Variance component linkage analysis indicates a QTL for femoral neck bone mineral density on chromosome 1p36

Marcella Devoto1,2,*, Claudia Specchia1,3, Hui-Hua Li1, John Caminis4, Alan Tenenhouse4, Hetty Rodriguez5 and Loretta D. Spotila5

1Department of Research, AI duPont Hospital for Children, Wilmington, DE 19899, USA 2Department of Oncology, Biology and Genetics, University of Genoa, Genoa, Italy, 3Department of Health Science, University of Genoa, Genoa, Italy, 4McGill Bone Centre, Montreal, Canada and 5Department of Bioscience and Biotechnology, Drexel University, Philadelphia, PA, USA

Received June 29, 2001; Revised and Accepted August 13, 2001

Osteoporosis is a common condition characterized by reduced skeletal strength and increased susceptibility to fracture. Eight million Americans over the age of 50 have osteoporosis of the femoral neck. The most important risk factor for osteoporosis is low bone mineral density (BMD), and several epidemiological studies have shown the importance of genetic factors in determining variability of BMD. An initial genome screen in seven large pedigrees suggested that a candidate region conferring susceptibility to low BMD of the femoral neck was located on chromosome 1p36. We have now confirmed and extended this finding by analyzing nine microsatellite markers spanning a 40 cM interval across the candidate region in an expanded sample of 42 families. Heritability of femoral neck BMD was estimated as 0.51 ± 0.13 in these families, after accounting for the effects of age, sex, body mass index, height and weight. Variance component linkage analysis yielded a maximum multipoint LOD score of 3.53 for linkage of femoral neck BMD to a quantitative trait locus (QTL) located near marker D1S214. The associated empirical P-value by simulation analysis was equal to 0.0001. The results strongly support the hypothesis that a major QTL controlling femoral neck BMD is located on chromosome 1p36.2–p36.3, and further analysis of candidate genes in this region is warranted.

INTRODUCTION

Osteoporosis is a disease characterized by low bone mineral density (BMD) and poor bone quality. During linear growth, new bone is synthesized and the maximum amount of bone mineral is accrued. After the third decade of life, bone is maintained by a balanced cycle of bone resorption and bone synthesis. However, as aging progresses, the resorption phase of bone metabolism outweighs the bone synthesis phase and results in absolute bone loss. Thus the risk of osteoporosis and fracture increases with age. The prevalence of osteoporosis at the femoral neck in US women 50 years and older is estimated at 18%, and in men is ~4% (1). In 1996, the population rate of hip fractures was 934 per 100 000 (2), and the estimated annual cost of medical care for hip fracture patients was over nine billion dollars (3).

The contribution of genetics to bone quality and BMD in particular is substantial (4–6). By comparing BMD in monozygotic and dizygotic twins, heritability of lumbar spine and femoral neck BMD was estimated at 92 and 70%, respectively (7). Similarly, heritability of bone mineral content of the lumbar spine was estimated as 0.88 in a study group younger than 25 years (8). Daughters of women with low bone mass have reduced bone mass at the lumbar spine and the femoral neck (9). Contrasting results have been obtained about the influence of genetic factors on the rate at which mineral is lost from bone (10,11). Segregation analysis has been used to evaluate the mode of transmission of BMD (12), and several studies have found evidence for a single major gene conferring susceptibility to low BMD in different populations (13–15).

A study of high bone mass in a single family indicated linkage to chromosome 11q12–q13 (16), consistent with the idea that a single locus can have a major effect on variance in bone density. That this locus may be generally important in determining variation in BMD was demonstrated by a linkage study of healthy female sibpairs (17,18). Several candidate genes, including parathyroid hormone receptor, interleukin-6, insulin-like growth factor I (IGF1), tumor necrosis factor α, vitamin D receptor (VDR), COL1A1, estrogen receptor (ER) and transforming growth factor β (TGFβ), have been analyzed for linkage or association in different populations with contrasting results (19–27).

Whole genome scans for linkage have been conducted to a limited extent. In a Chinese cohort selected for hypertension, forearm bone density was modestly linked to chromosomes 2p21 and 13q34 (28). The most extensive whole genome screens published to date present evidence of linkage of lumbar spine BMD to chromosomes 1q21–q23 and 6p11–p12, and femoral neck BMD to 5q33–q35 (18) and femoral structure to 5q, 4q and 17q (29).

*To whom correspondence should be addressed at: Department of Research, AI duPont Hospital for Children, AR 210A, PO Box 269, Wilmington, DE 19899, USA. Tel: +1 302 651 6838; Fax: +1 302 651 6895; Email: mdevoto@nemours.org
We conducted a whole genome screen in seven families with multiple individuals presenting with low BMD. The characteristics of these families and results of commingling analysis on the distribution of spine BMD (30) and femoral neck BMD (unpublished data) were consistent with a major autosomal dominant gene inheritance. Linkage to candidate regions containing COL1A1, COL1A2 and VDR was not demonstrated (30). However, linkage to several other chromosomal regions was suggested by both model-based and non-parametric sibpair analyses (31). Chromosomal regions suggested by these results were 1p36, 2p23–p24 and 4qter. In particular, analysis of allele-sharing by the sibpair Haseman–Elston test suggested linkage of femoral neck BMD to chromosome 1p36 with a multipoint LOD score of 2.29 (31).

Seeking to extend the initial positive observations, we recruited 36 additional families and analyzed nine markers on chromosome 1p36.2–p36.3 with an average spacing of 5 cM. In this study we describe results obtained by variance component analysis of both lumbar spine and femoral neck BMD in this expanded cohort of families.

RESULTS

A total of 42 families were ascertained on the basis of the presence of one individual with a spinal BMD Z-score of less than –2.00. 254 individuals from these families were evaluated for BMD at the lumbar spine (L2–L4) and femoral neck. Covariates of age, weight, height and body mass index (BMI) were also determined (Table 1). Values of skewness and kurtosis were 0.08 and –0.18 for spinal BMD, and 0.56 and 0.46 for femoral neck BMD. Variance component linkage analysis is known to be robust to deviation from the assumption of normality under a variety of scenarios (32,33), but extreme leptokurtosis of 2 or greater may lead to inflation of type I error.

We estimated heritability of spine and femoral neck BMD and the significance of the covariates (Table 1) using the likelihood method implemented in SOLAR (34). In addition to the covariates described in Table 1, terms defining ethnicity, age squared effects and sex-specific age effects (or sex–age interaction) were included in the quantitative analysis. The five different ethnic groups represented were French (n = 4 families), Greek (n = 17), Italian (n = 3), Jewish (n = 7) and Middle Eastern (n = 3). An additional group was defined for nine additional families that were of mixed ethnicity.

Heritability was 0.51 ± 0.13 for femoral neck BMD (P < 0.00001) and 0.79 ± 0.10 for spine BMD (P < 0.00001). Age, sex, BMI and weight showed a significant effect on variability of femoral neck BMD (all P-values < 0.05). Height was marginally significant (P = 0.07); whereas ethnicity, age squared and sex-specific age were not significant (P > 0.1). Overall, the proportion of phenotypic variance in femoral neck BMD explained by these covariates was 0.46. Only weight and sex-specific age had significant effects on spine BMD (P-values < 0.02), and explained ~27% of the spine BMD variance.

We then performed two-point linkage analysis with nine markers on chromosome 1p36 using the variance component procedure implemented in SOLAR (Table 2). Marker allele frequencies and inter-marker distances were estimated in our sample using maximum likelihood procedures implemented in SOLAR and ILINK (35), respectively. All covariates with P-values < 0.1 in the quantitative genetic analysis were included in the linkage analyses. A maximum two-point LOD score of 3.02 was obtained with marker D1S214 for femoral neck BMD. Two-point LOD scores >2 were obtained for femoral neck BMD for three additional markers (D1S468, D1S2870 and D1S450). The maximum two-point LOD score for spine BMD was 0.56 also for marker D1S214.

Multipoint variance component linkage analysis was then carried out at constant increments of 1 cM along the genetic map defined by the nine chromosome 1p36 markers (Fig. 1). The maximum LOD score observed for femoral neck BMD was 3.53 at 10 cM, corresponding to the position of marker D1S214. The total additive genetic variance explained by this locus was 0.56, with no residual additive genetic variance. The maximum LOD score for spine BMD was 0.63 at 11 cM (data not shown).

Table 1. Distributions of traits and covariates in individuals from 42 families

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD or total number (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
</tr>
<tr>
<td>No.</td>
<td>100</td>
</tr>
<tr>
<td>Spine BMD (g/cm²)</td>
<td>1.07 ± 0.18 (0.68–1.49)</td>
</tr>
<tr>
<td>Femoral neck BMD (g/cm²)</td>
<td>0.95 ± 0.16 (0.58–1.47)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>41.2 ± 16 (18–84)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.8 ± 15.1 (48–120)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173.3 ± 8.7 (151–193)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.6 ± 4.8 (17.2–40.5)</td>
</tr>
</tbody>
</table>

*Number of individuals for whom information on traits and covariates was available.

Table 2. LOD scores from two-point variance component linkage analysis

<table>
<thead>
<tr>
<th>Marker</th>
<th>cM*</th>
<th>Femoral neck BMD</th>
<th>Spine BMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1S468</td>
<td>6.6</td>
<td>2.14</td>
<td>0.08</td>
</tr>
<tr>
<td>D1S2870</td>
<td>3.1</td>
<td>2.22</td>
<td>0.00</td>
</tr>
<tr>
<td>D1S214</td>
<td>5</td>
<td>3.02</td>
<td>0.56</td>
</tr>
<tr>
<td>D1S2694</td>
<td>6.5</td>
<td>0.61</td>
<td>0.43</td>
</tr>
<tr>
<td>D1S450</td>
<td>5</td>
<td>2.18</td>
<td>0.30</td>
</tr>
<tr>
<td>D1S244</td>
<td>5.3</td>
<td>0.74</td>
<td>0.17</td>
</tr>
<tr>
<td>D1S2667</td>
<td>7.3</td>
<td>1.10</td>
<td>0.00</td>
</tr>
<tr>
<td>D1S434</td>
<td>4.7</td>
<td>0.04</td>
<td>0.00</td>
</tr>
<tr>
<td>D1S507</td>
<td>0.02</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>

All LOD scores are at a recombination fraction θ = 0 from the corresponding marker.

*Distance from the next marker estimated by ILINK in this sample of families.
DISCUSSION

Despite efforts in many laboratories, no major gene influencing susceptibility to osteoporosis has so far been identified. Various studies have focused on association analysis of candidate genes with BMD, one of the major factors in determining the risk of osteoporosis fractures. Although results of these studies have sometimes been controversial, and replication studies have often yielded contradictory results, several loci are undoubtedly important at least in some populations (4–6). These are VDR, COL1A1, IGF1, TGFβ, ER and others.

One limitation of population association studies, which may impact the ability to replicate an initial finding, is the risk of false positive results due to population stratification. In contrast, the pedigree-based linkage approach is not affected by population structure, and has the advantage of providing estimates of the heritability of the loci thus identified. We initially carried out a whole genome scan for genes affecting BMD in seven large pedigrees with a high prevalence of individuals affected with osteoporosis (31). Results of sibpair analysis indicated that chromosomes 1, 2 and 4 might contain genes involved in determining low BMD. In particular, a maximum LOD score of 2.29 was obtained by multipoint linkage analysis of femoral neck BMD near marker D1S450, located in 1p36 (31).

To follow up these findings, we recruited 36 additional families from the same Canadian population as six of the original families and typed a total of nine microsatellite markers spanning a 40 cM interval centered on D1S450. Variance component multipoint linkage analysis has confirmed and extended the significance of our initial results, with a maximum LOD score of 3.53 for linkage of femoral neck BMD to marker D1S214, which is located ∼10 cM distal to D1S450. To account for possible deviations of the trait distribution from the assumption of multivariate normality, which may inflate the probability of type I error, we estimated the significance of the maximum LOD score by means of an empirical null distribution obtained by Monte Carlo simulation. Of 40 000 unlinked markers simulated to segregate in the 42 families, only four had LOD scores larger than the one we observed, thus resulting in a P-value of 0.0001. In contrast, no linkage was found for spine BMD to the same chromosomal region, which is consistent with our previous finding (31). Although correlated traits can share common genetic determinants, they often are influenced by trait-specific loci as well (36,37).

The present analysis differs from the sibpair linkage analysis reported earlier in at least two aspects (31). First, we were able to analyze all large pedigrees without subdividing them into their nuclear components. Secondly, we have directly estimated the effects of several covariates, such as age, sex, weight, height and BMI, known to influence BMD values. Inclusion of the effects of these covariates in the linkage analysis has allowed the use of the uncorrected BMD measures instead of the standardized Z-scores, which are obtained from

![Figure 1. Multipoint LOD scores for linkage of femoral neck BMD to microsatellite markers on chromosome 1p36.](image-url)
population control values not necessarily appropriate to our population. In spite of these differences, the positive linkage result has been confirmed in an extended sample of 42 families including six previously studied families and an additional group of 36 new independent families from the same population.

The variance component approach has estimated the heritability of the 1p36 quantitative trait locus (QTL) as 0.56, with no residual genetic variance. It has been shown that estimates of locus-specific heritability from variance component linkage analysis of whole genome scans tend to be biased upward (38). It is possible that a similar effect also exists for estimates obtained in linkage studies of a single genomic region like the one presented here. The observed value of 0.56 is slightly larger than, but within 1 SE of the overall heritability for hip BMD of 0.51 ± 0.13. Although it is unlikely that this is the only QTL accounting for variability of hip BMD, this result seems to indicate that the QTL located in 1p36 has a major effect in determining variability of hip BMD in these families.

The 1-LOD-unit support interval for our candidate region extends beyond the most distal marker included in our analysis, D1S468 (which has a multipoint LOD score of 2.83), and proximally up to marker D1S450. These two markers are separated by ~20 cM. The physical distance represented by this interval is ~7 Mb. Positional candidate genes in 1p36.2–p36.3 are limited to genes that have functions in many different tissues such as isoprenylcysteine carboxyl methyltransferase (ICMT) and tumor necrosis factor receptor superfamily member 9 (TNFRSF9). There are at least 16 additional known genes as well as about 40 gene sequences that have not been characterized.

In conclusion, we have provided significant evidence that a gene located in 1p36.2–p36.3 may be responsible for variation in femoral neck BMD in families with osteoporosis. The same QTL does not seem to affect variability of spine BMD, at least in most of the families studied here. Further studies with candidate genes from this region may help us understand the complex mechanisms that regulate bone density and confer susceptibility to osteoporosis.

MATERIALS AND METHODS

Patient ascertainment and BMD measurements

Six large families recruited at Montreal General Hospital were described previously (30,31). Briefly, families were identified through the presence of a proband who had a spinal BMD Z-score of less than −2.00 as measured by dual energy X-ray absorptiometry (DEXA) using the Lunar DPX densitometer (Lunar, Madison, WI), and who reported having relatives who might also be affected with osteoporosis. In some cases, radiographic evidence of osteopenia was also available. All available relatives were evaluated by DEXA at both the lumbar spine (L2–L4) and the femoral neck, thus confirming the presence of osteoporosis in other family members. The six families thus collected consisted of 137 individuals of which 34 had osteopenia or osteoporosis. One family included in the previous studies (family 5) (30,31) was excluded from the present analysis because it had been recruited at a different clinical site, and BMD in members of this family had been determined using a densitometer from a different manufacturer (30). Additional independent families were collected at Montreal General Hospital by identifying probands with low BMD and evaluation of available first-degree relatives. In all cases except five, the presence of low bone density or osteoporosis was observed in probands’ relatives. In this way we collected a total of 30 nuclear families with two to four children, and six three-generation families with two sibs of two to four offspring.

All individuals were examined at Montreal General Hospital, and bone density determinations were made on the same instrument. A 5–15 ml blood sample was collected from each participating individual for either genomic DNA extraction or lymphocyte culture. All subjects gave written informed consent, and the study was approved by the Institutional Review Board of the participating institutions.

Genotyping analysis

DNA was extracted from blood or lymphoblasts using the Genepure 341 (Applied Biosystems, Foster City, CA). Primer pairs for genotyping were purchased from Research Genetics (Huntsville, AL). The loci selected for testing were based on the GB4 GeneMap 99 of the International Radiation Hybrid Consortium (http://www.ncbi.nlm.nih.gov/genemap/). PCR and gel electrophoresis conditions were as described previously (31).

Statistical analysis

Statistical genetic analysis was carried out by means of the computer program SOLAR (34). Using this program, we first estimated heritability of spine and femoral neck BMD in our sample of families as the proportion of the trait variance attributable to additive genetic factors, while simultaneously estimating the significance of the effect of the covariates sex, age, age squared, sex-specific age (or sex–age interaction), height, weight, BMI and ethnicity. All covariates with associated P-values ≤0.1 from the quantitative analysis were retained in the linkage analysis.

Two-point and multipoint linkage analyses of spine and femoral neck BMD to nine chromosome 1p36 markers were then carried out using the variance component approach (34). In this approach, the total trait phenotypic variance is partitioned into components attributable to the effects of the covariates, the effect of the specific locus under scrutiny and residual additive genetic effects. A test for linkage is carried out by testing whether the variance attributable to the QTL is significantly different from 0. One of the advantages of this method as implemented in the program SOLAR is its ability to analyze pedigrees of general structure, without limitation on the number of individuals. This option gave us the opportunity to re-evaluate the results originally obtained in our first sample of six large families using a sibpair approach (31).

Because families were ascertained through the presence of an individual with low spine BMD, all linkage analyses presented here included an ascertainment correction by conditioning on the likelihood of observing the BMD value of the proband. However, we also reran all the analyses for hip BMD without the ascertainment correction, to account for the fact that the correlation between the two traits is <100%. Differences observed between the results of the two kinds of analyses were trivial, with LOD scores differing by 0.1 at most.

Marker order used in the multipoint linkage analysis was as reported in GeneMap 99. The genetic distances between
adjacent markers were estimated in our sample using the ILINK program (35). Marker allele frequencies used in all linkage analyses were estimated in our families by the maximum likelihood procedure implemented in SOLAR (34).

ACKNOWLEDGEMENTS

The authors wish to thank Dr Laura Almasy, Tom Dyer and Charles Peterson for extensive technical advice, and Drs Michael Mahaney and Yin Yao for critical reading of the manuscript. In addition, the authors acknowledge the assistance of Richard Kosich and Dr Jarmo Korkko in the laboratory of Dr Darwin Prockop for analysis of marker D1S450. This work was supported by National Institutes of Health grant HD36606 (L.D.S.).

REFERENCES


