

Genetically Based Influences on the Site-Specific Regulation of Trabecular and Cortical Bone Morphology

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ABSTRACT: The degree of site-specificity by which genes influence bone quantity and architecture was investigated in the femur of three strains of mice. Morphological indices were highly dependent on both genetic makeup as well as anatomical location showing that the assessment of bone structure from a single site cannot be extrapolated to other sites even within a single bone.

Introduction: The identification of genes responsible for establishing peak BMD will yield critical information on the regulation of bone quantity and quality. Whereas such knowledge may eventually uncover novel molecular drug targets or enable the identification of individuals at risk of osteoporosis, the site-specificity by which putative genotypes cause low or high bone mass (and effective bone morphology) is essentially unknown.

Materials and Methods: μ CT was used to determine morphological and microarchitectural features of the femora harvested from three genetically distinct strains of 4-month-old female mice, each with distinct skeletal mass (low: C57BL/6J [B6], medium: BALB/cByJ [BALB], high: C3H/HeJ [C3H]). Two trabecular regions (distal epiphysis and metaphysis) were considered in addition to four cortical regions within the metaphysis and diaphysis.

Results and Conclusions: Comparing morphological properties of the different trabecular and cortical femoral regions between the three strains of mice, it was apparent that high or low values of specific parameters of bone morphology could not be consistently attributed to the same genetic strain. Trabecular metaphyseal bone volume, for instance, was 385% larger in C3H mice than in B6 mice, yet the two strains displayed similar bone volume fractions in the epiphysis. Similarly, BALB mice had 48% more trabecular bone than C3H mice in the epiphysis, but there were no strain-specific differences in cortical bone area at the diaphysis. These data suggest that the genetic control of bone mass and morphology, even within a given bone, is highly site-specific and that a comprehensive search for genes that are indicative of bone quantity and quality may also have to occur on a very site-specific basis.

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INTRODUCTION

THE INCIDENCE OF osteoporosis is strongly associated with the levels of peak bone mass typically reached in the third decade of life.⁽¹⁾ A variety of environmental factors, including mechanical loading⁽²⁾ and diet,⁽³⁾ influence peak bone mass, yet the predominant determinant of BMD is manifested within the genome.⁽⁴⁾ This link between genetics and the prevalence of osteoporosis has caused investigators to search for a specific gene, or set of genes, that is responsible for defining bone mass. To this end, polymorphisms in several genes, including those coding for estrogen receptor- α , vitamin D receptor, or transforming growth factor- β , have been associated with bone mass and a susceptibility to osteoporosis.^(5–9) While the comprehensive identification of genes predicting variations in bone mass may both unravel mechanisms by which the skeleton regu-

lates its morphology as well as lead to the discovery of novel drug targets to prevent and treat osteoporosis, the majority of genes defining bone morphology are still elusive.⁽⁵⁾

Gene searches have not been limited to humans⁽¹⁰⁾ and have been very effective in genetically distinct inbred strains of mice with different bone mass and architecture, readily facilitating the separation of genetic from environmental factors.⁽¹¹⁾ Most studies in both humans and mice have treated the quantitative trait of peak bone mass as one that is manifested at the organ level. In other words, it has been presumed that a skeleton with high bone mass will have a high bone mass at all skeletal regions for both trabecular and cortical bone. For example, C3H/HeJ mice have been labeled a high BMD strain based on pQCT measurements of the tibia and femur.⁽¹²⁾ Trabecular bone within the vertebral bodies of these mice, however, is associated with a rather low BMD,⁽¹³⁾ similar to site-specific gene–bone mass interactions found in other human and mouse studies.^(14–17)

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These data indicate that different sets of genes may control bone morphology at different skeletal sites. The degree of this site-specificity, which is yet to be established, will provide critical information on the required site-specificity when screening for gene candidates or assessing an individual's skeletal status. Here, we investigated six trabecular and cortical regions in the femur of three genetically distinct strains of mice to test the hypothesis that subtle variations in the genome will influence bone quantity and architecture not only across different bones within a given skeleton, but even within a given bone.

MATERIALS AND METHODS

Experimental design

Female mice of three genetically distinct strains, C57BL/6J (B6, $n = 8$), C3H/HeJ (C3H, $n = 8$), and BALB/cByJ (BALB, $n = 9$), were killed when they reached 4 months of age.⁽¹⁸⁾ These strains are commonly known as low, high, and medium BMD mice, respectively. All mice were housed individually in standard cages ($28 \times 17 \times 13$ cm) and had access to rodent chow (autoclaved diet National Institutes of Health-31 with 6% fat, 18% protein, Ca:P 1:1, vitamin and mineral fortified) and tap water ad libitum. On death, right femurs were harvested and preserved in 70% EtOH. All experimental procedures were approved by the Institutional Animal Care and Use Committee.

μ CT

High-resolution ($11 \mu\text{m}$) μ CT scanning (μ CT 20; Scanco Medical) was used to determine morphological indices of bone volume and architecture in the epiphyseal, metaphyseal, and diaphyseal regions of the distal femur (Fig. 1). The trabecular volume of interest in the epiphysis contained $444 \mu\text{m}$ and enclosed the secondary center of ossification, with the most distal slice defined as the plane where the condyles merge. The metaphyseal region spanned $1500 \mu\text{m}$, with the first slice starting $400 \mu\text{m}$ proximal of the physal-metaphyseal demarcation. The length of these regions was chosen to maximize the volume of interest given the space constraints within the distal mouse femur. Trabecular bone was separated from cortical bone with manually drawn contour lines. Cortical bone was analyzed from the metaphysis (surrounding the trabecular volume of interest) and from three $224\text{-}\mu\text{m}$ -long diaphyseal regions; the proximal diaphysis (defined at 40% of femoral length), the midshaft (at 50%), and the distal diaphysis (at 60%). Using these landmarks in a blinded study design (unpublished data), the root mean square CV⁽¹⁹⁾ associated with selecting the volume of interest for any given region was less than 2% for bone volume and slightly higher for bone architectural indices such as trabecular thickness (3.0%), trabecular number (4.3%), trabecular spacing (4.0%), and connectivity density (11.1%)—consistent with the previously suggested high repeatability of μ CT scanning.^(20,21)

A constrained 3D Gaussian filter partly suppressed the noise in the volumes; values for “support” and “sigma” were chosen as 1.0 and 0.6, respectively. Bone tissue was segmented from marrow and soft tissue using a thresholding

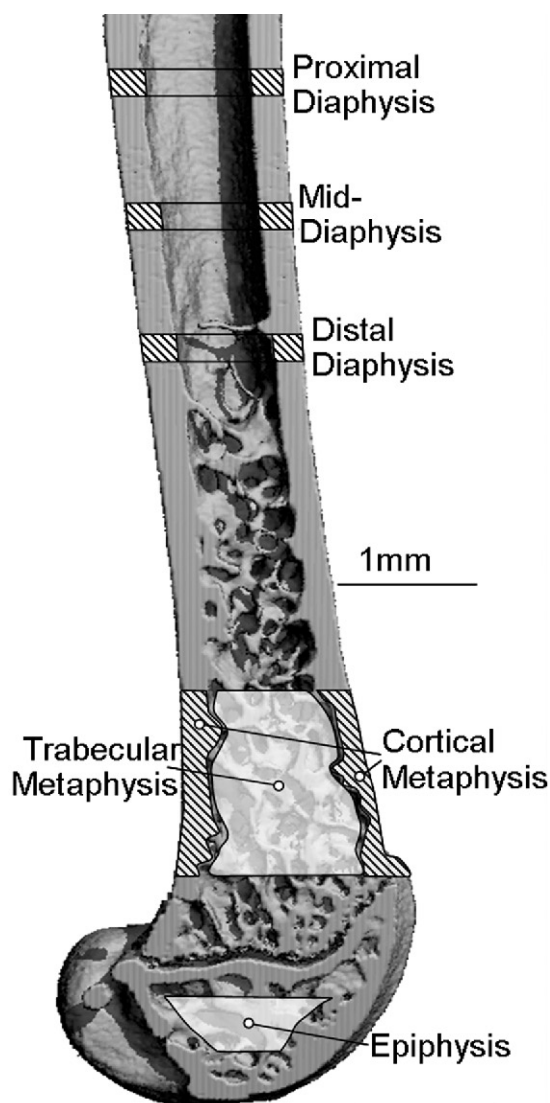


FIG. 1. Volumetrically rendered μ CT image of the femur of a BALB mouse depicting the different trabecular and cortical regions analyzed in this study.

procedure that used the same threshold for each analyzed region within each mouse strain. Thresholds had to be adjusted, however, across anatomical location to allow for accurate reconstructions of the scanned trabecular and cortical morphology (because absorption coefficients and beam hardening artifacts were influenced by trabecular and cortical geometry, in addition to different degrees of tissue mineralization). Threshold values chosen for the metaphysis and diaphysis were 143 for B6, 162 for BALB, and 200 for C3H, whereas thresholds for the epiphysis were 176 for B6, 184 for BALB, and 202 for C3H. These thresholds were chosen by a single evaluator a priori who averaged the “visually optimal” threshold from six bones of each strain that contained three slices for each region (i.e., a threshold for a given region was based on 18 measurements). We found in a preliminary study, that a blinded evaluator was able to identify the thresholds of 32 μ CT images (from eight

TABLE 1. MORPHOLOGICAL PARAMETERS OF TRABECULAR BONE QUANTITY AND MICROARCHITECTURE QUANTIFIED IN THREE REGIONS OF THE DISTAL FEMUR IN THREE STRAINS OF MICE

	<i>Index</i>	<i>B6</i>	<i>C3H</i>	<i>BALB</i>
Metaphysis	BV/TV (%)	3.37 ± 1.21 ^{a,b}	16.4 ± 3.2 ^a	18.0 ± 6.1 ^b
	Tb.Th (μm)	35.6 ± 4.1 ^{a,b}	60.2 ± 6.9 ^{a,c}	46.5 ± 6.3 ^{b,c}
	Conn.D (1/mm ³)	16.5 ± 17.6 ^{a,b}	71.6 ± 15.1 ^{a,c}	120.8 ± 38.2 ^{b,c}
	SMI	3.51 ± 0.31 ^{a,b}	1.95 ± 0.25 ^{a,c}	1.40 ± 0.58 ^{b,c}
	DA	1.26 ± 0.08 ^{a,b}	1.63 ± 0.07 ^{a,c}	1.48 ± 0.08 ^{b,c}
Epiphysis	BV/TV (%)	20.1 ± 1.5 ^b	19.2 ± 1.6 ^c	28.4 ± 3.0 ^{b,c}
	Tb.Th (μm)	50.9 ± 3.3	56.2 ± 3.0 ^c	48.2 ± 5.2 ^c
	Conn.D (1/mm ³)	124.7 ± 12.9 ^a	64.6 ± 17.3 ^{a,c}	133.3 ± 15.4 ^c
	SMI	1.17 ± 0.17 ^b	1.23 ± 0.18 ^c	0.40 ± 0.31 ^{b,c}
	DA	1.39 ± 0.11 ^a	1.56 ± 0.20 ^{a,c}	1.28 ± 0.11 ^c

Values are means ± SD.

^a Significant differences between B6 and C3H mice.

^b Significant differences between B6 and BALB mice.

^c Significant differences between C3H and BALB mice.

bone samples that were randomly repeated four times) with a high repeatability. The CV associated with selecting thresholds was 6% for the trabecular metaphysis and 5% for the trabecular epiphysis (variations for cortical bone were less). Inherently, a local thresholding procedure may introduce more bias than a global one⁽²²⁾; however, the site-specific analyses used here required an accurate reconstruction of all locations, which global thresholding was not able to achieve. While inaccuracies in threshold selection would affect absolute values of the morphological parameters, these variations are likely small compared with the large differences related to genetics and anatomical location.

For all trabecular regions, bone volume fraction (BV/TV), trabecular separation (Tb.Sp), trabecular thickness (Tb.Th), trabecular number (Tb.N), and connectivity density (Conn.D) were determined.^(23,24) In addition, the geometrical degree of anisotropy (DA), defined as the ratio between the maximal and minimal radii of the mean intercept length ellipsoid and the structural model index⁽²⁵⁾ (SMI), a measure indicating the composition of the trabecular structure with respect to the occurrence of plates and rods with SMI = 0 being a perfect plate and SMI = 3 being a perfect rod, were calculated. For all cortical regions, cortical bone area (Ct.Ar) and areas of the endocortical (Ec.En) and periosteal envelopes (Ps.En) were calculated as averages along the length of each region.

Statistics

One-way ANOVAs followed by Student-Neumann-Keul (SNK) tests evaluated differences in body mass, bone quantity and microarchitecture of mice across the three strains. Significance levels of 0.05, 0.01, and 0.001 were tested for the SNK test (SPSS for Windows 9.0). All data are presented as mean ± SD.

RESULTS

Body mass

At the time of death, there were no differences in body mass between B6 mice (22.7 ± 1.9 g) and C3H mice

(22.2 ± 1.6 g), but BALB mice were significantly ($p < 0.05$) heavier than the other two strains (24.7 ± 2.1 g). Weekly weight recordings indicated that the body mass of any given strain fluctuated by less than 3% during the 3 weeks before death, consistent with the completion of bone density accrual at 4 months of age reported previously.⁽¹²⁾

Metaphyseal trabecular bone

Metaphyseal trabecular bone quantity and architecture was significantly different between the B6, C3H, and BALB mouse strains. B6 mice had less bone fractional volume (BV/TV) than C3H (−79%, $p < 0.001$) and BALB (−81%, $p < 0.001$) mice, which was associated with a lower connectivity density (−77%, $p < 0.001$ and −86%, $p < 0.001$, respectively) and trabecular thickness (−41%, $p < 0.001$ and −23%, respectively, $p < 0.01$; Table 1; Fig. 2). The SMI of trabecular bone in B6 mice was 80% greater ($p < 0.001$) than in C3H mice and 150% greater ($p < 0.001$) than in BALB mice. Although there was no difference between C3H mice and BALB mice in trabecular bone quantity, there were differences in trabecular architecture: SMI and trabecular thickness were 39% ($p < 0.05$) and 29% ($p < 0.001$) larger in C3H mice, whereas connectivity density was greater in BALB mice (69%, $p < 0.01$).

Epiphyseal trabecular bone

In stark contrast to the metaphysis, there was no difference in bone volume between B6 and C3H mice in the trabecular epiphysis. Bone quantity of B6 mice, however, was 29% ($p < 0.001$) less than in BALB mice (Table 1; Fig. 3). Microarchitecturally, connectivity density was 93% ($p < 0.001$) greater in B6 mice than in C3H mice, while no other differences between these two strains were detectable. Both quantitative and structural indices were significantly different between C3H and BALB mice: BV/TV (48%, $p < 0.001$) and connectivity density (106%, $p < 0.001$) were greater in BALB mice, whereas trabecular thickness (16%, $p < 0.05$) was greater in C3H mice.

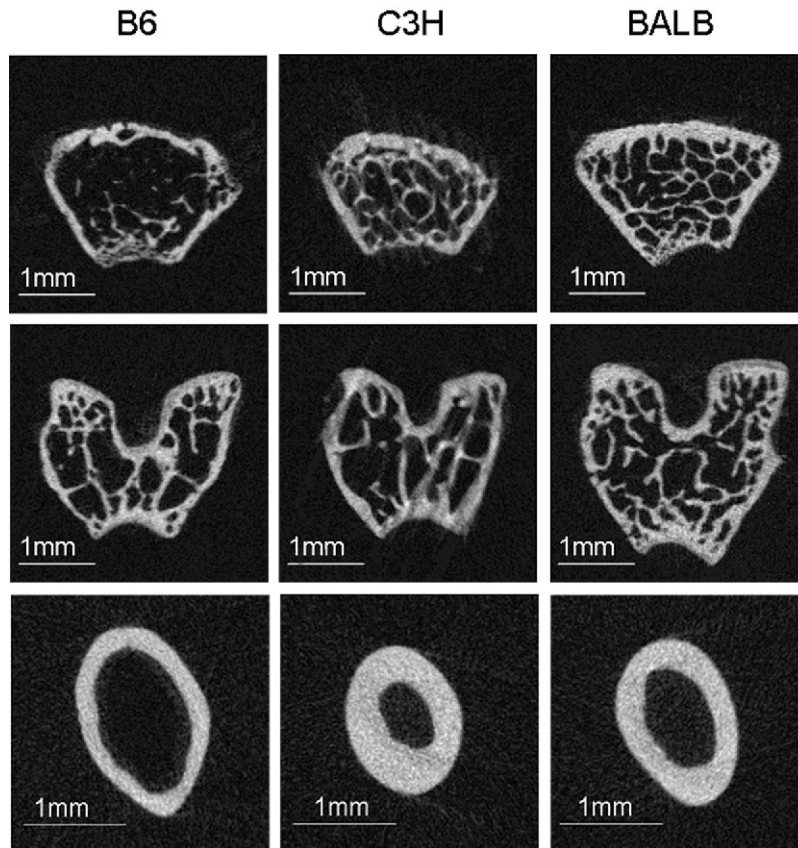


FIG. 2. Representative cross-sections of B6, C3H, and BALB mice displaying differential bone quantity and architecture in the metaphysis (top row), the epiphysis (middle row), and the mid-diaphysis (bottom row).

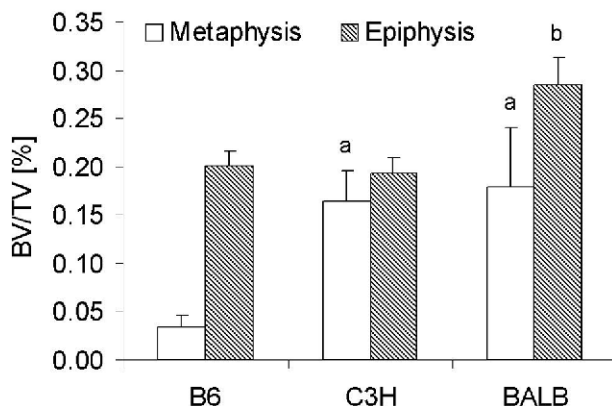


FIG. 3. Trabecular bone volume fraction of the metaphysis and epiphysis of the distal femur in mice pertaining to the three strains (mean \pm SD), showing the high degree of site-specificity by which bone accrues and maintains mineral. ^aSignificantly different metaphyseal bone volume than in B6 mice ($p < 0.001$); ^bsignificantly different epiphyseal bone volume than in B6 and C3H mice ($p < 0.001$).

Metaphyseal cortical bone

Genetic variations also influenced the morphology of cortical bone in the metaphysis. Compared with B6 mice, mean cortical bone area was significantly greater in C3H (12%, $p < 0.05$) and BALB (19%, $p < 0.01$) mice, which

was related to smaller endocortical envelopes (35%, $p < 0.001$ and 21%, $p < 0.001$, respectively) and smaller periosteal envelopes (20%, $p < 0.001$ and 8%, $p < 0.05$, respectively). There was no difference in cortical bone area between C3H and BALB mice, but BALB mice had significantly greater endocortical (22%, $p < 0.01$) and periosteal (15%, $p < 0.01$) envelopes (Table 2).

Diaphyseal cortical bone

Three regions within the diaphysis were compared between the different mouse strains. Cortical bone area in B6 mice was significantly smaller than in the C3H mice for all three regions, although the difference was more pronounced in the distal diaphysis (-17%, $p < 0.001$) than in the proximal diaphysis (-13%, $p < 0.05$; Table 2). Averaged across the three regions, B6 mice had a 182% ($p < 0.001$) larger endocortical and a 29% ($p < 0.001$) larger periosteal envelope than C3H mice, with the difference in endocortical envelope area being 239% at the distal diaphysis and only 129% at the proximal diaphysis. There was no difference in cortical area between C3H and BALB mice; however, BALB mice had significantly greater endocortical and periosteal envelopes than C3H mice. These differences amounted to 118% (Ec.En; $p < 0.001$) and 20% (Ps.En; $p < 0.01$) in the distal region but only to 60% (Ec.En; $p < 0.001$) and 10% (Ps.En; $p < 0.05$) in the proximal region (Table 2).

TABLE 2. CORTICAL BONE AREA (CT.AR), THE AREA ENCLOSED BY THE ENDOCORTICAL ENVELOPE (EC.EN), AND THE AREA ENCLOSED BY THE PERIOSTEAL ENVELOPE (PS.EN) MEASURED IN THE METAPHYSIS AND THREE DIAPHYSAL REGIONS

	Index	B6	C3H	BALB
Metaphysis	Ct.Ar (mm ²)	0.92 ± 0.04 ^{a,b}	1.03 ± 0.13 ^a	1.10 ± 0.09 ^b
	Ec.En (mm ²)	1.97 ± 0.10 ^{a,b}	1.27 ± 0.11 ^{a,c}	1.55 ± 0.25 ^{b,c}
	Ps.En (mm ²)	2.89 ± 0.10 ^{a,b}	2.31 ± 0.16 ^{a,c}	2.65 ± 0.26 ^{b,c}
Proximal diaphysis	Ct.Ar (mm ²)	0.89 ± 0.05 ^a	1.02 ± 0.12 ^a	0.96 ± 0.08
	Ec.En (mm ²)	0.76 ± 0.04 ^{a,b}	0.33 ± 0.03 ^{a,c}	0.53 ± 0.08 ^{b,c}
	Ps.En (mm ²)	1.65 ± 0.06 ^{a,b}	1.36 ± 0.12 ^{a,c}	1.49 ± 0.14 ^{b,c}
Mid-diaphysis	Ct.Ar (mm ²)	0.78 ± 0.04 ^{a,b}	0.93 ± 0.09 ^a	0.89 ± 0.08 ^b
	Ec.En (mm ²)	0.80 ± 0.03 ^{a,b}	0.27 ± 0.04 ^{a,c}	0.52 ± 0.07 ^{b,c}
	Ps.En (mm ²)	1.58 ± 0.06 ^{a,b}	1.20 ± 0.10 ^{a,c}	1.41 ± 0.13 ^{b,c}
Distal diaphysis	Ct.Ar (mm ²)	0.77 ± 0.03 ^{a,b}	0.93 ± 0.08 ^a	0.89 ± 0.08 ^b
	Ec.En (mm ²)	0.80 ± 0.04 ^{a,b}	0.24 ± 0.04 ^{a,c}	0.51 ± 0.09 ^{b,c}
	Ps.En (mm ²)	1.57 ± 0.05 ^{a,b}	1.17 ± 0.09 ^{a,c}	1.41 ± 0.16 ^{b,c}

Values are means ± SD.

^a Significant differences between B6 and C3H mice.

^b Significant differences between B6 and BALB mice.

^c Significant differences between C3H and BALB mice.

TABLE 3. RELATIVE DIFFERENCES IN BONE FRACTIONAL VOLUME (BONE AREA FOR CORTICAL BONE) BETWEEN A GIVEN STRAIN AND THE AVERAGE OF ALL THREE STRAINS FOR EACH OF THE CORTICAL AND TRABECULAR REGIONS

		B6	C3H	BALB
Trabecular	Epiphysis	↓	↓	↑ ↑
	Metaphysis	↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓	↑ ↑ ↑	↑ ↑ ↑ ↑
Cortical	Metaphysis	—	—	—
	Proximal diaphysis	—	—	—
	Mid-diaphysis	↓	—	—
	Distal diaphysis	↓	—	—

—, difference in BV/TV (Ct.Ar.) between the respective strain and the mean value of the three strains was between -10% and +10%; ↓, 10-19% lower; ↓ ↓, 20-29% lower; ↓ ↓ ↓, 30-39% lower, etc. Analogous to downward arrows, upward arrows denote larger BV/TV (Ct.Ar.) than the average value.

DISCUSSION

The site-specificity by which genetic variations influence the quantity and architecture of trabecular and cortical bone was examined in the femora of three genetically distinct strains that, based on whole bone density, have been previously labeled low, medium, and high BMD mice. Inter- and intrastain comparisons between six trabecular and cortical regions demonstrated that indices of bone volume and microarchitecture were strongly dependent on bone type and anatomic location, with no single strain consistently displaying small, large, or intermediate values of a specific parameter of bone morphology (Table 3). Such a relationship was not even found when solely considering trabecular bone. For example, trabecular connectedness in the metaphysis was 77% greater in C3H than in B6 mice, yet this relationship was reversed in the epiphysis, where B6 mice showed a 93% greater trabecular connectedness. These data indicate that a more complete identification of specific genes or gene sets that are responsible for defining bone

quantity and architecture will have to occur at a site-specific level.

This study focused on the assessment of bone morphology, and other parameters such as moments of inertia and mineral content of the tissue⁽²⁶⁾ could add another degree of site-specificity if bone strength, the most reliable indicator of fracture risk, was considered. As with any model, direct conclusions from this investigation are limited to the mouse strains under consideration. Mice lack primary haversian bone in addition to other systemic differences between the two species; nevertheless, the human and mouse genomes share a large degree of homology⁽²⁷⁾ and a similar putative number of genes.⁽²⁸⁾ Not surprisingly, many genes, not only those mapping for bone quantity, have been discovered in mice with homologous regions to the human genome,^(8,29,30) but data from non-human models will always have to be interpreted with care. Thus, extrapolations of our results from mice to humans, which may help to identify high bone mass genes and explain why some body types are more prone to osteoporosis than others,⁽³¹⁾ ultimately relies on the validity of this established model.^(30,32)

The complex interdependence between bone morphology, genetic variations, and anatomic location observed in this study raises the question of how such a site-specific regulation of bone morphology is achieved. At the level of the skeleton, the influence of genetic factors on bone formation and bone mass⁽³³⁻³⁵⁾ have been established long ago, consistently showing that epigenetic determinants of bone quantity or quality are small by comparison (although co-dependencies may exist). A recent study examined a large number of such genetic and epigenetic factors and found that even the combination of all parameters was only able to explain 14-18% of individual variation in peak bone mass,⁽⁵⁾ inferring that a large number of genes that affect bone morphology is yet to be uncovered.

If such a large number of bone-specific genes exist, it is conceivable that polymorphisms within different combinations of these genes determine site-specific differences in

morphology dependent on an individual genotype. For the three strains of mice used in this study, it can be assumed that they are polymorphic for ~50% of their genomes. While only a fraction of these polymorphisms is currently known⁽³⁶⁾ and an even smaller fraction of these polymorphisms will be specific to bone, the large number of possible combinations of genes with polymorphisms and even without polymorphisms might be capable of realizing the local control of bone quantity and quality within a bone. Nevertheless, such a relation seems overly complex given the great number of anatomical sites within and across bones, especially in light of the systemic, and not necessarily local, influence of most of the genes currently known to affect bone mass.

Alternatives to a multigene regulated control mechanism include the possibility of very few genes regulating the distribution of tissue within a bone, or more likely, the existence of local control mechanisms and strong gene-environment interactions.⁽³⁴⁾ Examples for such mechanisms comprise (genetically defined) intracellular networks modulated by gap-junctions⁽³⁷⁾ or autocrine and paracrine factors⁽³⁸⁾ to orchestrate the distribution of tissue quantity within a bone. Thus, an exogenous nonuniformly distributed factor, such as mechanical loading arising through load bearing, might interact with one such system very differently than another, although the physical signal is identical. In addition, differences in the genetically controlled growth and development of specific anatomical regions between the different strains (e.g., primary center of ossification versus secondary center of ossification) may have contributed to the observed site-specificity, a hypothesis that could be tested when using inbred strains at different developmental stages.

This work also implies inherent difficulties in approximating the skeletal status of an animal or human based on single-site measurements of bone quantity. Similarly to inaccuracies encountered when assessing the risk of osteoporosis from a single bone,⁽³⁹⁾ our current data may implicate that evaluating low bone mass from just one site within a bone may also be problematic because of the site-specific variations in bone morphology in heterogeneous populations. Thus, a measurement indicating high bone mass for a specific region may be poorly correlated with the relative mass of an adjacent region. Because of the current lack of means to determine which region of the bone presents the optimal measuring site (weakest link) based on an individual's genotype, the sensitivity of detecting osteoporosis may be enhanced by performing multiple measurements within a bone.

In summary, we investigated the extent by which genetic variations influence bone quantity and microarchitecture on a site-specific basis. The relative difference of any investigated index of bone morphology between the genetically distinct strains of mice differed greatly from one anatomical region to another, and often, even reversed in sign. Thus, optimal detection methods of osteoporosis as well as the comprehensive search for genes responsible for high bone mass may have to consider site-specific approaches that fully assess bone quantity and quality at the level of the tissue.

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REFERENCES

1. Nguyen TV, Maynard LM, Towne B, Roche AF, Wisemandle W, Li J, Guo SS, Chumlea WC, Siervogel RM 2001 Sex differences in bone mass acquisition during growth: The Fels Longitudinal Study. *J Clin Densitom* **4**:147-157.
2. Marcus R 2001 Role of exercise in preventing and treating osteoporosis. *Rheum Dis Clin North Am* **27**:131-141.
3. Ho SC, Chan SG, Yi Q, Wong E, Leung PC 2001 Soy intake and the maintenance of peak bone mass in Hong Kong Chinese women. *J Bone Miner Res* **16**:1363-1369.
4. Eisman JA 1999 Genetics of osteoporosis. *Endocr Rev* **20**:788-804.
5. McGuigan FE, Murray L, Gallagher A, Davey-Smith G, Neville CE, Van't Hof R, Boreham C, Ralston SH 2002 Genetic and environmental determinants of peak bone mass in young men and women. *J Bone Miner Res* **17**:1273-1279.
6. Jurada S, Marc J, Prezelj J, Kocijancic A, Komel R 2001 Codon 325 sequence polymorphism of the estrogen receptor alpha gene and bone mineral density in postmenopausal women. *J Steroid Biochem Mol Biol* **78**:15-20.
7. Lorentzon M, Lorentzon R, Nordstrom P 2001 Vitamin D receptor gene polymorphism is related to bone density, circulating osteocalcin, and parathyroid hormone in healthy adolescent girls. *J Bone Miner Metab* **19**:302-307.
8. Koller DL, Econs MJ, Morin PA, Christian JC, Hui SL, Parry P, Curran ME, Rodriguez LA, Conneally PM, Joslyn G, Peacock M, Johnston CC, Foroud T 2000 Genome screen for QTLs contributing to normal variation in bone mineral density and osteoporosis. *J Clin Endocrinol Metab* **85**:3116-3120.
9. Kobayashi S, Inoue S, Hosoi T, Ouchi Y, Shiraki M, Orimo H 1996 Association of bone mineral density with polymorphism of the estrogen receptor gene. *J Bone Miner Res* **11**:306-311.
10. Koller DL, White KE, Liu G, Hui SL, Conneally PM, Johnston CC, Econs MJ, Foroud T, Peacock M 2003 Linkage of structure at the proximal femur to chromosomes 3, 7, 8, and 19. *J Bone Miner Res* **18**:1057-1065.
11. Beamer WG, Shultz KL, Churchill GA, Frankel WN, Baylink DJ, Rosen CJ, Donahue LR 1999 Quantitative trait loci for bone density in C57BL/6J and CAST/EiJ inbred mice. *Mamm Genome* **10**:1043-1049.
12. Beamer WG, Donahue LR, Rosen CJ, Baylink DJ 1996 Genetic variability in adult bone density among inbred strains of mice. *Bone* **18**:397-403.
13. Turner CH, Hsieh YF, Muller R, Bouxsein ML, Baylink DJ, Rosen CJ, Grynpas MD, Donahue LR, Beamer WG 2000 Genetic regulation of cortical and trabecular bone strength and microstructure in inbred strains of mice. *J Bone Miner Res* **15**:1126-1131.
14. Duncan EL, Cardon LR, Sinsheimer JS, Wass JA, Brown MA 2003 Site and gender specificity of inheritance of bone mineral density. *J Bone Miner Res* **18**:1531-1538.
15. Carn G, Koller DL, Peacock M, Hui SL, Evans WE, Conneally PM, Johnston CC Jr, Foroud T, Econs MJ 2002 Sibling pair linkage and association studies between peak bone mineral density and the gene locus for the osteoclast-specific subunit (OC116) of the vacuolar proton pump on chromosome 11p12-13. *J Clin Endocrinol Metab* **87**:3819-3824.
16. Masinde GL, Li X, Gu W, Wergedal J, Mohan S, Baylink DJ 2002 Quantitative trait loci for bone density in mice: The genes determining total skeletal density and femur density show little overlap in F2 mice. *Calcif Tissue Int* **71**:421-428.
17. Beamer WG, Shultz KL, Donahue LR, Churchill GA, Sen S, Wergedal JR, Baylink DJ, Rosen CJ 2001 Quantitative trait loci for femoral and lumbar vertebral bone mineral density in C57BL/6J and C3H/HeJ inbred strains of mice. *J Bone Miner Res* **16**:1195-1206.
18. Judex S, Donahue LR, Rubin C 2002 Genetic predisposition to low bone mass is paralleled by an enhanced sensitivity to signals anabolic to the skeleton. *FASEB J* **16**:1280-1282.

19. Gluer CC, Blake G, Lu Y, Blunt BA, Jergas M, Genant HK 1995 Accurate assessment of precision errors: How to measure the reproducibility of bone densitometry techniques. *Osteoporos Int* **5**:262–270.
20. Muller R, Van Campenhout H, Van Damme B, Van Der Perre G, Dequeker J, Hildebrand T, Ruegsegger P 1998 Morphometric analysis of human bone biopsies: A quantitative structural comparison of histological sections and micro-computed tomography. *Bone* **23**:59–66.
21. Adams DJ, Diaz-Doran V 2001 Resolution sensitivity and precision of mouse trabecular bone morphometry measurements by micro-computed tomographic imaging. *J Bone Miner Res* **16**:S345.
22. Muller R, Ruegsegger P 1997 Micro-tomographic imaging for the nondestructive evaluation of trabecular bone architecture. *Stud Health Technol Inform* **40**:61–79.
23. Hildebrand T, Laib A, Muller R, Dequeker J, Ruegsegger P 1999 Direct three-dimensional morphometric analysis of human cancellous bone: Microstructural data from spine, femur, iliac crest, and calcaneus. *J Bone Miner Res* **14**:1167–1174.
24. Odgaard A 1997 Three-dimensional methods for quantification of cancellous bone architecture. *Bone* **20**:315–328.
25. Hildebrand T, Ruegsegger P 1997 Quantification of bone micro-architecture with the structure model index. *Comput Methods Biomech Biomed Engin* **1**:15–23.
26. Jepsen KJ, Akkus OJ, Majeska RJ, Nadeau JH 2003 Hierarchical relationship between bone traits and mechanical properties in inbred mice. *Mamm Genome* **14**:97–104.
27. Emes RD, Goodstadt L, Winter EE, Ponting CP 2003 Comparison of the genomes of human and mouse lays the foundation of genome zoology. *Hum Mol Genet* **12**:701–709.
28. Waterston RH, Lindblad-Toh K, Birney E, Rogers J, Abril JF, Agarwal P, Agarwala R, Ainscough R, Alexandersson M, An P, Antonarakis SE, Attwood J, Baertsch R, Bailey J, Barlow K, Beck S, Berry E, Birren B, Bloom T, Bork P, Botcherby M, Bray N, Brent MR, Brown DG, Brown SD, Bult C, Burton J, Butler J, Campbell RD, Carninci P, Cawley S, Chiaromonte F, Chinwalla AT, Church DM, Clamp M, Clee C, Collins FS, Cook LL, Copley RR, Coulson A, Couronne O, Cuff J, Curwen V, Cutts T, Daly M, David R, Davies J, Delehaunty KD, Deri J, Dermitzakis ET, Dewey C, Dickens NJ, Diekhans M, Dodge S, Dubchak I, Dunn DM, Eddy SR, Elnitski L, Emes RD, Eswara P, Eyraes E, Felsenfeld A, Fewell GA, Flicek P, Foley K, Frankel WN, Fulton LA, Fulton RS, Furey TS, Gage D, Gibbs RA, Glusman G, Gnerre S, Goldman N, Goodstadt L, Grafham D, Graves TA, Green ED, Gregory S, Guigo R, Guyer M, Hardison RC, Haussler D, Hayashizaki Y, Hillier LW, Hinrichs A, Hlavina W, Holzer T, Hsu F, Hua A, Hubbard T, Hunt A, Jackson I, Jaffe DB, Johnson LS, Jones M, Jones TA, Joy A, Kamal M, Karlsson EK, Karolchik D, Kasprzyk A, Kawai J, Keibler E, Kells C, Kent WJ, Kirby A, Kolbe DL, Korf I, Kucherlapati RS, Kulbokas EJ, Kulp D, Landers T, Leger JP, Leonard S, Letunic I, LeVine R, Li J, Li M, Lloyd C, Lucas S, Ma B, Maglott DR, Mardis ER, Matthews L, Mauceli E, Mayer JH, McCarthy M, McCombie WR, McLaren S, McLay K, McPherson JD, Meldrim J, Meredith B, Mesirov JP, Miller W, Miner TL, Mongin E, Montgomery KT, Morgan M, Mott R, Mullikin JC, Muzny DM, Nash WE, Nelson JO, Nhan MN, Nicol R, Ning Z, Nusbaum C, O'Connor MJ, Okazaki Y, Oliver K, Overton-Larty E, Pachter L, Parra G, Pepin KH, Peterson J, Pevzner P, Plumb R, Pohl CS, Poliakov A, Ponce TC, Ponting CP, Potter S, Quail M, Reymond A, Roe BA, Roskin KM, Rubin EM, Rust AG, Santos R, Sapojnikov V, Schultz B, Schultz J, Schwartz MS, Schwartz S, Scott C, Seaman S, Searle S, Sharpe T, Sheridan A, Showkeen R, Sims S, Singer JB, Slater G, Smit A, Smith DR, Spencer B, Stabenau A, Stange-Thomann N, Sugnet C, Suyama M, Tesler G, Thompson J, Torrents D, Trevaskis E, Tromp J, Ucla C, Ureta-Vidal A, Vinson JP, Von Niederhausern AC, Wade CM, Wall M, Weber RJ, Weiss RB, Wendl MC, West AP, Wetterstrand K, Wheeler R, Whelan S, Wierzbowski J, Willey D, Williams S, Wilson RK, Winter E, Worley KC, Wyman D, Yang S, Yang SP, Zdobnov EM, Zody MC, Lander ES 2002 Initial sequencing and comparative analysis of the mouse genome. *Nature* **420**:520–562.
29. Klein OF, Carlos AS, Vartanian KA, Chambers VK, Turner EJ, Phillips TJ, Belknap JK, Orwoll ES 2001 Confirmation and fine mapping of chromosomal regions influencing peak bone mass in mice. *J Bone Miner Res* **16**:1953–1961.
30. Korstanje R, Paigen B 2002 From QTL to gene: The harvest begins. *Nat Genet* **31**:235–236.
31. Aloia JF, Vaswani A, Mikhail M, Badshah M, Flaster E 1999 Cancellous bone of the spine is greater in black women. *Calcif Tissue Int* **65**:29–33.
32. Malakoff D 2000 The rise of the mouse, biomedicine's model mammal. *Science* **288**:248–253.
33. Kelly PJ, Hopper JL, Macaskill GT, Pocock NA, Sambrook PN, Eisman JA 1991 Genetic factors in bone turnover. *J Clin Endocrinol Metab* **72**:808–813.
34. Brown MA, Haughton MA, Grant SF, Gunnell AS, Henderson NK, Eisman JA 2001 Genetic control of bone density and turnover: Role of the collagen 1 α 1, estrogen receptor, and vitamin D receptor genes. *J Bone Miner Res* **16**:758–764.
35. Ralston SH 2002 Genetic control of susceptibility to osteoporosis. *J Clin Endocrinol Metab* **87**:2460–2466.
36. The Jackson Laboratory 2003 Mouse genome informatics. Available online at <http://informatics.jax.org/>. Accessed on August 2003.
37. Donahue HJ 2000 Gap junctions and biophysical regulation of bone cell differentiation. *Bone* **26**:417–422.
38. Karsenty G, Wagner EF 2002 Reaching a genetic and molecular understanding of skeletal development. *Dev Cell* **2**:389–406.
39. Sahota O, Pearson D, Cawte SW, San P, Hosking DJ 2000 Site-specific variation in the classification of osteoporosis, and the diagnostic reclassification using the lowest individual lumbar vertebra T-score compared with the L1–L4 mean, in early postmenopausal women. *Osteoporos Int* **11**:852–857.

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