Genetic Determinants of Systolic and Pulse Pressure in an Intercross Between Normotensive Inbred Rats

Bastien Llamas, Catherine Lau, William A. Cupples, Marie-Line Rainville, Emmanuelle Souzeau, Christian F. Deschepper

Abstract—By continuous monitoring of abdominal aortic blood pressure via telemetry in conscious rats, we have observed that systolic, diastolic, and pulse pressures of male Brown–Norway rats were all significantly lower than that of male Wistar–Kyoto rats, despite the fact that all of the values in both strains were within normotensive ranges. Further analyses performed in 166 animals from the progeny of an F2 intercross between Brown–Norway and Wistar–Kyoto rats revealed that, despite a high correlation between systolic blood pressure and diastolic blood pressure, there was no correlation between pulse pressure and diastolic blood pressure, and the value of the correlation between systolic blood pressure and pulse pressure was lower than that of systolic blood pressure with diastolic blood pressure. Two major and highly significant ($P<0.001$) quantitative trait loci linked to pulse pressure were found on chromosome 4 ($Pp1$) and 16 ($Pp2$). Only suggestive quantitative trait loci were found for systolic blood pressure, but the strongest one ($Sbp1$) had the same peak and linkage probability profile as $Pp1$. Altogether, these data show that genetic determinants affecting pulse pressure in normotensive animals are either stronger or independent from the ones affecting systolic blood pressure and are of interest in light of evidence showing that pulse pressure is highly heritable in humans and that elevated pulse pressure is a predictor of cardiovascular risk. (Hypertension. 2006;48:921-926.)

Key Words: blood pressure/analysis ■ blood pressure/genetics ■ quantitative trait loci/genetics ■ rats inbred bn/genetics ■ models ■ animal/genetics ■ vascular diseases/ultrastructure

Numerous studies have used crosses between inbred rat strains to dissect out quantitative trait loci (QTLs) linked to blood pressure. Although such crosses (maintained under different environmental conditions) have made it possible to identify up to now $\sim 273$ QTLs linked to blood pressure (http://rgd.mcw.edu), most of these studies (if not all) have used crosses where one of the two parental strain was hypertensive. Moreover, the majority of these QTLs concern only systolic blood pressure (SBP), because only a limited number of studies have measured blood pressure via intra-arterial devices.1–4 Such recordings are a requirement to measure diastolic blood pressure (DBP) and pulse pressure (PP). PP is of particular interest, because it is governed, in addition to the force of ventricular ejection, by intrinsic properties of the vascular tree. The principal vascular characteristics that influence PP are the cushioning capacity of large arteries and the timing and intensity of wave reflections,5 both of which are governed predominantly by large artery stiffness.6 Moreover, PP is a powerful and independent predictor of cardiovascular risk, particularly in older patients,7–10 and, thus, carries predictive information above and beyond that carried by SBP or DBP. Likewise, PP or surrogate measurements are markers of atherosclerosis,11–13 and elevated PP associates with low renal function in elderly patients.14

In the course of preliminary experiments where abdominal aortic blood pressure was continuously monitored via telemetry in conscious rats, we have observed that SBP, DBP, and PP of Brown–Norway (BN) rats were all significantly lower than that of Wistar–Kyoto (WKY) rats, despite the fact that all of the values in both strains were within normotensive ranges.15 We, therefore, proceeded to generate an F2 intercross between both strains to test to which extent the values of SBP, DBP, and PP would cosegregate and whether QTLs linked to each particular trait would be distinct or show overlap.

Methods

Two inbred strains of rats were used for the current studies. The WKY/Cfd rats originated from a colony maintained at the Institut de Recherches Cliniques de Montréal and were derived from WKY/Cr parents obtained from Charles River (St Constant, Quebec, Canada). BN/SsN rats were purchased from Harlan Sprague Dawley (Indianapolis, IN). Male and female WKY/Cfd rats were first mated to either male or female BN/SsN rats. The resulting F1 animals were further mated randomly to generate male F2 animals. This design
represents a “reciprocal cross,” which ensures that sex chromosomes of both parental origins are represented equally within the F2 population. All of the procedures on animals were approved by the University of Victoria Animal Care Committee and conducted according to guidelines issued by the Canadian Council on Animal Care.

Each rat was first operated at 10 to 12 weeks of age. Twenty minutes before induction of anesthesia, each animal received the analgesic buprenorphine (Temgesic, Schering-Plough, 0.02 mg/kg IP) and the antibiotic preparation Tribrisen 24% (0.25 mL/kg). Anesthesia was first induced by 4% isoflurane in inspired gas (30% O2 and 70% air) then maintained by reducing the concentration to ~2%. Each rat was transferred to a heated table for surgical implantation of the blood pressure PA-C40 telemetry implant (DataSciences). The cannula of the implant was advanced via the femoral artery into the abdominal aorta at about the level of the kidneys. The body of the transmitter was inserted into a subcutaneous pocket on the rat’s left flank and held in place by a purse string suture. The rats received 2 additional doses of buprenorphine at ~12-hour intervals after transmitter implantation. At least 10 days were allowed for recovery before acquiring one 24-hour control record of blood pressures, heart rate (HR), and locomotor activity. We occasionally acquired a second 24-hour record if the first was of poor quality or incomplete. Data were acquired at 250 Hz for 10 s every 2 minutes. Transmitters were zeroed before implantation, and the 0 was checked at explantation. If the 2 zeroes differed by >12 mm Hg, then a linear correction was applied to the data. All of the values for the above variables represent averages of values collected over the 24-hour period.

After recording baseline values in parental BN and WKY rats, a second surgery was performed to clip the right renal artery and, thus, generate 2 kidney-1 clip (2K-1C) hypertension. The right kidney was approached by a flank incision and a silver clip placed on its artery. The gap in the clip was initially set to 0.25 mm with a feeler gauge; after positioning, the gap was adjusted so that the diameter of the artery distal to the clip was less than that proximal to the clip. The incision was closed in layers, the abdominal muscle with interrupted sutures, and the skin with interrupted subcuticular sutures. Analgesia and anesthesia procedures were as described above. Blood pressure was monitored by brief sampling in the following days. Animals that developed malignant hypertension (defined as mean arterial pressure [MAP] >160 mm Hg accompanied by rapid weight loss and/or development of seizures) and those that failed to develop hypertension were culling (~11%). The effect of strains on the time-dependent evolution of SBP and PP was tested by 2-way ANOVA, followed by post hoc Fisher’s test to test for differences between strains at particular time points.

To monitor general health, rats were weighed daily from 5 days before the initial surgery until 12 days after the second surgery and then at weekly intervals. Blood pressure recordings after 2K-1C began 12 to 14 days after surgery. Twenty-four hour records were acquired weekly for 8 weeks, as described above. At the end of that period, all of the animals were euthanized by KCl injection under isoflurane anesthesia, and the spleens were collected for genomic DNA extraction.

### Microsatellite Markers and Genetic Analyses
Genomic DNA was obtained from frozen spleen samples using the DNeasy tissue kit (Qiagen). Analysis of simple sequence-length polymorphisms was carried out by PCR amplification of genomic DNA with M13-tailed microsatellite markers labeled with either dye IRD700 or dye IRD800 (LiCor Biotechnology) similarly as described previously. The products were separated and analyzed using a LiCor 4200 DNA analysis system.

The order of microsatellite markers was obtained from rat genome databases (http://www.niams.nih.gov/rbc/ratbase; http://rgd.mcw.edu; http://www.well.ox.ac.uk/rat_mapping_resources; and http://www-genome.wi.mit.edu/rat/public). Genotyping data were then analyzed with J/qtl (http://www.jax.org/staff/churchill/labsite/software/Jqtl/), which is a JAVA-enabled version of the R/qtl package. Markers misplaced or showing aberrant recombination were removed from the dataset. We used a total of 144 polymorphic microsatellite markers to obtain dense coverage of all of the autosomal chromosomes (7.6 ± 3.2 markers per chromosome, with distance between 2 markers averaging 14.3 ± 8.2 centimorgans). Linkage probability was examined by interval mapping, using the EM algorithm. Genomewide significance thresholds were set, as suggested previously, at the 37th (“suggestive”), 95th (“significant”), and 99.9th (“highly significant”) percentiles, which correspond to the chance of finding 1 false-positive linkage 0.63, 0.05, and 0.001 times, respectively. Threshold for values of the log-likelihood of the odds (LOD) ratios were calculated on the basis of permutation tests performed on the data sets themselves (using 1000 permutations). For QTLs of interest, the phenotypic data were grouped according to the origin of the allele closest to the peak of the LOD curve, and corresponding data were analyzed by 1-way ANOVA to determine the mode of inheritance. Confidence intervals (CIs) were estimated by the 1-LOD support interval method. Possible interactions between QTLs were investigated by 2-dimensional, 2-QTL genome scanning using the EM algorithm. The degree of genetic determination (ie, the ratio of genetic to total variance) was estimated as (Vg/Vt), where “Vg” was the total variance of the phenotype in the F2 cohort (explained by both environmental and genetic factors), and “Vt” was the pooled estimate of the phenotype variance in the parental and F1 cohorts (solely because of environmental factors).

### Results
The distribution of phenotypic values in the parental, F1, and F2 cohorts is shown in Table 1. It can be appreciated that the values of PP, SBP, and DBP were ~24%, 15%, and 11% lower in BN than in WKY rats, respectively. Phenotypic values in the F1 and F2 animals were intermediate between those of the 2 parental strains. The degree of genetic determination in the F2 cohort was estimated as 36%, 25%, and 20% for PP, SBP, and DBP, respectively. Other measured phenotypes (HR and locomotor activity) were not different between the 2 strains and did not segregate in the progeny of the cross.

Using results obtained in all 166 of the F2 male animals, we performed correlations among the values of SBP, DBP,
Not surprisingly (because each variable participates in the calculation of MAP), there was a high and significant correlation ($r^2 = 0.88; P < 0.0001$) of the values of SBP and DBP with that of MAP. However, the value of the correlation of SBP with DBP, although still highly significant ($P < 0.0001$), was lower ($r^2 = 0.625$) than that of SBP with MAP. Despite the high correlation between SBP and DBP, there was no correlation between PP and DBP, and the value of the correlation between SBP and PP was lower ($r^2 = 0.345; P < 0.0001$) than that of SBP with DBP.

The whole genome scan revealed 2 major and highly significant ($P < 0.001$) QTLs linked to PP on chromosome 4 ($Pp1$) and 16 ($Pp2$; Table 2 and Figure 2). Two-dimensional, 2-QTL genome scanning revealed that $Pp1$ and $Pp2$ interacted in an additive (nonepistatic) manner, the combined LOD score for $Pp1$ and $Pp2$ being 10.49. In contrast to PP, only suggestive QTLs were found for SBP. The highest of such QTLs ($Sbp1$) had an LOD score >3 but did not quite reach the significant level ($P < 0.08$; Table 2 and Figure 2). However, the peak of $Sbp1$ was identical to that of $Pp1$, and its profile of linkage probability on chromosome 4 was very similar to that of $Pp1$ (Figure 3). Other QTLs linked to either PP and SBP showed only suggestive linkage ($P < 0.63$), and none of these peaks showed significant overlap. Characteristics of all of the significant or suggestive QTLs (with exact location, CI, and mode of inheritance) are summarized in Table 2.

Although DBP was different in parental strains, we found no QTL with significant or suggestive linkage to DBP in the F2 progeny. To test whether the difference in PP observed between BN and WKY rats under basal normotensive conditions would

![Figure 1. Correlations between the values of DBP vs SBP, MAP vs DBP or SBP, and PP vs MAP, DBP, or SBP. The values of the $r^2$ correlation coefficients and the significance levels of each correlation are as indicated.](image)

### Table 2. Characteristics of QTLs

<table>
<thead>
<tr>
<th>QTL</th>
<th>Chromosome</th>
<th>Properties</th>
<th>Peak Marker</th>
<th>LOD</th>
<th>Position, Centimorgan</th>
<th>CI, Centimorgan</th>
<th>Dominant</th>
<th>BN/BN</th>
<th>BN/WKY</th>
<th>WKY/WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Pp1$</td>
<td>4</td>
<td>Highly significant</td>
<td>D4Wox2</td>
<td>5.51</td>
<td>59</td>
<td>50 to 65</td>
<td>BN</td>
<td>35.6±3.7*</td>
<td>37.2±3.8*</td>
<td>39.8±4</td>
</tr>
<tr>
<td>$Sbp1$</td>
<td>4</td>
<td>Suggestive</td>
<td>D4Wox2</td>
<td>3.18</td>
<td>58</td>
<td>48 to 100</td>
<td>BN</td>
<td>112.3±6.4†</td>
<td>114.1±5.9†</td>
<td>117.5±7.2</td>
</tr>
<tr>
<td>$Pp2$</td>
<td>16</td>
<td>Highly significant</td>
<td>D16Rat66</td>
<td>4.97</td>
<td>23</td>
<td>5 to 30</td>
<td>BN</td>
<td>35.2±4.0‡</td>
<td>38.3±4.0</td>
<td>38.9±3.6</td>
</tr>
<tr>
<td>$Pp3$</td>
<td>2</td>
<td>Suggestive</td>
<td>D2Mgh14</td>
<td>2.27</td>
<td>32</td>
<td>1 to 44</td>
<td>WKY</td>
<td>36.3±4.2‡</td>
<td>38.3±4.0</td>
<td>38.4±3.9</td>
</tr>
<tr>
<td>$Pp4$</td>
<td>5</td>
<td>Suggestive</td>
<td>D5Mit9</td>
<td>2.78</td>
<td>42</td>
<td>30 to 53</td>
<td>BN</td>
<td>38.1±3.7‡</td>
<td>38.6±4.3†</td>
<td>36.0±3.7</td>
</tr>
<tr>
<td>$Pp5$</td>
<td>9</td>
<td>Suggestive</td>
<td>D9Rat85</td>
<td>2.77</td>
<td>76</td>
<td>65 to 87</td>
<td>BN</td>
<td>38.7±4.3‡</td>
<td>38.3±4.0</td>
<td>36.2±3.1</td>
</tr>
<tr>
<td>$Pp6$</td>
<td>10</td>
<td>Suggestive</td>
<td>D10Mgh14</td>
<td>2.36</td>
<td>37</td>
<td>18 to 57</td>
<td>WKY</td>
<td>39.2±4.3‡</td>
<td>36.8±4.1</td>
<td>38.0±3.5</td>
</tr>
<tr>
<td>$Pp7$</td>
<td>15</td>
<td>Suggestive</td>
<td>D15Mgh2</td>
<td>2.90</td>
<td>38</td>
<td>20 to 56</td>
<td>BN</td>
<td>38.9±5.1‡</td>
<td>38.2±3.4†</td>
<td>36.0±3.9</td>
</tr>
<tr>
<td>$Pp8$</td>
<td>19</td>
<td>Suggestive</td>
<td>D19Rat7</td>
<td>2.99</td>
<td>59</td>
<td>16 to 66</td>
<td>WKY</td>
<td>35.9±3.7†</td>
<td>37.9±4.2</td>
<td>39.3±3.4</td>
</tr>
<tr>
<td>$Sbp2$</td>
<td>1</td>
<td>Suggestive</td>
<td>D1Mgh11</td>
<td>2.92</td>
<td>185</td>
<td>158 to 217</td>
<td>WKY</td>
<td>111.8±5.8†</td>
<td>115.2±6.1</td>
<td>116.5±6.8</td>
</tr>
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<td>$Sbp3$</td>
<td>6</td>
<td>Suggestive</td>
<td>D6Rat86</td>
<td>2.74</td>
<td>10</td>
<td>3 to 22</td>
<td>WKY</td>
<td>112.1±6.2†</td>
<td>116.4±6.6</td>
<td>115.3±6.7</td>
</tr>
<tr>
<td>$Sbp4$</td>
<td>10</td>
<td>Suggestive</td>
<td>D10Rat44</td>
<td>2.20</td>
<td>24</td>
<td>13 to 40</td>
<td>WKY</td>
<td>117.6±7.5†</td>
<td>113.5±6.4</td>
<td>115.4±6.1</td>
</tr>
</tbody>
</table>

* $P < 0.001$ vs WKY; † $P < 0.01$ vs WKY; ‡ $P < 0.05$ vs WKY.
hypertensive conditions, hypertension was induced by 2K-1C, and blood pressure was monitored weekly for several weeks after installing the clip of the renal artery. SBP increased in both BN and WKY rats in a time-dependent manner, but at most time points, both SBP and PP were lower in BN rats than in their WKY counterparts (Figure 4). By 2-way ANOVA, it was found that strains (P<0.05) and time (P<0.001) each had a significant effect on the values of SBP.
Correlation coefficient ($r$) of PP correlated significantly with SBP, the relatively low determination of PP was greater than that of either SBP or DBP. Although normotensive (LN) rats, other had reported previously a similarity lack of correlation between PP and DBP. In animals, our data are the first to identify a QTL linked to PP independently of blood pressure.

The mechanisms governing PP and aortic stiffness are complex and involve multiple factors, including the properties of the extracellular matrix, the composition and properties of cells constituting the vessel walls, and several humoral influences. Although some have reported that there were differences in collagen content and mechanical properties of conduit arteries from several normotensive and hypertensive strains of rats, it has not been verified whether these differences correlate with differences in PP in vivo. Interestingly, the vascular walls of BN rats have been reported to harbor several anomalies compared with other strains, including a high incidence of ruptures of the internal elastic lamina of large arteries and a decreased concentration of aortic elastin. Moreover, internal elastic lamina ruptures have been linked to 2 QTLs on chromosomes 5 and 10 in a cross between BN and the New Zealand Genetically Hypertensive rat. The aortic elastin content has been linked to 2 QTLs on chromosome 2 and 1 QTL on chromosome 14 in a cross between BN and Louvain rats. Although these 2 traits indicate that there is vascular fragility in BN rats, it is unknown whether they have an impact on PP. Moreover, none of these previously reported QTLs show any overlap with the ones identified in the present study.

### Perspectives

The continuation of the current studies by the generation of congenic animals should make it possible to identify candidate genes and/or vascular factors that are responsible for differences in PP. Although the current QTLs were identified under normotensive conditions, we also show that differences in PP were maintained when BN and WKY rats were made hypertensive.
Thus, the generation of congenic animals should have the additional utility of allowing one to test whether PP has pathophysiological consequences that are different or additional to that of blood pressure per se. There has been a lack of such a genetic animal model if one excepts models where more severe monogenic diseases (such as Marfan, Ehlers-Danlos, or Williams syndromes) have been mimicked by gene inactivation. For instance, despite having increased susceptibility to hypertension-induced renal disease and defective renal autoregulation, BN rats have both low incidence and slow progression of age-related glomerular disease and a greater life expectancy than Sprague–Dawley and Wistar rats. One plausible explanation would be that the lower PP in BN rats is partly responsible for their relative freedom from chronic progressive nephropathy, a hypothesis that could be formally tested in congenic strains. Finally, identification of genes linked to PP would be of interest in light of the evidences suggesting the adverse prognostic value of elevated PP in humans.

**Source of Funding**

This work has been supported by the National Institutes of Health/National Heart, Lung, and Blood Institute grant HL69122.

**Disclosures**

None.

**References**


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Hypertension. 2006;48:921-926; originally published online October 2, 2006; doi: 10.1161/01.HYP.0000244758.50351.29

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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