A genomewide scan for quantitative trait loci underlying areal bone size variation in 451 Caucasian families

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Background: Bone size is an important determinant of bone strength and is under strong genetic control.

Objective: To identify quantitative trait loci (QTL) for areal bone size variation, a large-scale genomewide linkage scan was carried out in 451 Caucasian families.

Participants and methods: Of 4124 people with phenotypes, 3899 were genotyped with 410 microsatellite markers. Multipoint linkage analyses were carried out in the entire sample, as well as in men and women separately. Potential epistatic interactions between identified genomic regions were also assessed.

Results: Several potentially important genomic regions were identified, such as 8q24 for hip bone size (logarithm of the ratio of the odds that two loci are linked (LOD) 3.27) and 2p24 (LOD 2.04) for spine bone size. 8q24 may also interact with 19p13 to affect hip bone size. Several sex-specific QTL were also detected, such as 14q21 (LOD 2.94) for wrist bone size in women and 16q12 (LOD 2.19) for hip bone size in men.

Conclusions: Together with previous findings, this study has further delineated the genetic basis of bone size and laid a foundation for future studies to eventually elucidate the mechanisms of bone size regulation and associated fracture risks.

Osteoporosis is a major public health problem and results in more than 1.3 million fractures per year in the US, with an estimated direct cost of more than US\$17 billion.^{1–2} Low bone mineral density (BMD) is an important risk factor for osteoporotic fractures and has been widely used in characterising osteoporosis.^{3–5} However, the pathogenesis of osteoporotic fracture is heterogeneous, and low BMD is not the only important risk factor for osteoporotic fractures.^{6–8} Many studies have suggested that bone size may also have an important role in determining bone strength and risks for fractures.^{9–13}

Being three-dimensional, bone size can be measured as bone volume (cm³), bone area (cm²) or bone length (or diameter, cm). All these measurements are legitimate and reflect various aspects of bone properties. Areal bone size measured by dual-energy x ray absorptiometry (DXA) may be interesting. Firstly, compared with other measurements (eg radiography), areal bone size measured by DXA is relatively precise and requires less radiation.^{14–15} Secondly, it is highly correlated to bone strength¹⁶ and osteoporotic fractures.^{12–17–18} However, genetic studies on areal bone size variation are rather rare so far. We have shown that areal bone size variation is under strong genetic control. Generally, the

heritability (h^2) of bone size variation at the spine, hip and wrist is more than 50%.^{19–20} A few candidate genes, such as the vitamin D receptor gene,²¹ low-density lipoprotein receptor-related protein 5 gene²² and type I collagen $\alpha 1$ gene,²³ have been shown to be associated with areal bone size variation.

We previously reported a pilot whole-genome linkage scan for areal bone size variation in 630 people from 53 pedigrees.²⁴ Several potentially important genomic regions have been suggested. Here, we report the results of a large-scale genomewide linkage scan for areal bone size in an extended sample of 451 Caucasian families with 4124 people.

PARTICIPANTS AND METHODS

Participants

The Creighton University institutional review board approved the study. All study participants signed documents giving informed consent before entering the project. All participants were Caucasians of European origin and were recruited by advertising. The sampling scheme and exclusion criteria have been detailed previously.²⁵ Briefly, patients with chronic diseases and conditions that might potentially affect bone mass, structure or metabolism were excluded.

The sample contained a total of 4124 phenotyped people from 451 families, including 630 people from our previous study.²⁴ Among the 4124 people, 1742 were recruited from 75 families, each ascertained through a single proband with either low or high BMD at the hip or spine (ie, BMD z score ≤ -1.28 or ≥ 1.28 , corresponding to the bottom or top 10% of the population distribution matched for age and sex). The remaining people, aged 25–40 years, were recruited from 376 families, each having two parents and at least two healthy children, without considering BMD values. As we have shown previously,^{26–27} such a sampling scheme will generally not change the identity of loci underlying bone size variation in the normal population and is thus unlikely to introduce heterogeneity in the loci identified.

Measurement

Areal bone size (cm²) of the lumbar spine (L1–4), total hip (femoral neck, trochanter and intertrochanteric region) and wrist (ultradistal region of the forearm) were measured using Hologic DXA scanners (Hologic Corp, Waltham, Massachusetts, USA). All scanners were calibrated daily, and long-term precision was monitored with external spine, hip and wrist phantoms. The short-term precision of areal bone size measurement was determined by Glüer's method²⁸

Abbreviations: BMD, bone mineral density; DXA, dual-energy x ray absorptiometry; LOD, logarithm of the ratio of the odds that two loci are linked; QTL, quantitative trait loci; SOLAR, sequential oligogenic linkage analysis routines

Table 1 Basic characteristics of the study participants

	Total (n = 4124)	Men (n = 1716)	Women (n = 2408)
Age (years)*	47.7 (16.0)	48.0 (16.1)	47.6 (16.0)
Height (m)*	1.70 (0.10)	1.78 (0.07)	1.64 (0.07)
Weight (kg)*	78.5 (18.2)	89.4 (15.8)	71.3 (16.0)
Bone size (cm ²)*			
Spine	63.40 (8.34)	70.39 (6.39)	58.60 (5.71)
Hip	38.15 (6.53)	44.59 (4.44)	33.70 (3.20)
Wrist	3.89 (0.54)	4.34 (0.43)	3.58 (0.36)
Heritability (bone size)†			
Spine	0.62 (0.03)	0.71 (0.05)	0.67 (0.06)
Hip	0.57 (0.03)	0.61 (0.05)	0.72 (0.06)
Wrist	0.58 (0.03)	0.55 (0.05)	0.57 (0.07)

*Phenotypic values are mean (SD) of the raw data without adjustment for covariates.

†Heritability values are mean (SE), estimated by sequential oligogenic linkage analysis routines, with adjustment for significant covariates.

with 121 random participants, each having three repeated measurements on the same day with re-positioning (ie, getting off the DXA bed, followed by getting on the DXA bed) between the measurements. The coefficients of variation of bone size measurement were 1.11%, 1.94% and 2.87% at the spine, hip and wrist, respectively. Weight (kg) and height (m) were measured at the same visit as the bone size measurement.

Low-trauma fractures of the spine, hip and wrist were ascertained through a nurse-administered questionnaire.

Self-reported symptomatic fractures are shown to be reliable, especially those involving extensive pain and requiring medical treatment, such as fractures at the wrist and hip.²⁹⁻³² Circumstances leading to fractures were ascertained by a research nurse, and cases of self-reported fractures that were not due to low trauma were excluded. The lower age limit for osteoporotic fractures was 35 years.

Genotyping

Among all the participants, 3899 were genotyped with 410 microsatellite markers (including 393 markers for 22 autosomes and 17 markers for the X chromosome) from the Marshfield screening set 14 by the Marshfield Center for Medical Genetics, Marshfield, Wisconsin, USA. The markers had an average population heterozygosity of 0.75 and were spaced on average 8.9 cM apart. PedCheck³³ was used for checking the Mendelian inheritance pattern at all the marker loci and for confirming the alleged relationships of family members within the pedigrees. In addition, we used MERLIN³⁴ to detect genotyping errors of unlikely recombination (eg, double recombination) in our sample. The overall genotyping missing and error rate was about 0.3%.

Statistical analyses

Variance component linkage analyses for quantitative traits were carried out using sequential oligogenic linkage analysis routines (SOLAR).³⁵ Age, sex, height, weight and sex-by-age interaction were tested for association with areal bone size variation, and significant factors were adjusted as covariates in linkage analyses. Multipoint logarithm of the ratio of the odds that two loci are linked (LOD) scores were calculated for

Table 2 Areal bone size in relation to fracture

Adjusted bone size (cm ²)	Spine fracture		Hip fracture		Wrist fracture	
	Absent (n = 1003)	Present (n = 37)	Absent (n = 993)	Present (n = 26)	Absent (n = 960)	Present (n = 134)
Spine	64.01 (5.03)	60.46 (5.97)				
Hip			39.65 (3.01)	40.99 (3.24)		
Wrist					3.66 (0.09)	3.70 (0.11)
p Value	<0.001		0.03		<0.001	

The groups with no fractures are made up of unrelated people, which are identified from the 451 families by selecting the founders and married-in people.

Values are means (SD) of bone size adjusted for sex, age, height and weight.

Table 3 Genomic regions with multipoint scores of the logarithm of the ratio of the odds that two loci are linked >1.5 detected in the total sample and in sex-specific subgroups

Skeletal site	Location (cM)*	Marker†	LOD‡		
			Total sample	Women	Men
Spine	2p24 (38)	GATA11H10	2.04 (2.39)	—	—
	5q23 (130)	GATA62A04	1.78 (1.93)	—	—
	15q21 (35)	GATA50C03N	—	1.63 (2.21)	—
Hip	8q24 (164)	UT721M	3.27 (4.01)	3.01 (4.07)	—
	14q11 (12)	GATA74E02Z	1.82 (1.37)	—	—
	16q12 (71)	GATA22F09P	—	—	2.19 (1.19)
	20q11 (58)	GATA42A03	—	—	2.18 (1.60)
Wrist	9p21 (34)	AGAT142P	—	2.60 (2.96)	—
	9q21 (76)	GATA89A11	—	2.45 (2.56)	—
	14q21 (51)	GATA90G11M	—	2.94 (2.62)	—
	21q22 (37)	ATA27F01	1.65 (1.59)	—	—

LOD, logarithm of the ratio of the odds that two loci are linked.

*The numbers in parentheses are the genetic distances of the LOD score peaks from the p-terminal.

†The nearest marker from the LOD score peak.

‡The numbers in parentheses are the two-point LOD scores for the nearest marker. —, the corresponding maximum LOD score is <1.50.

22 autosomes. As SOLAR cannot handle multipoint linkage analysis for chromosome X, we calculated only two-point LOD scores for markers on chromosome X. Other software, such as GENEHUNTER, capable of multipoint linkage analysis for chromosome X, unfortunately cannot handle large pedigrees that made up the major part of our sample. Breaking down the large pedigrees into smaller ones might be an option, but this procedure would result in a considerable loss of statistical power.

To estimate the empirical *p* values for observed LOD scores, we carried out 10 000 simulations using the procedure "lodadj" implemented in SOLAR.³⁶ For each replicate, this procedure simulated a fully informative marker, unlinked to bone size, and computed an LOD score for the simulated marker. The results of 10 000 replicates sampled the null distribution of LOD scores (ie, the distribution of LOD scores obtained under the hypothesis of no linkage), and the proportion of those LOD scores greater than an observed one was the empirical *p* value of the observed score.

Tests for potential epistatic interaction effects were carried out for loci with LOD ≥ 2 on the genome-wide screen, with any other loci showing LOD > 1 for the corresponding trait. Two levels of modelling in addition to single-locus modelling were carried out: (1) two-locus models with only additive effects for each pair of loci; and (2) two-locus models with additive effects as well as an epistatic term for interaction between the two loci. The increase in LOD scores under the epistatic model over that under the additive model suggested genetic interactions. One-tailed *p* values were generated using the χ^2 test with *df* = 1 for all interactions tested.³⁷ Significance of a single test on any one variable was assessed at a type I error rate of 0.05/*n* according to the Bonferroni adjustment for multiple comparisons, where *n* is the number of independent tests conducted for each trait.

As gender differences exist in bone size and geometry,^{38–39} it is expected that the genetic determinants of bone size variation would be influenced by sex. Thus, we also conducted linkage analyses for areal bone size variation in sex-specific subgroups. In the sex-specific analyses, the areal bone size values for people of the opposite sex were recorded as missing data. Age, height and weight were also tested and relevant factors were adjusted as covariates in the sex-specific linkage analyses.

RESULTS

Table 1 summarises the basic characteristics of the study participants. After adjusting for age, height, weight and sex, we found significant difference in areal bone size between people with and without fractures (table 2). Those with hip and wrist fractures have significantly larger areal bone size at the hip (*p* = 0.03) and wrist (*p* < 0.001), respectively. In contrast, those with spine fractures have significantly smaller spine bone size (*p* < 0.001). These results are consistent with previous reports.^{9–18–40–41} All three bone size variables showed strong genetic determination in our sample, with *h*² ranging from 0.55 to 0.72 (table 1).

Baseline linkage analyses

Figure 1 shows the multipoint LOD scores for baseline linkage analyses in the whole study population. Table 3 and fig 2 show detailed information of genomic regions with multipoint LOD > 1.5 . As SOLAR cannot handle multipoint linkage analysis for chromosome X, we conducted only two-point linkage analyses on chromosome X and all those two-point LOD scores were < 0.5 (data not shown).

The highest LOD score was achieved on chromosome 8q24 for hip bone size (multipoint LOD 3.27, pointwise empirical *p* < 0.001; table 3, fig 2C). We also detected a suggestive

Figure 1 Multipoint linkage results for the spine, hip and wrist bone size in the baseline linkage analyses. Chromosomes showing logarithm of the odds that two loci are linked (LOD) > 1.0 are marked with LOD score scales.

linkage evidence on chromosome 2p24 for spine bone size (multipoint LOD 2.04, pointwise empirical *p* = 0.001; table 3, fig 2A). Other interesting findings included 5q23 for spine bone size (multipoint LOD 1.78) and 14q11 for hip bone size (multipoint LOD 1.82; table 3, fig 2B,D).

Figure 2 Genomic regions with logarithm of the odds that two loci are linked (LOD) >1.5 detected in baseline linkage analyses. Dashed line, published 53 families; solid line, current 451 families. (A,B) Spine bone size. (C,D) Hip bone size.

Epistatic interaction analyses

For hip bone size, epistatic interaction tests were conducted between 8q24 and three other genomic regions, including 5q23, 14q11 and 19p13. After applying the Bonferroni correction for multiple comparisons ($n=3$, significant threshold $0.05/3 = 0.017$), we detected a significant interaction between 8q24 and 19p13 ($p = 0.011$) on hip bone size (fig 3). For spine bone size, we tested the potential epistatic interaction between the region 2p24 and three other regions that showed multipoint LOD ≥ 1.0 for spine bone size in the baseline linkage analyses, including 5q23, 10q21 and 11q22. However, we detected no significant interaction for spine bone. As no genomic regions achieved multipoint LOD ≥ 2.0 for wrist bone size in the baseline linkage analyses, we did not carry out epistatic interaction analyses for wrist bone size.

Sex-specific linkage analyses

Table 3 and fig 4 summarise the results of sex-specific linkage analyses. In the female subgroup, we detected a strong suggestive linkage evidence on 8q24 (multipoint LOD 3.01)

for hip bone size (table 3, fig 4A). Interestingly, this region also showed strong evidence for hip bone size in the whole study population (ie, baseline linkage analyses), but not in men (table 3, fig 4A), suggesting that the signal observed in the baseline linkage analyses is largely driven by female-specific quantitative trait loci (QTL). Additionally, we detected three potential female-specific QTL on chromosome regions 9p21 (LOD 2.60), 9q21 (LOD 2.45) and 14q21 (LOD 2.94) for wrist bone size, which also showed weak linkage evidence in the baseline linkage analyses (table 3, fig 4B,C). Similarly, male-specific linkage analyses identified two potential male-specific QTL for hip bone size, which were mapped on regions 16q12 (LOD 2.19) and 20q11 (LOD 2.18; table 3, fig 4D,E).

DISCUSSION

Although BMD is the most commonly used characteristic in clinical practice for osteoporosis, many studies have shown that areal bone size is also an important determinant of bone

Figure 3 Significant epistatic interaction detected between chromosome regions 8q24 and 19p13. Solid line, baseline linkage analyses; dashed line, additive two-locus model; dotted line, epistatic two-locus model.

strength^{16, 17} and may have the potential to improve fracture risk assessment.^{12, 18, 42, 43} Consistent with previous reports,^{9, 18, 40, 41} we also detected considerable difference in areal bone size between people with and without fractures. As such, people with hip fractures have markedly larger hip bone size, whereas those with spine fractures have considerably smaller spine bone size. The reason for such dissimilarity is not clear, but previous studies suggested that larger bone size in patients with hip fractures is mainly due to greater periosteal apposition during growth, whereas reduced vertebral bone size in those with spine fractures is largely caused by reduced periosteal apposition during ageing.^{40, 41}

Previously, we reported a pilot whole-genome linkage scan for areal bone size variation in 53 Caucasian pedigrees with 630 people. Several genomic regions were suggested to be linked to areal bone size variation. To substantiate our previous findings and to detect novel genomic regions for areal bone size, we conducted a second genomewide linkage scan in an extended sample of 451 families. We detected a strong linkage evidence for hip bone size on chromosome 8q24. Two prominent candidate genes reside in this region, the osteoprotegerin gene and the multiple exostoses 1 gene. Osteoprotegerin is a member of the tumour necrosis factor receptor superfamily and acts as an osteoblast-secreted decoy receptor that functions as a negative regulator of bone resorption.⁴⁴ The multiple exostoses 1 gene is associated with hereditary multiple exostosis⁴⁵ and may have an additional role in bone biology and osteoporosis.⁴⁶ Although 8q24 showed little evidence of linkage with hip bone size variation in our previous genomewide scan²⁴ (fig 2), we did not detect significant genetic heterogeneity at this region in our sample ($p > 0.10$). In addition, the fact that the newly recruited 398 families (which comprised about 84% of the entire sample) contributed to about 80% of the detected linkage signal further suggested that genetic heterogeneity is unlikely to be the main reason underlying the discordant observations at this region between our previous and present studies. Instead, the discordant results at this region may be due to different marker sets and genetic maps, genotyping errors and the relatively low statistical power of the previous genomewide scan.

On the other hand, the linkage signals on genomic regions 2p24, 5q23 and 14q11 all showed similar patterns between our previous²⁴ and current scan, with higher LOD scores detected in the present study (fig 2). The higher LOD scores in the present study may reflect the increased sample size and statistical power to detect linkage. Although the linkage signals detected at the three regions barely reached or did not reach suggestive linkage,⁴⁷ the generally consistent patterns detected across our two studies suggested that these regions may actually contain QTL for bone size variation, each with small or modest effects.

Recent studies suggested that gene–gene interactions are the norm rather than the exception in genetic control of complex traits or diseases.^{48, 49} Therefore, we further assessed potential epistatic interaction effects between genomic regions identified in the baseline linkage analyses. To control for the untoward effects of multiple comparisons, we tested only the possible interactions between regions showing LOD > 2 and any other regions showing LOD > 1 for the corresponding trait, and adjusted the significance thresholds via the conservative Bonferroni correction. A marked epistatic interaction was detected between regions 8q24 and 19p13 for hip bone size variation. 19p13 also showed suggestive linkage evidence for hip bone size variation (LOD 2.83) in our previous genomewide scan.²⁴ A notable candidate gene located in this region is the multiple exostoses 3 gene.⁵⁰ The exact epistatic interaction mechanisms remain unknown at present. A potential mechanism is the interactions between the multiple exostoses 3 gene and the multiple exostoses 1 gene at region 8q24, as previous studies suggested that the products of the multiple exostoses gene family may interact with each other.^{51, 52}

We also identified several potential sex-specific QTL for areal bone size variation, such as 9p21 and 14q21 in women, and 16q12 and 20q11 in men. On the one hand, these findings may reflect genuine aetiological heterogeneity due to enhanced sample homogeneity. This is probably the case for loci such as on 9p21 and 16q12, where we detected only weak evidence in the total sample but stronger evidence in specific sexes. On the other hand, these results should be treated with caution because of the inflated false-positive or false-negative rates due to increased multiple comparisons and insufficient power in individual subgroups. This may be true for potential male-specific QTL on chromosome 20q11 for spine bone size, which is not reflected in the total sample analyses.

Replication of genetic studies for complex traits is indeed difficult^{53, 54}; thus, it is not surprising that the genomic regions identified in this study and in previous genetic studies for osteoporosis⁵⁵ are largely inconsistent. Despite this, several genomic regions have been linked to BMD or phenotypes related to bone size. At the exact position of our LOD score peak on chromosome 8q24, Karasik *et al*⁵⁶ detected suggestive linkages with Ward's area BMD and a combined phenotype of BMD and quantitative ultrasound,⁵⁷ suggesting that QTL located in this region may contribute to both BMD and bone size variation. 19p13 has also been linked to femoral neck axis length and femoral head width.⁵⁸ These findings may substantiate the potential importance of these genomic regions on bone size variation.

In summary, we reported a genomewide linkage scan on areal bone size in an extended sample of 451 Caucasian families. Chromosome 8q24 may harbour QTL underlying hip bone size variation. These QTL may also interact with QTL on 19p13 to regulate hip bone size variation. In addition, sex-specific linkage analyses showed several potential sex-specific QTL for hip and wrist bone size. The obtained knowledge would be crucial to eventually identify the functional variants and elucidate the mechanisms for bone size regulation and osteoporotic fractures.

Figure 4 Genomic regions with logarithm of the ratio of the odds that two loci are linked (LOD) > 1.5 detected in sex-specific linkage analyses. Solid line, the entire sample; dashed line, women; dotted line, men. (A,D,E) Hip bone size. (B,C) Wrist bone size.

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