# QUANTITATIVE TRAIT LOCI INFLUENCING BONE QUALITY IN YOUNG AND OLD MICE

[1,2] Dean H. Lang, [1]Neil A. Sharkey [1]George P. Vogler, [1]David J. Vandenbergh, [1]David A. Blizard, [1]Joseph T. Stout, and [1]Gerald E. McClearn
[1]The Pennsylvania State University, University Park, PA, USA
[2] email: tcl133@psu.edu

## INTRODUCTION

C57BL/6J and DBA/2 inbred mouse strains have been shown to differ in their skeletal response to aging. This study was designed to increase our understanding of the genetic architecture responsible for the maintenance of bone quality across the lifespan. Our primary aim was to identify quantitative trait loci (QTL) linked to bone quality in young (200 day) and old (800 day) C57BL/6J X DBA/2 (BXD) recombinant inbred (RI) mice. Identifying QTLs in both cohorts adds confirmation of the locus while the identification of QTLs in either the 200 or 800 day old cohort suggests that these loci are operating on bone quality at specific ages.

## METHODS

Skeletal measures of strength and architecture were assessed in C57BL/6J X DBA/2 recombinant inbred (RI) mouse strains at both 200 and 800 days of age. The young adult (200 day) population included 23 RI strains with 10 male and 10 female mice per strain. A second population was bred to achieve similar numbers of mice at old age (800 days of age). The original study was designed to target 10 male and 10 female mice per 23 strains at 800 days of age taking into account known attrition rates. Despite these precautions, adequate numbers of mice per strain were unable to be maintained to 800 days of age. Those strains that did not have at least 3 mice for each male and female strain were not included in the QTL analyses. This resulted in 15 strains in the female analysis and 18 strains in the male analysis. The average number of 800 day old mice included in the QTL analyses was 6 per strain. Strain means were used in quantitative trait loci analysis to search for chromosomal regions influencing skeletal phenotypes at 200 and 800 days of age.

A three-point bending test was used to assess the mechanical integrity of the mid-shaft of femora and tibiae and a shear test was performed on the femoral neck to assess the mechanical integrity of this functionally significant skeletal site. Gross morphological measurements were made on both bones. Percent water, organic, ash, and mineralization were also measured. Separate sex-specific QTL analyses were performed using QTL Cartographer. Genotypes for the RI lines consisted of 672 microsatellite markers in each BXD strain. LOD scores of 3.3 or greater were considered significant while scores between 1.9 and 3.3 were considered suggestive.

## RESULTS AND DISCUSSION

The QTL results from the sex-specific interval mapping analyses are presented in Table 1 (cM corresponds to peak centimorgan position of the maximum LOD score or logarithm of the odds that linkage between a marker and a trait did not occur by chance). Significant LOD scores are highlighted with grey shading. Numerous QTLs for skeletal phenotypes mapped throughout the mouse genome. Table 1 summarizes the QTL results for those traits that had a LOD score of 3.3 or greater in either the 200 or 800 day old analyses.

**Table 1:** Interval mapping results from QTL analyses of 200 and 800 day BXD RI mice. R indicates QTLs detected using data adjusted for animal size.

| Chr. | Trait | Sex | 200 Day | | 800 Day | |
|---|---|---|---|---|---|---|
| | | | cM | LOD | cM | LOD |
| 1 | Femur Length | F | 89 | 2.9 | 90 | 1.9 |
| 1 | Femur Length | M | 90 | 3.3 | | |
| 1 | Femur Length - R | M | 90 | 4.7 | | |
| 1 | Femur Stiffness - R | F | 85 | 4.3 | | |
| 1 | Tibia Length - R | M | 78 | 1.6 | 62 | 3.3 |
| 1 | Tibia Length - R | M | 89 | 4.0 | 90 | 2.0 |
| 4 | Femur Coronal Width | F | | | 7 | 3.3 |
| 4 | Femur Coronal Width - R | F | | | 7 | 3.8 |
| 5 | Femur Epiphyseal Width | F | 44 | 3.5 | 43 | 1.6 |
| 5 | Tibia Length - R | M | 20 | 2.1 | 3 | 3.5 |
| 9 | Femur Stiffness | F | 21 | 3.5 | | |
| 9 | Femur Stiffness - R | F | 21 | 4.5 | | |
| 9 | Tibia Length - R | F | 21 | 3.3 | | |
| 10 | Femur Neck Ult. Work | F | 4 | 2.7 | 2 | 4.0 |
| 13 | Femur Yield Load | M | 70 | 3.3 | | |
| 13 | Femur Yield Work | M | 68 | 3.8 | | |
| 13 | Femur Yield Work - R | M | 68 | 3.7 | | |
| 15 | Tibia Length | M | 54 | 3.4 | | |
| 15 | Tibia Length | F | 48 | 3.5 | 48 | 2.5 |
| 15 | Tibia Length - R | F | 48 | 3.8 | 48 | 2.5 |
| 15 | Tibia Yield Work | M | 41 | 3.3 | | |
| 15 | Tibia Yield Work - R | M | 41 | 3.3 | | |
| 16 | Femur Stiffness | F | | | 16 | 3.8 |
| 16 | Femur Stiffness - R | F | | | 16 | 3.5 |
| 16 | Femur Ult. Load - R | F | 12 | 1.5 | 18 | 3.3 |
| 16 | Femur Ult. Work | F | | | 21 | 4.1 |
| 16 | Femur Ult. Work - R | F | | | 21 | 3.6 |
| 17 | Tibia Length | F | 23 | 2.8 | 24 | 3.8 |
| 17 | Tibia Length - R | F | 23 | 2.3 | 24 | 4.0 |
| 18 | Femur Stiffness - R | F | 8 | 4.0 | | |
| 18 | Tibia Stiffness | M | | | 49 | 3.3 |
| 20 | Femur Ult. Displacement | F | | | 30 | 3.5 |

Of primary interest are the QTLs that were identified in either the 200 or 800 day analyses suggesting these loci are operating at specific time points throughout the lifespan on chromosomes 1, 4, 9, 13, 15, 16, 18, and 20. These data illustrate the complex nature of genetic influence on bone quality and suggest that some genes exert their effects in an age dependent manner.

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