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Bivariate Whole Genome Linkage Analyses for Total Body Lean Mass and BMD

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ABSTRACT: A genome-wide bivariate analysis was conducted for TBLM and BMD at the spine and hip in a large white sample. We found some QTLs shared by TBLM and BMD in the entire sample and the sex-specific subgroups, and QTLs with potential pleiotropy were disclosed.

Introduction: Previous studies suggested that total body lean mass (TBLM) and BMD are highly genetically correlated. However, the specific shared genetic factors between TBLM and BMD are unknown.
Materials and Methods: To identify the specific quantitative trait loci (QTLs) shared by TBLM and BMD at the spine (L_1–L_4) and total hip, we performed bivariate whole genome linkage analysis (WGLA) in a large sample involving 4498 white subjects of European origin.
Results: Multipoint bivariate linkage analyses for 22 autosomes showed evidence of significant linkage with an LOD score of 4.86 at chromosome region 15q13 for TBLM and spine BMD in women, and suggestive linkage findings (LOD > 2.2) at 7p22 for TBLM and spine BMD for the entire sample, at 7q32 for TBLM and BMD at both spine and hip in women, and at 7q21 and 13p11 for TBLM and BMD at both spine and hip in men. Two-point linkage analyses for chromosome X also showed significant linkage signals at several regions such as Xq25. Complete pleiotropy (a single locus influencing both traits) was suggested at 7q32 and 13q11 for TBLM and BMD. Additionally, complete co-incident linkage (separate tightly clustered loci each influencing a single trait) was detected at 7p22 for TBLM and spine BMD.
Conclusions: We identified several genomic regions shared by TBLM and BMD in whites. Further studies may focus on fine mapping and identification of the specific QTLs in these candidate genomic regions.
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Key words: BMD, total body lean mass, genetic correlation, bivariate linkage analysis, whole genome linkage scan

INTRODUCTION

Osteoporosis is a major public health problem that predisposes to subsequent bone fractures and results in an estimated direct cost of more than $17 billion per year in the United States.[1,2] BMD is the most powerful, measurable determinant of osteoporosis,[3–5] and it is estimated that 50~90% of BMD variation is genetically determined.[6–9]

Total body lean mass (TBLM), mainly composed of muscle, has been highly phenotypically correlated to BMD.[10] Studies reported that lean mass exerts a significant effect on BMD variation in young women[11,12] and that high lean mass is favorable to BMD.[13] More than 50% of the correlation between TBLM and BMD at the femoral neck can be explained by genetic factors,[14] and our previous study also validated the high genetic correlation between TBLM and BMD at the lumbar spine.[15] These findings suggest that some genetic factors that influence both TBLM and BMD are shared; however, these specific shared genomic regions have not been identified.

Bivariate linkage analysis, extended from univariate linkage analysis, is a powerful method that can be used to identify genomic regions influencing two correlated traits by simultaneously considering their genetic and environmental correlations. Bivariate variance component linkage analysis permits identification of loci whose effects are too small to be detected by univariate linkage analysis and improves the statistical power for detecting common quantitative trait loci (QTLs).[16,17] Furthermore, bivariate linkage analysis

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provides a solution to the difficult problem of differentiating pleiotropic effects of a single locus influencing two related traits from co-incident linkage of tightly clustered loci that each influences a different trait.[18]

The major aim of this study was to identify the genomic regions shared by TBLM and BMD at the spine and hip through bivariate whole genome linkage analysis (WGLA). The results will lay a foundation for further fine mapping and identification of QTLs common to the underlying variation in TBLM and BMD.

MATERIALS AND METHODS

Subjects

The study was approved by necessary Institutional Review Boards. All study subjects signed informed consent documents before entering the project. The sample contains a total of 4498 subjects from 451 pedigrees, of whom 4126 were genotyped. This large sample provides an exceedingly large number of relative pairs (>150,000) informative for linkage analysis.[19] The pedigree size varied from 4 to 416 individuals, with a mean of 11.6 ± 28.5 (SD). All study subjects were whites of European origin. The sampling scheme and exclusion criteria have been detailed in a previous publication.[20] Briefly, patients with chronic diseases or conditions that might potentially affect bone mass, structure, or metabolism were excluded.

Measurements

TBLM (g) and BMD (g/cm^2) at the lumbar spine (L_1–L_4) and total hip were measured by Hologic 1000, 2000+, or 4500 DXA scanners (Hologic, Bedford, MA, USA) in the Osteoporosis Research Center at Creighton University. All scanners are calibrated daily, and long-term precision is monitored with external phantoms. Data obtained from different machines were transformed to a compatible measurement using the transformation formula.[21] Members of the same pedigree were usually measured on the same type of machine. The CVs obtained on the Hologic 2000+ DXA scanner were 1.0%, 0.9%, and 1.4% for TBLM, spine BMD, and hip BMD, respectively.[20]

Genotyping

Whole genome DNA was extracted from peripheral blood samples using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN, USA). A total of 4126 subjects were genotyped for 410 microsatellite markers from the Marshfield screening set 14 by the Marshfield Center for Medical Genetics (Marshfield, WI, USA). This set of markers had an average population heterozygosity of 0.75 and spaced on average 8.9 cM. The detailed genotyping protocol is available on http://research.marshfieldclinic.org/genetics/Lab_Methods/methods.html.

A genetic database management system (GenoDB)[22] was used to manage the phenotype and genotype data for linkage analyses. PedCheck[23] was performed to ensure that the genotype data conformed to a Mendelian inheritance pattern at all the marker loci. In addition, we used MERLIN[24] to detect genotyping errors of unlikely recombination (e.g., double recombination) in our sample. The genotyping error rate was ~0.03%.

Statistical analyses

Basic characteristics of the study sample were calculated with the SAS package (SAS Institute, Cary, NC, USA). Multipoint bivariate linkage analyses for 22 autosomes and two-point linkage analysis for the X chromosome were performed using Sequential Oligogenic Linkage Analysis Routines (SOLAR) version 3.0.4, which is available at http://www.sfbr.org/solar/.[18,25] Age and sex were used as the covariates in analysis for the entire sample, and age was used as the covariate in analyses for the sex-specific samples.

Using the variance component model, we tested the null hypothesis of no linkage (i.e., $\sigma^2_m = 0$, where σ^2_m is the additive genetic variance caused by a major locus) by comparing the likelihood of this restricted model with that of a model in which σ^2_m was estimated. The difference between the two $\log_{10}$ likelihoods is LOD score. The bivariate LOD scores have 2 df in contrast to the univariate LOD scores (that have 1 df). To compare with the univariate LOD scores, all the 2 df bivariate multipoint LOD scores in this study were transferred into 1 df univariate LOD scores with equal p values. The p values can be calculated from the univariate test statistic ($2 \times \mathrm{Ln}_{10} \times \mathrm{LOD}$) following a mixture distribution of 1/2 χ_1^2 and 1/2 χ_0^2. The bivariate test statistic ($2 \times \mathrm{Ln}_{10} \times \mathrm{LOD}$) follows a mixture distribution of 1/2 χ_1^2, 1/4 χ_2^2, and 1/4 χ_0^2,[26] and the corresponding two-trait LOD score can be calculated by command "-clod" in SOLAR software.

Considering the potential sex-specific effects, we also performed bivariate analyses in the women and men separately, with age as covariate. The Marshfield sex-specific genetic maps were used in this subgroup analysis instead of the sex-averaged map (The Marshfield map database is publicly accessible at http://research.marshfieldclinic.org/genetics/).

Because two trait pairs were adopted in our bivariate linkage analyses, correction for multiple testing was required. Following the method described by Camp and Farnham,[27] we calculated the linear correlation coefficient between the two sets of whole genome bivariate LOD scores by estimating the number of effectively independent tests. The independent test numbers were 1.78, 1.83, and 1.78 in the entire sample, women, and men, respectively. According to the following formula[27–29]:

$$\mu\,(X) = N[C + 2\rho GX]\,\alpha(X),$$

the genome-wide "suggestive" and "significant" LOD thresholds of linkage ($p = 0.0005$ and 0.000017, respectively) for the two studied trait pairs were determined to be 2.15 and 3.57 in the entire sample, 2.16 and 3.58 in women, and 2.15 and 3.57 in men.

To further explore if the QTLs disclosed by bivariate analyses have pleiotropic effect or are coincidentally linked, we used the likelihood-based tests proposed by Almasy et al.[18] and implemented in SOLAR. Briefly, the likelihood of the linkage model in which ρ_m (a measure of shared

Table 1. Basic Characteristics of the Study Subjects Stratified by Age and Sex

Age group	N	Age (yr)	TBLM (kg)	Spine BMD (g/cm^2)	Hip BMD (g/cm^2)
Male					
19~	278	24.26 ± 2.97	67.04 ± 0.60	1.08 ± 0.13	1.11 ± 0.15
30~	327	35.61 ± 2.85	67.45 ± 8.35	1.07 ± 0.11	1.06 ± 0.13
40~	445	45.13 ± 2.85	68.15 ± 8.21	1.06 ± 0.14	1.05 ± 0.14
50~	333	54.59 ± 2.96	67.14 ± 8.40	1.07 ± 0.15	1.04 ± 0.14
60~	222	64.88 ± 2.77	66.07 ± 8.74	1.07 ± 0.18	1.01 ± 0.15
70~	211	76.08 ± 4.80	61.62 ± 7.69	1.11 ± 0.22	0.97 ± 0.15
Female					
19~	393	24.80 ± 3.15	45.98 ± 6.92	1.06 ± 0.14	0.98 ± 0.13
30~	545	35.46 ± 2.85	46.41 ± 6.78	1.06 ± 0.12	0.96 ± 0.13
40~	676	44.98 ± 2.85	46.58 ± 6.76	1.05 ± 0.13	0.95 ± 0.13
50~	439	54.27 ± 2.91	46.13 ± 6.81	1.00 ± 0.16	0.92 ± 0.15
60~	331	64.91 ± 2.83	45.43 ± 7.04	0.92 ± 0.20	0.86 ± 0.16
70~	298	76.00 ± 4.80	43.39 ± 5.82	0.91 ± 0.22	0.79 ± 0.17

The values are presented as mean ± SD.

major gene effect near the region at which linkage is being assessed) was estimated was compared with the likelihood of the restricted model in which ρ_m was constrained to 0 (no shared major gene effect in the region, i.e., complete coincident linkage) and to 1 (complete pleiotropy). For test of complete coincident linkage, twice the difference in the likelihoods follows a χ^2 distribution. For test of complete pleiotropy, twice the difference in the likelihoods follows a 1/2:1/2 mixture of χ^2 distribution.[18,26] The possibility of complete pleiotropy and coincident linkage was denoted as p_{pl} and $p_{co\text{-}l}$ in the Results section, respectively; $p < 0.05$ was used for rejection of complete pleiotropy or co-incident linkage.

RESULTS

Basic characteristics

Basic characteristics of the study subjects are summarized in Table 1. It is shown that BMD at different sites generally decline with aging in women, and in men at the hip but not at the spine. In addition, both TBLM and BMD values are higher in men than in age-matched women.

Bivariate linkage analyses

Multipoint analyses on 22 autosomes: Using the thresholds corrected for multiple testing, a suggestive linkage was detected at 7p22 (LOD = 2.53) for TBLM and spine BMD in the entire sample (Table 2). In women, a peak LOD score of 4.86 ($p < 0.0001$) was found at chromosome region 15q13 for TBLM and spine BMD (Table 2), and suggestive bivariate linkage was found at 7q32 for TBLM and hip BMD (LOD = 2.67) and for TBLM and spine BMD (LOD = 2.44). In men, suggestive linkage signals were detected at 7q21 for TBLM and hip BMD (LOD = 2.52) and at 13p11 for TBLM and spine BMD (LOD = 3.25; Table 2).

Two-point linkage analyses on chromosome X: In Fig. 1, we plotted all the "suggestive" or "significant" results of bivariate two-point linkage analyses on chromosome X for the entire sample and the two sex subgroups. In the entire sample, the strongest linkage signal was detected at the marker ATCT003 (Xq25) for TBLM and spine BMD (LOD = 5.26). Evidence of significant linkage (LOD > 3.5) in the entire sample was also found at the markers GATA52B03 (Xp22.3), GATA144D04 (Xp11.4-Xq11.1), GATA31D10 (Xq13.3), GATA165B12 (Xq23-Xq24), and CTAT014 (Xq27.1) for TBLM and spine BMD (LOD = 5.26, 5.20, 4.54, 4.31, 3.78, and 3.68, respectively). Female-specific significant linkage presented at the markers GATA31D10 (Xq13.3), GATA52B03 (Xp22.3), and GATA52B03 (Xq22.33) for TBLM and spine BMD (LOD = 3.94, 3.92 and 3.92, respectively). Male-specific significant linkage presented at the marker GATA72E05 (Xq11.1) for TBLM and spine BMD (LOD = 3.78).

Pleiotropy versus co-incident linkage

Presented also in Table 2 are p values for discrimination analysis of complete pleiotropy versus co-incident linkage for chromosome regions with "suggestive" or "significant" linkage signals. In women, chromosome region 7q32 may include QTLs with pleiotropic effects on TBLM and hip BMD (197cM, $p_{pl} = 0.424$, $p_{co\text{-}l} = 0.003$), and on TBLM and spine BMD (198cM, $p_{pl} = 0.339$, $p_{co\text{-}l} = 0.021$). In men, chromosome region 13p11 may harbor QTL influencing TBLM and spine BMD (6cM, $p_{pl} = 0.397$, $p_{co\text{-}l} = 0.040$). In the entire population, co-incident linkage was suggested for TBLM and spine BMD at 7p22 (9cM, $p_{pl} = 0.049$, $p_{co\text{-}l} = 0.220$). In addition, we failed to reject both complete pleiotropy and complete co-incident linkage effect for chromosome regions 15q13 on TBLM and spine BMD (18 cM, $p_{pl} = 0.138$, $p_{co\text{-}l} = 0.234$) and 7q21 on TBLM and hip BMD (71cM, $p_{pl} = 0.417$, $p_{co\text{-}l} = 0.186$).

DISCUSSION

This study showed several genomic regions shared by TBLM and BMD through bivariate WGLA conducted in both entire and sex-specific white sample populations. The implicated chromosome regions include 7p22 and Xq25 (entire sample), 7q32 and 15q13 (women), and 7q21 and 13p11 (men). Furthermore, our data suggest that chromosome regions near 7q32 and 13p11 may harbor loci with pleiotropic effects.

In previous univariate WGLA for TBLM and BMD performed by our group,[15] significant or suggestive linkage was detected on chromosome regions 5q23, 15q13, Xq27, and 11q23 for BMD variation and on 5q35, 5q23, 7q32, and 15q12 for TBLM variation. Among these regions, linkage signals at chromosome regions Xq27, 7q32, and 15q13 (very close to 15q12) were consistently found in the current bivariate analysis, which highlights the importance of these loci for both TBLM and BMD variation. Genomic regions detected in our previous univariate WGLA but not in this bivariate analysis, such as 5q23, 15q13, 11q23, and 5q35, suggest that these regions may have individual effects on either TBLM or BMD but not both.[15] On the other hand, linkage signals at new genomic regions that were detected in our bivariate WGLA but not in univariate analysis, such as 7p22, 7q21, 13p11, and Xq25, may reflect the greater

TABLE 2. AUTOSOMAL REGIONS OF SUGGESTIVE OR SIGNIFICANT LINKAGE TO TBLM AND BMD

Bivariate	*Genomic region**	*Nearest marker*[†]	*LOD score*[‡]	p_{pl}	$p_{co\text{-}l}$	*p*
Entire sample						
TBLM and spine BMD	7p22 (9 cM)	GATA119B03	2.53	0.049	0.220	0.00032
Female sample						
TBLM and hip BMD	7q32 (197 cM)	MFD442-GTTT002	2.67	0.424	0.003	0.00023
TBLM and spine BMD	15q13 (18 cM)	GATA88H02N	**4.86**	0.138	0.234	<0.0001
	7q32 (198 cM)	MFD442-GTTT002	2.44	0.339	0.021	0.0004
Male sample						
TBLM and hip BMD	7q21 (71 cM)	GATA24D12P	2.52	0.417	0.186	0.00033
TBLM and spine BMD	13p11 (6 cM)	GATA23C03P	3.25	0.397	0.040	<0.0001

p_{pl} value >0.05 means complete pleiotropy; $p_{co\text{-}l}$ value >0.05 means co-incident linkage.

* The numbers in parentheses are chromosomal positions with LOD peaks, distant from the most p-terminal of the chromosome according to the Marshfield genetic map.

[†] The nearest marker from the LOD score peak.

[‡] This is the multivariate adjusted LOD scores, converted from a mixed distribution to a 1 df distribution, thus comparable to the univariate LOD scores. LOD marked in bold is the evidence of significant linkage corrected for multiple genome-wide tests.

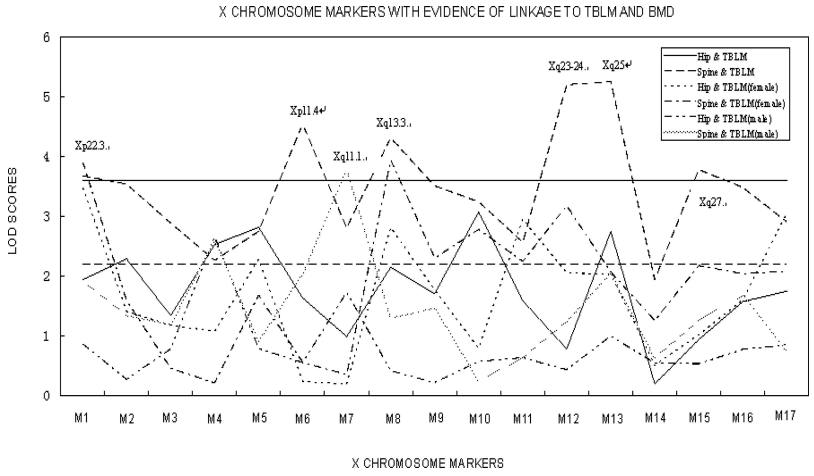

FIG. 1. X chromosome markers with evidence of linkage to TBLM and BMD. M1–M17 denotes markers sequentially located on chromosome X from p- to q-terminal (i.e., GATA52B03, GATA175D03, ATA28C05, GATA124E07, GATG011, GATA144D04, GATA72E05M, GATA31D10M, GATA31F01P, GATA172D05, GATA48H04, GATA165B12P, ATCT003, GATA31E08, CTAT014, TATC043, and TTTA062, respectively), The horizontal solid line represents significant threshold level (LOD = 3.6). The horizontal dotted line represents suggestive threshold level (LOD = 2.2).

statistical power of bivariate linkage analyses, compared with univariate analyses, for detection of QTLs with modest effect.[17]

Genomic regions with suggestive or significant linkage signals detected in the entire sample were generally different from those implicated in sex-specific subgroups. Sex-specific QTLs disclosed in this study may contribute to variations in TBLM and BMD observed between the two subgroups. Because of the admixture of subjects of different sexes, the genomic regions disclosed in the subgroups might not be found in the entire sample. For example, a significant female-specific genomic region 15q13 (LOD = 4.86) was not found in either the men or the entire sample. On the other hand, some genomic regions disclosed in the entire sample, such as 7p22 (LOD = 2.53), were not detected in either subgroup. Considering the smaller sample size in subgroup analysis and consequent decrease in statistical power, however, we cannot exclude the significance of genome regions identified in the entire subpopulation on variation of TBLM and BMD in subgroups.

In women, chromosome region 7q32 showed pleiotropic effects on TBLM and BMD. *LEP* (leptin), an interesting candidate gene located nearby in 7q31.3, is a powerful inhibitor of bone formation that regulates bone resorption through the sympathetic nervous system.[30,31] Leptin also seems to be an important mediator influencing the relationship between fat mass and BMD.[32] Circulating leptin levels have been negatively correlated with BMD,[32]and leptin was also shown to be associated with TBLM.[33] These findings suggest that leptin may contribute to the pleiotropic effect of chromosome region 7q32 on TBLM and BMD observed in this study.

In the entire sample, chromosome region 7p22 showed co-incident linkage for TBLM and BMD, suggesting at least two loci in this region independently influencing the corresponding traits. Two candidate genes reside in this region, e.g., *TWIST* (twist homolog 1) and *IL6* (interleukin 6). *TWIST* is an integrator of SHH (sonic hedgehog homolog), FGF (fibroblast growth factor), and BMP (bone morphogenetic protein) signaling,[34] and it acts as a negative regulator of osteoblast differentiation.[35] *IL6* was found associated with both BMD[36] and lean mass,[37,38] *IL6* has a role essential to the regulation of bone resorption,[36] and Roth et al.[38] found that IL6 G-174C polymorphism is significantly associated with FFM. Although the hypothesis of complete pleiotropic effect was rejected for chromosome

region 7p22, partial or minor pleiotropic effects of the underlying loci on both TBLM and BMD might exist.

The strongest linkage signal (LOD = 4.86) detected in this study was in women, on chromosome region 15q13, for TBLM and spine BMD. Zhao et al.[15] and Xiao et al.[19] consistently detected linkage signals on 15q13 in their univariate WGLA for TBLM and BMD. In light of these consistent findings, chromosome region 15q13 may harbor potential QTLs important for both TBLM and BMD. *GREM1* (gremlin 1), located at this region, is a BMP antagonist that is expressed in osteoblasts, and opposes BMP's effects on osteoblast differentiation and function in vitro.[39] Moreover, transgenic mice overexpressing *GREM1* have decreased osteoblast number and function, leading to osteopenia and spontaneous fractures.[39] *GREM1* is also highly expressed in human fetal skeletal muscle cells, and regulates myogenic progenitor proliferation.[40] Combined with these known functions, our findings suggest that *GREM1* is an important candidate gene for both TBLM and BMD variation.

For chromosome regions 15q13 and 7q21, both complete pleiotropy and complete co-incident linkage effects were suggested. We propose that there could be multiple functional variants underlying the linkage signal, with some variants influencing both TBLM and BMD (pleiotropic effects) and some influencing either TBLM or BMD but not both (effects of co-incident linkage).

In summary, this study represents our pioneering effort to map the QTLs shared by TBLM and BMD at the spine and hip. We were successful in identifying several significant genomic regions whose effects on TBLM and BMD were sex-specific, pleiotropic, or caused by co-incident linkage. This study paves the way for further fine mapping and identification of QTLs underlying variation in both TBLM and BMD and for unraveling the mechanisms underlying genetic correlation of TBLM and BMD.

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