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GENETIC FACTORS AND DIET AFFECT LONG BONE LENGTH IN THE F_{34} LG,SM ADVANCED INTERCROSS

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Abstract

Previous studies on the LG,SM advanced intercross line have identified ~40 quantitative trait loci (QTL) for long bone (humerus, ulna, femur, and tibia) lengths. In this study, long bone length QTL were fine-mapped in the F_{34} generation (n=1,424) of the LG,SM advanced intercross. Environmental effects were assessed by dividing the population by sex between high fat and low fat diet, producing eight sex/diet cohorts. We identified 145 individual bone length QTL comprising 45 pleiotropic QTL; 69 replicated QTL from previous studies, 35 were new traits significant at previously identified loci, and 41 were novel QTL. Many QTL affected only a subset of the population based on sex and/or diet. Eight of ten known skeletal growth genes were up-regulated in 3-week-old LG/J male proximal tibial growth plates, relative to SM/J. The sequences of parental strains LG/J and SM/J indicated the presence of over half a million polymorphisms in the confidence intervals of these 45 QTL. We examined 526 polymorphisms and found that 97 represented radical changes to amino acid composition while 40 were predicted to be deleterious to protein function. Additional experimentation is required to understand how changes in gene regulation or protein function can alter the genetic architecture and interact with the environment to produce phenotypic variation.

Keywords

quantitative genetics; genetic architecture; long bone length; polymorphisms; environmental variation

INTRODUCTION

Skeletal growth is a complex process subject to both genetic and environmental influences (Forriol and Shapiro 2005; Karsenty 2003; Kronenberg 2003; Mariani and Martin 2003; Nilsson et al. 2005; Provot and Schipani 2005). Previous studies of long bone length in early generations of the LG,SM advanced intercross have detected ~40 quantitative trait loci (QTL) influencing bone length, including several sexually dimorphic QTL (Kenney-Hunt et al. 2006; Norgard et al. 2009; Norgard et al. 2008). The QTL have complex genetic properties, with almost all acting additively and nearly half having significant dominance effects. The numerous pleiotropic and epistatic interactions identified in the advanced intercross population suggest the presence of complex, integrated regulatory networks that

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influence variation in the population (Norgard et al. 2009; Norgard et al. 2008). However, previous studies have not focused specifically on environmental components affecting bone length in this population. Additionally, the QTL confidence intervals mapped in these early generations represent large genomic areas, up to 25 cM or ~20 Mb in length, each containing hundreds of genes.

As later generations of this intercross are born, offspring accumulate additional recombination events (Darvasi 1998; Darvasi and Soller 1995), allowing fine-mapping of previously identified QTL and corresponding reductions in confidence interval size and candidate gene number. In this study, QTL for humerus, ulna, femur, and tibia lengths are mapped in the F_{34} generation of the LG,SM advanced intercross (Wustl:LG,SM-G34). The effects of isocaloric diets with varying fat content on bone length are analyzed here to directly assess one environmental component of long bone elongation. Additionally, the expression levels of known skeletal growth genes located within fine-mapped QTL confidence intervals are determined for the LG/J and SM/J parental strains and the sequences of positional candidate genes are analyzed to identify potential regulatory changes and mutations associated with the QTL.

MATERIALS AND METHODS

Animals

The F_{34} generation of the LG,SM advanced intercross (n=1,424) was used in this study. The details of the line can be found in (Norgard et al. 2009; Norgard et al. 2008). All animals were raised in accordance with Washington University in St. Louis IACUC standards. After weaning, each F_{34} litter was divided so that half of each sex was fed high-fat (42% energy from fat; #TD88137, Harlan Teklad) or low-fat (15% energy from fat; #D12284, Research Diets) isocaloric diets, for a total of eight cohorts (male, female, high-fat, low-fat, high-fat male, high-fat female, low-fat male, and low-fat female). The mice were weighed for 20 weeks before necropsy, the right-side humerus, ulna, femur, and tibia were removed immediately and measured to the nearest 0.01 mm with digital calipers before storage.

SNP Genotypes

The F_{34} animals were genotyped at 2,842 polymorphic autosomal SNPs selected from the Oxford/CTC set (http://www.well.ox.ac.uk/mouse/INBREDS/). SNPs were scored with the Illumina GoldenGate Bead Array (Illumina, San Diego) by the Center for Inherited Disease Research (http://www.cidr.jhmi.edu/). SNPs were mapped for each autosome using R/QTL (Broman and Sen 2009). Over short distances, the F_{34} map is related to the F_2 map by an order of 17X. There is about one SNP for every 8.5 cM (equivalent to ~0.5 F_2 cM). The combination of some genomic regions lacking any polymorphisms between LG/J and SM/J and the large amount of accumulated recombination led to parts of some autosomes being virtually unlinked to other, neighboring segments on the same chromosome. In these cases, the chromosome was divided into segments for analysis.

Quantitative Genetic Analysis

The F_{34} population was analyzed for the effects of sex, diet, and family membership on individual bone lengths (humerus, ulna, femur or tibia) using the ANOVA model:

$$Y_{ijk}=\mu+Sex_i+Diet_j+Family_k+Sex_i \times Family_k+\varepsilon_{ijkl},$$

where μ is the population mean, Sex is either male or female, Diet is either the high fat or low fat diet, Family identifies the random effect of sibship membership, Sex x Family is the

interaction between sex and family, and ε is the error term. The Family effect was used to calculate heritability (Falconer and Mackay 1996) for each trait using the full population, pooled over different sex-diet cohorts. Diet x Fam and Sex x Diet x Fam were not significant for this analysis and were left out of the final model. The presence of a significant Sex x Family term for femur and tibia suggests sex differences in genetic effects, so ANOVAs modeling only Sex, Diet, and Family were performed on males and females separately. Heritability estimates for the separate sexes were similar to those for pooled sexes.

Quantitative Trait Locus Analysis

QTL mapping proceeded according to the equations described by (Haley and Knott 1992). Imputing and calculations of additive and dominance genotypic scores are summarized in (Norgard et al. 2009; Norgard et al. 2008). QTL were mapped using a mixed model (SAS version 9.1; SAS Institute, Cary, NC) that included sex, diet, and additive and dominance genotype scores. Two- and three-way interactions between genotype and sex and diet were modeled as fixed effects, while two- and three-way interactions between family and sex and diet were modeled as random effects. The full model used the linear equation:

$$Y_{ijkl}=\mu+\mathrm{Sex}_i+\mathrm{Diet}_j+sd(\mathrm{Sex}_i\times\mathrm{Diet}_j)+\mathrm{a}X_{ak}+\mathrm{d}X_{dl}+\mathrm{a}s(X_{ak}\times\mathrm{Sex}_i)+\mathrm{d}s(X_{dl}\times\mathrm{Sex}_i)+\mathrm{a}d(X_{ak}\times\mathrm{Diet}_j)+\mathrm{d}d(X_{dl}\times\mathrm{Diet}_j)+\mathrm{a}sd(X_{ak}\times\mathrm{Sex}_i\times\mathrm{Diet}_j)+\mathrm{d}sd(X_{dl}\times\mathrm{Sex}_i\times\mathrm{Diet}_j)+\varepsilon_{ijkl},$$

where μ, Sex, Diet, and ε_i are defined as above; ‘a’ and ‘d’ are the regression coefficients for additive and dominance genotype scores, respectively; ‘*s*’, ‘*d*’, and ‘*sd*’ are the interacting terms for sex, diet, or both sex and diet together (found in term number 4 and term numbers 7–12 in the equation above). The probabilities of the null hypothesis were adjusted for deflation caused by the inclusion of non-independent family members in the analysis, by including the random effects of family and the interactions between family, sex, and diet. The -2ln(likelihood) of both the full model and a reduced model lacking interaction terms were compared to a base model with sex, diet and sex by diet interaction using a chi-square test with either 8 (model including interactions) or 2 (model with only marginal effects) degrees of freedom. As in previous generations, probabilities obtained from the likelihood ratio tests above were log-transformed into a linear scale using the equation LPR = $-\log_{10}$(Probability).

The significance thresholds were adjusted to reflect the large number of tests performed, as 5% of the independent regressions were expected to be significant at a 5% level under the null model. A Bonferroni correction using the number of independent tests (Li and Ji 2005) was applied utilizing the eigenvalues of the intermarker correlation matrix for each chromosome. This resulted in a corrected threshold for the entire genome (LPR > 4.72) as well as for individual chromosomes (LPRs listed in Tables 3 and 4). With corrected chromosome-wise thresholds, one false positive chromosome across the whole genome is expected under the null model. QTL surpassing genome- and chromosome-wise thresholds are listed in Table 3. Previously identified QTL were considered protected and thus only needed to surpass the point-wise significance threshold (LPR > 1.3); protected QTL are presented in Table 4.

If a QTL peak fell within confidence intervals from previous generations, the QTL was considered a replicate of the previously identified QTL. Pleiotropy was not formally tested due to programming limitations, but given the great extent of recombination, QTL separated by more than 50 cM were considered distinct. In some cases, there were multiple peaks for a trait that were of similar size and fell near established QTL peaks for other traits. In these cases, multiple QTL testing was used to determine whether the multiple peaks represented separate QTL. If the model predicted that multiple QTL were not likely, the peak with the

highest LPR score was chosen as the position. In some cases, this lead to a QTL position for one trait that was quite distant from the QTL for the rest of the traits (such as *Lbn6.2* for humerus or *Lbn8.1a* for ulna). As these positions fell within previous QTL boundaries, they are counted as part of the same QTL, although they may represent linked genomic elements.

Loci with significant interactions with sex, diet, or sex and diet had additional analyses performed. When the full or reduced model had a point-wise significant interaction term (LPR > 1.3), the individual sex and/or diet cohorts were analyzed separately to identify cohort-specific effects of loci. In these cases, cohort-specific genotypic values are presented in Tables 3 and 4.

Candidate Gene Analysis

Potential candidate genes associated with bone growth were identified within $F_{9\text{-}10}$ QTL confidence regions (Norgard et al. 2009). To determine whether LG/J and SM/J differentially express these candidate genes, RT-PCR was conducted using a relative quantitation strategy. Growth plate tissue was grossly dissected from the proximal tibiae of 3-week-old LG/J and SM/J animals (9 individuals per sex per strain; 8 LG/J males). While tibiae were submerged in RNAlater (Ambion), the proximal tibiae were split longitudinally and excess muscle and bone were dissected away from the growth plate cartilage. Dissected cartilage was transferred to clean RNAlater and stored at −20°C.

RNA was extracted using the RNaqueous kit (Ambion), cDNAs were synthesized using the High Capacity RNA-to-cDNA kit (Applied Biosystems), and Taqman assays (Applied Biosystems) were performed for each candidate gene listed in Table 1 using 1 μg cDNA and Taqman gene expression master mix (Applied Biosystems), with four reactions per sample. *Hprt1*, *Col2a1*, and *Bglap1* were used as controls for amount of tissue loaded, amount of cartilage, and amount of bone contamination, respectively. The use of controls for the levels of cartilage and bone tissue were necessary because the gross dissection of growth plate tissue and different growth plate sizes led to varying amounts of actual growth plate tissue in any given sample. Reactions were run on an Applied Biosystems 7300 Real Time PCR system using default cycle parameters (50°C for 2 minutes, 95°C for 10 minutes, and 40 cycles of 95°C for 15 seconds followed by 60°C for 1 minute) and raw C_t values were obtained using the "Auto C_t" option of the SDS Software 1.3.1.

Rather than employing the traditional $\Delta\Delta C_t$ method to identify increased or decreased expression of samples relative to a single reference sample and then comparing candidate genes to the multiple control genes, a regression strategy was used to correct all samples for interfering factors at once. This strategy eliminates amplification of error that would be created by introducing ratios when comparing candidate gene expression to control gene expression. Ratios of raw C_t values for the candidate gene relative to the C_t value for *Hprt1* only removes the effect of total cellular material from inter-sample comparisons if the slope of the regression between the scores is 1.0, otherwise, the ratio remains correlated with its denominator. The same difficulty occurs when correcting for amounts of bone versus cartilage. Raw C_t values were regressed in Systat12.0 using the model:

$$C_t \text{ values} = \mu + Hprt1 + \text{plate} + Col2a1 + Bglap1 + Col2a1 * Bglap1 + \varepsilon,$$

where C_t values are the raw C_t values output by the SDS software, μ is the population mean, *Hprt1* accounts for variation in the amount of total cellular material loaded in each reaction, plate accounts for different levels of fluorescence on different plates, *Col2a1* accounts for the amount of cartilage in the sample, *Bglap1* accounts for the amount of bone in the sample, *Col2a1*Bglap1* accounts for variation due to the interaction of *Col2a1* and *Bglap1*

(when the effect of the amount of cartilage in a sample depends on the amount of bone and vice versa), and ε is the error, or residual variation left in the data after removing the variation associated with the other factors in the model. Outliers were removed from analysis at this stage. The residual C_t values (ε) from this regression show no variation in the total amount of cellular material in the sample, no variation in the amount of bone and cartilage cells, and no direct effect of plate. The analysis of corrected data proceeded using the residuals (ε) from this analysis.

The corrected data was analyzed in Systat12.0 by ANOVA with strain, plate, and strain*plate interaction. Plate was added back in to the model to detect cases with significant strain*plate interaction (when the effect of strain is dependent on which plate is observed). Two such cases were identified, indicating the presence of "bad" reactions. When the reactions were repeated and the new data substituted for the "bad" reaction data, the strain*plate interaction disappeared. Initially, data included both sexes and the ANOVA model included sex and sex*strain terms. Significant sex*strain interactions were observed for several candidate genes and indicated male-specific candidate gene expression differences. When the data were reanalyzed separately by sex, significant strain effects were only observed in the male population. Thus, the data from the two sexes were ultimately analyzed separately (including separate data correction steps) to increase the accuracy of the final estimates. Final values reported here are from a model with corrected data regressed on strain only.

Sequence analysis

Sequences for LG/J and SM/J were obtained from The Genome Sequencing Center at Washington University (http://genome.wustl.edu). The sequencing data identified 4,299,800 autosomal polymorphisms between LG/J and SM/J, with 20X coverage for LG/J and 14X coverage for SM/J. All the SNPs used in this study are available in dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/) and can be cross-referenced with the physical distances provided in the tables below. Candidate gene RefSeq mRNA coding regions were downloaded in FASTA format from the University of California, Santa Cruz Genome Bioinformatics website (http://genome.ucsc.edu, NCBI m37 mouse assembly). Non-translated DNA regions for the genes listed in Table 1, including introns, 3′ UTR, 5′ UTR, 10 Kb upstream, and 10 Kb downstream, were also downloaded. These sequences were then compared with our list of LG/J and SM/J polymorphisms to identify changes to the protein coding regions as well as to non-protein coding regions. Nonsynonymous coding region polymorphisms were analyzed using the PANTHER (http://www.pantherdb.org/) cSNP analysis tool (Brunham et al. 2005; Thomas et al. 2003; Thomas et al. 2006), which proceeded on proteins manually translated from the RefSeq mRNA coding regions. Noncoding polymorphisms were analyzed in the Genome Bioinformatics website for 30-Way Multiz Alignment and Conservation, using the phastCons program (Siepel et al. 2005), as well as for the presence of ORegAnno regulatory elements (Griffith et al. 2008; Montgomery et al. 2006).

RESULTS

The genetic architecture controlling long bone lengths is complex

Long bone lengths were highly heritable, with heritabilities ranging from 0.68 to 0.85 (Table 2). This is slightly lower than estimates from the F_3 population (Norgard et al. 2008), but higher than estimates from the F_{10} population (Norgard et al. 2009). The genetic correlations between skeletal traits (values off the diagonal) were also high, ranging from 0.74 to 0.90, and are similar to genetic correlation estimates from the previous generations.

We identified 145 individual-trait QTL for humerus, ulna, femur, and tibia in this population, including QTL on all autosomes except 5, 16, and 17. The absence of QTL on chromosome 17 is caused by difficulties analyzing the data due to large stretches of homogeneity at the proximal end of the chromosome, where QTL have been identified in previous generations (Norgard et al. 2009; Norgard et al. 2008). Of the 145 QTL identified here, 69 were identified previously, 35 were new individual-trait loci discovered near the positions of previously identified QTL, and 41 represent newly discovered QTL (*Lbn2.4*, *Lbn4.3*, *Lbn4.4*, *Lbn4.5*, *Lbn6.3*, *Lbn7.3*, *Lbn7.4*, *Lbn12.2*, *Lbn12.3*, *Lbn12.4*, *Lbn12.5*, *Lbn15.3*, and *Lbn18.2*). Although *Lbn2.4* did not surpass the chromosome-wise significance threshold, it was included as a new QTL due to the strong cohort-specific effects observed (see Table 4). The 145 individual-trait QTL were condensed into 45 pleiotropic QTL; of these, 34 had individual-trait elements that surpassed chromosome-wide or genome-wide thresholds (Table 3), while the remaining 11 were in protected regions and surpassed only the point-wise threshold (Table 4). Three previously identified QTL, *Lbn2.1*, *Lbn2.3*, *Lbn8.1*, and *Lbn18.1*, were resolved into multiple loci. *Lbn2.1* was divided into three loci (*Lbn2.1a*, *Lbn2.1b*, and *Lbn2.1c*), each with diverse additive and dominance effect patterns (see Tables 3 and 4). *Lbn18.1a* had significant dominance effects on the humerus, ulna, femur, and tibia, while *Lbn18.1b* had significant additive effects on the ulna, only.

Significant additive (**a**) effects were observed in 74% of the loci examined. The average difference in bone lengths between the LG/J and SM/J homozygotes caused by additive effects of QTL (2 × the average additive effect) was 0.16 mm for the humerus, 0.24 mm for the ulna, 0.25 mm for the femur, and 0.26 mm for the tibia. The average effect size of significant |**a**/SD| values for all the long bones was 0.20 SD units, with absolute values of standardized additive effects ranging 0.08 to 0.87 SD units. Additive effects were distributed such that most were small in effect size (0.1 - 0.2 SD units) with fewer loci having large effect sizes (0.3 - 0.5 SD units) and only *Lbn8.2* for ulna in males having |**a**/SD| values greater than 0.7 SD units. For 56 loci, the SM/J allele led to longer bone lengths than the LG/J allele, as indicated by negative **a**/SD values in Tables 3 and 4. Most of these cases were either identified in previous analyses or are in newly discovered QTL. *Lbn9.2* had negative additive effects in the $F_{2\text{-}3}$ analysis, positive additive effects in the $F_{9\text{-}10}$ analysis, and has negative additive effects here. When the sign of the effects was taken into account, the average effect size of significant **a**/SD values was 0.03 SD units, indicating that the LG/J and SM/J alleles counterbalance each other, leading to an overall neutral effect on additive variation in this population.

Significant dominance (**d**) effects were observed at 40% of the loci. Of the 27 loci with significant **a** and **d** values, there were four cases of underdominance (**d/a** < −1.5), seven cases of SM/J dominance (−1.5 < **d/a** < −0.5), ten cases of LG/J dominance (0.5 < **d/a** < 1.5), and four cases of overdominance (1.5 < **d/a**). The 55 remaining significant loci were considered co-dominant. The average difference in bone lengths between the midpoint of LG/J and SM/J homozygotes and the mean for heterozygotes caused by dominance effects of QTL was 0.26 mm for the humerus, 0.30 mm for the ulna, 0.35 mm for the femur, and 0.28 mm for the tibia. The average normalized dominance effect size (**d**/SD) for all the long bones was 0.26 SD units, but with directionality factored in, this dropped to 0.02, suggesting that overall, dominance effects average to zero and neither LG/J nor SM/J alleles are more commonly dominant.

Sex and diet can alter QTL genetic architecture

Sex and diet had significant effects on a major component of the genetic architecture: additive and dominance effect patterns. Of the reported **a** and **d** effects for individual-trait QTL, 49% had significant interactions with sex, diet, or both. There were 40 additive loci

with significant interaction effects: 17 interacted with diet, 26 interacted with sex, and 16 interacted with sex and diet. Significant interaction effects occurred at 15 dominant loci: 23 interacted with diet, 7 interacted with sex, and 19 interacted with sex and diet. When specific cohorts were examined to identify the sources of these interactions, 129 cohort-specific QTL were identified. Of these, 12% were female-specific (16), 10% were male-specific (13), 11% were high fat diet-specific (14), 10% were low fat diet-specific (13), 16% were high fat male-specific (21), 9% were high fat female-specific (12), 9% were low fat male-specific (12), and 22% were low fat female-specific (28). Two QTL were resolved into multiple QTL based on their effects in different subpopulations. *Lbn2.3a* had significant additive and dominance effects in various subpopulations, while *Lbn2.3b* acted additively on all four long bones in the full population. *Lbn8.1a* had significant negative additive effects on humerus, ulna, femur, and tibia in the low fat population, while *Lbn8.1b* had a significant dominance effect on ulna and femur in high fat males and a significant positive additive effect on ulna in low fat males.

While some cohort-specific QTL affected bone length in a single subpopulation in the same way (as at *Lbn8.1a*), many cohort-specific QTL had different effects in different subpopulations. Cohort-specific QTL acted additively in some subpopulations (*Lbn4.5* for tibia in high fat males) and via dominance in others (*Lbn4.5* for tibia in high fat females). Directionality for different traits also changed between some subpopulations (at *Lbn2.3a*, **a**/SD is negative for humerus and femur in low fat females, while it is positive for ulna and tibia in males, animals on high fat diets, and high fat males). For *Lbn8.2* and *Lbn12.1*, which had positive additive effects in previous analyses but negative additive effects here, the presence of significant interactions may be responsible for the changing additive effect directionality. Interestingly, for the low fat male population, the average additive effect size with effect sign taken into account was −0.14 SD units, suggesting an overall positive influence of the SM/J allele on bone length in this subpopulation. Similarly, in several subpopulations, the average dominance effect sizes with sign taken into account were less than −0.1 (low fat, −0.12; low fat male, −0.23; and high fat female, −0.18).

Known skeletal growth genes are differentially regulated in LG/J and SM/J growth plate cartilage

As an initial step to determine whether expression differences in skeletal growth genes were responsible for the observed length differences in the parental strains and thus might underlie some of the QTL, ten candidate genes chosen for their known roles in endochondral ossification and their presence in F_9-F_{10} confidence intervals were tested for expression differences in the growth plate (see Table 1). Of these, only *Runx2* and *Tgfb1* did not show significant expression differences between strains, as shown in Supplemental Table 1. Variation in expression levels of these genes between strains (R^2 values in Supplemental Table 1) accounted for 3–37% of the phenotypic variation. In the cases of *Igf1*, *Ihh*, *Pthlh*, *Smad1*, *Sox5*, *Sox9*, and *Vegfa*, the average C_t value for LG/J was lower than that for SM/J, with the average C_t difference ranging from 0.2 to 0.7 cycles. This indicates that relative to SM/J, these genes are significantly up-regulated in LG/J. Conversely, for *Comp*, the average C_t value was greater for SM/J, indicating that SM/J expresses more *Comp* than LG/J. A significant strain*sex interaction for *Smad1* ($p = 0.015$) indicated the presence of a sex-specific effect. LG/J males expressed more *Smad1* than SM/J males, but no significant difference was observed between the strains in females. These genes likely play a role in the bone length and growth rate differences observed in LG/J and SM/J (Sanger et al. 2011) as well as in the LG,SM advanced intercross.

Coding region polymorphisms occur in many positional candidate genes

The confidence intervals of the 45 pleiotropic F_{34} QTL contained 186 positional candidate genes, comprising family members of known skeletal growth regulators as well as genes known to regulate cellular growth, embryonic patterning, programmed cell death, or other cellular processes with potential for modifying chondrocyte growth (listed in Table 5). These potential candidate genes have not all been tested for effects on cellular morphology or skeletal growth phenotypes. When examined for sequence polymorphisms between LG/J and SM/J, 111 of the genes were found to harbor polymorphisms and 526 SNPs were found. This represents only one-thousandth of the total number of SNPs present within the QTL confidence intervals (see Supplemental Table 2).

Only 152 of these polymorphisms altered the amino acid residue of the resulting protein; these changes are listed in Supplemental Table 2. The amino acid variants produced by these polymorphisms were examined to assess their potential for altering protein function. Using the amino acid classifications defined in (Hanada et al. 2006), the reference and altered amino acids were categorized to determine whether the alteration was radical enough to cause a shift in the residue's chemistry category. About 65% (97) of the polymorphisms caused a change in at least one amino acid chemistry category, with around one-third of these causing a change in all three categories. These polymorphisms affected 55 separate gene products.

In and of itself, radical changes to amino acid chemistry category do not provide strong evidence of the importance of the change to the protein. To determine potential impact on protein function, the cSNP tool in PANTHER was used to determine the likelihood of a protein change being deleterious based on evolutionarily conserved sequences across protein family members (Brunham et al. 2005; Thomas et al. 2003; Thomas et al. 2006). Only about 50% (74) of the amino acid changes could be analyzed, and these results are summarized in the P_{del} column of Supplemental Table 2. About half of the noted residue changes were predicted to have greater than a 50% chance of being deleterious to protein function (SNPs with $P_{del} > 0.50$). This suggests that the other half of the analyzed residues have a smaller chance of causing a deleterious change to protein function, but does not mean that they have no effect at all. Future experimental verification will be needed to confirm these predictions.

All ten genes tested for differential expression in the growth plate had polymorphisms within their coding regions (Supplemental Table 3). Of the >4,000 polymorphisms identified in Supplemental Table 3, only 170 had PhastCon scores greater than 0.95, indicating extreme conservation of these residues across mammals. *Sox5* had the most noncoding DNA polymorphisms, with introns that were relatively divergent between LG/J and SM/J. The *Sox5* intron polymorphisms affected multiple *Foxa2* and *Esr1* regulatory regions, and additional polymorphisms affecting a *Foxa2* regulatory region were identified within the sequence 10 Kb upstream of the gene. On the other end of the spectrum, *Igf1* had only 5 intronic polymorphisms and one highly conserved exonic SNP in a *Foxa2* regulatory region. The ORegAnno database is highly enriched for *Foxa2* and *Esr1* regulatory regions (Lin et al. 2007; Wederell et al. 2008), and this may be why polymorphisms in regulatory regions have not yet been discovered in any of the other genes examined.

DISCUSSION

Similarly to previous studies (Norgard et al. 2008; Norgard et al. 2009), a total of 45 pleiotropic QTL were identified here. About 70% of the original $F_{2\text{-}3}$ and $F_{9\text{-}10}$ individual-trait QTL (69/101) were replicated in this study. The probability of QTL replication varied with the LPR scores from the $F_{2\text{-}3}$ and $F_{9\text{-}10}$ analyses. If the LPR score in the previous generations was low (LPR < 9.0; scores from previous generations are inflated by familial

autocorrelation) in the original analyses, only 57% (36 of 63) of the QTL replicated, while 87% (33 of 38) of QTL with higher LPR scores (LPR ≥ 9.0) replicated. This is likely the result of the Beavis effect, which operates randomly in different generations and can result in QTL with small effects near the detection threshold either exceeding or failing to reach the significance threshold by chance in different studies (Beavis 1994).

While we expected to identify QTL with confidence intervals of ~0.5 F_2 cM, actual confidence intervals averaged ~1.7 F_2 cM (corresponding to ~4.5 Mb). In terms of physical distances, the confidence intervals ranged from 14 Mb for the largest QTL (*Lbn1.2*) to 0.6 Mb for the smallest QTL (*Lbn2.4*), with about half of the QTL having confidence intervals between 2 and 5 Mb (see Table 5). There are several possible reasons for the apparent lack of increased resolution between the $F_{9\text{-}10}$ and F_{34} generations. First, as opposed to the previous studies on which the 0.5 F_2 cM estimate was based, the parental generation (F_{33}) was not included in the mapping population because it was not subject to the same dietary treatments. Familial autocorrelation inflated QTL peak size and impacted how confidence intervals were set in earlier generations. While all F_{34} individuals are related and subject to familial autocorrelation, the absence of the parental generation decreased QTL peak inflation relative to previous generations. A second possibility is that some QTL are caused by multiple linked genomic elements. While several of the QTL reported above have short confidence intervals with few (≥10) or no candidate genes (*Lbn2.1b*, *Lbn2.3a*, *Lbn2.4*, *Lbn3.3*, *Lbn8.1a*, *Lbn11.1*, *Lbn12.2*, *Lbn14.1*, and *Lbn18.2*), the majority have between 20 and 70 candidate genes (listed in Table 5).

Fewer additive effects were observed in the full F_{34} population than in previous generations, but this is likely caused by dietary variation resulting in a large number of cohort-specific QTL observed in the population. As in previous generations, between one-half and one-third of the observed QTL had significant dominance effects. Interestingly, in the F_{16} generation, which underwent the same dietary treatments as this generation, more sex and diet interactions were detected, most likely because the phenotypes under investigation in that population were adiposity and obesity-related traits that are more subject to the effects of sex hormones and dietary influences (Cheverud et al. 2011). Interestingly, only five adiposity-related QTL (*Dob1a*, *Dob6b*, *Dob7d*, *Dob8c*, and *Dob11a*) overlapped with the confidence intervals of the long bone length QTL identified here (*Lbn1.1a*, *Lbn6.3*, *Lbn7.1*, *Lbn8.1b*, and *Lbn11.2*, respectively), indicating that most of the QTL identified alter long bone length independently of body size. Although fewer interactions are observed for long bone length than obesity-related traits, the presence of multiple cohort-specific long bone length QTL implies a complex picture of how the hormonal and dietary environment can alter bone elongation.

Frequently, only one or two subpopulations were responsible for the presence of QTL in the full population. This may be why the majority of individual-trait QTL in the full population only surpassed the point-wise threshold. Most cohort-specific QTL affected female animals on a low-fat diet or male animals on a high-fat diet. Previous studies in rats on the effects of extremely high fat diets low in carbohydrates and proteins indicate that long bone growth is inhibited by very large amounts of dietary fat (94.5% energy from fat), perhaps through decreased levels of circulating IGF-1 (Bielohuby et al. 2009). *Igf1* is a candidate gene for *Lbn10.1* (Table 5), and SM/J shows decreased levels of *Igf1* mRNA in the proximal tibial growth plate (Supplemental Table 1). Molecular analysis of the effect of the very high fat diet in rats also suggests that reductions in bone length may be the result of reduced osteoblast activity and decreased expression of transcription factors, such as *Runx2*, that drive mesenchymal stem cells to become osteoblasts, leading to an overall decrease in bone formation (Bielohuby et al. 2009). Although no expression differences between strains were observed in the growth plate, *Runx2* is a potential candidate gene for a QTL that was

identified previously (*Lbn17.1*, located on chromosome 17 and not analyzed here; see Results) (Norgard et al. 2009). Future efforts will use cohort-specific data to elucidate the molecular and biochemical mechanisms leading to skeletal growth differences and help determine how differences in sex and diet interact with and alter established genetic patterns.

The presence of 97 polymorphisms that change amino acid classification as well as the presence of altered amino acids in evolutionarily conserved regions or motifs suggests that changes to translated proteins may cause some QTL. However, most of the polymorphisms identified between LG/J and SM/J have not been associated with changes in protein sequence or function, suggesting that polymorphisms in the protein coding regions of the candidate genes are only partially responsible for the bone length variation observed. Further analysis of non-translated sequence may lead to the identification of polymorphisms that impact the activity of regulatory regions or the expression and activity of the translated protein. The observation of eight known skeletal growth regulators with differential mRNA expression in the LG/J and SM/J strains supports the hypothesis that differences in SM/J and LG/J regulatory regions exist, especially given the absence of protein coding polymorphisms in any of these genes (except *Pthlh*) and the large number of non-translated polymorphisms observed in and adjacent to those genes (see Supplemental Table 3). Indeed, the presence of relatively few radical amino acid changes in the protein coding region suggests that more variation may be explained by sequence alterations in non-translated genomic elements, such as promoters, enhancers, introns, or other genetic modifier elements. Future studies will investigate differences in noncoding regions to identify additional sources of variation in the population.

Most of the polymorphisms identified here have not yet been associated with any specific change in growth or cellular processes. One candidate gene, *Pthlh*, does have a polymorphism that has been previously shown to alter cellular dynamics. Relative to reference strain C57BL/6J and LG/J, SM/J harbors the mutation P166T. Both of the residues at this position have been previously described as skin cancer modifier elements (Benelli et al. 2003; Gianni-Barrera et al. 2006; Manenti et al. 2000). Previous studies have shown that the proline variant is associated with clustered growth in cell culture, increased growth rates, tumor growth, and increased cell migration. The threonine variant is associated with the opposite effects—flat growth in cell culture, normal growth rate, decreased tumor growth, and normal cell migration. Additionally, the threonine and proline variants display differential cDNA expression profiles, with the proline variant displaying higher expression of *Igfbp3* and *Igfbp5* (Gianni-Barrera et al. 2006). These cellular changes may have important implications in the growth plate, in which changes in cellular morphology are a primary mechanism of longitudinal elongation. This polymorphism does not preclude the *Pthlh* regulatory region polymorphisms from altering its expression level in the two strains. Additional experimental data on the *Pthlh* P166T SNP as well as the other polymorphisms reported here will be necessary to confirm phenotypic effects on chondrocyte biology or bone lengths.

Ultimately, this study has replicated previously identified QTL for long bone length with greater accuracy and precision, detected QTL specific for sex and diet, determined specific gene products up-regulated in LG/J animals relative to SM/J, and identified 526 SNPs that fall within QTL confidence intervals. The absence of most of the known major endochondral ossification regulators within the confidence intervals supports the idea that genes controlling normal cellular processes such as metabolism, motility, or cell division have a measurable cumulative effect on cellular dynamics. Given the nature of bone elongation via the growth plate, this finding is not unreasonable. Future work will include

experimental testing of SNPs to identify the specific mechanisms by which the QTL act and how environmental and genetic components interact to produce variation in bone length.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Taqman Assays

The symbols, names, and Taqman assay numbers of the genes examined for mRNA expression differences are listed.

Gene	Gene Name	Taqman Assay
Bglap1	Osteocalcin	Mm03413826_mH
Col2a1	Collagen II, alpha 1	Mm01309565_m1
Hprt1	Hypoxanthine guanine phosphoribosyltransferase 1	Mm00446968_m1
Comp	Cartilage oligomeric matrix protein	Mm00489490_m1
Igf1	Insulin-like growth factor 1	Mm00439560_m1
Ihh	Indian hedgehog	Mm01259021_m1
Pthlh	Parathyroid hormone-like hormone	Mm00436057_m1
Runx2	Runt-related transcription factor 2	Mm01269515_mH
Smad1	Homolog of drosophila mothers against decapentaplegic 1	Mm00484721_m1
Sox5	SRY-box 5	Mm00488381_m1
Sox9	SRY-box 9	Mm00448840_m1
Tgfb1	Transforming growth factor, beta-1	Mm03024053_m1
Vegfa	Vascular endothelial growth factor a	Mm00437308_m1

Table 2

Heritabilities and Genetic Correlations

Mean values and SDs are listed in millimeters for each long bone trait (H, humerus; U, ulna; F, femur; T, tibia). Heritabilities (diagonal) and genetic correlations (off-diagonal) were calculated from the F_{34} population using the full sibship method (Falconer and Mackay 1996).

	H	U	F	T	Mean	SD
H	0.83				12.51	0.50
U	0.74	0.68			14.18	0.56
F	0.90	0.78	0.81		16.09	0.61
T	0.84	0.87	0.88	0.85	17.87	0.61

Table 3

Genome- and Chromosome-wise Significant F_{34} QTL

QTL harboring individual traits that surpass genome- or chromosome-wise significance thresholds are listed by chromosome (with chromosome-wise significance thresholds indicated), pleiotropic QTL name, and trait affected (H, humerus; U, ulna; F, femur; T, tibia). Bold QTL names indicate newly identified QTL; italicized traits surpass only the point-wise threshold. The position of the QTL peak is given in bp, followed by the LPR score. Proximal and distal QTL confidence interval (CI) boundaries are given in bp. The "Sig. Model Terms" column lists whether **a** and **d** were significant for an individual trait, as well as any other significant interaction terms from the full mapping model (ad, dd, as, ds, asd, or dsd; see Materials and Methods). For each trait, gene effect estimates—**a**, **a**/SD, **d**, **d**/SD—are listed with their respective standard errors (SE). Positive '**a**' values indicate the LG/J allele leads to longer bone lengths; negative '**a**' values indicate the SM/J allele leads to longer bone lengths. The values for **d/a** indicate: underdominance (**d/a** < −1.5), SM/J dominance (−1.5 < **d/a** < −0.5), LG/J dominance (0.5 < **d/a** < 1.5), or overdominance (1.5 < **d/a**). When appropriate, gene effect estimates are given by cohort. Bold values in gene effect estimate columns indicate significance at the $p < 0.05$ level.

QTL	Trait	Peak (bp)	LPR	Proximal CI Boundary (bp)	Distal CI Boundary (bp)	Sig. Model Terms	a (mm)	a SE	a/SD	d (mm)	d SE	d/a
Chromosome 1							Chromosome-wise Threshold: 3.66					
Lbn1.1a	*H*	73,934,976	2.14	73,006,099	74,948,766	**a**	**0.07**	**0.02**	**0.14**	0.01	0.03	0.02
	F	76,922,763	1.38	74,798,862	78,164,165	ad						
				Low Fat-Female			**0.16**	**0.06**	**0.26**	0.06	0.08	0.10
	T	77,315,320	4.32	76,593,188	78,164,165	**a**, ad						
				Low Fat			**0.20**	**0.04**	**0.33**	0.06	0.05	0.10
				Female			**0.19**	**0.04**	**0.32**	0.03	0.05	0.04
				High Fat-Male			0.10	0.05	0.16	**0.16**	**0.07**	**0.26**
	U	77,565,875	2.96	77,158,297	80,883,467	**a**	**0.09**	**0.03**	**0.17**	−0.01	0.03	−0.02
Lbn1.1b	H	88,597,036	4.52	84,456,111	91,061,680	**a**	**0.13**	**0.03**	**0.25**	0.05	0.03	0.10
	F	89,141,109	3.73	84,118,557	90,389,263	**a**	**0.14**	**0.04**	**0.22**	0.02	0.04	0.03
	T	89,290,553	12.75	87,687,979	89,738,884	**a**, **d**	**0.26**	**0.03**	**0.42**	**0.09**	**0.04**	**0.15**
Chromosome 2							Chromosome-wise Threshold: 3.62					
Lbn2.1c	*U*	126,005,696	1.87	115,432,770	127,531,124	**a**	**0.07**	**0.02**	**0.12**	−0.02	0.03	−0.03
	T	126,101,854	6.74	124,466,590	126,871,119	**a**	**0.13**	**0.03**	**0.22**	−0.04	0.03	−0.06
	H	126,294,170	3.61	124,466,590	127,483,445	**a**	**0.07**	**0.02**	**0.13**	−0.05	0.02	−0.09
	F	126,678,803	3.42	125,123,509	127,578,804	**a**, **d**	**0.06**	**0.03**	**0.09**	**−0.11**	**0.03**	**−0.17**
Lbn2.3a	*U*	138,895,272	2.41	137,366,816	139,824,730	dd						

QTL	Trait	Peak (bp)	LPR	Proximal CI Boundary (bp)	Distal CI Boundary (bp)	Sig. Model Terms	a (mm)	a SE	a/SD	d (mm)	d SE	d/a
				High Fat			**0.07**	**0.03**	**0.13**	**0.11**	**0.04**	**0.20**
				Male			**0.06**	**0.03**	**0.12**	0.03	0.04	0.06
	F	138,895,272	4.04	137,275,419	139,629,055	ad, dd, as, dsd						
				Low Fat-Female			**−0.15**	**0.06**	**−0.24**	**−0.24**	**0.07**	**−0.39**
				High Fat-Female			0.02	0.05	0.04	**0.17**	**0.06**	**0.27**
	T	138,895,272	2.66	137,618,158	139,824,730	dd, as, dsd						
				High Fat			0.06	0.04	0.10	**0.10**	**0.04**	**0.16**
				High Fat-Male			**0.11**	**0.05**	**0.17**	0.09	0.06	0.14
				Low Fat-Female			−0.07	0.05	−0.12	**−0.14**	**0.07**	**−0.23**
	H	138,895,272	1.64	137,092,625	140,264,999	dd, as, dsd						
				Low Fat-Female			**−0.09**	**0.04**	**−0.18**	**−0.14**	**0.05**	**−0.28**
Chromosome 4							Chromosome-wise Threshold: 3.55					
Lbn4.3	*(U)*	67,407,538	1.59	64,461,467	71,268,867	**a**, dsd						
				Low Fat-Male			**0.16**	**0.05**	**0.29**	**0.18**	**0.08**	**0.32**
	(F)	67,849,078	2.62	65,227,791	71,268,867	**a**, dsd						
				High Fat			**0.10**	**0.05**	**0.16**	0.02	0.06	0.04
				Female			**0.10**	**0.05**	**0.17**	−0.03	0.07	−0.05
				Low Fat-Male			**0.19**	**0.06**	**0.31**	0.12	0.08	0.19
	H	68,290,618	4.48	66,377,278	70,203,958	**a**	**0.11**	**0.02**	**0.21**	0.00	0.03	0.01
	(T)	68,437,798	1.93	63,889,889	73,202,145	**a**, dsd						
				Low Fat-Male			**0.16**	**0.06**	**0.26**	0.08	0.09	0.13
				High Fat-Female			0.11	0.06	0.19	**0.18**	**0.09**	**0.29**
Lbn4.4	*(H)*	117,457,828	3.04	117,193,104	118,520,283	**d**	−0.01	0.02	−0.02	**−0.09**	**0.03**	**−0.19**
	F	117,457,828	3.55	117,166,987	118,591,114	**d**	−0.05	0.03	−0.09	**−0.12**	**0.04**	**−0.20**
	(T)	117,953,641	2.89	117,198,327	118,945,265	**d**	−0.02	0.03	−0.03	**−0.15**	**0.04**	**−0.24**
Lbn4.5	T	124,258,562	3.58	123,438,287	125,542,213	as, dsd						
				High Fat-Male			**0.17**	**0.06**	**0.27**	0.03	0.08	0.06
				High Fat-Female			−0.02	0.05	−0.03	**−0.18**	**0.08**	**−0.29**
	(F)	124,517,596	2.88	123,783,666	125,603,506	as, dsd						

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QTL	Trait	Peak (bp)	LPR	Proximal CI Boundary (bp)	Distal CI Boundary (bp)	Sig. Model Terms	a (mm)	a SE	a/SD	d (mm)	d SE	d/a
				High Fat-Male			**0.14**	**0.05**	**0.23**	−0.02	0.07	−0.03
Lbn4.2	F	133,662,611	3.85	130,441,117	134,441,572	**a**, **d**	**−0.09**	**0.03**	**−0.14**	**0.12**	**0.04**	**0.20**
	T	133,954,721	2.55	133,045,137	134,831,052	**a**, **d**	**−0.08**	**0.03**	**−0.13**	**0.12**	**0.05**	**0.20**
	H	134,279,288	1.92	133,420,403	135,285,446	**a**	**−0.07**	**0.03**	**−0.13**	0.08	0.04	0.15
	U	134,279,288	4.78	133,300,318	134,895,966	**a**, **d**	**−0.10**	**0.03**	**−0.17**	**0.21**	**0.05**	**0.37**
Chromosome 6							Chromosome-wise Threshold: 3.59					
Lbn6.1a	*F*	23,014,524	2.08	21,554,570	24,899,551	**a**, ds						
				Low Fat-Female			**−0.20**	**0.06**	**−0.32**	0.10	0.08	0.16
	T	23,153,567	3.61	21,624,092	23,691,979	**a**, as, ds						
				Low Fat			**−0.13**	**0.03**	**−0.21**	0.02	0.04	0.03
				Female			**−0.16**	**0.03**	**−0.26**	0.08	0.04	0.13
Lbn6.2	*H*	142,951,483	1.95	142,672,872	146,336,656	**a**, asd						
				High Fat			**0.05**	**0.03**	**0.11**	0.04	0.03	0.09
				Female			**0.07**	**0.03**	**0.14**	**0.08**	**0.03**	**0.15**
				Low Fat-Female			**0.09**	**0.04**	**0.19**	0.08	0.05	0.16
	U	146,070,667	2.80	146,070,667	147,203,378	**a**, dd						
				Low Fat			0.08	0.04	0.14	**−0.15**	**0.07**	**−0.27**
				High Fat-Female			**0.13**	**0.06**	**0.23**	−0.04	0.08	−0.07
	F	146,159,330	4.28	146,159,330	147,203,378	**a**, asd						
				High Fat-Male			**0.18**	**0.05**	**0.29**	−0.09	0.09	−0.15
				Female			**0.15**	**0.05**	**0.25**	0.05	0.07	0.08
	T	146,225,827	7.14	146,225,827	147,203,378	**a**	**0.14**	**0.03**	**0.23**	−0.05	0.04	−0.07
Chromosome 7							Chromosome-wise Threshold: 3.43					
Lbn7.3	*(F)*	74,271,309	3.09	72,380,138	75,173,597	**a**, **d**	**0.12**	**0.03**	**0.19**	**0.08**	**0.04**	**0.14**
	T	74,440,336	4.43	72,837,779	75,173,597	**a**	**0.13**	**0.03**	**0.22**	0.06	0.03	0.09
	(U)	74,609,363	1.96	72,707,024	75,437,068	**a**	**0.08**	**0.03**	**0.15**	0.04	0.03	0.07
	(H)	74,778,390	1.95	72,445,515	75,173,597	**a**	**0.06**	**0.02**	**0.13**	0.04	0.03	0.09
Lbn7.4	*(F)*	122,192,753	2.07	121,909,230	122,877,125	**a**, dd						

QTL	Trait	Peak (bp)	LPR	Proximal CI Boundary (bp)	Distal CI Boundary (bp)	Sig. Model Terms	a (mm)	a SE	a/SD	d (mm)	d SE	d/a
				Low Fat-Female			0.05	0.06	0.09	**−0.21**	**0.08**	**−0.35**
	H	122,242,786	3.78	122,025,975	122,810,349	**a**, dd						
				High Fat-Male			**−0.08**	**0.04**	**−0.16**	0.06	0.05	0.12
				Low Fat-Female			0.00	0.04	0.00	**−0.17**	**0.05**	**−0.34**
	(T)	122,259,464	1.96	121,975,941	123,010,677	**a**, dd						
				High Fat			**−0.10**	**0.04**	**−0.17**	0.00	0.04	0.00
				Female			**−0.07**	**0.04**	**−0.12**	−0.07	0.04	−0.12
				Low Fat-Female			−0.02	0.05	−0.04	**−0.17**	**0.06**	**−0.28**
	(U)	122,543,245	1.76	122,025,975	130,717,933	dd						
				Low Fat			−0.02	0.03	−0.03	**−0.10**	**0.05**	**−0.18**
				High Fat-Female			**−0.09**	**0.04**	**−0.15**	0.03	0.06	0.06
Chromosome 8							Chromosome-wise Threshold: 3.20					
Lbn8.2	*H*	17,721,221	2.43	17,047,047	17,891,745	**a**, as						
				Female			**−0.23**	**0.08**	**−0.46**	−0.14	0.09	−0.28
	U	17,778,062	3.46	17,607,539	18,756,716	**a**, **d**, asd						
				High Fat-Male			**−0.41**	**0.16**	**−0.73**	**−0.34**	**0.17**	**−0.62**
				Low Fat-Male			**−0.49**	**0.20**	**−0.87**	−0.39	0.22	−0.70
Chromosome 9							Chromosome-wise Threshold: 3.45					
Lbn9.2	H	70,160,776	4.16	69,012,301	70,821,832	**a**	**−0.07**	**0.02**	**−0.14**	0.04	0.02	0.07
	T	70,160,776	1.58	69,012,301	70,617,356	**a**, ad	**−0.12**	**0.03**	**−0.20**	0.01	0.04	0.01
				High Fat								
	U	70,675,778	2.15	69,230,291	72,470,441	**d**, dd						
				Low Fat			0.01	0.03	0.01	**0.14**	**0.04**	**0.25**
				High Fat-Female			**−0.10**	**0.04**	**−0.17**	0.03	0.06	0.06
	F	70,880,254	1.93	68,794,312	72,600,465	**a**	**−0.08**	**0.03**	**−0.13**	0.02	0.03	0.02
Chromosome 12							Chromosome-wise Threshold: 3.46					
Lbn12.1	*T*	56,769,820	2.48	55,811,620	57,905,811	**a**, dd						
				High Fat-Male			**−0.18**	**0.05**	**−0.29**	**−0.17**	**0.06**	**−0.28**

QTL	Trait	Peak (bp)	LPR	Proximal CI Boundary (bp)	Distal CI Boundary (bp)	Sig. Model Terms	a (mm)	a SE	a/SD	d (mm)	d SE	d/a
	F	58,426,609	2.32	56,022,449	59,755,563	**a**, dd, as						
				Male			**−0.11**	**0.03**	**−0.18**	−0.01	0.04	−0.02
	H	58,773,265	3.67	55,952,173	59,830,628	**a**, as						
				Male			**−0.10**	**0.03**	**−0.19**	0.01	0.03	0.02
				Female			−0.04	0.03	−0.08	**0.07**	**0.03**	**0.14**
	U	58,773,265	4.53	57,737,098	59,830,628	**a**	**−0.11**	**0.02**	**−0.19**	−0.01	0.03	−0.01
Lbn12.2	U	66,802,612	3.67	65,018,595	67,260,571	**a**, ad, asd						
				High Fat			**−0.14**	**0.03**	**−0.25**	0.00	0.04	0.00
				Low Fat-Female			**−0.12**	**0.05**	**−0.21**	0.09	0.06	0.16
Lbn12.3	*F*	69,316,047	2.33	69,125,068	70,704,785	**a**, **d**, as, asd						
				Male			**−0.17**	**0.04**	**−0.28**	**0.11**	**0.05**	**0.18**
				High Fat-Male			**−0.21**	**0.06**	**−0.35**	**0.14**	**0.07**	**0.23**
	H	70,007,545	4.23	69,125,068	70,356,165	**a**	**−0.09**	**0.02**	**−0.18**	0.04	0.02	0.08
	T	70,007,545	3.05	69,125,068	70,704,785	**a**, as						
				Male			**−0.20**	**0.04**	**−0.32**	0.06	0.05	0.10
	(U)	70,356,165	1.63	69,125,068	73,020,609	**a**, asd, dsd						
				Female			**−0.07**	**0.03**	**−0.13**	0.04	0.04	0.07
				High Fat-Male			**−0.17**	**0.05**	**−0.31**	0.11	0.07	0.20
Lbn12.4	T	77,363,594	6.00	77,069,144	80,917,072	**a**, **d**	**−0.12**	**0.02**	**−0.20**	**0.06**	**0.03**	**0.09**
	H	77,880,194	3.10	76,993,654	80,955,183	**a**	**−0.07**	**0.02**	**−0.14**	0.03	0.03	0.07
	F	78,120,247	5.02	77,031,399	80,955,183	**a**, **d**	**−0.12**	**0.03**	**−0.20**	**0.08**	**0.04**	**0.13**
	U	78,680,372	5.80	77,144,633	80,878,961	**a**, **d**	**−0.10**	**0.03**	**−0.18**	**0.14**	**0.04**	**0.24**
Lbn12.5	T	87,515,792	3.90	83,412,699	88,054,770	**a**	**−0.11**	**0.03**	**−0.18**	0.07	0.03	0.11
Chromosome 13							Chromosome-wise Threshold: 3.40					
Lbn13.1	*U*	101,657,022	2.04	101,328,947	102,063,211	**a**, **d**	**0.13**	**0.05**	**0.22**	**0.22**	**0.07**	**0.39**
	F	105,132,937	2.56	101,906,985	106,699,649	**a**	**0.10**	**0.03**	**0.17**	0.03	0.04	0.04
	H	105,200,250	2.74	102,063,211	106,437,202	**a**	**0.07**	**0.02**	**0.13**	0.04	0.02	0.08
	T	105,200,250	3.57	104,930,999	106,787,132	**a**	**0.10**	**0.03**	**0.17**	0.04	0.03	0.07

QTL	Trait	Peak (bp)	LPR	Proximal CI Boundary (bp)	Distal CI Boundary (bp)	Sig. Model Terms	a (mm)	a SE	a/SD	d (mm)	d SE	d/a
Chromosome 15							Chromosome-wise Threshold: 3.43					
Lbn15.3	U	55,079,296	3.56	54,512,296	56,378,204	**a**, **d**	**0.07**	**0.02**	**0.12**	**−0.10**	**0.03**	**−0.18**
	(H)	56,637,986	1.62	54,906,109	57,761,491	**a**	**0.05**	**0.02**	**0.10**	0.00	0.02	0.00
Chromosome 18							Chromosome-wise Threshold: 3.34					
Lbn18.2	*(H)*	14,536,869	2.37	13,649,082	15,363,405	**a**, asd						
				Low Fat			**0.06**	**0.03**	**0.12**	0.02	0.04	0.04
				High Fat-Male			**0.10**	**0.04**	**0.21**	0.01	0.06	0.02
	(F)	14,689,061	2.87	14,054,928	15,284,430	**a**, asd						
				Low Fat-Female			**0.12**	**0.06**	**0.20**	0.03	0.07	0.04
	T	14,889,556	3.50	14,029,562	15,284,430	**a**, ds, asd						
				Female			0.04	0.04	0.06	**−0.10**	**0.04**	**−0.16**
				High Fat-Male			**0.16**	**0.05**	**0.27**	0.03	0.06	0.05
Chromosome 19							Chromosome-wise Threshold: 2.97					
Lbn19.1	*U*	5,833,042	2.49	5,029,790	8,587,050	**a**, asd						
				Low Fat-Male			**0.19**	**0.05**	**0.33**	−0.08	0.08	−0.15
				High Fat-Female			**0.11**	**0.05**	**0.20**	−0.02	0.07	−0.03
	F	6,292,043	3.28	5,029,790	9,046,051	**a**	**0.11**	**0.03**	**0.19**	−0.03	0.04	−0.05
	H	7,210,046	2.34	5,029,790	9,505,052	**a**	**0.07**	**0.02**	**0.15**	−0.02	0.04	−0.04
	T	7,210,046	1.67	5,029,790	9,505,052	**a**	**0.08**	**0.03**	**0.13**	−0.03	0.05	−0.05

Table 4

Protected F_{34} QTL

Protect QTL surpassing only the point-wise significance thresholds are listed by chromosome (with chromosome-wise significance thresholds indicated), pleiotropic QTL name, and trait affected (H, humerus; U, ulna; F, femur; T, tibia). Bold QTL names indicate newly identified QTL (only *Lbn2.4*; see Results). The position of the QTL peak is given in bp, followed by the LPR score. Proximal and distal QTL confidence interval (CI) boundaries are given in bp. The "Sig. Model Terms" column lists whether **a** and **d** were significant for an individual trait, as well as any other significant interaction terms from the full mapping model (ad, dd, as, ds, asd, or dsd; see Materials and Methods). For each trait, gene effect estimates—**a**, **a**/SD, **d**, **d**/SD—are listed with their respective standard errors (SE). Positive '**a**' values indicate the LG/J allele leads to longer bone lengths; negative '**a**' values indicate the SM/J allele leads to longer bone lengths. The values for **d/a** indicate: underdominance (**d/a** < −1.5), SM/J dominance (−1.5 < **d/a** < −0.5), LG/J dominance (0.5 < **d/a** < 1.5), or overdominance (1.5 < **d/a**). When appropriate, gene effect estimates are given by cohort. Bold values in gene effect estimate columns indicate significance at the $p < 0.05$ level.

QTL	Trait	Peak (bp)	LPR	Proximal CI Boundary (bp)	Distal CI Boundary (bp)	Sig. Model Terms	a (mm)	SE	a/SD	d (mm)	SE	d/SD
Chromosome 1							Chromosome-wise Threshold: 3.66					
Lbn1.2	U	10,552,679	1.40	5,176,059	18,911,762	**a**, **d**, dsd						
				Low Fat			**−0.11**	**0.04**	**−0.20**	**−0.11**	**0.05**	**−0.19**
				Low Fat-Female			**−0.11**	**0.05**	**−0.20**	−0.13	0.07	−0.23
				High Fat-Male			−0.01	0.05	−0.01	**−0.16**	**0.07**	**−0.29**
	F	11,871,997	1.49	10,021,088	18,911,762	**d**, dsd						
				Low Fat-Female			−0.07	0.06	−0.11	**−0.20**	**0.08**	**−0.32**
Lbn1.3	F	153,331,100	3.57	153,167,981	157,305,905	**a**, dd						
				Low Fat			**−0.13**	**0.04**	**−0.21**	−0.04	0.05	−0.07
				Female			**−0.16**	**0.04**	**−0.26**	0.00	0.05	0.00
	T	153,343,974	1.88	153,105,243	156,160,451	**a**, asd						
				High Fat-Female			**−0.18**	**0.05**	**−0.30**	0.12	0.06	0.20
	H	153,343,974	1.52	150,895,076	159,993,765	**a**	**−0.05**	**0.02**	**−0.10**	0.01	0.02	0.01
Chromosome 2							Chromosome-wise Threshold: 3.62					
Lbn2.1a	U	109,092,100	1.46	106,526,798	110,200,694	ad, as						
				Low Fat-Female			**−0.17**	**0.06**	**−0.31**	0.06	0.08	0.10
	T	110,200,694	2.04	108,295,972	110,654,338	**d**, ad, dd						
				High Fat-Male			0.05	0.05	0.08	**−0.21**	**0.07**	**−0.34**

QTL	Trait	Peak (bp)	LPR	Proximal CI Boundary (bp)	Distal CI Boundary (bp)	Sig. Model Terms	a (mm)	SE	a/SD	d (mm)	SE	d/SD
Lbn2.1b	T	112,915,087	3.04	112,450,335	113,537,970	**d**, dd, ds						
				High Fat			0.03	0.03	0.05	**−0.16**	**0.04**	**−0.27**
				Male			0.02	0.03	0.03	**−0.15**	**0.04**	**−0.25**
	F	114,120,349	2.12	113,667,509	115,248,630	**d**, ds						
				Male			0.02	0.04	0.04	**−0.16**	**0.05**	**−0.26**
				High Fat-Male			**0.12**	**0.06**	**0.20**	**−0.15**	**0.07**	**−0.24**
	H	114,132,531	2.01	113,756,356	115,248,630	**d**	0.04	0.02	0.07	**−0.05**	**0.03**	**−0.11**
	U	114,169,075	2.64	114,022,898	115,248,630	ad, ds						
				Male			0.04	0.04	0.07	**−0.12**	**0.05**	**−0.21**
				High Fat			**0.08**	**0.03**	**0.14**	−0.06	0.04	−0.11
				Low Fat-Female			−0.01	0.05	−0.02	**0.13**	**0.06**	**0.23**
Lbn2.4	H	135,758,395	2.04	135,541,887	136,178,035	as, dsd						
				Male			**0.05**	**0.02**	**0.10**	0.05	0.03	0.09
				Low Fat-Female			−0.05	0.04	−0.10	**−0.15**	**0.05**	**−0.29**
	U	135,758,396	2.10	135,628,490	136,058,138	dd, as						
				High Fat-Male			**0.10**	**0.04**	**0.17**	**0.11**	**0.05**	**0.19**
				Low Fat-Female			−0.04	0.04	−0.08	**−0.15**	**0.06**	**−0.27**
	F	135,758,397	3.21	135,570,755	136,208,009	dd, as, dsd						
				Male			**0.08**	**0.03**	**0.14**	0.07	0.04	0.11
				Low Fat-Female			−0.10	0.06	−0.16	**−0.23**	**0.07**	**−0.37**
				High Fat-Female			0.02	0.05	0.04	**0.13**	**0.06**	**0.21**
	T	135,758,398	1.91	135,556,321	136,178,035	as, dsd						
				Male			**0.07**	**0.03**	**0.11**	**0.10**	**0.04**	**0.16**
				Low Fat-Male			0.05	0.05	0.08	**0.15**	**0.06**	**0.24**
				Low Fat-Female			−0.05	0.05	−0.08	**−0.13**	**0.06**	**−0.21**
Lbn2.3b	U	152,901,677	2.30	150,443,412	153,546,994	**a**	**0.08**	**0.03**	**0.14**	0.00	0.03	0.00
	T	152,968,434	2.71	151,822,704	153,814,022	**a**	**0.10**	**0.03**	**0.17**	0.02	0.04	0.03
	H	153,057,443	2.85	151,960,633	153,791,769	**a**	**0.09**	**0.02**	**0.17**	0.02	0.03	0.03
	F	153,124,200	2.45	151,477,881	153,658,255	**a**	**0.12**	**0.04**	**0.20**	0.04	0.05	0.07

Chromosome 3 — Chromosome-wise Threshold: 3.63

QTL	Trait	Peak (bp)	LPR	Proximal CI Boundary (bp)	Distal CI Boundary (bp)	Sig. Model Terms	a (mm)	SE	a/SD	d (mm)	SE	d/SD
Lbn3.2a	H	90,491,261	3.41	89,446,772	96,365,229	**a**	**0.07**	**0.02**	**0.15**	−0.02	0.02	−0.04
	U	90,564,824	1.36	89,446,772	96,365,229	**a**, dsd						
				High Fat			**0.07**	**0.03**	**0.12**	0.00	0.04	0.00
				Male			**0.07**	**0.03**	**0.12**	−0.02	0.04	−0.04
				Low Fat-Female			**0.10**	**0.05**	**0.17**	−0.06	0.06	−0.10
	T	95,750,438	2.10	89,446,772	96,365,229	**a**	**0.07**	**0.02**	**0.12**	−0.03	0.03	−0.04
	F	95,955,368	3.05	89,446,772	96,365,229	**a**	**0.10**	**0.03**	**0.16**	−0.04	0.03	−0.06
Lbn3.3	U	146,988,827	2.14	146,691,940	147,713,479	**d**, dsd						
				High Fat-Male			**−0.11**	**0.04**	**−0.19**	−0.01	0.06	−0.02
				High Fat-Female			−0.03	0.04	−0.06	**0.23**	**0.06**	**0.40**
Chromosome 4							Chromosome-wise Threshold: 3.55					
Lbn4.1	H	100,113,776	2.04	98,437,152	102,525,229	**a**, ad						
				High Fat			**0.08**	**0.03**	**0.17**	0.05	0.03	0.10
	F	100,963,991	1.45	98,437,152	104,771,452	**a**	**0.06**	**0.03**	**0.10**	0.04	0.03	0.07
	U	103,634,668	3.03	100,113,776	104,719,249	**a**	**0.09**	**0.02**	**0.15**	0.02	0.03	0.03
	T	104,131,509	1.68	99,636,848	104,928,060	**a**	**0.06**	**0.03**	**0.10**	0.03	0.03	0.06
Chromosome 6							Chromosome-wise Threshold: 3.59					
Lbn6.3	T	91,551,568	2.97	90,689,058	92,506,805	**a**, **d**, as						
				Low Fat-Female			0.06	0.06	0.10	**0.31**	**0.12**	**0.51**
				High Fat-Female			**0.16**	**0.07**	**0.26**	0.16	0.12	0.26
	H	91,583,410	2.53	90,550,500	92,411,281	**a**, **d**, as						
				Low Fat-Female			0.06	0.05	0.13	**0.24**	**0.09**	**0.48**
				High Fat-Female			**0.14**	**0.05**	**0.28**	0.02	0.09	0.03
	U	91,647,092	2.81	90,755,538	92,443,122	**a**, **d**, as						
				Female			**0.15**	**0.04**	**0.27**	0.09	0.08	0.16
	F	91,678,933	2.52	90,671,738	92,379,440	**a**, **d**, ds						
				Low Fat-Female			0.06	0.07	0.10	**0.45**	**0.14**	**0.73**
Chromosome 7							Chromosome-wise Threshold: 3.43					

QTL	Trait	Peak (bp)	LPR	Proximal CI Boundary (bp)	Distal CI Boundary (bp)	Sig. Model Terms	a (mm)	SE	a/SD	d (mm)	SE	d/SD
Lbn7.1	T	86,871,151	2.11	85,011,051	91,287,407	**a**, as, asd						
				Low Fat-Female			**0.17**	**0.06**	**0.28**	0.01	0.09	0.02
	F	87,177,593	3.29	86,114,896	88,108,213	**a**, as						
				Female			**0.20**	**0.05**	**0.32**	0.05	0.09	0.08
	H	87,279,741	1.62	85,869,597	88,568,954	**a**	**0.05**	**0.02**	**0.11**	−0.04	0.04	−0.08
	U	89,430,611	1.35	87,647,472	91,287,407	dd						
				Low Fat-Male			−0.03	0.05	−0.05	**0.20**	**0.06**	**0.35**
				Low Fat-Female			**0.11**	**0.04**	**0.20**	0.07	0.06	0.12
Chromosome 8							Chromosome-wise Threshold: 3.20					
Lbn8.1a	U	61,956,909	2.69	61,329,788	63,775,969	**a**	**−0.07**	**0.02**	**−0.13**	0.05	0.03	0.10
	F	70,431,892	1.87	69,790,876	70,845,435	ad						
				Low Fat			**−0.15**	**0.04**	**−0.25**	0.04	0.05	0.07
	H	70,431,892	1.59	69,719,652	70,845,435	ad						
				Low Fat			**−0.10**	**0.03**	**−0.21**	0.03	0.03	0.06
	T	70,431,892	1.55	69,648,428	70,845,435	ad						
				Low Fat			**−0.15**	**0.04**	**−0.24**	0.04	0.05	0.06
Lbn8.1b	F	81,172,331	2.67	80,549,384	83,318,039	dd						
				High Fat-Male			−0.06	0.10	−0.11	**0.36**	**0.14**	**0.59**
	U	82,210,577	2.34	81,172,331	85,158,365	dd, asd, dsd						
				Low Fat-Male			**0.24**	**0.08**	**0.43**	−0.13	0.13	−0.24
				High Fat-Male			−0.07	0.08	−0.13	**0.32**	**0.13**	**0.58**
Chromosome 9							Chromosome-wise Threshold: 3.45					
Lbn9.3	T	96,831,731	3.29	94,380,583	99,238,806	**a**, as						
				Female			**−0.19**	**0.05**	**−0.31**	0.02	0.06	0.04
	U	97,193,859	1.38	95,199,986	98,823,436	**d**	−0.01	0.03	−0.01	**−0.08**	**0.04**	**−0.15**
	H	97,374,924	2.45	94,483,008	99,814,189	as						
				Female			**−0.09**	**0.04**	**−0.19**	−0.04	0.05	−0.08
	F	98,280,244	3.24	94,636,647	99,515,719	as						

QTL	Trait	Peak (bp)	LPR	Proximal CI Boundary (bp)	Distal CI Boundary (bp)	Sig. Model Terms	a (mm)	SE	a/SD	d (mm)	SE	d/SD
				High Fat			0.00	0.05	−0.01	**−0.12**	**0.06**	**−0.20**
				Low Fat-Female			**−0.21**	**0.07**	**−0.35**	0.04	0.09	0.06
Chromosome 10							Chromosome-wise Threshold: 3.35					
Lbn10.1	T	85,585,850	1.49	85,418,835	85,914,425	ad, dd						
				High Fat			−0.01	0.03	−0.02	**−0.10**	**0.04**	**−0.17**
	H	85,771,566	1.71	85,439,712	88,398,880	**a**, ad						
				Low Fat			**0.08**	**0.03**	**0.17**	0.02	0.04	0.04
	F	87,488,435	2.30	85,728,709	88,343,701	**a**	**0.09**	**0.03**	**0.14**	0.04	0.03	0.06
Lbn10.2	T	126,891,649	1.54	126,295,292	128,381,379	**a**	**0.05**	**0.02**	**0.08**	−0.04	0.03	−0.07
	U	126,891,649	1.34	126,310,367	128,553,075	**d**	0.00	0.02	0.00	**−0.07**	**0.03**	**−0.12**
Chromosome 11							Chromosome-wise Threshold: 3.49					
Lbn11.1	U	15,462,308	2.06	13,413,103	16,763,472	ad, asd, dsd						
				High Fat-Male			**0.11**	**0.05**	**0.20**	0.13	0.07	0.23
				Low Fat-Female			0.03	0.05	0.06	**0.16**	**0.07**	**0.29**
	H	16,472,331	1.41	13,413,103	16,763,472	as, dsd						
				High Fat-Male			**0.08**	**0.04**	**0.15**	0.07	0.05	0.14
				Low Fat-Female			−0.03	0.04	−0.07	**0.12**	**0.05**	**0.24**
Lbn11.2	F	51,188,546	1.50	47,798,023	52,319,440	ad						
				Low Fat-Male			0.05	0.05	0.08	**−0.15**	**0.06**	**−0.25**
	H	51,188,546	1.61	48,161,168	52,250,744	ad, as						
				High Fat			**0.05**	**0.03**	**0.11**	−0.02	0.03	−0.05
				Male			**0.06**	**0.03**	**0.11**	−0.06	0.03	−0.13
	U	55,804,486	1.34	50,486,110	56,637,878	dd						
				Low Fat			0.06	0.04	0.11	**−0.12**	**0.05**	**−0.22**
				Female			0.02	0.03	0.03	**−0.10**	**0.05**	**−0.18**
				Low Fat-Male			**0.11**	**0.05**	**0.20**	−0.12	0.07	−0.22
	T	56,462,949	2.27	55,244,588	59,130,714	**a**, **d**	**0.10**	**0.03**	**0.16**	**−0.13**	**0.05**	**−0.21**
Chromosome 14							Chromosome-wise Threshold: 3.26					

QTL	Trait	Peak (bp)	LPR	Proximal CI Boundary (bp)	Distal CI Boundary (bp)	Sig. Model Terms	a (mm)	SE	a/SD	d (mm)	SE	d/SD
Lbn14.1	F	84,554,168	1.62	83,392,386	86,419,639	**a**	**0.06**	**0.03**	**0.11**	0.04	0.03	0.06
	T	86,739,348	2.322731	86,419,639	88,843,283	**a**	**0.08**	**0.03**	**0.13**	0.01	0.03	0.02
	H	86,852,479	3.17	86,483,581	88,843,283	**a**	**0.07**	**0.02**	**0.15**	0.00	0.02	0.00
	U	87,191,874	1.76	86,419,639	88,493,111	**a**	**0.07**	**0.02**	**0.12**	0.01	0.03	0.02
Chromosome 15							Chromosome-wise Threshold: 3.43					
Lbn15.2	T	91,385,562	1.81	90,674,653	93,782,312	**d**	−0.05	0.03	−0.08	**0.07**	**0.03**	**0.12**
	F	92,014,070	1.99	90,759,412	93,624,072	**a**	**−0.07**	**0.03**	**−0.12**	0.03	0.03	0.05
	U	92,076,446	3.08	91,147,489	92,753,385	**a**	**−0.08**	**0.02**	**−0.15**	0.02	0.03	0.03
	H	92,476,975	1.82	91,147,489	93,844,354	**a**	**−0.04**	**0.02**	**−0.09**	0.04	0.02	0.07
Chromosome 18							Chromosome-wise Threshold: 3.34					
Lbn18.1a	T	42,051,753	2.03	39,445,587	42,842,537	**d**	−0.02	0.03	−0.03	**0.11**	**0.04**	**0.18**
	H	42,381,247	2.41	40,020,882	43,886,598	**d**, asd						
				Low Fat-Male			−0.07	0.04	−0.15	**0.17**	**0.06**	**0.34**
	U	42,414,196	1.38	39,821,487	43,440,607	**d**	−0.03	0.03	−0.06	**0.10**	**0.04**	**0.17**
	F	42,480,095	1.48	39,195,395	44,587,379	**d**, asd						
				Low Fat-Male			−0.06	0.06	−0.10	**0.22**	**0.08**	**0.35**
Lbn18.1b	U	46,357,853	1.49	40,020,882	49,262,246	**a**	**−0.05**	**0.02**	**−0.09**	0.06	0.03	0.10

Table 5

QTL Positional Candidate Genes

Positional candidate genes are listed for each QTL by QTL name and trait affected (H, humerus; U, ulna; F, femur; T, tibia). The length of the pleiotropic QTL confidence interval (CI; given in bp), the number of genes in the confidence interval, and the number of polymorphisms between LG/J and SM/J that fall in the CI are listed. The genes of interest include genes from families known to contribute to skeletal growth or cellular morphology (see Results). Underlining indicates that a SNP was identified in the coding region of the gene, while boldface indicates the presence of an amino-acid changing mutation(s).

QTL	Trait	CI	Genes	SNPs	Genes of Interest
Lbn1.2	UF	13,735,703	61	22,548	*Rdh10*
Lbn1.3	HFT	9,098,689	75	36,819	
Lbn1.1a	UFT	6,084,606	53	16,044	***Atg9a***, ***Chpf***, *Cul3*, *Ihh*, *Pax3*
Lbn1.1a	H	1,941,869	33	4,079	***Rufy4***, *Tmbim1*, *Wnt10a*, *Wnt6*
Lbn1.1b	HFT	6,943,123	69	8,323	*Akp5*, *Atg16l1*, *Cab39*, *Dner*, ***Trip12***
Lbn2.4	HUFT	666,122	5	1,161	*6330527O06Rik*, *Pak7*, *Plcb4*
Lbn2.1a	UT	4,127,540	20	5,245	*Fibin*, ***Kif18a***
Lbn2.1b	T	1,087,635	4	1,886	***Aven***, ***Fmn1***, *Ryr3*
Lbn2.1b	HUF	1,581,121	8	2,696	*Actc1*, *Aqr*, ***Arhgap11a***, *Atpbd4*, *Gjd2*, *Scg5*, ***Zfp770***
Lbn2.1c	HUFT	12,146,034	178	35,894	*1500003O03Rik*, *Bmf*, *Capn3*, *Ccndbp1*, ***Dnajc17***, ***Mapkbp1***, *Meis2*
Lbn2.3a	HUFT	3,172,374	8	7,841	***2310003L22Rik***, *Btbd3*, ***Esf1***, *Ism1*, ***Macrod2***, ***Sel1l2***, ***Sptlc3***, *Tasp1*
Lbn2.3b	HUFT	3,370,609	74	9,308	*Bcl2l1*, *Gm1006*, *Kif3b*, ***Rya3***, *Sox12*
Lbn3.3	U	1,021,539	0	1,203	
Lbn3.2a	HUFT	6,918,457	180	2,980	*Adamts14*, *Bnipl*, *Ecm1*, *Npr1*, *Snapin*, *Tnfaip8l2*
Lbn4.1	HUFT	6,490,909	50	15,426	***Insl5***, ***Lepr***, ***Leprot***, *Oma1*
Lbn4.2	HUFT	4,844,329	101	25,700	***Gmeb1***, ***Map3k6***, *Matn1*, ***Rcan3***, *Runx3*
Lbn4.3	HUFT	9,312,256	13	18,282	*Megf9*, *Tlr4*, *Tnfsf15*, *Tnfsf8*
Lbn4.4	HFT	1,778,278	48	4,398	***Cdc20***
Lbn4.5	HFT	2,165,219	29	5,894	***Snip1***
Lbn6.2	UFT	3,663,784	20	10,087	***1700023A16Rik***, ***1700034J05Rik***, ***2210417D09Rik***, ***4933424B01Rik***, ***Arntl2***, ***EG545893***, ***Fgfr1op2***, ***Itpr2***, *Klhdc5*, *Med21*, ***Mrps35***, ***Ppfibp1***, ***Pthlh***, *Skt38l*, ***Tm7sf3***
Lbn6.2	H	1,132,711	15	31,262	*Sox5*
Lbn6.3	HUFT	1,956,305	20	537	*Hdac11*, *Wnt7a*
Lbn6.1a	FT	3,344,981	24	7,316	*Cadps2*, *Hyal4*, *Hyal5*, *Hyal6*, *Ing3*, *Spam1*, *Wnt16*
Lbn7.1	HUFT	6,276,356	89	15,784	***Hapln3***, *Mesdc1*, *Mesdc2*, *Mesp1*, *Mesp2*, *Rlbp1*

QTL	Trait	CI	Genes	SNPs	Genes of Interest
Lbn7.3	HUFT	3,056,930	20	7,935	*Adamts17*, *Chsy1*, *Igf1r*
Lbn7.4	HUFT	8,808,703	93	6,125	***Sox6***
Lbn8.2	HU	1,709,669	21	950	
Lbn8.1a	HFT	1,197,007	9	496	***Cilp2***, *Comp*, *Csgalnact1*, ***Gdf1***, ***Gdf15***, ***Insl3***, *Jund*
Lbn8.1a	U	2,446,181	9	5,184	
Lbn8.1b	UF	4,608,981	23	18,965	*Hapln4*, ***Hhip***
Lbn9.2	HUFT	3,806,152	31	4,574	*Adam10*, *Aldh1a2*, *Bnip2*
Lbn9.3	HUFT	5,433,606	50	40,323	***Pcolce2***, *Rbp1*, *Rbp2*, *Sox14*
Lbn10.1	HFT	2,980,044	33	6,438	*Igf1*, *Timp3*
Lbn10.2	UT	2,257,783	105	8,839	*Cdk2*, *Gdf11*, ***Hsd17b6***, ***Itga7***, ***Mmp19***, ***Os9***, *Rdh1*, ***Rdh5***, ***Rdh7***, *Rdh9*, *Rdh16*, *Rdh19*, ***Stat2***, *Stat6*
Lbn11.1	HU	3,350,369	5	7,092	*Egfr*, *Meis1*
Lbn11.2	HUF	8,839,855	131	14,294	*Adamts2*, ***Col23a1***, ***Flt4***, ***Gdf9***, ***Kif3a***, *Mapk9*
Lbn11.2	T	3,886,126	74	9,493	*Sap30l*
Lbn12.1	HUFT	4,019,008	27	7,320	*Mbip*, *Nfkbia*, *Nkx2-1*, ***Nkx2-9***, *Pax9*
Lbn12.2	U	2,241,976	10	5,557	
Lbn12.3	HUFT	3,895,541	40	10,749	*Cdkl1*, *Map4k5*
Lbn12.4	HUFT	3,961,529	33	2,730	*Esr2*, *Rdh11*, ***Rdh12***
Lbn12.5	T	4,642,071	63	1,352	*Fos*, *Ltbp2*, *Pgf*, *Tgfb3*
Lbn13.1	HUFT	5,458,185	35	7,926	***Adamts6***, *Ccnb1*, *Rad17*
Lbn14.1	HUFT	5,450,897	5	21,697	***Diap3***, *Pcdh17*, ***Pcdh20***, ***Tdrd3***
Lbn15.2	HUFT	3,169,700	15	7,953	***Kif21a***
Lbn15.3	HU	3,249,196	18	4,716	***Col14a1***, *Has2*
Lbn18.2	HFT	1,635,348	8	1,348	
Lbn18.1a	HUFT	5,391,984	35	10,052	*Nr3c1*
Lbn18.1b	U	9,241,364	51	18,924	*Atg12*
Lbn19.1	HUFT	4,475,262	161	1,395	*Atg2a*, *Bad*, *Capn1*, *Efemp2*, *Esrra*, *Fibp*, ***Fosl1***, *Kat5*, ***Ltbp3***, *Map3k11*, ***Map4k2***, *Vegfb*

Interestingly, while we expected to identify QTL with confidence intervals of ~0.5 F_2 cM, actual confidence intervals averaged ~1.7 F_2 cM (corresponding to ~4.5 Mb). In terms of physical distances, the confidence intervals ranged from 14 Mb for the largest QTL (*Lbn1.2*) to 0.6 Mb for the smallest QTL (*Lbn2.4*), with about half of the QTL having confidence intervals between 2 and 5 Mb (see Table 5). There are several possible reasons for the apparent lack of increased resolution between the $F_{9\text{-}10}$ and F_{34} generations. First, as opposed to the previous studies on which the 0.5 F_2 cM estimate was based, the parental generation (F_{33}) was not included in the mapping population because it was not subject to the same dietary treatments. As discussed in the previous studies, familial autocorrelation inflated QTL peak size and this did impact how confidence intervals were set in earlier generations. While all F_{34} individuals are related and thus subject to familial autocorrelation, the absence of the parental generation should have decreased QTL peak inflation relative to previous generations. A second possibility is that some QTL are caused by multiple linked genomic elements. While several of the QTL reported above have short confidence intervals with few (≥ 10) or no candidate genes (*Lbn2.1b*, *Lbn2.3a*, *Lbn2.4*, *Lbn3.3*, *Lbn8.1a*, *Lbn11.1*, *Lbn12.2*, *Lbn14.1*, and *Lbn18.2*), the majority have between 20

and 70 candidate genes (listed in Table 5). The absence of most of the known major endochondral ossification regulators within the confidence intervals supports the idea that genes controlling normal cellular processes such as metabolism, motility, or cell division have a measurable cumulative effect on cellular dynamics. Given the nature of bone elongation via the growth plate, this finding is not unreasonable.