INTRODUCTION: Previous studies have shown that genetic background affects bone characteristics, particularly bone mineral density in mouse. Much less is known about the polymorphic genes on bone shape. In our previous study using SAMP2 and SAMP6 strains, we reported a quantitative trait locus (QTL) on chromosome 11 (Chr11) that has significant linkage to peak relative bone mass. We named it Phdl [1]. The aim of this study was to clarify the effects of Phdl for skeletal phenotype and narrow the QTL region.

MATERIALS AND METHODS: A congenic strain named P6.P2-Phd1b that carried a 39 cM SAMP2-derived Chr11 interval onto a SAMP6 background was generated as previously described [2]. Furthermore, 16 sub-congenic strains that have smaller overlapping intervals on SAMP6 background were generated from P6.P2-Phd1b. Femora of SAMP6 and sub-congenic mice were scanned by microphotodensitometry (MD) or micro-CT for morphological analyses (Fig. 1). For the index of relative bone mass, we used cortical thickness index (CTI) (Fig. 1A) or bone area fraction (BA/TA). Quantitative histology was performed using right femurs at 8 weeks of age. Results were tested statistically using one-way ANOVA to detect major differences, followed by the Scheffe test to assess the individual strain difference. The time-course of oblateness (OBL, Fig. 1B) of the femur was analyzed using two-way ANOVA. For other parameters, an unpaired two-tailed t test was used. All mice were handled according to the Guideline for Animal Experiments of Kyoto University. Male mice without macroscopically detectable pathological changes at autopsy were evaluated.

RESULTS: CTI of P6.P2-Phd1b at 16 weeks was significantly higher than that of SAMP6 and significantly lower than that of SAMP2 (Fig. 2A). To narrow down the interval, we first generated seven sub-congenic strains from P6.P2-Phd1b. As shown in Fig. 2A, the values of CTI of S4, S5, S6 were significantly higher than that of SAMP6. Therefore, the Phdl locus should be seated on the region between D11Mit59 and D11Mit167. Next, we generated an additional 9 sub-congenic strains from the S5 strain. These mice were examined by BA/TA instead of CTI, because BA/TA of S5 at 16 weeks was significantly higher than that of SAMP6. As shown in Fig. 2B, BA/TA of S5, S6, S7, S8, S9 and S10 were respectively significantly higher than that of SAMP6, indicating that Phdl is located in the 3.0 cM region (physical distance is 3.7 Mb) between D11Mit10 and D11Mit224. In the morphological analyses of femora at 16 weeks, the cross-sectional shape of S5 at midshaft was more compressed than that of SAMP6 in the anteroposterior direction (OBL) was significantly higher in S5, p<0.001, while the long axis length (LongAxL) was not different. Cortical thickness (Ct.Th) was not different between the two groups. We then examined the time-course change of OBL between SAMP6 and S5. Interestingly, OBL was not different at 5 days of age (Fig. 3). However, the difference in OBL became remarkable as they grew. The difference was greatest after 16 weeks, and two-factor ANOVA showed significant age x strain interactions for OBL from 5 days to 16 weeks, indicating that the difference was formed in the process of bone modeling. Because the shape of the growth plate may influence the cross-sectional shape of the midshaft, we also examined Dist-LongAxL, Dist-ShrtAxL, and Dist-OBL (Fig.1C) at 16 weeks of age. We did not find any significant difference between SAMP6 and S5 in these parameters. No trabecular bone parameters at the metaphyseal region of femora were different between SAMP6 and S5. To investigate the mechanisms that alter the cross-sectional shape of the femur, dynamic histomorphometrical parameters at 8 weeks (growth phase) were measured (Table 1).

Ps.MAR was significantly higher in SAMP6, whereas Ec.MAR was significantly higher in S5. Ec.BFR/BS was also significantly higher in S5, however, Ps.BFR/BS was not different. These differences may produce the difference of cross-sectional shape seen between SAMP6 and S5.

DISCUSSION: We have uncovered the effect of a QTL on Chr11, and found it is different to any previously described QTL on Chr11 for bone characteristics. We also showed that the QTL alters the cross-sectional shape of femur during growth without changing the cortical thickness. We could not fully exclude the possibility that there might exist more than one QTL that affects the bone properties on Chr11 in this study. However, from the morphological properties of congenic and sub-congenic strains using MD or micro-CT, all the sublines were classified into two types, suggesting that there exists only one locus that produces the difference between SAMP6 and P6.P2-Phd1b. In Phdl between D11Mit10 and D11Mit224, 49 genes have been identified. To detect the candidate gene, analyses of mRNA expression in various tissues and organs and gene’s sequence of all 49 genes will be required.

Table 1. Dynamic histomorphometrical parameters at the midshaft. (n=9)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SAMP6</th>
<th>S5</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ps.MAR (µm/day)</td>
<td>4.07 (1.35)</td>
<td>2.39 (0.59)</td>
<td>0.0058</td>
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<tr>
<td>Ps.BFR/BS (µm/day)</td>
<td>1.83 (0.67)</td>
<td>1.60 (0.28)</td>
<td>0.38</td>
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<tr>
<td>Ec.MAR (µm/day)</td>
<td>3.16 (0.76)</td>
<td>5.25 (1.02)</td>
<td>0.0002</td>
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<tr>
<td>Ec.BFR/BS (µm/day)</td>
<td>1.62 (0.51)</td>
<td>2.34 (0.68)</td>
<td>0.025</td>
</tr>
</tbody>
</table>

REFERENCES:

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