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Bone Microstructure and its Associated Genetic Variability in 12 Inbred Mouse Strains: μ CT Study and In Silico Genome Scan

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Abstract

MicroCT analysis of 12 inbred strains of mice identified 5 novel chromosomal regions influencing skeletal phenotype. Bone morphology varied in a compartment- and site-specific fashion across strains and genetic influences contributed to the morphometric similarities observed in femoral and vertebral bone within the trabecular bone compartment.

Introduction—Skeletal development is known to be regulated by both heritable and environmental factors, but whether genetic influence on peak bone mass is site- or compartment-specific is unknown. This study examined the genetic variation of cortical and trabecular bone microarchitecture across 12 strains of mice.

Materials and methods—MicroCT scanning was used to measure trabecular and cortical bone morphometry in the femur and vertebra of 12 strains of 4-month-old inbred male mice. A computational genome mapping technique was used to identify chromosomal intervals associated with skeletal traits.

Results—Skeletal microarchitecture varied in a compartment- and site-specific fashion across strains. Genome mapping identified 13 chromosomal intervals associated with skeletal traits and 5 of these intervals were novel. Trabecular microarchitecture in different bone sites correlated across strains and most of the chromosomal intervals associated with these trabecular traits were shared between skeletal sites. Conversely, no chromosomal intervals were shared between the trabecular

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and cortical bone compartments in the femur, even though there was a strong correlation for these different bone compartments across strains, suggesting site-specific regulation by environmental or intrinsic factors.

Conclusion—In summary, these data confirm that there are distinct genetic determinants that define the skeletal phenotype at the time when peak bone mass is being acquired, and that genomic regulation of bone morphology is specific for skeletal compartment.

Keywords

cortical bone; trabecular bone; SNPs; genomic mapping; inbred strains

Introduction

Fractures due to age-related osteoporosis are a major public health problem in developed countries. The strength of bone and its ability to resist fracture is dependent on many factors including intrinsic properties of the bone itself. One of the most established and identifiable risk factors for osteoporotic fractures is a low bone mineral density (BMD). The proliferation of newer tools to measure bone mass has resulted in widespread testing and has also led to the realization that BMD is a complex trait normally distributed across various populations.

BMD in mammalian models as well as in humans follows a pattern of increase during growth and puberty, followed by a steady state after skeletal maturity (peak BMD) and then decline in later life. Peak BMD is the single most important determinant of fracture risk in later life and is quite variable among individuals [1]. Based on family, twin and large sib cohort studies, it has been estimated that between 50% to 70% of the variance in peak BMD is attributable to heritable factors [2]. In attempting to identify an underlying genetic component of osteoporosis most investigators have proposed that the genetic influences on BMD are oligogenic. Fueled by this concept, the last ten years have been characterized by a flood of candidate-gene association studies [2-5] and numerous linkage-based genome scans [6-9]. Neither approach in humans is likely to reveal novel genes of small to modest effect underlying the complex and polygenic nature of peak BMD variation. The ultimate goal of defining the role of multiple genes that individually, or in concert, regulate the acquisition and maintenance of peak bone mass has yet to be achieved.

Using rodent models for testing hypotheses related to skeletal disorders is not a new approach. The Lewis and Fischer inbred rat strains have been used as a model of human aging-related traits [10-12] including an intercross genome screening study of bone properties [13]. The power of the mouse as a genetic tool has been utilized to uncover genes whose normal allelic variation regulates BMD and other skeletal traits such as bone geometry, microarchitecture and strength [14-28]. An inbred strain survey is a useful step in a thorough genetic analysis of a complex quantitative trait like BMD in mice [23]. The ability to use skeletal phenotypic values from inbred strains to map likely genomic locations of bone related quantitative trait loci (QTLs) constitutes a major advance in the investigation of the genetics of skeletal biology. QTL analysis of various bone properties of inbred mouse strains and their crosses has uncovered an unambiguous association between genetic polymorphism across the inbred strains and the bone phenotypes of these strains. Furthermore, the genes underlying variance in a specific trait can be identified within associated chromosomal regions following a segregation analysis. Thus QTL mapping ultimately allows the determination of genetic factors influencing the specific complex trait under investigation [29]. Most QTL studies for skeletal phenotypes have concentrated on BMD, thus resulting in the development of the genetic maps encompassing loci which are likely responsible for the genetic regulation of bone morphometry and microarchitecture [16-18,21,22,24,30-36].

It has been shown that BMD measured by dual-energy X-ray absorptiometry (DXA) highly correlates with trabecular volumetric BMD (vBMD, mg/cm³) measured by peripheral quantitative computed tomography (pQCT), and that both these bone indices highly correlate with trabecular bone volume fraction (BV/TV) measured by microcomputed tomography (μCT) [37-42].

In the current study we examined the potential role of genetic factors in modulating peak bone acquisition using μCT to measure various skeletal microstructural parameters including the trabecular BV/TV in the distal femur and L5 vertebra, and the cortical bone area of the midfemur shaft in twelve inbred mouse strains. Trabecular and cortical bone are present in different proportions at the various skeletal sites prone to fracture and we hypothesized that bone morphology varies among inbred mouse strains in a compartment- and site- specific fashion. Specifically, we asked whether skeletal morphology for different bone sites (femur and vertebra) were correlated within a specific bone compartment (eg. trabecular bone) across the species. We also tested for the correlation of different bone compartments (trabecular and cortical bone) within a given skeletal site (femur). The goal of the correlation analysis was to identify structurally related skeletal traits and to seek how these interactions contribute to variability across the inbred strains. The issue of inter-strain variability related to genetic differences has been highlighted in previous reports showing genetic effects on traits like biomechanical properties, morphological and compositional traits of the femur diaphysis [19], as well as biomechanical, morphological and compositional evaluation of vertebral bodies [25] in inbred mouse strains.

To test the hypothesis that different skeletal sites and compartments as well as body weight are genetically related, we conducted *in silico* genome scan analysis. In addition, *in silico* genome scan allowed us to identify chromosomal regions containing genetic factors regulating inter-strain skeletal phenotype variability [43,44]. The advent of *in silico* genetic mapping may allow investigators to gain rapid insight into the genetic underpinnings of inter-strain trait differences [43].

Materials and Methods

Animals

All animal experiments were done after approval of protocols by our Institutional Animal Care and Use Committee. Inbred mouse strains were obtained from Jackson Labs (Bar Harbor, ME) at 7–8 weeks of age. Mice were kept under pathogen-free conditions and were provided food and water ad libitum with a 12:12 h light–dark cycle. Group sizes of eight mice at 4 months of age were used for μCT scanning. All mice were male. The strains we used were: 129S1/SvImJ (129SV), A/J (A), AKR/J (AKR), B10.D2-H2/oSNJ (B10), BALB/cByJ (BcBy), BALB/cJ (Bc), C3H/HeJ (C3H), C57BL/6J (C57), DBA/2J (DBA), MRL/MpJ (MRL), NZW/LaCJ (NZW), and SM/J (SMJ) (12 strains). Catalog numbers for these strains can be found at http://mousesnp.roche.com/mouse_strain.htm.

μCT

We assessed trabecular and cortical bone architecture using a vivaCT 40 μCT scanner (Scanco Medical AG, Basserdorf Switzerland). Specifically, trabecular bone architecture was evaluated at the fifth lumbar vertebra and distal femur, whereas cortical bone morphology was evaluated at the femur midshaft. CT images were reconstructed in 2048 × 2048-pixel matrices for vertebral and distal femur samples while midfemur CT images were reconstructed in 1024 × 1024-pixel matrices and stored in 3-D arrays. In a preliminary experiment femur and vertebral samples were processed for μCT measurements (both *in vivo* and *ex vivo*) and then for conventional histomorphometry. The scanned acquired images were reprocessed to test the

threshold giving the best fit with histomorphometric BV/TV data. We therefore defined the optimal threshold used in the remaining studies. All grayscale images were segmented using a constrained Gaussian filter to remove noise, and a fixed threshold (17.5% of the maximal grayscale value for vertebrae, 27.6% for distal femur and 35% for midfemur cortical bone) was used to extract the structure of the mineralized tissue. μ CT parameters were set as below: threshold = 175, sigma = 0.8, support = 1 for vertebral samples, threshold = 276, sigma = 1.0, support = 1 for distal femur, and threshold = 350, sigma = 1.2, and support = 1 for midfemur evaluation analysis. A single operator outlined the trabecular bone region within distal femur and vertebral body, and cortical bone region in midfemur shaft.

Distal and midshaft femurs were scanned *in vivo*, whereas fifth lumbar vertebrae were harvested and cleaned of soft tissue, and then were wrapped in saline-soaked gauze and stored frozen at -20°C for *ex vivo* μ CT scanning. *In vivo* scanning was done by anesthetizing the mouse with isoflurane and right hindlimb was used for distal and midshaft femur scans. The anesthetized animals were placed inside the μ CT, and the right hindlimb was fixed inside a carbon tube and measured. A scout view scan was obtained for selection of the examination volume of the samples, by automatic positioning, measurement, and offline reconstruction [37].

For L5 vertebrae scanning all spinous processes were removed and 8 samples were fixed in a notched plexiglas holder for batch scanning. Each vertebral body was scanned using 235 transversely oriented $19\text{ }\mu\text{m}$ thick slices ($19\text{-}\mu\text{m}$ isotropic voxel size). The trabecular bone region was manually outlined on each slice and all slices containing trabecular bone were included for analysis.

In the distal femur, 160 transverse slices of $10.5\text{ }\mu\text{m}$ thickness ($10.5\text{-}\mu\text{m}$ isotropic voxel size) encompassing a length of 1.68mm were acquired, but only 100 slices encompassing 1mm of the distal femur were evaluated, starting where the growth plate bridge across the middle of the metaphysis ends. The region of interest (ROI) was manually outlined on each CT slice, excluding the primary spongiosa, and extending proximally from the growth plate.

The vertebral and distal femur trabecular bone basic structural parameters, including bone volume fraction (BV/TV, %), trabecular number (Tb.N, mm^{-1}), trabecular thickness (Tb.Th, μm), trabecular separation (Tb.Sp, μm), structure model index (SMI), and connectivity density (ConnDens, mm^{-3}) were measured in three dimensions using direct 3D morphometry based on the distance transformation [45,46], without assumptions regarding the underlying bone architecture [45,47]. The connectivity of a two-component system, i.e., bone and marrow, was defined directly if all trabeculae and bone marrow cavities are connected without isolated marrow cavities inside the bone [48]. It was normalized by examined tissue volume and reported as ConnDens.

At the femur midshaft, 26 transverse CT slices were obtained, each $21\text{ }\mu\text{m}$ thick totaling 0.55mm in length ($21\text{-}\mu\text{m}$ isotropic voxel size) and used to compute the total cross-sectional area (TtAr, mm^2), cortical bone area (BAr, mm^2), cortical thickness (CtTh, μm), and bone area fraction (BAr/TtAr, %). One of the most important properties determining the ability of a long bone to resist fracture is its cross-sectional area [49]. For this reason we used the BAr μ CT parameter for cortical bone correlation analysis in this study.

In silico genome mapping

A computational genome mapping approach has been recently used by our group in identification of chromosome regions associated with different type of phenotypic responses [43,44]. This program uses a murine genome single nucleotide polymorphism (SNP) database developed by Roche Bioscience (Palo Alto, CA) to identify chromosomal regions associated

with different phenotypes complex traits. This approach has been used to correctly predict the chromosomal regions for several mouse traits including airway hyperresponsiveness, alcohol withdrawal, and the major histocompatibility complex K locus [43,44]. In this study we utilized μ CT skeletal phenotypic results of eleven inbred strains. SMJ inbred mouse strain phenotypic results were not included in this analysis since the software does not contain SNP data for this particular strain. The key μ CT parameters for trabecular and cortical bones (BV/TV, TbTh, TbN, and BAr), as well as body weight phenotype, were studied in this analysis. The average size of a predicted genome interval was 10 mega base pairs (10Mbp) and a total of 250 genome intervals were analyzed for examined traits. For each genomic interval, the computational technique calculated genotypic distances for each pair of mouse strains using the allelic distribution across inbred strains SNP data, and a genotypic distance matrix is generated for the interval. Similarly, phenotypic differences for each pair of strains are calculated and a phenotypic distance matrix is generated for the trait studied. Standard linear regression methods are applied to calculate the degree of correlation between the phenotypic distance matrix and the genotypic distance matrix of the interval. The degree of correlation was then expressed as SD above or below the average for all intervals analyzed. These phenotypic-genotypic correlations across the genome allowed us to identify 10Mbp genome intervals with correlated SNPs in strong linkage disequilibrium. The genome-wide threshold of 5% which corresponded to $SD \geq 2$ was used to distinguish the highly correlated 10Mbp chromosomal intervals from the rest of the genome [43].

Statistical analysis

Variables were evaluated using standard descriptive statistics. All data are expressed as means $\pm$ SEM. To test whether skeletal parameters differed among these inbred strains, we used one-factor ANOVA analysis, followed by post hoc Tukey testing. To test whether skeletal parameters differed between two trabecular sites – vertebral and distal femur within any given strain, values for the variable of interest in distal femur and L5 vertebra were compared using an unpaired Student's *t*-test. Pearson correlation analysis was performed in all 12 inbred mouse strains to estimate for association between; 1) distal femur and vertebral trabecular traits, 2) distal femur trabecular and midfemur cortical traits, and 3) vertebral trabecular and midfemur cortical traits. All tests were two tailed, and differences were considered statistically significant at $p < 0.05$ level.

Results

Strain-related skeletal site- and compartment-specific differences

Consistent with other studies, we found differences in skeletal morphology among inbred strains that depended on the skeletal site and compartment (Table 1, Figs. 1,2). For distal femur trabecular bone, the BV/TV ratio was highest in the MRL and NZW strains, intermediate in the B10 and 129SV strains, and lowest in the A and DBA strains ($p < 0.0001$). Distal femur trabecular bone in the MRL strain was characterized by the highest trabecular number, an intermediate increase in trabecular thickness, and the lowest trabecular separation compared with other strains. The A strain had the lowest trabecular number and thickness and consequently the greatest trabecular separation (Table 1; Fig. 1).

In the fifth lumbar vertebrae, the BV/TV ratio was highest in the NZW, intermediate in the B10 and C57, and lowest in the A strain ($p < 0.0001$, Table 1; Fig. 2). Vertebral trabecular bone in the NZW strain was characterized by increased trabecular number and thickness with reduced trabecular separation. In comparison with other strains, vertebral trabecular bone in the A strain was characterized by reduced trabecular number and thickness, but increased trabecular separation (Table 1). Figure 3 illustrates that vertebral trabecular BV/TV was

consistently greater than distal femur trabecular BV/TV across all 12 mouse strains ($p < 0.0001$).

At the femur midshaft, BAR was highest in MRL, intermediate in Bc and BcBy and lowest in SMJ and A strains ($p < 0.0001$, Table 1; Fig. 1 and Fig. 2). Cortical thickness and the ratio of cortical bone to total area (BA/TA) were highest in C3H, intermediate in Bc, BcBy and AKR, and lowest in C57 strain.

In order to better understand the effects of genetic background, we performed a linear regression and Pearson correlation analysis for skeletal traits measured by μ CT and body weight. Correlation analysis of μ CT indexes identified a strong association between the vertebral and distal femur trabecular sites for BV/TV, TbN, and TbSp parameters in the 12 inbred mouse strains (Fig.4). Thus mouse strains with higher values of vertebral BV/TV tended to have a relatively greater distal femur BV/TV values than strains that had lower vertebral BV/TV values. There was also a correlation between the trabecular bone compartment in the distal femur and the cortical bone compartment in femur midshaft (Fig.5). All distal femur μ CT parameters (BV/TV, connectivity, TbN, TbTh, and TbSp) demonstrated correlation with cortical midfemur BAR. Conversely, the vertebral trabecular bone parameters did not correlate with midshaft femur BAR ($p > 0.05$ for all comparisons). There was a positive correlation between cortical BAR, and body weight, as well as between distal femur BV/TV and body weight across 12 inbred mouse strains (Fig.6).

In silico genome scan

One of the goals in collecting strain-specific data for mouse skeletal morphology is to allow subsequent genome mapping studies to be undertaken. A total of 25 10Mbp intervals were identified for each skeletal trait, (cutoff genome wide threshold of 10%, Fig. 7). However, we arbitrarily set the level of significance at $SD \geq 2$ (less than 5% of the genome, Table 2) for strongest correlated loci emphasis.[43,44]

The distal femur trabecular BV/TV was strongly associated with 8 genome intervals on 5 chromosomes, including chromosome 1 at intervals 170-180 and 180-190 Mbp, chromosome 7 at intervals 20-30, 30-40, and 110-120 Mbp, chromosome 13 at interval 100-110 Mbp, and chromosome 19 at intervals 0-10 and 10-20 Mbp (Fig. 7A, Table 2). These intervals are in close approximation with chromosomal loci previously associated with various skeletal traits using QTL mapping approach, with the exception of the intervals observed in chromosome 19 at 0-10 and 10-20 Mbp, which are novel (Table 3).

Vertebral trabecular BV/TV was strongly associated with 6 genome intervals on 5 chromosomes, including *chromosome 1 at intervals 170-180 and 180-190 Mbp*, chromosome 5 at interval 40-50, *chromosome 7 at intervals 30-40, and 110-120 Mbp*, *chromosome 13 at interval 100-110 Mbp*, and chromosome 18 at interval 60-70 Mbp (Fig. 7B, Table 2). The italicized intervals overlapped with the distal femur BV/TV genome intervals. All these intervals were in close approximation with chromosomal loci previously identified by the QLT approach, with the exception of chromosome 5 at interval 40-50, which is novel (Table 3).

In silico mapping also identified 4 genome intervals on 3 chromosomes associated with midfemur cortical BAR, including chromosome 5 at interval 20-30 Mbp, chromosome 7 at interval 0-10 Mbp, and chromosome 17 at intervals 10-20 and 30-40 Mbp (Fig. 7C, Table 2). These genome intervals are in close approximation with loci identified using QTL genome mapping of various skeletal phenotypes, with the exception of the intervals observed in chromosome 5 at 20-30 Mbp and chromosome 7 at 0-10 Mbp, which are novel (Table 3). None of the genome intervals associated with midfemur cortical BAR correspond with trabecular bone intervals.

Body weight strongly associated with 7 intervals on 6 chromosomes, including chromosome 1 at 20-30 and 180-190 Mbp, chromosome 7 at 0-10 Mbp, chromosome 11 at 20-30 Mbp, chromosome 12 at 90-100 Mbp, chromosome 15 at 80-90 Mbp, and chromosome 17 at interval 30-40 Mbp (Fig. 7D, Table 2). These intervals are in close approximation with chromosomal loci previously associated with body weight using QTL mapping, with the exception of the intervals observed in chromosome 12 at 80-90 Mbp and chromosome 15 at 80-90 Mbp, which are novel (Table 3). Several body weight associated intervals corresponded to intervals associated with skeletal traits, including chromosome 1 at 180-190 (distal femur and vertebral trabecular BV/TV), chromosome 7 at 0-10 Mbp (midfemur cortical BAR), and chromosome 17 at 30-40 Mbp (midfemur cortical BAR).

Discussion

In this study μ CT imaging was employed to detect differences in trabecular and cortical bone parameters among twelve inbred mouse strains. We examined whether genetic factors influence bone morphology by studying the skeletal traits in these strains. We found that bone morphology varies among inbred mouse strains in both a compartment- and site-specific fashion. We also confirmed and extended previous observations regarding differences in trabecular and cortical bone morphology among inbred mouse strains. In addition, we refined our understanding of the genetics affecting skeletal traits using a novel computational genome mapping technique.

With regard to skeletal traits, the effect of mouse genetic background on bone morphology depended on the skeletal site and compartment, with strain-dependent variation in the trabecular bone volume fraction and cortical bone area traits ($p < 0.0001$; Table 1, Fig. 2). The vertebral trabecular BV/TV was consistently greater than the distal femur trabecular BV/TV across all strains (Fig. 3). Correlation analysis showed statistically significant association between vertebral and distal femur trabecular traits (Fig. 4), as well as distal femur trabecular and midfemur cortical traits (Fig. 5), but there was no correlation between vertebral trabecular traits and midfemur cortical bone area. Our findings support clinical observations that distinct bone compartments acquire different peak bone mass and that there can be very disparate BMD values between spine and hip densities within any individual. We hypothesize that despite some degree of compartment specificity with respect to genetic regulation of bone density and structure, different bone compartments may respond in similar fashion if these compartments are localized in anatomical proximity and are subjected to similar mechanical influences.

There is considerable evidence that skeletal traits positively correlate with body size phenotypes [32,50,51]. We found a positive correlation between body weight and the midfemur cortical bone area ($r^2 = 0.63$) and distal femur trabecular BV/TV ($r^2 = 0.45$), but there was no correlation between body weight and trabecular BV/TV in the lumbar vertebra (Fig. 6). The mechanisms involved in bone growth are complex and mediated through muscle, body size and weight, and activity [33]. The association of increased body weight with higher skeletal trait values could stem either from the impact of weight loading on BMD, or may be genetically modulated.

Wide variation in bone morphology among inbred mouse strains indicates that the genome enables regulation of the skeleton on a site-specific basis, with combinations of genes modulating the response within each bone region. A strategy that enlists a distinct set of genes for each skeletal location would be very complex and genetically improbable. It is likely that gene environment interactions also contribute to the observed site-specificity. Taken together, our results are consistent with other's observations that skeletal traits are governed by complex relationships between genetic background, skeletal site, and compartment [14,17,20,21,52].

An additional aim of this study was to identify chromosomal loci associated with specific skeletal traits. We approached this question with a novel computational-analysis method – *in silico* genome scan mapping [43,44]. This strategy allowed rapid identification of chromosomal loci associated with various skeletal phenotypes.

In silico genome scanning identified chromosomal loci where the patterns of genomic variation had the strongest association with the skeletal trait distribution among these strains (Fig.7, Table 2). Distal femur trabecular BV/TV was associated with 8 genome intervals on 5 chromosomes and vertebral trabecular BV/TV associated with 6 genome intervals on 5 chromosomes. There were 5 shared chromosomal intervals for these trabecular sites (Table 2) consistent with the high correlation observed for these trabecular traits across strains (Fig.4). The areas of strongest association between the examined trabecular traits were found on chromosomes 1, 7, and 13 (Table 2). We postulate that these genetic determinants modulate trabecular bone morphometry through out the skeleton.

Correlation analysis between cortical and trabecular femur traits revealed an association between these structurally different compartments (Fig.5), but genome scanning failed to identify any shared chromosomal intervals associated with these phenotypes (Table 2, Fig. 7). Based on these data we postulate that the correlation between these distinct bone compartments might not be driven directly by genetic determinants, but rather by the complex interaction of environmental and intrinsic factors. Inevitably, genetically regulated differences in baseline morphology produce distinct milieus within each specific skeleton site and compartment. For example, distribution of mechanical signals across a bone is nonuniform and related not only to differences in bone morphology between distinct intraosseous sites, but also to the imposed mechanical environment (e.g., as influenced by potential differences in muscle–bone interactions or body mass distribution between the strains). We suggest that genetic modulation of skeletal morphology is strongly site-specific, greatly limiting our ability to label solely one main skeletal gene. Further structural evaluation of the murine skeleton may allow us to identify genes controlling site-, compartment-, and environment-specific relations.

Several novel chromosomal intervals strongly associated with specific bone traits were identified in this study (Tables 2, 3), including intervals 0-10 Mbp and 10-20 Mbp on chromosome 19 (distal femur trabecular BV/TV), interval 40-50 Mbp on chromosome 5 (vertebral trabecular BV/TV), interval 20-30 Mbp on chromosome 5 (midfemur cortical BAr) and interval 0-10 Mbp on chromosome 7 (midfemur cortical BAr). The principles underlying SNP-based computational analysis of inbred strains are different from conventional QTL analysis using intercross progeny or recombinant inbred strains, and this may explain our ability to identify these regions. First, our analysis included utilization of 11 inbred strains of mice. Conventional QTL studies use the progeny or recombinant inbred strains deriving from only two founding strains. The use of a broader panel of strains potentially allows the identification of a larger number of genetic factors influencing bone-related traits in mice [53,54]. Second, we had available a large SNP database providing relatively high power to detect associations with 10 Mbp genomic segments [43]. This power and resolution is superior to what has been used in most available QTL studies [54]. Even so, far denser SNP maps and the use of alternative types of analysis like haplotypic approaches will be necessary to identify specific genes involve in mediating these effects [54]. However, more dense marker sets and large panels of recombinant inbred strains are becoming available for use in conventional QTL paradigms which should improve the power of those techniques significantly.

With regard to the body weight genome analysis, we identified 7 intervals on 6 chromosomes strongly associated with body weight, including 2 novel intervals, chromosome 12 at 80-90 Mbp, and chromosome 15 at 80-90 Mbp (Fig. 7). We also observed that body weight correlated with the distal femur BV/TV and the midfemur cortical bone area across strains, but not with

the vertebral trabecular BV/TV (Fig. 6). However, only a few chromosomal intervals were strongly associated with both body weight and bone traits (Table 2), including chromosome 1 at 180-190 Mbp (distal femur and vertebral trabecular BV/TV), chromosome 7 at 0-10 Mbp (midfemur cortical BAr), and chromosome 17 at 30-40 Mbp (midfemur cortical BAr). The association of chromosomal segments linked to both cortical bone parameters and weight (Table 2) is consistent with previous studies (Table 3) [24,30,33,51]. Body weight is one of the biggest intrinsic factors of bone strain [50], which is directly related to cortical area [33]. Based on these data, we postulate that the correlation between body weight and femur bone traits across strains may be at least partially due to skeletal loading.

It has been previously proposed that during growth net bone apposition is adapted to load-bearing demands, e.g. body weight and muscle tension, through a process that is referred to as mechanostat [55,56]. Strain differences in bone density and morphology could be explained by inter-strain variability of mechanostat set point that might be lower in SMJ and greater in MRL strains (Table 1). Therefore, the osteotrophic effect of body weight bearing during growth may be lower in SMJ and greater in MRL strains resulting in weight-related increase of bone density in MRL strain.

One important finding of the *in silico* genome scanning was the association of several loci to individual traits (Table 2). An interaction of these loci, which represents a general epistatic effect, might have an important role in regulation of skeletal and body weight traits, as was shown by Li et al., (2002) for complex gene action on femur breaking strength trait [33]. Thus, the multiple chromosomal intervals that were identified in our study may represent epistatic loci containing regulatory genes common for multiple pathways [57]. Identification of chromosomal loci involved in multiple interactions is crucial since they could have a more prominent role on complex traits by combined interaction rather than acting as single locus. We suggest that some of these loci may encompass genes regulating putative mechanostat modulation. These findings emphasize the need for epistatic analysis in the genetic dissection of complex skeletal traits.

Another interesting observation in this study was that different traits have shared chromosomal loci. This finding might be explained by a pleiotropic effect of shared chromosomal loci on different bone traits. For example, we found a chromosomal locus in interval 30-40Mb on chromosome 17 for the cortical BAr trait, which was associated with other traits such as distal femur BV/TV and TbTh, vertebral BV/TV, and body weight (Table 2, Fig.7). This chromosomal region was previously identified as controlling one of the most important cortical bone related traits – femur breaking strength (Table 3) [33]. It has been hypothesized that this region includes a general regulatory gene influencing multiple bone metabolism pathways represents a common genetic mechanism [33]. Several chromosomal loci on genome scan analysis had a weak association with multiple traits (Table 2, Fig.7), which may still represent a true chromosomal intervals [43]. These loci might underlie a complex epistatic or/and pleiotropic mechanism of action for one or more phenotypes. Pleiotropy is a more complex mechanism than epistasis as it involves different genes interacting to influence the biological outcome in more than one phenotype.

Previous studies have identified QTLs associated with peak BMD in mice and several candidate genes within the QTLs have been proposed as key regulators of skeletal traits. One of the QTLs identified by Ishimori et al., (2006) on chromosome 7 - BMD9 is located in the vicinity of the marker D7Mit300 [58] and overlaps with chromosomal interval 110-120 Mbp which was identified by our study (Table 2 and 3). This region contains numerous genes, including *Pth* (113 Mbp) coding for PTH, *Calca* (114.5 Mbp) coding for a calcitonin, and *Sox6* (115.5 Mbp) coding for a chondrocyte-specific Sry-related HMG box transcriptional factor. Subsequent haplotype analysis based on 129SV, C3H, DBA, and C57 inbred mouse strains excluded the

candidate genes *Pth* and *Calca*, but *Sox6*, which mediates differentiation and proliferation of chondrocytes [59,60], was included in one haplotypic block.[58] These findings make *Sox6* an excellent gene candidate as a determinant of trabecular bone volume. Given that our own data were obtained using a SNP database containing information from 11 inbred mouse strains it is possible that strains not tested in these other studies possess *Pth* and *Calca* gene polymorphisms that contribute to the skeletal phenotypes we observed.

We also compared our genome scan findings with QTL markers that previously were reported for skeletal and body weight traits (Table 3). Although the genetic approach was different [43,44] and we have used inbred male mice as opposed to female RI and F2 intercrosses with different skeletal measures (skeletal and/or whole body BMD measured by DXA or pQCT), we have identified chromosomal intervals that correspond to reported in the QTL literature (Table 3). Orwoll et al., (2001) have demonstrated that some chromosomal loci appear to be associated with peak BMD in one but not both genders [32]. Comparing this study with our genome scan results we noted the highest correlation between QTL markers for whole body BMD in male RI mice versus our chromosomal loci for skeletal (trabecular and cortical both) traits in inbred male mice. It is important to remember in comparing our results with those of previous studies that the current study was performed utilizing 12 inbred strains of male mice and that gender effects may be important factors underlying differences between prior QTL analyses and our *in silico* genome scan outcomes.

In summary, the data presented in the current study confirm that there are distinct genetic determinants within a given strain that define the skeletal phenotype at the time when peak bone acquisition occurs. Bone morphology varied among inbred mouse strains in a compartment- and site- specific fashion. Genome mapping identified 13 chromosomal intervals strongly associated with skeletal traits in trabecular and/or cortical bone, including 5 novel intervals. Skeletal morphology for different bone sites (vertebra and femur) highly correlated within a specific bone compartment (e.g. trabecular bone) across species. Genomic analyses indicate that this correlation across strains was due to genetic determinates. Correlation of different bone compartments within a given skeletal site (e.g. femur) was also observed, but this correlation might not be attributable to genetic factors, as there were no chromosomal intervals in common for these compartments. Despite the technical difficulties in determining the specific contribution of genetics, intrinsic factors, and environmental influences on skeletal phenotype, we postulate that genetic studies will eventually identify a number of genes involved in the site- and compartment-specific regulation of bone morphology.

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Figure 1.

These are μ CT images (2-D) of the distal femur (A) and midfemur shaft (B) in representative mice from six inbred strains. μ CT images are listed in according to the ascending magnitude order of trabecular bone volume fraction (A) and midfemur cortical bone area (B) in 6 inbred mouse strains. The white bar represents 1mm.

Figure 2.

Genetic variability of distal femur trabecular bone volume fraction (A), vertebra trabecular bone volume fraction (B), and midfemur cortical bone area (C) in 12 inbred mouse strains. Strains are listed in ascending trait magnitude order. The data were expressed as means $\pm$ SEM, and analyzed by one-way ANOVA with post hoc Tukey testing.

Figure 3. Vertebral vs. distal femur trabecular bone volume fraction (BV/TV) in 12 inbred mouse strains. Strains are listed in ascending trait magnitude order for distal femur BV/TV. The data were expressed as means $\pm$ SEM, and analyzed by unpaired Student's t-test; $P < 0.0001$ for all 12 mouse strains.

Figure 4.

Linear regression analysis between two trabecular bone sites, the distal femur (DF) and the L5 vertebra (L5) for 12 inbred mouse strains. Each graph represents the comparison between similar μ CT parameters in 12 inbred mouse strains. There was a positive correlation for the bone volume fraction (A), trabecular number (C), and trabecular separation (E) between the two skeletal sites, but not for connectivity density (B) or trabecular thickness (D).

Figure 5.

Linear regression analysis between two different bone compartments, trabecular bone in the distal femur (DF) and cortical bone in midfemur (MF). Each graph represents comparison between cortical bone area (mm²) and five different trabecular μ CT indexes in 12 inbred mouse strains. Four of the trabecular indices (bone volume fraction (A), connectivity density (B), trabecular number (C), and trabecular thickness (D)) positively correlated with cortical bone area. Trabecular separation (E) negatively correlated with cortical bone area.

Figure 6.

Linear regression between body weight and μ CT skeletal parameters. Body weight positively correlated with the distal femur (DF) bone volume fraction (A), and the midfemur (MF) cortical bone area (C), but not with the L5 vertebra (L5) bone volume fraction (B).

Figure 7.

Graphic presentation of the results of in silico genome mapping displaying chromosomal loci involved in skeletal traits and body weight determination, as based on the analysis of 11 inbred mouse strains. Each bar represents a 10Mbp interval. The dotted line represents the cutoff threshold, while 10% of the highly correlated loci are above this line and are presented as black bars. y-Axis is SD of the correlation value above average for all loci analyzed. Panel A represents distal femur trabecular BV/TV analysis, panel B represents vertebral trabecular BV/TV analysis, panel C represents midfemur cortical BAr analysis, and panel D is a body weight analysis.

Table 1
 μ CT skeletal parameters and body weight data from 12 inbred mouse strains (MEAN $\pm$ SE). $p < 0.0001$, one-way ANOVA for strain variability for every parameter, including bone volume fraction (BV/TV, %), connectivity density (ConnDens, mm^{-3}), trabecular number (Tb.N, mm^{-1}), trabecular thickness (Tb.Th, μm), trabecular separation (Tb.Sp, μm), cortical bone area (BAr, mm^2), total cross-sectional area (TtAr, mm^2), cortical thickness (CtTh, μm), and bone area fraction (BAr/TtAr, %). Strains are listed in ascending trait magnitude order (BV/TV for trabecular and BAr for cortical bone).

A	DBA	C57	SMJ	I29SV	B10	Bc	BcBy	C3H	AKR	NZW	MRL
12.5 $\pm$ 0.6	14.7 $\pm$ 0.6	19.6 $\pm$ 1.4	21.4 $\pm$ 0.8	23.4 $\pm$ 1.1	23.5 $\pm$ 1.1	25.2 $\pm$ 0.7	26 $\pm$ 0.6	26.3 $\pm$ 1	27.4 $\pm$ 2.1	30.5 $\pm$ 1.2	34.5 $\pm$ 1.8
147 $\pm$ 8.9	156.3 $\pm$ 10.4	185.1 $\pm$ 19.6	249.1 $\pm$ 9.9	284.7 $\pm$ 15.2	209.8 $\pm$ 7.6	300.9 $\pm$ 9.4	336.6 $\pm$ 11.9	202.9 $\pm$ 6	239.6 $\pm$ 11	237.3 $\pm$ 5.9	380.9 $\pm$ 13.4
4 $\pm$ 0.1	4.3 $\pm$ 0.1	5.4 $\pm$ 0.3	5.1 $\pm$ 0.1	6 $\pm$ 0.1	5.3 $\pm$ 0.1	6 $\pm$ 0.09	6.2 $\pm$ 0.1	5.2 $\pm$ 0.1	5.4 $\pm$ 0.2	5.8 $\pm$ 0.1	7.3 $\pm$ 0.1
47.4 $\pm$ 0.8	58.8 $\pm$ 0.6	56.1 $\pm$ 1.3	53.5 $\pm$ 0.9	54 $\pm$ 0.7	63.6 $\pm$ 1.9	54 $\pm$ 0.4	54 $\pm$ 0.9	64.3 $\pm$ 1	65.5 $\pm$ 2.7	67.4 $\pm$ 1.1	61.1 $\pm$ 2.2
259.2 $\pm$ 10.1	230.8 $\pm$ 6.1	186.9 $\pm$ 9.8	195.3 $\pm$ 3.7	163.7 $\pm$ 4	188.8 $\pm$ 3.8	160.2 $\pm$ 2.8	158.5 $\pm$ 2.8	184.9 $\pm$ 3.9	182.9 $\pm$ 7	170.6 $\pm$ 4	131.6 $\pm$ 2.5
A	C3H	DBA	AKR	C57	B10	BcBy	SMJ	I29SV	MRL	Bc	NZW
24.5 $\pm$ 0.6	38.2 $\pm$ 0.4	37.7 $\pm$ 1.6	38.1 $\pm$ 0.9	40.1 $\pm$ 1.7	41.9 $\pm$ 0.8	48.7 $\pm$ 1.4	49.5 $\pm$ 1.1	49.7 $\pm$ 1.8	53.4 $\pm$ 1.8	56.8 $\pm$ 0.7	59.7 $\pm$ 0.5
95 $\pm$ 3.3	95.4 $\pm$ 1.5	105.3 $\pm$ 4.2	158.1 $\pm$ 3.1	145.7 $\pm$ 4.4	117.6 $\pm$ 8.9	137.2 $\pm$ 3.6	138.6 $\pm$ 4.3	129.8 $\pm$ 6.8	151.5 $\pm$ 11.4	133.3 $\pm$ 6.2	103.4 $\pm$ 7.8
4.1 $\pm$ 0.1	4 $\pm$ 0.07	4.8 $\pm$ 0.2	5.9 $\pm$ 0.1	6 $\pm$ 0.08	5.8 $\pm$ 0.2	6.8 $\pm$ 0.2	6.5 $\pm$ 0.1	5.9 $\pm$ 0.2	6.9 $\pm$ 0.1	7.3 $\pm$ 0.1	6.7 $\pm$ 0.09
64.3 $\pm$ 1.2	88.0 $\pm$ 1.2	83.2 $\pm$ 1.8	73.1 $\pm$ 1	75.2 $\pm$ 2.3	83.8 $\pm$ 2.5	80.7 $\pm$ 2.3	84.3 $\pm$ 1	89.9 $\pm$ 2.8	86.4 $\pm$ 3.3	90.7 $\pm$ 1.1	99.7 $\pm$ 1.9
255.9 $\pm$ 8.2	267.7 $\pm$ 6.4	211.2 $\pm$ 10	159 $\pm$ 5.3	154.1 $\pm$ 3.8	158.7 $\pm$ 5.2	131.8 $\pm$ 4.5	139.3 $\pm$ 5.4	172.7 $\pm$ 11.1	131.5 $\pm$ 4.5	117.7 $\pm$ 2.1	124.8 $\pm$ 3.9
SMJ	A	DBA	B10	C57	I29SV	NZW	Bc	BcBy	AKR	C3H	MRL
0.70 $\pm$ 0.01	0.78 $\pm$ 0.01	0.82 $\pm$ 0.02	0.90 $\pm$ 0.02	0.92 $\pm$ 0.02	0.94 $\pm$ 0.03	0.99 $\pm$ 0.01	1.01 $\pm$ 0.02	1.03 $\pm$ 0.04	1.14 $\pm$ 0.01	1.23 $\pm$ 0.02	1.46 $\pm$ 0.04
1.22 $\pm$ 0.02	1.09 $\pm$ 0.03	1.29 $\pm$ 0.03	2.04 $\pm$ 0.06	2.09 $\pm$ 0.04	1.64 $\pm$ 0.04	1.86 $\pm$ 0.03	1.70 $\pm$ 0.05	1.70 $\pm$ 0.06	2.05 $\pm$ 0.03	1.65 $\pm$ 0.03	2.68 $\pm$ 0.10
224.5 $\pm$ 1.5	252.7 $\pm$ 1.9	244.5 $\pm$ 4.6	200.7 $\pm$ 3.4	199.2 $\pm$ 4.7	249.6 $\pm$ 3.9	240.8 $\pm$ 2.5	260.2 $\pm$ 3	262.7 $\pm$ 4.5	266.3 $\pm$ 2	358.8 $\pm$ 3.2	250.5 $\pm$ 4.6
58 $\pm$ 0.5	66.9 $\pm$ 0.4	63.6 $\pm$ 0.7	44.4 $\pm$ 0.5	44.2 $\pm$ 0.6	57.3 $\pm$ 0.4	53.2 $\pm$ 0.5	59.9 $\pm$ 0.5	60.6 $\pm$ 0.6	55.4 $\pm$ 0.4	75 $\pm$ 0.4	54.7 $\pm$ 0.5
SMJ	A	C3H	B10	I29SV	Bc	C57	BcBy	DBA	NZW	AKR	MRL
18.75 $\pm$ 0.39	19.93 $\pm$ 0.45	21.50 $\pm$ 0.33	21.85 $\pm$ 0.31	22.13 $\pm$ 0.60	23.33 $\pm$ 0.28	23.5 $\pm$ 0.27	23.78 $\pm$ 0.38	24.8 $\pm$ 0.43	26.75 $\pm$ 0.46	28.35 $\pm$ 0.46	38.50 $\pm$ 0.88

Table 2

Association of chromosomal intervals (Mbp) with μ CT parameters, and body weight across 11 inbred mouse strains (129/SvImJ, A/J, AKR/J, B10.D2-H2/6SNJ, BALB/cByJ, BALB/cJ, C3H/HeJ, C57BL/6J, DBA/2J, MRL/MpJ, NZW/LaCJ). Highlighted numbers represent significant power $\geq 2SD$, which corresponds to genome threshold cutoff of less than 5%. MF: midfemur, BW: body weight

Chr.	Distal femur			L5 vertebra			MF	BW
	BV/TV	TbTh	TbN	BV/TV	TbTh	TbN		
1	20-30, 160-170, 170-180, 180-190	170-180, 180-190	20-30, 50-60, 80-90, 160-170, 180-190	170-180, 180-190	170-180, 180-190	80-90, 130-140, 160-170	20-30, 180-190	20-30, 50-60, 90-100, 180-190
2	20-30, 60-70	120-130	20-30, 60-70, 130-140	20-30, 60-70	20-30	130-140, 180-190	60-70	60-70
3	100-110	100-110	100-110, 140-150	100-110	100-110	10-20, 100-110, 120-130	10-20, 130-140	100-110, 130-140, 140-150
4	130-140						130-140	
5		0-10, 40-50		40-50	0-10, 40-50	100-110	20-30	0-10
6				130-140	130-140	60-70	110-120, 140-150	110-120, 140-150
7	20-30, 30-40, 40-50, 110-120	20-30, 30-40, 40-50, 110-120, 120-130	0-10, 20-30, 110-120	20-30, 30-40, 40-50, 110-120, 120-130	10-20, 30-40, 40-50, 110-120, 120-130	20-30, 60-70, 80-90, 110-120	0-10, 20-30, 110-120	0-10, 40-50, 110-120
8				120-130	70-80	120-130	120-130	
9	90-100		50-60, 90-100	70-80	70-80	120-130	50-60, 90-100	90-100
10		90-100					90-100	90-100, 120-130
11	20-30, 100-110	20-30, 50-60, 100-110		20-30, 50-60, 90-110	20-30, 50-60, 100-110	100-110		20-30
12			90-100					90-100
13	70-80, 100-110	100-110	70-80, 100-110	100-110	100-110	100-110	100-110	
14	40-50	40-50						
15	80-90		80-90				80-90	80-90
16	80-90		10-20, 80-90	80-90		10-20, 80-90		

Chr.	Distal femur				L5 vertebra				MF	BW
	BV/TV	TbTh	TbN		BV/TV	TbTh	TbN			
17	20-30, 30-40	10-20, 30-40 , 40-50, 70-80	10-20		40-50, 70-80	20-30, 30-40, 40-50, 70-80	10-20		Bar 10-20 , 20-30, 30-40	10-20, 30-40
18		60-70	60-70		60-70	60-70	60-70			
19	0-10 , 10-20	0-10, 10-20	0-10, 10-20		0-10	0-10	0-10, 10-20		0-10, 20-30, 40-50	20-30, 40-50

Table 3

Comparison of chromosomal intervals identified by In Silico Genome Scan for trabecular and cortical μ CT bone traits, and body weight (BW) across 11 inbred mice strains with previously identified QTL's for various bone and BW phenotypes. I: Position of peak or nearest QTL marker in Mbp (highlighted in bold) from NCBI database (<http://www.ncbi.nlm.nih.gov/mapview/>). G: Gender; males (M), females (F), and combined males and females (C). FBS: femur breaking strength. WC-FCSA: weight corrected femoral cross sectioned area. DF: distal femur. L5: L5 vertebra. MF: midfemur.

Present study				Previous studies				
Chr	Trait	Interval (Mbp) $\geq$ 2SD	¹ Position (Mbp)	Trait	G	Age	Method	Reference
1	DF BV/TV &	180-190	Mit14 (158.5)	Femur BMD	F	4m	pQCT	Beamer et al., 2001 [18]
	TbTh	170-180, 180-190		Vert BMD				
	DF TbN	180-190		VertBV/TV, TbN	F	4m	μ CT	Bouxsein et al., 2004 [21]
	L5 BV/TV	170-180, 180-190						
	L5 TbTh	170-180, 180-190	Mit106 (164.2)	Midtibia	F	10w	pQCT	Kesavan et al., 2006 [36]
	L5 TbN	130-140	Mit15 (170.2)	Femur BMD	F	4m	pQCT	Beamer et al., 1999 [30]
			Mit291 (186.5)	Total BMD	C	4m	DXA	Klein et al., 2001 [22]
				Midfemur, FBS	F	7w	pQCT, FBS	Li et al., 2002 [33]
			Mit362 (191)	Midfemur	C	7w	pQCT, DXA	Masinde et al., 2002 [34]
				Total BMD				
2			Mit406/115(157.5/179.5)	Total BMD	F	20w	DXA	Ishimori et al., 2006 [58]
	BW	20-30,	Mit20 (32)	Vert BMD			DXA	
				BW	C	10w		Cheverud et al., 1996 [61]
	BW	180-190	Mit116 (180)	BW	C	8w, 10w		Zhang et al., 2003 [62]
				FBS	F	7w	FBS	Li et al., 2002 [33]
				Total BMD	C	7w	DXA	Masinde et al., 2002 [34]

Present study					Previous studies			
Chr	Trait	Interval (Mbp) $\geq$ 2SD	¹ Position (Mbp)	Trait	G	Age	Method	Reference
3	L5 BV/TV&	100-110	Rik75 (127)	Total BMD	M	12w	DXA	Orwoll et al., 2001 [32]
	TbN	10-20	Mit23 (10.2)	Femur BMD	F	4m	pQCT	Beamer et al., 1999 [30]
4	DF BV/TV	130-140	Mit251 (136.2)	Midtibia	F	10w	pQCT	Kesavan et al., 2006 [36]
			Mit124 (123.8)	Femur BMD	F	4m	pQCT	Beamer et al., 2001 [18]
			Mit312 (141)	Vert BMD				
			Mit204 (132.5)	Total BMD	C	4m	DXA	Klein et al., 2001 [22]
				Midfemur	F	7w	pQCT	Li et al., 2002 [33]
				Midfemur	C	7w	pQCT	Masinde et al., 2002 [34]
			Mit42 (150.5)	Total BMD	F	20w	DXA	Ishimori et al., 2006 [58]
7	DF BV/TV	20-40, 110-120	Mit332 (134)	Vert	F	4m	pQCT	Beamer et al., 2001 [18]
	DF TbTh	20-30, 30-40	Mit238 (119)	Vert TbTh	F	4m	μ CT	Bouxsein et al., 2004 [21]
	DF TbN	20-30	Mit300 (90.5)	Vert BMD	F	20w	DXA	Ishimori et al., 2006 [58]
	L5 BV/TV	30-40, 110-120	Mit297 (65.5)	WC-FCSA	F	4m	Dig	Klein et al., 2002 [24]
	L5 TbTh&	30-40	Mit155 (31)	Total BMD	M	12w	Image	
	TbN MF Bar	20-30, 60-70 0-10					DXA	Orwoll et al., 2001 [32]

Present study					Previous studies			
Chr	Trait	Interval (Mbp) $\geq$ 2SD	¹ Position (Mbp)	Trait	G	Age	Method	Reference
	BW	0-10	Mit22 (24)	Lean BM	C	7w	DXA	Masinde et al., 2002 [63]
11	L5 TbTh	20-30, 50-60	Mit242 (63.2)	Femur BMD	F	4m	pQCT	Beamer et al., 2001 [18]
			Mit349 (55.5)	Total BMD	C	4m	DXA	Klein et al., 2001 [22]
	BW	20-30	Mit227/231 (17.5/35.5)	BW	F	10w		Kirkpatrick et al.,1998 [64]
13	DF BV/TV & TbN	100-110	Ncvs41 (113)	Total BMD	M	12w	DXA	Orwoll et al., 2001 [32]
	L5 BV/TV, TbTh&TbN	100-110	Ncvs41 (113)	Total BMD	M	12w	DXA	Orwoll et al., 2001 [32]
17	DF TbTh	30-40	Mit180 (50.9)	Total BMD	F	20w	DXA	Ishimori et al., 2006 [58]
	MF BA _r	10-20, 30-40	Mit175(31.5)	Midfemur	F	7w	pQCT, FBS	Li et al.,2002 [33]
			Mit175/176(31.5/42)	FBS				
				Midfemur	C	7w	pQCT	Masinde et al., 2002 [34]
			Tu36 (16.5)	Total BMD	M	12w	DXA	Orwoll et al., 2001 [32]
	BW	30-40	Mit49 (44.8)	BW	C	6w		Keightley et al.,1996 [65]
18	L5 BV/TV	60-70	Mit185 (69.1)	Midfemur	F	7w	pQCT	Li et al.,2002 [33]
			Mit152 (62)	Midfemur	C	7w	pQCT	Masinde et al., 2002 [34]
			Ncvs23 (72.5)	Total BMD	F	12w	DXA	Klein et al., 1998 [16]

Present study				Previous studies				
Chr	Trait	Interval (Mbp) $\geq$ 2SD	¹ Position (Mbp)	Trait	G	Age	Method	Reference
			Ncvs21 (78.6)	Total BMD	M	12w	DXA	Orwoll et al., 2001 [32]