Bone 46 (2010) 1251–1259

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Linkage mapping of femoral material properties in a reciprocal intercross of HcB-8 and HcB-23 recombinant mouse strains

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ARTICLE INFO

Article history:
Received 22 September 2009
Revised 15 January 2010
Accepted 15 January 2010
Available online 25 January 2010

Edited by: David Burr

Keywords:
Recombinant congenic mice
Biomechanics
Linkage mapping
Systems biology
Bone quality

ABSTRACT

Skeletal fragility is an important health problem with a large genetic component. We performed a 603 animal F2 reciprocal intercross of the recombinant congenic strains HcB-8 and HcB-23 to genetically map quantitative trait loci (QTLs) for tissue-level femoral biomechanical performance. These included elastic and post-yield strain, Young's modulus, elastic and maximum stress, and toughness and were calculated from 3-point bend testing of femora by the application of standard beam equations. We mapped these with R/qtl and QTL Cartographer and established significance levels empirically by permutation testing. Significant QTLs for at least one trait are present on chromosomes 1, 6, and 10 in the full F2 population, with additional QTLs evident in subpopulations defined by sex and cross direction. On chromosome 10, we find a QTL for post-yield strain and toughness, phenotypes that have not been mapped previously. Notably, the HcB-8 allele at this QTL increases post-yield strain and toughness, but decreases bone mineral density (BMD), while the material property QTLs on chromosomes 1, 6, and at a second chromosome 10 QTL are independent of BMD. We find significant sex × QTL and cross direction × QTL interactions. A robust, pleiotropic chromosome 4 QTL that we previously reported at the whole-bone level showed no evidence of linkage at the tissue-level, supporting our interpretation that modeling capacity is its primary phenotype. Our data demonstrate an inverse relationship between femoral perimeter and Young's modulus, with $R^2 = 0.27$, supporting the view that geometric and material bone properties are subject to an integrated set of regulatory mechanisms. Mapping QTLs for tissue-level biomechanical performance advances understanding of the genetic basis of bone quality.

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Introduction

Skeletal fragility is an important health problem (for review, see [1]). In developed countries, the burden of fracture-related morbidity and mortality is increasing as the population ages. Multiple risk factors for fracture have been identified in epidemiological studies (e.g. [2,3]). Moreover, it is well-established that the genetic contribution to fracture risk is substantial (for review, see ([4–6]).

For these reasons, there has been substantial interest in identifying the genes and alleles that underlie the genetic contribution to differences in fracture risk. The majority of this literature is devoted to bone mineral density as the phenotype of interest, although an increasing fraction now also addresses bone size as well. Biomechanical performance has been studied as a phenotypic endpoint in only a small number of rodent genetic mapping experiments [7–17]. The relative dearth of biomechanical genetic investigations reflects the very real barriers to their successful completion: greater technical difficulty, lesser robustness of the phenotypes, and ultimately lesser statistical power to find quantitative trait loci (QTLs). Thus, in striking the balance between the similarity of the outcome measures to the clinical endpoint of fracture and experimental feasibility, the balance has generally favored the pragmatic issues.

Yet, to achieve the ultimate goal of identifying the genes responsible for variation in bone's biomechanical performance will require that we understand the biology underlying the phenotypes studied. These are generally remote from the actions of individual genes at the cellular level, so that identifying a QTL with a specific gene is challenging even under the most favorable circumstances. When confronted with a set of positional candidate genes identified by linkage mapping or whole genome association mapping, it is necessary to consider the mechanisms that might lead from a gene to the measured phenotype to decide which among them is likeliest to be the responsible gene. Moreover, clinically important phenotypes are not equally well represented by surrogate measures such as bone

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8756-3282/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.bone.2010.01.375

Table 1
Femoral biomechanical performance and geometry in HcB-8 and HcB-23[a].

	HcB-8		HcB-23	
	Female ($N=6$)	Male ($N=7$)	Female ($N=14$)	Male ($N=16$)
Body mass (g)[b,c]	19.9 ± 2.2	22.6 ± 1.6	23.9 ± 2.2	27.9 ± 1.8
Body length (cm)[b,f]	8.8 ± 0.4	9.0 ± 0.3	9.5 ± 0.4	9.6 ± 0.3
CSA (mm^2)[b]	0.90 ± 0.12	0.91 ± 0.08	1.02 ± 0.08	1.15 ± 0.12
Perimeter (mm)[b,c]	4.18 ± 0.32	4.42 ± 0.24	4.52 ± 0.19	4.96 ± 0.23
Inner minor axis (mm)[c]	0.50 ± 0.06	0.61 ± 0.06	0.53 ± 0.03	0.58 ± 0.04
Inner major axis (mm)[b,c,d]	0.68 ± 0.06	0.83 ± 0.08	0.80 ± 0.07	0.86 ± 0.07
Outer minor axis (mm)[b,e]	1.08 ± 0.10	1.12 ± 0.05	1.15 ± 0.04	1.16 ± 0.04
Outer major axis (mm)[b,c]	1.43 ± 0.12	1.55 ± 0.10	1.58 ± 0.08	1.81 ± 0.11
Shape factor (unitless)[b,c,d]	1.35 ± 0.07	1.40 ± 0.07	1.37 ± 0.08	1.56 ± 0.07
CSMI (mm^4)[b,c]	0.080 ± 0.02	0.090 ± 0.02	0.107 ± 0.01	0.118 ± 0.01
Femoral length (mm)	15.51 ± 0.45	15.36 ± 0.23	15.32 ± 0.38	15.36 ± 0.28
Slenderness (unitless)[b,c]	3.73 ± 0.21	3.48 ± 0.19	3.39 ± 0.14	3.11 ± 0.16
Yield displacement (mm)	0.14 ± 0.02	0.14 ± 0.02	0.14 ± 0.02	0.15 ± 0.02
Post-yield displacement (mm)[b,e]	0.40 ± 0.18	0.35 ± 0.04	0.18 ± 0.03	0.14 ± 0.03
Total displacement (mm)[b,e]	0.54 ± 0.16	0.49 ± 0.05	0.32 ± 0.04	0.28 ± 0.05
Stiffness (N/mm)[b]	122 ± 27	125 ± 16	137 ± 15	142 ± 14
Yield load (N)[b]	14.1 ± 2.3	13.9 ± 2.0	16.3 ± 1.5	16.4 ± 0.9
Maximum load (N)[b]	17.3 ± 3.3	17.9 ± 1.9	19.7 ± 1.6	19.9 ± 1.4
Energy (N-mm)[b,e]	7.14 ± 3.23	6.46 ± 0.87	4.28 ± 0.73	3.54 ± 0.65
Yield strain (unitless)[e]	0.016 ± 0.001	0.017 ± 0.002	0.018 ± 0.002	0.019 ± 0.004
Post-yield strain (unitless)[b,f]	0.049 ± 0.026	0.042 ± 0.004	0.023 ± 0.006	0.017 ± 0.004
Total strain (unitless)[b,f]	0.066 ± 0.025	0.059 ± 0.006	0.039 ± 0.005	0.035 ± 0.006
Modulus (MPa)[b,c]	13480 ± 570	12230 ± 1040	11330 ± 1260	10680 ± 1510
Yield stress (MPa)[b,c]	181 ± 6	159 ± 9	165 ± 15	153 ± 14
Maximum stress (MPa)[b,c,e]	222 ± 15	209 ± 7	199 ± 17	186 ± 23
Toughness (MPa)[b,f]	10.75 ± 4.58	8.85 ± 0.85	5.44 ± 1.00	4.19 ± 0.64
Areal BMD (mg/cm^2)[b,c,d]	59.9 ± 4.1	54.8 ± 3.3	60.9 ± 3.3	59.9 ± 2.0

[a] Data shown as mean ± SD.
[b] Significant difference between strains.
[c] Significant difference between sexes.
[d] Significant interaction between strain and sex.
[e] Data transformed to satisfy ANOVA assumptions of normality and equal variance.
[f] Data analyzed by non-parametric rank sum test.

mineral density (BMD). BMD is a reasonably useful marker for the load that a bone can tolerate, but it provides no insight into how much a bone can bend, or how well a damaged bone is able to resist completion of a fracture. These bone properties are both aspects of bone quality, and are difficult to measure noninvasively, and are therefore appropriate targets for study in a model system.

It is useful to distinguish the relative contributions of bone tissue's inherent mechanical behavior, bone size, and bone architecture to whole-bone strength and to map genes for each of them. However, these conceptual components of bone biomechanical performance do not correspond to the usual measurements that are obtained in real experimental settings. This is particularly true in mice, whose bones are too small to allow uniform specimens to be prepared for mechanical testing. A standard approach to achieve this end is to test whole bones and then apply beam theory to "factor out" the contribution of bone size to its mechanical performance. Simple algebraic transformations calculate material, *i.e.* tissue-level, properties (strain, stress, toughness, modulus) from biomechanical tests of whole bones (displacement, load, energy, and stiffness) and measures of whole-bone geometry [18]. Here, we report application of beam theory to map genes for tissue-level biomechanical performance in a reciprocal intercross of the recombinant congenic strains HcB-8 and HcB-23 that we recently analyzed at the whole-bone level [19]. HcB-8 and HcB-23 were chosen as parental strains because we previously found that they differ greatly in tissue-level mechanical performance of humeral cortical bone, and that these differences are accompanied by differences in collagen cross-linking and apatite crystallinity [20,21]. We find that most of the QTLs identified in that prior study also include material-level mechanical performance phenotypes. We report here successful linkage mapping of QTLs for post-yield strain and toughness, phenotypes that have not previously been mapped genetically. We also note an important negative result: a robust pleiotropic chromosome 4 QTL affecting bone size, strength and bone mineral density, which yielded the strongest evidence of linkage for whole-bone biomechanics [19], was not detected for any aspect of tissue-level biomechanical performance.

Materials and methods

Mice

The parental mice in this study were the recombinant congenic strains HcB-8 and HcB-23, produced by inbreeding N3 C3H/DiSnA (C3H) × C57BL/10ScSnA (B10) mice to fixation [22]. This breeding program resulted in inbred strains harboring alleles of B10 origin at approximately 1/8 of the genome on a C3H background. Therefore, only approximately 1/4 of the genome segregates in an intercross of HcB-8 and HcB-23, with the remaining portions of the genome fixed for the same allele in both parental strains. We performed a reciprocal F2 intercross, producing 603 F2 mice, and maintained the animals to an age of 17 ± 1 weeks, as this is the age at which mice achieve peak bone mass [23]. Mice were housed 2–5 mice per 500 cm^2 cages, with 12 h light–dark cycling, given autoclaved tap water and fed laboratory rodent chow 5001 (PMI Nutrition International, Richmond, IN) *ad lib*. Animals were euthanized by CO_2 inhalation, following AVMA recommendation. Immediately following sacrifice, animals were weighed and measured (rostro-anal length), viscera were harvested for DNA isolation (Puregene), and femora and humeri were dissected free of soft tissue for additional phenotyping. Bones were wrapped in phosphate buffered saline-saturated gauze and stored frozen at −70°. The animal protocol was approved by the University of Wisconsin and the William S. Middleton Memorial Veterans' Hospital IACUCs.