Assessment of a possible relation between osteoporosis and hypertension in spontaneously hypertensive rats and recombinant inbred strains.

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Aims:

Osteoporosis and hypertension are two age related diseases that could be associated with accelerated aging. Our project focuses on studying the common genetic determinants related with the progression of hypertension and the changes in bone density and the bone structural parameters associated to osteoporosis.

Methods:

The spontaneously hypertensive rats (SHR) exhibit a gradual increase in blood pressure during puberty and young adulthood that resembles what is seen in human essential hypertension. It thus makes it a good genetic model for studying hypertension and its complications (1). In addition to high blood pressure, several abnormalities in metabolic pathways have been reported in the SHR. Many of these defects are related to calcium handling and several groups have reported abnormal bone mineralization (2,3). Hence, SHR is also a good model to study osteoporosis.

Recombinant inbred strains (RIS) are inbred replica of F₂ segregating population obtained after several round of brother-sister mating (>20) of progeny of fixed (selected) F₂ pairs (Figure 1). As such, RIS are a unique opportunity to study genetically identical animals at different ages, and are considered as a very valuable tool for genetic studies since the mapping of their genome is practically permanent and they allow longitudinal studies (they are inbred strains) (4). They possess 50% of each of the two original ancestral genomes but in different combinations. This allows the dissection and mapping of the polygenic phenotypes segregating in the RIS along with the various combinations of the two progenitor genomes. One of the largest set of RIS for study of cardiovascular and metabolic traits was developed by the crossing of two genetically distant parental strains: the SHR and the normotensive Brown Norway rat (BN-Lx). Reciprocal crosses of SHR and BN-Lx are represented as follows: HxB and BxH (H representing SHR and B representing BN-Lx). The subsequent inbreeding of rats for over 35 generations has produced the strains available today and the present work was performed using these HxB/BxH RIS (5-9). Using those strains we have proposed that hypertension is a case of accelerated aging. Here we are reporting initial studies
of another aging related phenotype, osteoporotic bone changes as a parallel of pleotropic characteristic of hypertension (8). In Study 1, we assessed major bone structure characteristics to initiate exploration of strain distribution pattern: BxH9, BxH11, BxH2, HxB23 and HxB3. The bone mineral density (BMD) was measured by DXA and histomorphometric analysis was performed on cancellous bone of decalcified L4 vertebra. Bones were obtained from male rats of fifteen weeks of age and kept in 70 % ethanol. Statistical analysis was done using one way ANOVA. As the onset of osteoporosis and hypertension is gradual and both conditions evolve over time, in Study 2 we assessed the intra-strain temporal dynamics of blood pressure (assessed by telemetry) and bone parameters (microCT). Therefore, we studied 3 different RIS (HxB3, HxB13 and HxB17) and the SHR parental strain which served as a positive control for hypertension and bone disorders. This longitudinal study was performed over a year so that we could follow the growth of rats and assess the possible morphological changes at different ages. Systolic, diastolic and mean blood pressures as well as heart rate were assessed by telemetry measurements at 3, 6, 9 and 12 months of age. Radiotelemetry transducers (Data Science International, St.Paul, MN) were placed in the lower abdominal cavity and connected to catheters implanted in the lower abdominal aorta of anesthetized rats under aseptic conditions following standard operating procedures at the CRCHUM. The measurements were performed after two weeks of recovery. The structural parameters of the left tibial bone were obtained from \textit{in vivo} microCT scans (skyscan 1076: Skyscan, Antwerp, Belgium) at the four different ages (3, 6, 9 and 12 months) in the same animals to assess the morphological changes. Three-dimensional analysis was then performed following the standard bone package analysis developed at Skyscan.

**Results**

In study 1, HxB3 strain showed a higher BMD then BxH9 (p<0.01), BxH11 (p<0.001) and BxH2 (p<0.001), and a greater trabecular bone volume (BV/TV) then BxH9 (p<0.001) and BxH11 (p<0.001). Resorption parameters showed that eroded surfaces (ES/BS) were significantly lower in HxB3 compared with the other groups (vs BxH9 p<0.01; vs BxH11, BxH2 and BxH23, p<0.001). The number of osteoclasts per bone perimeter (N.Oc/Bpm) was significantly lower in HxB3 compared with BxH9 (p<0.01) (Figure 2). Finally, the results showed that HxB3, compared to the other RIS, possesses genetic factors which have positive effects on trabecular bone mass, possibly by lowering bone resorption.

In study 2, the results showed that HxB17 rats have extreme phenotypes as compared to the other strains. For instance, highest diastolic blood pressure and systolic blood pressure detected in the RIS was HxB17 (p<0.0001) (Figure 3). In addition, our scans showed that HxB17 rats have more trabecular weaknesses than the other strains for example the trabecular thickness was the lowest in HxB17 (p<0.0001) compared to the other strains. And the HxB 17 showed the lower trabecular bone volume (BV/TV) (p<0.0001) then the other rats (Figure 4).

**Conclusion**

The data from study 1 indicated the existence of strain differences in bone parameters, from which we conclude that the HxB3 has the most “robust” bone of all
the tested strains. The observed differences in BV/TV and resorption parameters between the tested RIS are large enough to potentially enable us to determine the loci related to these parameters of bone mass and remodelling (analysis is underway). The strain distribution pattern of the traits will then serve to map the genetic loci responsible for the observed differences, using the publicly available genetic map of all HXB BXH RIS containing over > 20,000 single nucleotide polymorphisms. In the second study, we observed that HxB17 shows extreme phenotypes compared to the other RIS, displaying weak trabecular architecture and high blood pressure.

Taken together, our data unveiled two strains, HxB3 and HxB17 which display opposite phenotypes. Fine mapping and identification of causal polymorphisms participating in the genetic architecture responsible for the observed bone density and blood pressure-related traits will be achieved by combination of genetic (advanced intercross lines) and bioinformatic (genetical genomics, network analysis) approaches.
**Figures**

**Figure 1:** The construction of the recombinant inbred strain panel (SHRxBN-Lx).

<table>
<thead>
<tr>
<th>RI strain</th>
<th>Age (week)</th>
<th>BMD L1-L4 (g/cm²)</th>
<th>BV/TV (%)</th>
<th>ES/BS (%)</th>
<th>N.OC/BPm (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BxH9, n=3</td>
<td>15</td>
<td>0.183 ± 0.009</td>
<td>16.8 ± 1.7</td>
<td>10.7 ± 0.3</td>
<td>0.97 ± 0.13</td>
</tr>
<tr>
<td>BxH11, n=4</td>
<td>15</td>
<td>0.178 ± 0.007</td>
<td>17.5 ± 4.5</td>
<td>12.2 ± 2.6</td>
<td>0.66 ± 0.29</td>
</tr>
<tr>
<td>BxH2, n=3</td>
<td>15</td>
<td>0.173 ± 0.01</td>
<td>25.5 ± 5.2</td>
<td>11.3 ± 0.8</td>
<td>0.56 ± 0.21</td>
</tr>
<tr>
<td>HxB23, n=3</td>
<td>16</td>
<td>0.203 ± 0.012</td>
<td>25.4 ± 4.6</td>
<td>17.1 ± 0.5</td>
<td>0.61 ± 0.35</td>
</tr>
<tr>
<td>HxB3, n=5</td>
<td>15</td>
<td>0.212 ± 0.002</td>
<td>31.9 ± 1.4</td>
<td>6.4 ± 1</td>
<td>0.29 ± 0.03</td>
</tr>
</tbody>
</table>

**Figure 2:** Bone parameters of five recombinant inbred strains of male rats at fifteen weeks of age assessed by DXA. (BMD = Bone Mineral Density, BV/TV = Bone Volume/ Tissue Volume, ES/BS = Eroded Surface/Bone Surface, N.OC/BPm = Number of osteoclast per bone perimeter)
Figure 3: Diastolic, systolic, mean arterial pressure and heart rate in SHR rats and recombinant inbred strains (HxB3, HxB23 and HxB17).

Figure 4: Trabecular thickness and bone trabecular bone volume ratio in HxB3, HxB13, HxB17 and SHR rats.
References


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Hypertension at nexus of thrifty, thirsty and accelerated ageing genotypes: and evolution of complex disease.