Genome-Wide Linkage Screen of Bone Mineral Density (BMD) in European Pedigrees Ascertained through a Male Relative with Low BMD Values: Evidence for Quantitative Trait Loci on 17q2123, 11q1213, 13q1214, and 22q11

Jean-Marc Kaufman, Agnès Ostertag, Aude Saint-Pierre, Martine Cohen-Solal, Anne Boland, Inge Van Pottelbergh, Kaatje Toye, Marie-Christine de Vernejoul and Maria Martinez

Genome-Wide Linkage Screen of Bone Mineral Density (BMD) in European Pedigrees Ascertained through a Male Relative with Low BMD Values: Evidence for Quantitative Trait Loci on 17q21–23, 11q12–13, 13q12–14, and 22q11

Jean-Marc Kaufman, Agnès Ostertag, Aude Saint-Pierre, Martine Cohen-Solal, Anne Boland, Inge Van Pottelbergh, Kaatje Toye, Marie-Christine de Vernejoul, and Maria Martinez

Department of Endocrinology and Unit for Osteoporosis and Metabolic Bone Diseases (J.-M.K., I.V.P., K.T.), Ghent University Hospital, 9000 Ghent, Belgium; Institut National de la Santé et de la Recherche Médicale Unit 606 (A.O., M.C.-S., M.-C.d.V.), 7547 Paris, France; Institut National de la Santé et de la Recherche Médicale Unit 563 (A.S.-P., M.M.), 31024 Toulouse, France; Centre National de Génotypage (A.B.), 91057 Evry, France; and Department of Endocrinology (I.V.P.), Onze-Lieve Vrouweziekenhuis Ziekenhuis Aalst, 9300 Aalst, Belgium

Context: Bone mass is under strong genetic control, with heritability estimates greater than 50% and is likely determined by complex interactions between genetic and environmental factors.

Objective: The objective of the study was to localize genes contributing to bone mineral density (BMD) variation.

Design: An autosomal genome-wide scan for BMD at the lumbar spine and femoral neck was conducted with variance components linkage methods.

Participants: A total of 103 pedigrees (Network in Europe on Male Osteoporosis Family Study) ascertained through a male relative with low (Z-score ≤ –2) BMD values at either lumbar spine or femoral neck.

Main Outcome Measures: Nonparametric multipoint logarithm of the odds ratio scores for lumbar spine and femoral neck BMD values adjusted for age, gender, and body mass index.

Results: We identified a total of eight chromosomal regions with logarithm of the odds ratio score of 1.5 or greater (P ≤ 5 × 10⁻⁴): on 1q42–43, 11q12–13, 12q23–24, 17q21–23, 21q22, and 22q11 for lumbar spine and on 5q31–33 and 13q12–14 for femoral neck BMD.

Conclusions: Four of our detected quantitative trait loci (QTL) reached the genome-wide criteria for significant (17q21–23, P ≤ 2 × 10⁻⁴) or suggestive (11q12–13, 22q11, and 13q12–14, P ≤ 7 × 10⁻³) linkage. Apart from 22q11, which is a novel QTL, all other loci provide consistent replication for previously reported QTLs for BMD and other bone-related traits. Finally, several of our specific linkage areas encompass prominent candidate genes: type 1 collagen (COL1A1) and the sclerosteosis/van Buchem disease (SOST) genes on 17q21–23; the low-density lipoprotein receptor-related protein 5 (LRP5) gene on 11q12–13; and the rank ligand gene on 13q12–14. Further analysis of these positive regions by fine linkage disequilibrium mapping is thus warranted. (J Clin Endocrinol Metab 93: 3755–3762, 2008)

Osteoporosis is a common multifactorial disease characterized by reduced bone mass and high susceptibility to low-trauma fractures. Low bone mineral density (BMD) is a major risk factor for osteoporotic fracture. Bone mass is under strong genetic control, with heritability estimates greater than 50% and is likely determined by complex interactions between genetic and environmental factors.
environmental factors, throughout fetal development childhood and adult life. Several genes may be involved in BMD regulation and/or BMD loss. Evidence from studies in animals and humans suggests that the genetic control of BMD may differ by skeletal sites and between genders and/or ethnic populations (1–3). Numerous molecular association or linkage studies aiming to identify genes for BMD determination have been performed, but to date, no clear consensus has been reached. Candidates might be genes involved in cytokine-signaling pathways, the hormonal regulation of calcium homeostasis, or the function of bone cells. Several positive associations for various BMD phenotypes have been reported with different candidate genes and/or polymorphisms, but the role and effect size of the associated polymorphisms/genes remain unclear (1, 2). Similarly, a large number of chromosomal regions have been reported as positively linked to BMD (4–13), but for the majority of these linkage signals, the contributing specific genes have not been identified. Indeed, few of the genomic regions thus far revealed meet the criteria for genome-wide significance, and/or there has been limited replication between studies. A number of factors may have confounded the studies (small sample size, clinical or genetic heterogeneity), making interpretation of the results difficult. Replication and confirmation of the findings are essential to enable conclusions to be drawn.

Here we undertook a full genome-wide screen for BMD variation in a sample of 103 pedigrees recruited within the thematic Network in Europe on Male Osteoporosis (NEMO) and ascertainment through a male with low BMD values (Z-score ≤ −2) at either the lumbar spine or femoral neck. This family sample offers the possibility to investigate the genetics of BMD in a rather unique collection of families collected through a male younger than 67 yr with idiopathic osteoporosis.

Subjects and Methods

Family data

The NEMO family study includes 103 Caucasian pedigrees selected through a male relative with idiopathic osteoporosis. Probands were ascertained from 1995 to 2003 in Belgium and France. The sampling scheme and inclusion/exclusion criteria have been elaborated elsewhere (14–16). Briefly, to be eligible as a proband, the subject had to be a male; needed to have a low bone mass, arbitrarily defined by a bone densitometry Z-score of −2.0 or less at the lumbar spine or femoral neck, secondary causes of osteoporosis having been excluded; and aged between 19 and 67 yr. Z-scores are BMD values expressed as units of SDs from the mean for an age- and gender-matched general referent healthy population. Family information was collected on all living first-degree relatives (parents, siblings, children), the proband’s spouse, and second or more distant relatives. Relatives, aged between 19 and 85 yr and who agreed to participate, underwent similar clinical investigation as for probands. Gender, age at examination, weight, and height were measured on the visit when the BMD measurements were performed. From all participants a written informed consent was obtained for the study, which was approved by the Ethical Committee of the Ghent University Hospital and the Lariboisière Hospital, respectively.

Measurements of phenotypes

BMDs (grams per square centimeter) of spine and femoral neck were measured using dual-energy x-ray absorptiometry with a QDR 2000 device (software version 7.2; Hologic Inc., Bedford, MA) in Belgium and a DPX-L densitometer (Lunar Corp., Madison, WI) in France. Machines were calibrated daily, and in both participating centers, the coefficient of variation for measurement of a phantom was less than 1%. For the spine, the quantitative phenotype was combined BMD of L2-L4 and L1-L4 for Lunar and Hologic measures, respectively. Members of the same pedigree were measured in the same center and on the same type of machine; data obtained from the two different osteodensitometers were made compatible by linear regression. Phenotypes analyzed. Before linkage analyses each BMD trait was adjusted for relevant risk factors, including age, gender, and body mass index (BMI), using multiple regression. We used a quadratic function to investigate BMD variations with age. BMI was calculated as weight in kilograms divided by height in meters squared. To remove the effects of these variables, and all possible interactions among them, regression models were built separately in three groups of family members (probands, male and female relatives), as explained in detail elsewhere (17). From the fitted model, a residual value was derived for each subject and each BMD. The distributions of the residuals displayed significant skewness and kurtosis: $P = 7 \times 10^{-5}$ and $P = 4 \times 10^{-5}$ for lumbar spine-BMD and femoral neck-BMD, respectively. To achieve a normal distribution, we removed the effect of outliers and any residual phenotypic data beyond 3σ and used a natural logarithm to transform the residual levels. The new logarithmically transformed residuals of lumbar spine BMD and femoral neck BMD exhibited nonsignificant kurtosis: $P = 0.07$ and $P = 0.052$, respectively, and for these new traits, the linkage tests can be assumed to follow the standard distribution of logarithm of the odds ratio (LOD) scores (18).

Molecular analyses

Genotyping was carried out at the Centre National de Génotypage (Evry, France). From the whole NEMO sample, 103 families with at least two siblings with DNA available were initially genotyped with a panel of 441 autosomal markers. The Linkage Marker Set MD 10 (Applied Biosystems, Foster City, CA) formed the core marker set for the genome-wide screen. These microsatellite markers, labeled with fluorescent dyes (FAM, HEX, NED), are distributed at an average marker density of 7.9 cM and have an average heterozygosity of 75%. The Centre National de Génotypage has developed a protocol allowing the coamplification of up to six of these markers in a single reaction to be robust using dual 384-well GeneAmp PCR 9700 cyclers (Applied Biosystems) and an automated procedure for PCR and purification setup. Automatic genotyping was performed based on a series of Genetic Profiler software (version 1.1, American Biosciences, Buckinghamshire, UK).

Genotype interpretation and quality control

Before statistical analysis, rigorous genotype quality assurance was performed to ensure accurate binning of alleles. Automatic genotyping was performed based on a series of software processes implemented in Genetic Profiler software (version 1.1) applied to the raw MegaBACE data: trace processing, fragment sizing, allele calling, and assigning genotype quality scores. Consistency of the data with Mendelian inheritance and lack of recombination between loci was evaluated using Pedcheck (19) and other purpose-written software. Allele frequencies for the 441 markers were estimated from our family data by Vitesse 2.0 program (20). Marker order and intermarker distances were obtained from the published Marshfield maps (http://research.marshfieldclinic.org/genetics). We used the sex-average genetic map in all our linkage analyses.

Statistical linkage methods

Multiscore genomic scans for quantitative traits were performed using variance-components linkage method (21), as implemented in SOLAR (22). We estimated the genetic variance attributable to the region around a specific genetic marker ($\sigma^2_m$) by specifying the expected genetic covariances between arbitrary relatives as a function of the identity-by-descent relationships at a given marker locus assumed to be tightly linked to a locus influencing the quantitative trait. Linkage is evaluated by compar-
ing a model incorporating both a genetic additive variance and a polygenic component with a purely polygenic model (no linkage, \( \sigma^2_m = 0 \)). Minus twice the natural logarithm of this likelihood ratio is assumed to follow a one-sided \( \chi^2 \) distribution. The LOD score is the \( \chi^2 \) divided by 2 \( \ln \)10. True multilocus identity-by-descent probabilities were computed using the Markov chain Monte Carlo algorithm implemented in LOKI (23). The ascertainment scheme of pedigrees based on low BMD values of the probands was accounted for in the analyses by computing the likelihood of the pedigrees conditional on the likelihoods of their respective probands (24).

Results

Sample characteristics

The NEMO sample includes 103 extended pedigrees, ascertained in Belgium (n = 72) or France (n = 31). The family size ranged from four to 64 members in a pedigree (up to four generations), with a mean size of eight members (for a full description of the NEMO data, see Ref. 17). Table 1 shows the main characteristics of the NEMO sample. BMD phenotypic and genotypic data were available for a total of 589 and 610 individuals, respectively. Of a total of 3269 relative pairs, 566 (17%) are siblings or cousins. Probands had a mean age of 47.0 yr and a mean BMI of 24.2 kg/m² and, as expected, had on average lower BMD values than their relatives. Male and female relatives had similar age and BMI distributions. Initial analyses of the full cohort revealed significant relationships between each BMD trait and the covariates gender, age, and BMI. Together, these variables accounted for 16 and 25% of the total variation of lumbar spine-BMD and femoral neck-BMD, respectively. In the relatives in our sample, the mean (SE) of bone densities adjusted for age, sex, and BMI at lumbar spine and femoral neck are, respectively, 0.89 (0.13) and 0.88 (0.11) g/cm². In the probands, the mean (SE) of bone densities adjusted for age and BMI at the lumbar spine and femoral neck are, respectively, 0.71 (0.08) and 0.76 (0.09) g/cm². The adjusted bone densities are significantly (\( P < 10^{-15} \)) lower at both sites in the probands, reflecting our sampling scheme through low BMD values. Subsequent analyses were conducted using the log-transformed residual values of lumbar spine-BMD and femoral neck-BMD. Both adjusted traits were found to have high heritability estimates: 61 ± 0.07% (\( P < 2 \times 10^{-18} \)) and 42 ± 0.08% (\( P < 6 \times 10^{-15} \)), respectively.

Genome-wide linkage analysis

Multipoint linkage analyses were performed across all 22 autosomes. The genome-wide linkage test results for the adjusted lumbar spine and femoral neck BMD phenotypes are shown in Fig. 1. We identified eight chromosomal regions with multipoint LOD score greater than 1.5 (Table 2), on chromosomes 1 (LOD = 1.75, position = 252 cm, close to marker D1S2800), 11 (LOD = 2.64, position = 60 cm at D11S4191), 12 (LOD = 1.65, position = 118 cm close to marker D12S78), 17 (LOD = 3.63, position = 76 cm, at marker D17S787), 21 (LOD = 2.05, position = 44 cm, close to marker D21S266), and 22 (LOD = 2.74, position = 24 cm, close to marker D22S315) for lumbar spine-BMD and on chromosomes 5 (LOD = 1.53, position = 163 cm at D5S422) and 13 (LOD = 2.71, position = 36 cm close to D13S218) for femoral neck-BMD.

Each of the loci detected on these regions appears to affect primarily either spine or hip BMD, not both of these two skeletal sites. Overall, our linkage scan results do not reveal loci with substantial effect on BMD (i.e. LOD score above 1) at both skeletal sites (Fig. 1).

We conducted secondary analyses aiming to evaluate, within our eight identified regions, the hypothesis of quantitative trait locus (QTL) with gender-specific effects on BMD variability. We used the strategy, which has been mostly used to identify, in humans, gender-specific QTLs on BMD variation. Gender-specific linkage results were obtained by setting the BMD values of either women or men as missing values, when building the men-strata or women-strata, respectively. Phenotypes of probands were, however, not altered to compute gender-specific LOD scores corrected for ascertainment. The men-strata was slightly smaller than the women-strata: number of informative pedigrees (86 vs. 88) and subjects with known phenotypes (301 vs. 354), respectively. The results of gender-specific linkage analyses in the eight identified linkage peaks are shown in Fig. 2. Overall, within five regions, similar linkage trends were obtained in each gender strata. The most notable differences in the gender-specific LOD scores were observed on chromosomes 1q42–43 (LOD score = 0.96 vs. 0.11), 5q31–33 (LOD score = 0.06 and 1.26), and 22q11 (LOD score = 3.54 vs. 0.52) in women and men, respectively. The evidence for linkage was found increased within one region only: on 22q11 (LOD score = 3.54, in women vs. LOD score = 2.74 in the full data).

### TABLE 1. Characteristics of the NEMO sample

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Probands</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>821</td>
<td>103</td>
<td>332</td>
<td>386</td>
</tr>
<tr>
<td>With DNA</td>
<td>610</td>
<td>103</td>
<td>219</td>
<td>288</td>
</tr>
<tr>
<td>Measured covariates and BMD phenotypes</td>
<td>589</td>
<td>103</td>
<td>215</td>
<td>271</td>
</tr>
<tr>
<td>Mean age (yr)</td>
<td>43.3 ± 15.8</td>
<td>47.0 ± 11.7</td>
<td>40.2 ± 15.9</td>
<td>43.8 ± 16.7</td>
</tr>
<tr>
<td>Range</td>
<td>19–82</td>
<td>19–67</td>
<td>19–80</td>
<td>19–82</td>
</tr>
<tr>
<td>Mean BMI (kg/m²)</td>
<td>24.5 ± 4.1</td>
<td>24.2 ± 3.4</td>
<td>24.6 ± 3.6</td>
<td>24.6 ± 4.7</td>
</tr>
<tr>
<td>Range</td>
<td>14.8–45.2</td>
<td>14.8–34.5</td>
<td>16.8–37.0</td>
<td>16.4–45.2</td>
</tr>
<tr>
<td>Mean BMD (g/cm²)</td>
<td>0.89 ± 0.15</td>
<td>0.75 ± 0.10</td>
<td>0.93 ± 0.13</td>
<td>0.92 ± 0.14</td>
</tr>
<tr>
<td>Lumbar spine</td>
<td>0.75 ± 0.13</td>
<td>0.67 ± 0.11</td>
<td>0.79 ± 0.13</td>
<td>0.74 ± 0.12</td>
</tr>
</tbody>
</table>
Discussion

Our multipoint genome scan identified eight chromosomal regions positively linked to lumbar spine-BMD or femoral neck-BMD with LOD score values of 1.5 or greater. Based on the theoretical genome-wide thresholds (25), we obtained one region with significant (i.e. point-wise \( P = 2.2 \times 10^{-5} \)) and three regions with suggestive (i.e. point-wise \( P = 7.4 \times 10^{-4} \)) evidence for linkage. It is worth noting that several of our eight positive findings overlap with major QTL identified in previous genome-wide scans for BMD and/or encompass prominent candidate genes for BMD variation.

We found little overlap between QTL for lumbar spine and femoral neck. This is consistent with previous whole-genome studies (4, 6, 8, 11, 13) that have reported linkage on different chromosomal regions to BMD at the spine or hip.

Previous genome-wide linkage studies have also suggested that some of the genes regulating BMD may act in a gender-specific manner. Only one of our identified linkage regions supports this hypothesis: evidence for linkage on 22q11 was obtained in women (LOD score = 3.54) but not men (LOD score = 0.52).

**FIG. 1.** Multipoint results of the genome-wide linkage scan for adjusted lumbar spine and femoral neck BMD values in 103 NEMO pedigrees.
Our highest linkage peak was found on 17q21–23 (LOD = 3.63, at marker D17S787). The same region has been previously identified but for other bone-related phenotypes: wrist bone size (26) and femur head width (27), with a multipoint LOD score of 3.01 and 3.6, respectively. The one-unit support interval surrounding our peak has a chromosomal location in the range of 67–80 cM. It encompasses two prominent candidate genes for BMD: type 1 collagen (COL1A1) and the sclerosteosis/van Buchem disease (SOST) gene. COL1A1 is one of the most widely studied candidate genes. It has been significantly associated with osteoporotic fracture risk, but its role on BMD variation remains unclear (2, 28–30). So far, and to our knowledge, two association studies investigated the impact of polymorphisms in the SOST gene on BMD and came to contradicting results. In a sample of 619 women, lumbar spine-BMD was not found to be associated with SOST sequence variants (31). The second study used a larger cohort (1939 men and women) and showed that the polymorphisms associated with BMD differed in women and men and that the association was mainly observed in the older subjects (32). It also reported significant interaction effects between polymorphisms at the SOST and COL1A1 genes. Altogether it is possible that either one or both of these two candidate genes explain our linkage signal on 17q21. However, the associated polymorphisms, so far identified by association studies, seem to have very modest effects on BMD variation. Under such conditions, it is striking to observe such a relatively high linkage peak as we obtained on 17q21. We plan to further explore the contribution of these two candidate genes on BMD variation through linkage disequilibrium mapping and also to estimate the amount of the linkage signal that can be explained by these candidate genes’ polymorphisms.

We obtained suggestive evidence for a QTL affecting lumbar spine-BMD variation on chromosomes 22q11–12 (LOD score = 2.74, close to D22S315) and 11q12–13 (LOD score = 2.64, close to D11S4191). The QTL on 22q11 is novel and does not overlap with major QTLs reported by other studies. Linkage for lumbar spine-BMD to the 11q12–13 genomic region is supported by two previous studies (6, 33). The first study (33) studied a single large kindred with autosomal dominant high lumbar spine-BMD, and found a linkage peak (maximum LOD score = 5.74) at D11S987, which is about 7 cM telomeric to our peak. The second study reported a multipoint LOD score of 1.97 close to D11S1313 (~2 cM centromeric to our peak) for lumbar spine-BMD in a sample of 835 normal premenopausal Caucasian and Afro-American sisters (6). Other studies identified different regions on chromosome 11: at 47 cM, close to D11S4148, in a sample of Irish families selected through a proband with low BMD (34); and at 109 cM, close to D11S908 (12). The 11q12-13 contains several putative candidate genes, as the T cell immune regulator 1 (TCIRG1) and the low-density lipoprotein receptor-related protein 5 (LRP5) gene. Mutations in LRP5 have been shown to lead to severe Mendelian bone phenotypes and can cause either markedly high or low bone mass traits (35, 36). Thereafter a number of studies have demonstrated that common polymorphic variants in LRP5 are associated with normal bone mineral density (37–41). Some studies suggested, however, that the contribution of LRP5 might depend on gender or be limited to women (42, 43). Our secondary linkage analyses within the 11q12–13 region did not favor the hypothesis of a QTL acting in a gender-specific manner on lumbar spine-BMD variation (Fig. 2).

Our third suggestive QTL was obtained on chromosome 13q12–14 (LOD score = 2.71, near D13SS218) and for femoral neck-BMD. Previous genome-scan studies have identified linkage peaks in the 13q14 region for different BMD phenotypes. A linkage peak (multipoint LOD score = 1.67, position = 46–55 cM) for distal forearm BMD has been obtained in a Chinese sample of 96 nuclear families (5). The same region has also been identified by gender-specific linkage analyses only but in the opposite gender-strata (9, 44). The study of 29 Mexican-American families (9) revealed, in men only, 13q linkage peaks for neck (LOD = 2.51, position 50 cM) and trochanter (LOD = 3.46, position 45 cM) BMD. Conversely, the study of 941 Asian nuclear families (44) found, in women only, significant evidence for a QTL on 13q for lumbar spine-BMD (LOD score = 3.62, position 40 cM). Potential candidate genes include TNF ligand superfamily, member 11 (TNFSF11) gene, which encodes receptor activator of nuclear factor-κB ligand (RANKL), the receptor of TRANCE (TNF-related activation-induced cytokine), a TNF family member. Both are critical regulators of dendritic cell and osteoclast function, bone resorption, and calcium homeostasis. A few studies have investigated the association of RANKL polymorphisms with BMD and come to conflicting results. A genetic

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Position (cm)</th>
<th>LODmax</th>
<th>Marker</th>
<th>Pointwise P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>2.75</td>
<td>D152800</td>
<td>2.25×10⁻³</td>
</tr>
<tr>
<td>11</td>
<td>60</td>
<td>2.64</td>
<td>D11S4191</td>
<td>2.46×10⁻⁴</td>
</tr>
<tr>
<td>12</td>
<td>118</td>
<td>1.65</td>
<td>D12S278</td>
<td>2.93×10⁻³</td>
</tr>
<tr>
<td>17</td>
<td>76</td>
<td>3.63</td>
<td>D17S787</td>
<td>2.19×10⁻⁵</td>
</tr>
<tr>
<td>21</td>
<td>44</td>
<td>2.05</td>
<td>D21S266</td>
<td>1.05×10⁻³</td>
</tr>
<tr>
<td>22</td>
<td>24</td>
<td>2.74</td>
<td>D22S315</td>
<td>1.90×10⁻⁴</td>
</tr>
<tr>
<td>FN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>163</td>
<td>1.53</td>
<td>D5S422</td>
<td>3.98×10⁻³</td>
</tr>
<tr>
<td>13</td>
<td>36</td>
<td>2.71</td>
<td>D13S218</td>
<td>2.05×10⁻⁴</td>
</tr>
</tbody>
</table>

* Marker positions using sex-average genetic maps from the Center for Medical Genetics, Marshfield Medical Research Foundation (http://research.marshfieldclinic.org/genetics).
screen of the promoter region identified several polymorphisms, and some were found positively associated with femoral neck-BMD in a sample of postmenopausal women (45). On the contrary, positive association with hip BMD has been found in men but not women (46). These association studies are, however, based on a limited number of polymorphisms in RANKL, which could explain their conflicting results. A recent family-based association study used a more systematic approach to screen the underlying genetic variation and showed strong positive association with hip-BMD (47). Additional sex-stratified analyses sug-

![FIG. 2. Linkage peaks identified in the NEMO data: multipoint LOD scores for lumbar spine (LS) and femoral neck (FN) BMD in the combined and by gender-specific strata.](image)
gested, however, that the observed association was mainly driven by the male subjects. In our NEMO data, gender-specific linkage analyses showed a slightly higher LOD score value in the men (LOD score = 1.42) than the women (LOD score = 0.75) strata (Fig. 2), but evidence for linkage is weaker in the men strata than the combined data, suggesting that both strata contribute to the linkage peak on 13q12–14.

All our next linkage peaks are consistent with previous studies. Our finding for lumbar spine on 12q23–24 (LOD score = 1.65, close to D12S78) overlaps with the linkage peak (LOD = 1.63, close to D12S79, position = 125 cm) reported for femoral neck in a sample of 40 multiplex Caucasian pedigrees ascertained through a proband with osteoporosis (48). Our finding on 1q42–43 coincides with the linkage peak (LOD score = 2, position = 255 cm, close to D1S2800) identified in a sample of 715 European pedigrees (13). Similarly, our linkage peak on 5q31–33 for femoral neck-BMD was previously identified in a sample of 595 sister-pairs for the same skeletal site and close to the same marker D5S422 (LOD score = 2.23) (6). This 5q31–33 peak encompasses the cytokine cluster and the reversion-induced LIM gene (RIL), which has been shown associated with radial BMD in adult Japanese women (49). Our 21q22 peak has also been previously identified. The first study observed suggestive linkage (LOD score = 3.14, close to D21S1446) of trochanteric BMD (8). A more recent work performed gender-specific linkage analyses and obtained, in men only, linkage evidence at 21q22 (LOD score = 3.36) for spine BMD (50). Possible candidates from this region are collagen VI, α1- and α2 (COL6A1, COL6A2) polypeptide genes.

In conclusion, our study is the first genome-wide linkage screen for genes underlying BMD variability performed, to date, in European pedigrees ascertained through a male relative with low BMD levels. Our results show novel linkage regions and also support some of the linkage regions previously reported. The site-specific differences in the heritability of BMD are already well acknowledged. Similar to other studies, we found differential linked regions for BMD at specific skeletal sites, supporting the view that different genes regulate BMD at different skeletal sites and that BMD as measured by dual-energy x-ray absorptiometry is a complex phenotype, a composite reflection of volumetric BMD and bone geometry. These observations highlighted the complexity of the interplay between genetic and environmental factors to determine the final BMD variance at specific sites. We found significant linkage on chromosome 17q21–23 and suggestive linkage on chromosomes 11, 13, and 22 for QTLs contributing to BMD variation at the lumbar spine or femoral neck. Further analysis of these positive regions by fine association, by identifying additional QTLs, not revealed by linkage approaches.

Acknowledgments
Address all correspondence and requests for reprints to: Dr. Maria Martinez, Ph.D., Institut National de la Santé et de la Recherche Médicale Unit 563, Hôpital Purpan, BP 31024 Toulouse, France. E-mail: maria.martinez@toulouse.inserm.fr.

This work was supported in part by the Network in Europe for Male Osteoporosis (European Commission Grant QL6-CT-2002-00491), the Flemish Fund for Scientific Research (FWO Vlaanderen Grants G.0331.02 and G.0662.07); the Société Française de Rhumatologie, and the French National Agency of Research.

Disclosure Statement: The authors have nothing to disclose.

References


51. Semenov MV, He X 2006 LRP5 mutations linked to high bone mass disease cause reduced LRP5 binding and inhibition by SOST. J Biol Chem 281:38276–38284


3762