Chromosomal Mapping of Quantitative Trait Loci Controlling Elastin Content in Rat Aorta

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Abstract—Extracellular matrix molecules such as elastin and collagens provide mechanical support to the vessel wall. In addition to its structural role, elastin is a regulator that maintains homeostasis through biologic signaling. Genetically determined minor modifications in elastin and collagen in the aorta could influence the onset and evolution of arterial pathology, such as hypertension and its complications. We previously demonstrated that the inbred Brown Norway (BN) rat shows an aortic elastin deficit in both abdominal and thoracic segments, partly because of a decrease in tropoelastin synthesis when compared with the LOU rat, that elastin gene polymorphisms in these strains do not significantly account for. After a genome-wide search for quantitative trait loci (QTL) influencing the aortic elastin, collagen, and cell protein contents in an F2 population derived from BN and LOU rats, we identified on chromosomes 2 and 14, 3 QTL specifically controlling elastin levels, and a further highly significant QTL on chromosome 17 linked to the level of cell proteins. We also mapped 3 highly significant QTL linked to body weight (on chromosomes 1 and 3) and heart weight (on chromosome 1) in the cross. This study demonstrates the polygenic control of the content of key components of the arterial wall. Such information represents a first step in understanding possible mechanisms involved in dysregulation of these parameters in arterial pathology. (Hypertension. 2005;45:460-466.)

Key Words: aorta ■ elastin ■ genetics ■ rats

Elastin and collagens are the main extracellular matrix proteins of blood vessels. The functional properties of vessels, particularly of the major arteries and veins, are largely dependent on the absolute and relative quantities of these 2 constituents.

Elastic fibers composed of an elastin core surrounded by microfibrils are designed to maintain elastic function in tissues such as blood vessels, lungs, and skin. The biology of elastic fibers is complex because of their multiple components, tightly regulated developmental pattern of expression, multistep hierarchical assembly, unique elastomeric properties, and influence on cell phenotype.

The elastin-null mice (ELN⁻/⁻) die of obstructive arterial disease, whereas the ELN⁺⁺ mice have reduced absolute quantities of elastin and are hypertensive, phenotypes similar to those observed in patients with supravalvular aortic stenosis and Williams syndrome. In these patients, the elastin gene is mutated or deleted.

Investigating the genetic basis and pathophysiological consequences of the modulation of elastin levels, rather than the impact of absolute elastin deficit, is central to our understanding of mechanisms involved in arterial pathologies. The composition of the arterial extracellular matrix and the adaptive synthesis of elastin and collagen in hypertension may also influence the degree of reversibility of arterial remodeling in response to antihypertensive treatment and the development of hypertensive arteriopathy. Genetic studies of the aortic content of extracellular matrix molecules are impossible in humans, for obvious reasons, and remain impractical in the mouse because of the relatively small amount of aortic tissue that can be obtained, thus making the accurate quantification of these molecules difficult. In this context, rat models provide essential tools for such genetic studies.

The inbred Brown Norway (BN) rat strain shows an aortic elastin deficit (by 5.8% dry weight when compared with the inbred LOU rat) and is also highly susceptible to spontaneous rupture of the internal elastic lamina (IEL) in its abdominal aorta. The aortic elastin deficit in the BN rat is, at least in part, caused by decreased tropoelastin synthesis during the period of rapid growth. Because the elastin gene polymorphism explains only a small part of differences between parental strains, it is thus probable that other genes account for the major proportion of the interstrain differences in aortic elastin content. Identification of genetic loci underlying the elastin content is the first step toward understanding which facet of elastin biology leads to a deficit of this parameter in the BN rat.
In this study, we have performed a genetic analysis in an F2 cohort of 161 rats derived from BN and LOU rat strains to detect genetic loci contributing to quantitative variations of aortic elastin, collagen, and cell protein contents and to test a possible relationship between these loci and those linked to susceptibility to aortic IEL ruptures.8

Methods

Animals and Phenotype Analysis
Inbred BN rats were obtained from Ifa Credo (Domaine des Oncins, l’Arbesle, France) and inbred LOU rats were from our own breeding stock. Female LOU rats and male BN rats were mated to produce an F1 population. Rats of the F1 generation were mated to produce the previously described 161 F2 hybrids (119 males and 42 females).3 Animal care complied with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication 86 to 23, revised 1985). The studies were performed under authorization 006235 of the Ministère de l’Agriculture, France.

Rats were studied at 18 weeks of age. Body and heart weights were recorded, and the composition of the thoracic aorta (dry weight, cell protein, elastin, and collagen contents) was measured as previously described.8 Results are expressed as percent of aortic dry weight or mg/cm of aorta length.

Genotype Characterization and Genetic Map Construction
Genomic DNA of the 161 BN×LOU F2 rats was prepared by phenol extraction from tail tips. After polymorphism assays between BN and LOU rat strains, genetic screening of the LOU×BN F2 hybrids was performed using a selection of rat microsatellite markers exhibiting allele variations between the parental strains. All markers used in this study were chosen in existing linkage and/or radiation hybrid maps of the rat9,10 to cover the 21 rat chromosomes with an average spacing of 10 to 15 cM between adjacent loci. Information regarding primer sequences and optimized polymerase chain reaction conditions for all markers used in this study are publicly available (http://www.well.ox.ac.uk/rat_mapping_resources). Genetic maps were constructed using the JoinMap version 2.0 suite of programs.11 Further details on standardized methods for genotype determination and genetic map production and verification are available in an online supplement available at http://www.hypertensionaha.org.

Genetic Linkage Analysis
Before quantitative trait loci (QTL) mapping, all phenotypes were regressed for sex and the standardized residuals were tested for departures from normality by the Kolmogorov–Smirnov and Shapiro–Wilk tests using the SPSS version 12.0 package (SAS Institute Inc, Cary, NC). In all further linkage analyses, the standardized residuals that best fit the normal distribution were used. Linkage between marker genotypes and phenotypes was initially evaluated by an ANOVA test followed by a permutation test \((n=10,000)\) to calculate the threshold of significance for each pair of genetic-phenotypic marker.12,13 We used R/qtl14 to generate the genome wide scans. MAPMAKER/QTL computer package was subsequently used for interval mapping15 in the chromosomal regions exhibiting evidence of significant genetic linkage. The probability values reported in the text along with logarithm of odds (LOD) scores account for multiple testing. Correlations between marker genotypes and phenotypes were calculated using the SPSS version 12.0 package.

Results

Phenotype Analyses
The distribution of elastin (percent dry weight), collagen (percent dry weight), and elastin/collagen values, as well as the correlation between elastin (percent dry weight) and collagen (percent dry weight) are presented in Figure 1A. There is no correlation between elastin (percent dry weight) and collagen (percent dry weight). The distribution of body weight and heart weight/body weight are shown separately for males and females in Figure 1B.

Genetic Maps
The main characteristics of the genetic maps constructed with the 159 markers typed in the BN×LOU F2 cross, including the number of markers typed for each chromosome, marker spacing, and chromosome length, are available in the online supplement (Table I). Both individual chromosome length and total genome length obtained in this cross were consistent with our published consensus linkage maps of the rat.10 The percentage of total genomic length that each chromosome represents was also very similar to our previous observations.10

To carefully test the possible effect of variants in the elastin gene on aortic elastin phenotypes, genotypes at the locus Eln containing the gene encoding elastin were determined by 2 markers, D12Wox14, which was located within the elastin gene sequence,10 and D12Rat51, which maps 400 kb away from the gene (http://www.ensembl.org; http://www.well.ox.ac.uk/rat_mapping_resources). We confirmed that these markers are localized in the linkage map constructed in the BN×LOU F2 cross approximately 0.5 cM apart on rat chromosome 12 at the genetic position 30.6 cM.

QTL Mapping
Statistical analyses demonstrated the absence of significant linkage between marker locus D12Wox14 (Eln) and the aortic elastin content (mg/cm) \((\text{LOD}=1.39; P=0.04)\) (data not shown), the percentage of aortic elastin \((\text{LOD}=1.40; P=0.04)\) (Figure 1), and the ratio elastin/collagen \((\text{LOD}=1.26; P=0.06)\) (data not shown) in the BN×LOU F2 cross. This locus thus explains only a small proportion of the phenotypic variance of these traits (3.8%, 3.9%, and 3.2%, respectively).

Evidence of genetic linkage to aortic elastin phenotypes in the BN×LOU F2 cross was detected with markers mapped to rat chromosomes 2 and 14 (Figure 2A and 2B). These loci were named aortic elastin 1 (Ael1) and aortic elastin 2 (Ael2). The pattern of the chromosome 2 QTL Ael1 and Ael2 strongly suggests that 2 independent loci mapped \(\approx 60\) cM apart control the percentage of aortic elastin and the elastin/collagen ratio in the cross. Markers showing the strongest evidence of linkage to these variables were D2Got24 (LOD 3.1; \(P=9.10^{-4}\)) and D2Wox26 (LOD 4.2; \(P=7.10^{-5}\)). These loci explain 9.2% (D2Got24) and 11.3% (D2Wox26) of the variance of aortic elastin content and elastin/collagen ratio, respectively (Table II). The effect of the LOU allele on these traits appears to be dominant in the region of marker D2Got24 but recessive at marker locus D2Wox26 (Figure 2A). The QTL Ael3 identified in the region of marker D14Rat36 (maximum LOD 3.7; \(P=5.10^{-4}\)) in rat chromosome 14 is also marginally linked to the percentage of aortic elastin and the ratio elastin/collagen in the cross (Figure 2B) and accounts for up to 9.7% of the variance of these traits (Table II). Altogether, these 3 loci explain >30% of the
Figure 1. Distribution of the aortic phenotypes, body weight, and heart weight quantified in the BN×LOU F2 cross. A, Distribution of elastin and collagen values (expressed as percent of dry weight), elastin-to-collagen ratios, and correlation between elastin and collagen contents in the aorta of 18-week-old male and female rats, taken together. B, Distribution of body weight and heart weight to 100 grams of body weight ratio (cardiac mass index) for 18-week-old male and female rats, plotted separately.
variance of the aortic elastin content in the BN×LOU F2 cross. At each of these 3 QTL, the BN allele was associated with a significant reduction in aortic elastin content (Figure 2A and 2B). These loci did not show statistically significant linkage to any other phenotypes quantified in the cross (Figure 2C, D, E, and F). Aortic collagen content was not significantly linked to any marker tested.

Highly significant linkage between markers in a broad region of rat chromosome 17 and the aortic content in cell proteins (percent) was identified and the QTL was named aortic cell protein 1 (Acp1) (Figure 2C). Marker locus D17Got80 was the most significantly linked to the phenotype (LOD 6.1; P<0.001 to P<10^{-5}) after 10,000 permutations. Black bars indicate the 1.5-LOD confidence intervals around the peak of linkage. Phenotype means±SE were calculated for each genotype at the marker locus showing the strongest evidence of linkage, B/B indicates homozygous for the BN allele; B/L, heterozygous; L/L, homozygous for the LOU allele. Number of animals carrying each of the 3 genotypes is reported. *Significantly different from L/L homozygotes. †Significantly different from B/L heterozygotes.

Figure 2. MAPMAKER LOD score curves from the BN×LOU F2 whole genome scan for aortic elastin in chromosomes 2 (A) and 14 (B) (QTL Ael1–3) and cell proteins in chromosome 17 (C) (QTL Acp1). LOD scores are plotted against map distance in centimorgans (cM), as determined in the BN×LOU cross. The dashed lines indicate genome wide thresholds of significance (P<0.001 to P<10^{-5}) after 10,000 permutations. Black bars indicate the 1.5-LOD confidence intervals around the peak of linkage. Phenotype means±SE were calculated for each genotype at the marker locus showing the strongest evidence of linkage, B/B indicates homozygous for the BN allele; B/L, heterozygous; L/L, homozygous for the LOU allele. Number of animals carrying each of the 3 genotypes is reported. *Significantly different from L/L homozygotes. †Significantly different from B/L heterozygotes.

Discussion
In the present study, we have identified 3 QTL (Ael1–3) mapped to chromosomes 2 and 14 specifically controlling elastin content in the thoracic aorta of the rat. Interval
mapping showed that the strongest evidence of linkage to aortic elastin in rat chromosome 2 was obtained with markers localized nearly 60 cM apart, strongly suggesting the existence of 2 QTL, Ael1 and Ael2, on this chromosome. At all 3 QTL Ael1–3, BN alleles were associated with a reduction in elastin levels with different genetic effects (dominant, recessive, or additive), which further support the existence of 2 QTL in rat chromosome 2. In our experimental conditions, polymorphisms at the elastin locus itself do not significantly contribute to the control of aortic elastin phenotypes. Results from genetic interaction (epistasis) tests between Ael loci were inconclusive, probably because of the relatively modest size of the F2 cohort (data not shown). The fact that they only account for up to 30% of the total variance of the aortic elastin phenotypes, which is, however, within the range of most QTL studies addressing the genetic control of vascular phenotypes in the rat, suggests an involvement of other loci that the application of stringent thresholds of statistical significance did not allow the detection.

Regions of rat chromosome 2 have been previously characterized for QTL linked to altered vascular phenotypes in hypertensive rat strains, and this chromosome is now a classical example for QTL replication. In our QTL mapping study, we chose 2 normotensive strains for the production of the F2 cohort to exclude or minimize the confounding effects of arterial pressure on arterial elastin and collagen. However, it is still possible that genes regulating arterial blood pressure may also contribute to aortic elastin and collagen synthesis in normotensive rats. The absence of correlation between elastin and collagen contents in the BN×LOU F2 population and the lack of significant effects of the QTL Ael1 and Ael2 on variations in the collagen content argue against such a role for blood pressure.

Highly significant linkage was found between aortic cell proteins and a broad region of rat chromosome 17 (locus Acp1). Cell proteins, which were measured after extraction by 0.3% SDS, represent an index of total cell mass. This parameter is dependent on cell size and cell number and in the aorta largely reflects the medial smooth muscle cells as endothelial cells and adventitial fibroblasts are proportionately minor constituents. This parameter has been shown to increase with aging in a manner similar to medial cell area measured by histomorphometry and to decrease in the normotensive rat after inhibition of the renin-angiotensin system. This phenotype, and the demonstration of its genetic control by gene(s) at the locus Acp1, is thus important in the context of vascular remodeling.
The BN strain has been widely used as a control normotensive strain in genetic studies related to hypertension. According to our results, it is possible that BN alleles leading to a depletion in aortic elastic content may impact on blood pressure QTL detection and significance. In addition, the BN rat shows evidence of vascular abnormalities, including susceptibility to spontaneous ruptures of the abdominal aortic IEL, which the LOU strain is devoid of. We particularly focused on the genome-wide scan in the BN×LOU cross on regions of rat chromosomes 5 and 10, which were previously associated with susceptibility to IEL rupture in a BN×GH cross, to test a possible relationship between this phenotype and variations in components of the aortic extracellular matrix. The absence of significant linkage between these chromosomal regions and aortic elastin, collagen, and cell protein levels in the BN×LOU cross provides strong evidence against this hypothesis.

In addition to QTL linked to components of the arterial wall, we also identified highly significant linkages to body weight in chromosomes 1 (QTL Bw1) and 3 (QTL Bw2) and cardiac mass in chromosome 1 (QTL Cmi1) in the BN×LOU cross. Interestingly, the BN and LOU alleles at the loci Bw1 and Bw2 showed opposite effects on body weight. Although the 95% confidence intervals for the QTL Bw1 and Cmi1 largely overlap, the markers showing the strongest evidence of linkage to these traits map 35 cM apart on rat chromosome 1, thus suggesting the involvement of different genes. The QTL Bw2 colocalizes with a locus associated with body weight in a SS/jrMNS cross. The position of the locus Cmi1 coincides almost exactly with a QTL linked to aortic weight in a SS/jrSHR cross and a congenic interval associated with both blood pressure and heart weight in a SS/jr×Lew strain combination. It is, however, nearly 100 cM distal to a QTL linked to cardiac mass in a SS/SHR cross.

Along the same line, previous studies have consistently shown the implication of QTL on chromosome 1 for the control of body weight, heart weight, and cardiac mass index in different rat crosses primarily designed to investigate the genetic control of complex disorders, including type 1 diabetes, type 2 diabetes, and hypertension. However, the clustering of QTL for body weight and cardiac mass index in a similar region of rat chromosome 1 in these crosses and in the BN×LOU cross suggests the effect of gene variants that are not necessarily involved in the pathophysiology of these diseases but would instead reflect strain differences. In our study, evidence of strongly significant linkage between cardiac mass and chromosome 1 loci, which is independent of significant variations in elastin levels in the BN×LOU F2 cohort, strongly suggests the effect of gene(s) at the QTL that primarily controls heart weight regardless of influences from altered metabolic and hormonal phenotypes. However, BN variants at Cmi and Ael QTL may have a significant impact on blood pressure variability in experimental crosses derived from BN and hypertensive strains and may eventually affect blood pressure QTL significance.

As generally observed in studies designed to map complex quantitative traits, the 95% confidence intervals of the QTL detected in the BN×LOU cross span large chromosomal regions ranging from 14 to 35 cM (31 to 70 Mb), thus making difficult and relatively subjective the selection of candidate genes. Nevertheless, anchoring the QTL in the rat genome sequence assembly allows their annotation for hundreds of known or predicted genes and expressed sequence tags localized at these loci, as well as the definition of conserved regions in the human and mouse genomes through comparative genome mapping. Among the genes of interests mapped at the QTL Ael1, Atp1a1, which encodes the alpha subunit of the Na+/K+-ATPase pump, is a potential candidate through the role of K+ on elastin synthesis.

In conclusion, our study demonstrates that numerous genes control the elastin and cell protein contents in the rat aorta. The identification of QTL in this study should contribute to our understanding of the role of components of the arterial wall in the pathogenesis of vascular disease.

**Perspectives**

Our genetic results provide important information on chromosomal targets for the production of congenic strains, which are primarily designed to validate and fine-map QTL. Furthermore, congenics carrying BN alleles at the Ael and Cmi loci will represent new powerful tools for accurately characterizing the functional relevance of these QTL. In particular, the impact of reduced elastin level on blood pressure regulation can be tested in congenics carrying either a single or a combination of Ael loci of BN origin introgressed onto the genetic background of a hypertensive strain. The availability of the annotated rat genome sequence and the ongoing study of sequence and haplotype conservations between rat strains will provide essential resources for the selection of candidate genes underlying the QTL effects in congenic intervals and identifying causative gene variants.

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