.e., ruptures of the internal elastic lamina (RIEL) in the abdominal aorta (AA), iliac (IAs), and renal arteries, aortic elastin deficit and higher frequency of persistent ductus arteriosus (PDA) than other strains. We investigated the genetic basis of these phenotypes. We established a backcross between BN and the LOU reference strain and performed a genome-wide scan on 104 males and 105 females with 193 microsatellite markers followed by linkage analysis. RIEL in AA and IAs showed highly significant linkage to a locus on chromosome 5 and suggestive linkage to a locus on chromosome 10, which is syntenic to one linked to a syndrome of thoracic aortic aneurysms with PDA in humans. In contrast, renal artery RIEL mapped to a chromosome 3 locus and thoracic aortic elastic content to two loci on chromosome 2. PDA was significantly linked to two different quantitative trait loci (QTLs) on chromosomes 8 and 9. This is the first study in rats to identify genetic loci for PDA.

We identified 21 candidate genes by functional relevance or integration of our mapping data with global expression analysis. Sequencing these genes identified 47 single nucleotide polymorphisms, but no functionally relevant amino acid changes. By expression analysis, myosin heavy chain 10, nonmucle, in the chromosome 10 QTL emerged as a candidate for RIEL in AA and IAs. Furthermore, production of a congenic line for the chromosome 5 QTL proved implication of this locus in RIEL formation.

internal elastic lamina; abdominal aorta; elastin; ductus arteriosus; quantitative trait loci

THE INBRED BROWN NORWAY (BN) rat presents several interesting arterial phenotypes which are not observed in other common strains of laboratory rat. All BN rats develop spontaneously, during growth and aging, large numbers of ruptures in the internal elastic lamina (RIEL) in the abdominal aorta (AA) and the iliac arteries (IAs), whereas most other strains do not (2, 5, 16). Most strains develop a few RIEL in the renal artery and many in the caudal artery (26), but again, the BN is more affected than other strains, such as the Long Evans (LE) (5) and LOU (unpublished data). Although the formation of these RIEL may be influenced by many factors, including hemodynamic factors (27) and various compounds such as β-amino-propionitrile (BAPN) (5) and inhibitors of the renin-angiotensin system (12), it is nevertheless strongly genetically determined. In view of the fact that in many arterial pathologies, rupture of the elastic lamellae is observed (e.g., aneurysms, dissections, fibromuscular dysplasia etc.), any genetic influence on elastic lamellar rupture may be of pathophysiological interest.

The adult BN also presents a lower elastin content and a proportionally higher collagen content in the aorta, both in the thoracic segment, which is unaffected by the above-mentioned RIEL, and in the abdominal segment, presenting numerous ruptures (31). The elastin deficit is at least in part due to a deficiency in tropoelastin synthesis in the BN rat, compared with LE and LOU, during the period of rapid growth (31, 32).

The genetic basis for the spontaneous RIEL in abdominal aorta was recently studied by another group, in an F2 population originating from a cross between BN and New Zealand genetically hypertensive (GH) rat strains (16, 11). This latter strain exhibits very low levels of aortic RIEL, similarly to the LE and LOU strains. The authors concluded from their phenotypic data that the character for RIEL was autosomal, dominant, and possibly a single gene effect (16). Subsequent linkage studies on the same population (11) identified two major quantitative trait loci (QTLs) for aortic RIEL, on chromosomes 5 and 10, exhibiting respectively semidominant and dominant modes of inheritance, with the BN alleles being associated with increased lesion number. These results are at variance with our phenotypic data in BN × LE and BN × LOU crosses because, in our case, no F1 rats and very few F2 rats presented significant numbers of RIEL. The BN, LE, and LOU strains are all normotensive, and adult male rats of these strains show no significant difference in systolic BP (2, 32).

In view of the divergence in the phenotypic data for RIEL incidence obtained by crossing BN rats with normotensive LE or LOU and with hypertensive GH rats, we have undertaken a genome-wide scan to locate QTLs for this character in a large cohort of rats originating from a BN × LOU backcross (BC). We chose to use BC rats [(BN × LOU) × BN], a population more suited to the study of RIEL than the F2 population, because of the previously observed recessive character of the phenotype in F2 rats. We included in our study the quantification of aortic elastin and collagen, the major aortic extracellular matrix (ECM) proteins, and of renal artery RIEL, a phenotype whose genetic basis has not yet been studied, and whose relation with aortic RIEL is unclear.

In addition to the above-mentioned phenotypes, the BN rat also presents another character of pathophysiological interest, namely persistence of the ductus arteriosus (PDA). PDA is quite frequently observed in the BN rat and never in the LOU or other strains (unpublished data). We thus included this phenotype in the present linkage analysis, which represents the first quantitative genetic investigation for PDA. Our data also enables us to determine whether RIEL, decreased aortic elas-
Materials and Methods

Animals

Inbred BN rats were from Elevage Janvier (Le Genest St Isle, France) and inbred LOU/M rats from our own breeding stock. F1 rats were produced by reciprocal mating of BN with LOU rats, and F1 were mated with female BN to produce the BC generation (n = 244). All rats were kept in standard conditions until 18 wk of age. In addition, male and female BN and LOU rats were used for determining parental quantitative traits not previously reported: i.e., RIEL in LOU rats and female BN at 18 wk of age and scores for PDA. Other parental phenotypes were data taken from our previous studies (2, 32).

Determination of Quantitative Traits

We phenotyped 41 F1 rats (20 male and 21 females) and 209 BC rats (104 males and 105 females) at 18 wk of age for RIEL, ductus arteriosus (DA) status, and thoracic aortic (TA) elastin and collagen content as described below. A further 36 BC rats were phenotyped later for fine mapping of QTL intervals and were integrated in the final linkage analysis presented here.

RIEL

RIEL were quantified on “en face” arterial preparations as previously described (2). For the AA, renal arteries, common IAs, and proximal 5 mm of the caudal artery, the total number of RIEL was recorded. Then, for AA and IAs only, each rupture was graded on a semiquantitative scale according to its size, and a score, taking into account the severity of the phenomenon, was allotted to each rat.

Scoring for PDA

Immediately after thawing the thoracic aortas to proceed to biochemical analysis, we evaluated the persistence and state of closure of the DA under a dissecting microscope (magnification ×12.5) and graded it by a semiquantitative score, taking into account its various features.

Composition of the Thoracic Aorta

Biochemical analysis of the descending thoracic aorta was performed as previously described (2, 32). Elastin, collagen, and cell proteins were all expressed both as a percentage of aortic dry weight and as mg/cm of aorta.

Genetic Studies

Microsatellite markers. We selected 175 microsatellite markers that exhibited allelic variation between the parental strains and that cover the 20 autosomal chromosomes of the rat genome. The primer pair information of these markers was obtained from RGD (http://www.rgd.mcw.edu/). Fine mapping with 18 additional markers was performed on 36 extra BC rats. All markers are listed in Suppl. Table S1. (The online version of this article contains supplemental material.)

Genotyping. DNA extraction followed by fluorescence-based semiautomated genotyping was performed (see online supplement). The genetic map of the 193 microsatellite markers was established using MAPMAKER/EXP V3.0 (21).

Linkage analysis. Linkage analysis was conducted using Mapmaker software (21) using all 209 BC progeny and separately for the subsets of 105 females and 104 males. Both parametric and nonparametric linkage analyses were performed using Mapmaker/QTL V3.0 (20) and V1.9 (19) and are reported as logarithm of the odds ratio (LOD) or Z-scores respectively.

RESULTS

RIEL

AA and IAs. Large numbers of RIEL were present in the AA and IAs in BN rats, as previously described (2, 5, 16), but this trait was totally absent in LOU rats (Fig. 1A, Suppl. Tables S2 and S3). Such RIEL in BC rats are illustrated in Fig. 2, A–C. The score for RIEL in AA and IAs (see Suppl. Tables S2 and S3), taking into account rupture size and thus the severity of the phenomenon, and numbers of RIEL in the proximal caudal artery (results not shown), closely paralleled the situation for RIEL counts in the AA and IAs across the different rat populations, suggesting that are all manifestations of the same underlying anomaly.

RIEL in AA and IAs mapped to a highly significant QTL on chromosome 5 (Suppl. Fig. S1, A–H) with a peak LOD score of 27.4 in the cumulative population (Fig. 1A, Table 1). This QTL is identical to that previously described (11), and at this locus the BN allele is associated with an increased number of RIEL (P = 1.2 × 10−23, Fig. 1A).

We identified 5 positional candidate genes in this QTL based on functional relevance: Rdh10, Kcnb1, Sulf1, Lyn, and Mmp16, none of which were differentially expressed. Sequencing of the exonic regions of these genes identified several synonymous SNPs (not leading to any amino acid changes), except for one SNP (2859G/A) in Sulf1, which led to a change of valine to isoleucine (Suppl. Table S4).

Large RIEL in AA and IAs showed suggestive linkage to chromosomes 2, 8, and 10 (Suppl. Fig. S1, E and F). The chromosome 10 QTL, between markers D10Rat96 and D10Rat27, (Z-score 3.3 at peak marker D10Rat64) overlapped with that previously described in rats (11) and is syntenic to the recently published locus in humans linked to a pathophysiological entity associating TA aneurysm and aortic dissection with PDA (18). Possible candidate genes in this QTL were the glycoprotein Sprc and three myosin heavy chain genes: Myh17, implicated in the above human syndrome (40), Myh3, and Myh10. Mutation screening was negative for these genes,
but both \textit{Sparc} and \textit{Myh10} were differentially expressed on the chip (Suppl. Table S4). Quantitative PCR confirmed a 2.7-fold downregulation of \textit{Myh10} in the AA of BN vs. LOU, making it a strong candidate for RIEL in AA and IAs. We thus sequenced, in addition to the exonic region of \textit{Myh10}, regions 5 \textit{kb} upstream and 1 \textit{kb} downstream to screen for variations in the regulatory regions, but no mutation was found. Only one synonymous SNP in exon 17 was identified (Suppl. Table S4).

\textbf{Renal artery.} Renal artery RIEL showed a slightly different distribution in the various rat populations from RIEL in the AA and IAs, as a few were present in male LOU and some BC rats showed more than BN rats (Suppl. Tables S2 and S3, Fig. 1B). A renal artery of a BC rat is illustrated in Fig. 2D.

Interestingly, unlike RIEL in the AA and IAs, those in the renal arteries did not map to chromosome 5, but to chromosome 3, with a peak LOD score of 4.0 (Fig. 1B, Table 1, Suppl. Fig. S1i). Renal artery RIEL were more numerous in the heterozygous than in the homozygous BC progeny (\(P = 0.0005\)), suggesting an effect of the LOU allele here also. \textit{Fbn1} was the only potential candidate gene in this QTL, but it was not significantly differentially expressed and sequencing revealed no variations leading to amino acid changes (Suppl. Table S4).

\textbf{PDA}\

PDA in adult rats of the BN strain it is not rare to observe a PDA (Suppl. Table S2, Fig. 1, C and D). The most affected BN rats exhibit a diverticulum, wide open on the aortic side and nearly closed on the pulmonary side (Fig. 3, A–C). This observed anatomy suggests that the aorto-pulmonary shunt effect is absent or minimal, explaining the lack of overt symptoms in affected BN rats. The mildness of the phenotype implies that complete DA closure is probably polygenic and only some of the implicated genes are affected here. A continuous variation of this trait was observed within the BN strain, ranging from a grossly visible diverticulum (as in Fig. 3, A–C; 18\% of the total BN population), via various intermediate stages of involution, to the presence of a normal fibrous ligamentum arteriosus (14\% of the total BN population), as consistently observed in the LOU strain (Fig. 3D). These observations are reflected by the significantly different scores between BN and LOU (Suppl. Table S2). BC rats showed a wide range of scores, as shown in Fig. 3, E–G, with only 3.7\% overall showing a macroscopically visible diverticulum as shown in Fig. 3E, and 70\% with scores lying close to those observed in LOU rats (Suppl. Table S3).

PDA was significantly linked to two distinct QTLs (Table 1), one on chromosome 8 in the female BC progeny only (Fig. 1C) and one on chromosome 9 in the cumulative population (Fig. 1D). In both cases, the BN allele contributed significantly to the increased PDA scores (Fig. 1, C and D). We also identified suggestive QTLs on chromosomes 3, 5, 7, and 12 (Suppl. Fig. S1j). The loci on chromosomes 8, 9, and 12 for PDA harbor eight different myosin genes, namely: \textit{Myo9a}, \textit{Tpm1a}, \textit{Myo1e}, \textit{Myo5a}, \textit{Myi3}, \textit{Myolb}, \textit{Mlc3}, and similar to \textit{Mlc2}, and two collagen genes, \textit{Col3a1} and \textit{Col5a2}, but no mutations were found (Suppl. Tables S4 and S5).

\textbf{TA Composition}\

LOU rats had higher TA elastin content (% of aortic dry weight) and elastin-collagen ratio than BN rats, and the F1 population showed intermediate values, as previously described (32). In the F1 and BC cohorts, females had significantly higher values than males, and the same tendency was observed in the parental strains (Suppl. Tables S2 and S3).

TA elastin content (\%) (Fig. 1E) and elastin-collagen ratio (Fig. 1F) were significantly linked to chromosome 2 (Table 1; Suppl. Fig. S1, l–n) although elastin-collagen linkage was observed in male progeny only and the pattern of the LOD score curve suggested two independent loci (Fig. 1F), with highest LOD scores at D2Rat205 and D2Rat51 respectively (Table 1). These two loci overlap with the QTL linked to TA % elastin. The BN allele was associated with lower values at all these three loci (Fig. 1, E and F).

TA dry weight (mg/cm) was significantly linked to chromosomes 7 and 19, showing respective LOD scores of 4.0 and 3.3 (Suppl. Table S6, Suppl. Fig. S1K). Both elastin and collagen contents, when expressed in mg/cm, also mapped to these loci (Suppl. Fig. S1, M and O), as these parameters are linked to aortic dry weight. Suggestive linkage was also observed for TA elastin content (expressed as both % and mg/cm) to chromosome 17 (Suppl. Fig. S1, M and N). Other suggestive loci were found for the various parameters expressing TA elastin and collagen contents on chromosomes 1 and 3 (Suppl. Fig. S1, K–P).

The QTL on chromosome 2 for % elastin and elastin-collagen span a relatively large interval and harbor numerous known and predicted genes. We chose to sequence \textit{Kcnjd3}, due to a possible role of K\(^+\) on elastin synthesis, We have detected eight synonymous SNPs in this gene (Suppl. Table S4).

Results of linkage analysis for other weight-related phenotypes are summarized in Suppl. Table S6.

No correlation was found between any of the major phenotypes, i.e., RIEL, PDA, and aortic elastin content, nor did we identify any epistatic interaction between the different loci. No significant lineage effects were detected in the different subgroups (from male Y-BN and Y-LOU matings, respectively), which we tested for at all significant QTL regions.

\textbf{Congenic Line LOU.BN.D5Rat59-D5Rat131}\

The details of the congenic strain LOU.BN.D5Rat59-D5Rat131, with the chromosomal segment of interest from BN introgressed into the LOU genetic background, are shown in Fig. 4. The number of RIEL in this congenic line compared with parental BN, LOU, F1, and BC (homozygous BN/BN at the locus of interest) are shown in Fig. 4A. The congenic rats present ~30\% of the BN value for this quantitative trait, confirming our linkage data and providing us with a useful tool for future studies.

\textbf{DISCUSSION}\

This study shows that the inbred BN rat presents several arterial phenotypes, which represent components of human multigenic cardiovascular pathologies, but are also of interest in fundamental vascular biology. The arterial wall elastic fiber network is essential for normal large artery function, and so any factor that affects it may render the arteries more susceptible to various pathologies, in view of the important hemodynamic stress to which they are constantly exposed. Interestingly, a recent study (17) has shown that IEL defects are an early feature of aortic lesions in ApoE\(^{-/-}\) mice, widely
considered as a model of human atherosclerosis. Furthermore, the AA and IAs in man are particularly prone to developing atheromatous lesions, leading to such complications as aneurysm formation and intermittent claudication, and so the BN model may provide some clue to the reason for this susceptibility. PDA is a congenital heart defect not uncommon in humans, and its genetic components have not yet been elucidated. Thus, although the BN rat appears to have a normal lifespan, as the anomalies described here are minor and do not evolve adversely in standard laboratory conditions, suggesting that in each case only one of a series of causal genes is affected, this model may nevertheless lead to the identification of genes which may be of relevance to human pathology.

The first major observation in this study is that the principal phenotypes are not correlated in the BC population and map to separate QTLs, indicating that they are each under independent genetic control and so do not represent a “syndrome.”

The major QTL on chromosome 5, linked to RIEL in AAs and IAs, was responsible for a large part (45%) of the phenotype and is identical to that described by Harris et al. (11) in a BN-GH cross. However, these authors also found a significant QTL on chromosome 10, the two loci together determining only 33.5% of the phenotype. Among several suggestive loci we found for this phenotype, our chromosome 10 locus overlapped with that previously described (11) and thus can be regarded as a confirmed QTL.

The major chromosome 5 locus contained no obvious potential candidate genes, such as lysyl oxidase or a known component of elastic fibers, and no genes in this region were differentially expressed between BN and LOU in the microarray analysis. However, considering that RIEL is likely to be due to some other underlying ECM anomaly or a dysfunction of smooth muscle or endothelial cells, we chose five genes for mutation analysis. Sulphatase-FP (Sulf1) (25), matrix metalloproteinase 16 (Mmp16), and Yamaguchi Sarcoma viral (v-yes-1) oncogene homolog (Lyn), via its participation in the regulation of uPA (11), may be involved in degradation of components of the ECM, retinol dehydrogenase 10 (all-trans) (Rdh10) in arterial smooth muscle signaling (1, 6), and potassium voltage-gated channel, Shab-related family, member 2 (Kcnb2) may influence elastin synthesis (36). However, no significant mutations were found in the coding regions of any of these genes.

The chromosome 10 locus harbors several myosin heavy chain genes. Myh11, a smooth muscle form (SM-MHC) was an interesting candidate in view of its recently described implication in a human syndrome associating PDA and aneurysms (40). Moreover, a transgenic mouse strain lacking SMC-MHC gene expression presents delayed closure of the ductus arteriosus (24). Myh3 encodes for an embryonic myosin form and Myh10 for the nonmuscle myosin heavy chain IIB (NMHC IIB), which is present in all mammalian cells and is involved in physiological functions such as cell motility, morphology and cytokinesis (34, 35). In mice this gene is required for normal cardiac development (35, 37). However, sequencing these three genes did not provide evidence for their implication in RIEL. In contrast, Myh10, but not Myh11, was downregulated in BN AA compared with LOU. This finding merits further investigation by a more integrated approach to minimize the limitations of genetic mapping and differential expression analysis, as previously discussed for hypertension (39).

To validate our findings using congenic approaches we are currently establishing several congenic lines for some of the loci identified. Here we represent a congenic line for the chromosome 5 locus, in which we were able to capture part of the phenotype of interest. This represents an essential tool to further the search for genes involved in this striking vascular phenotype of spontaneous RIEL. Indeed, looking at functionally relevant genes has shown its limitations here, and using congenics and derivation of sublines will provide an unbiased view. Although the above-mentioned myosin genes merit further investigation, it is possible that some developmental or remodeling genes or an as yet unknown, minor constituent of elastic fibers may be implicated. Indeed, the steps involved in IEL formation during arterial growth and development are poorly understood, and the role, if any, of the endothelial cells in this process is unclear. Discovery of the gene(s) present in this QTL with such a major influence on RIEL may provide...
insight into fundamental mechanisms involved in development of the vascular elastic fiber network.

The tight correlation that we observed between RIEL in the AA, IAs, and the proximal caudal artery within the BC population is not surprising, as these are all closely related elastic arteries and the common iliac and caudal arteries take origin in the affected AA. However, renal artery RIEL were much less significantly correlated with those in AA and IAs in males and did not correlate at all in females (results not shown). Indeed the upper AA, where the renal arteries take origin, is unaffected by RIEL in the BN rat, as this phenotype occurs only in the subrenal aorta (unpublished observations). Moreover, we found a distinct QTL for renal artery RIEL on chromosome 3, which harbors the gene for fibrillin-1 (Fbn1), a potential candidate gene for elastic fiber anomalies in view of its implication in the Marfan syndrome (22). However, no mutation was found.

Surprisingly, the LOU allele was associated with increased RIEL in the renal artery. We have previously shown that renal artery RIEL, unlike those in AA and IAs, occur in all rat strains, to varying degrees (5, 26, 27), and they may be influenced by many local factors, such as blood pressure and flow and thus, indirectly, kidney size (26, 27). We did find QTLs for kidney size on chromosomes 3 and 7 (Suppl. Table S6), but the one on chromosome 3 did not localize to the same area as that for renal artery RIEL.

It thus appears that the presence of RIEL is a complex phenotype, as superposed on a genetically determined susceptibility, which may or may not be the same throughout the arterial tree, local factors undoubtedly play a role in inducing
these ruptures. The fact that the BN is the only rat strain to present the AA/IA/proximal caudal artery phenotype (2), whereas all strains present RIEL in their renal arteries and in the more distal, muscular part of the caudal artery, albeit to different degrees (5, 26), is intriguing. Thus, either the two phenotypes are distinct, as suggested by the present linkage analysis, or local factors so greatly influence the phenotype that in the adult the effect of the original determining gene may be masked in the renal artery. Indeed, RIEL, once formed in rat arteries are never completely repaired since, despite rapid replacement of the cellular elements, the gap in the IEL remains throughout life. The effect of any local factors on numbers of RIEL is thus cumulative and would be proportionately greater the older the population studied.

One of the aims of this study was to investigate whether the aortic elastin deficit in the BN rat could be the cause of RIEL formation. Previous studies have indirectly suggested that this was not the case (2, 9), and here we found no correlation between these two phenotypes in the BC population in either sex, suggesting no causal relationship. Indeed, aortic RIEL correlated with none of the aortic biochemical parameters we measured in this study. Furthermore, RIEL and aortic elastin content are controlled by genes on different chromosomes. The QTLs we found on chromosome 2, for aortic elastin content and elastin-collagen ratio, overlap with those previously described (9) and confirm that the BN allele is linked to decreased elastin content and that many different genes control this phenotype. None of our QTLs for elastin content mapped to the position of *Eln*, confirming that the elastin gene itself controls a very minor part of the variation in aortic elastin content (32).

Identification of genes controlling ductal closure in a quantitative manner has never been attempted before. Studies in both dog (28) and human (10) suggest a genetic component, but the exact mode of transmission and the number of genes involved remain unknown. In the rat it appears to be a rare phenotype, which we only discovered because of high frequency sampling of BN thoracic aortas. Recently, another group has confirmed our observations (3). We found two significant QTLs for PDA, on chromosomes 8 and 9, with the BN allele giving the highest scores. Among genes present in these QTLs are a large number of myosin genes, which is of interest in view of the recently reported involvement of *Myh11* in a human syndrome with PDA (40). However, we found no mutations with possible functional repercussions in these various myosin genes, although several synonymous SNPs were found. Three collagen genes (*Col5a3* on Chr8, *Col3a1*, and...
Col5a2 on Chr9) were also present in these loci, which merited further exploration as we found a weak correlation ($P = 0.047$) between this phenotype and collagen content in males. However, this is may be a secondary phenomenon, as sequencing of two of these genes did not show any significant mutations in the BN rat.

A recent study in an Iranian population has reported a recessive component to PDA, implicating a single locus on chromosome 12q24 (23). Sequencing of selected candidate genes in this locus (nNOS, paxillin, and PTPIN1) did not provide evidence of their implication in the phenotype. In our study, one of our three suggestive loci was in the syntenic region on the rat genome (peak marker D12Rat80, LOD score 3.5). This QTL also contained a myosin gene (Mlc2), but no mutation was found. Ductal closure is a complex process involving smooth muscle cell contraction, synthesis of ECM molecules, neointima formation etc., implicating a multitude of possible genes. Further studies are thus required, but, in any event, the finding of a rat strain with incomplete ductal closure opens up new perspectives into the study of genes intervening in the control of this process, a crucial event at birth for subsequent normal cardiovascular function.

In conclusion, we have shown here that the BN rat presents several arterial phenotypes that are of pathophysiological and of fundamental interest. Here we provide considerably detailed data concerning the various phenotypes in the same population. We conclude that these phenotypes appear to be independent, controlled by different genes, and, in this respect it is surprising to find them united in one strain, which, moreover, appears to have a normal lifespan. The BN rat has also been shown to present other vascular abnormalities not studied here, such as poor survival in hypertension, related to susceptibility to cerebrovascular hemorrhage (5, 7), impaired myogenic autoregulation in the kidney (38), and a defective flow-induced arterial wall remodeling (8, 15). In addition, the BN rat also presents hydropnephrosis, already reported by others (29, 33), for which we have found linkage to another distinct genetic locus to be reported elsewhere. Obviously further studies will be necessary to unravel the mechanisms underlying this accumulation of subclinical abnormalities of a predominantly vascular nature in one strain, a strain that appears to be a genetic outlier compared with most other commonly used laboratory rats (4). Lastly, these data should be of particular interest to the scientific community as the BN rat has been widely used as a “reference” strain in genetic studies and was chosen to sequence the rat genome (30).

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