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Exclusion mapping of chromosomes 1, 4, 6 and 14 with bone mineral density in 79 Caucasian pedigrees

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Abstract

Low bone mineral density (BMD) is a major determinant of osteoporosis and is under strong genetic control. A large number of linkage and association studies for BMD variation have been conducted, with the results being largely inconsistent. Linkage exclusion analysis is a useful tool for gene mapping but has never been used on BMD. In the present study, we conducted a linkage exclusion mapping for BMD variation on chromosomes 1, 4, 6 and 17 in 79 Caucasian pedigrees. For hip BMD variation, several genomic regions were excluded for effect sizes of 10% or greater, including regions of 61–77 cM at 1p35–p34, 167–196 cM at 1q21–q23 and 261–291 cM at 1q42–q44; 85–112 cM at 4q21–q25 and 146–150 cM at 4q31; and 77–85 cM at 6p12–q13. For spine BMD, we were able to exclude the regions of 168–189 cM at 1q21–q23, 92–94 cM at 4q21 and 106–107 cM at 4q24 and 56–103 cM at 17q12–q25, as having effect sizes of 10% or greater. These results suggest that a number of candidate genes located in the excluded regions, such as interleukin 6 receptor (IL6R) gene, type I collagen α 1 (COL1A1) gene and bone morphogenetic protein-3 (BMP3) gene are unlikely to have a substantial effect on BMD variation in this Caucasian population. Along with previous studies searching for genes underlying BMD variation, the current study has further delineated the genetic basis of BMD variation and provided valuable information for future genetic studies.

Keywords: Linkage exclusion analyses; Bone mineral density (BMD); Osteoporosis

Introduction

Low bone mineral density (BMD) is a major determinant for osteoporosis, which is a serious public health problem [1]. BMD variation is under strong genetic control with heritability ranging from 0.5 to 0.9 [2–4]. Several segregation studies have suggested that there exists at least one major gene for BMD variation, with effect size up to >30% [5–8]. Though a large number of linkage and association studies on BMD variation have been reported, the results are largely inconsistent [9,10]. This lack of consistency may be due to a number of confounding factors, such as limited statistical power, genetic heterogeneity, phenotype difference and multiple testing [11,12]. While studies searching *for* quantitative trait loci (QTL) are imperative for genetic dissection of complex traits, testing *against* genomic regions as putative QTL–exclusion mapping–can also provide useful information to constrict genomic regions which may contain QTLs [13,14]. So far, no exclusion mapping study has been conducted for BMD variation. In this study, we performed the first linkage exclusion mapping for BMD variation in a large sample of 79 Caucasian pedigrees.

Based on the results from our previous whole genome linkage scans [15,16] and studies from other groups, we selected chromosomes 1, 4, 6 and 17 for exclusion analyses. On chromosome 1, several groups have detected significant/suggestive linkage at 1p36 with BMD [17,18] or quantitative ultrasound [19]. Region on 1q21–23 also showed significant linkage with spine BMD variation [20,21]. On chromosome 4, BMD has been linked to several genomic regions, including

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4p15 [22] and 4q34 [17], and region containing the epidermal growth factor (EGF) gene [23]. Furthermore, in our first whole genome linkage scan [15], logarithm of odds (LOD) scores of 3.08 and 2.26 was achieved at 4q31 for spine BMD and wrist BMD, respectively. Chromosomes 6 and 17 contain several prominent candidate genes, such as tumor necrosis factor α (TNFA) gene and estrogen receptor α (ER-α) gene on chromosome 6 and type I collagen α 1 (COL1A1) gene on chromosome 17. These genes have all been linked or associated with BMD variation [9,10]. Given the above evidence, a number of genomic regions on the four chromosomes are promising candidates for enclosing QTLs for BMD variation. However, in our recent genome-wide linkage scan for BMD variation [16], LOD scores achieved on chromosomes 1, 4, 6 and 17 were all ≤1.5. Because of these contradictory results, we performed linkage exclusion analyses on the four chromosomes to exclude some genomic regions that do not actually contain a QTL underlying BMD variation in our sample of 79 Caucasian pedigrees.

Materials and methods

Subjects

This study was approved by Institutional Review Board at Creighton University. All the study subjects signed informed consent documents before entering the project. 1816 subjects from 79 pedigrees were used in the present study. All the subjects are Caucasians of European origin. Among the 79 pedigrees, 50 pedigrees were ascertained through probands having low BMD (Z score ≤ −1.28 at the hip or spine, which belongs to the bottom 10% in the distribution of the age-matched population), and 25 pedigrees were ascertained through probands with high BMD (Z score ≥ +1.28 at the hip or spine, which belongs to the top 10% in the distribution of the age-matched population). The remaining four pedigrees were recruited without regard to BMD values of the probands.

Only healthy subjects with no apparent non-genetic cause for low or high BMD were included in the analyses. The exclusion criteria have been detailed in our previous genome scan study [15]. Briefly, patients with chronic diseases and conditions that may potentially affect susceptibility to osteoporosis were excluded from the study. These diseases or conditions include chronic disorders involving vital organs (heart, lung, liver, kidney, brain), serious metabolic diseases (diabetes, hypo- and hyperparathyroidism, hyperthyroidism, etc.), other skeletal diseases (Paget's disease, osteogenesis imperfecta, rheumatoid arthritis, etc.), chronic use of drugs affecting bone metabolism (corticosteroid therapy, anti-convulsant drugs) and malnutrition conditions (chronic diarrhea, chronic ulcerative colitis, etc.), etc. The exclusion criteria were assessed by nurse-administered questionnaires or medical records.

Measurement

Areal BMDs (g/cm^2) at the lumbar spine (L1–L4) and hip (femoral neck, trochanter and intertrochanteric region) were measured by Hologic 1000, 2000+ or 4500 dual energy X-ray absorptiometry (DXA) scanners (Hologic Corp., Waltham, MA). Data obtained from different machines were transformed to a compatible measurement using the transformation formula described in Genant et al. [24]. This transformation was based on linear regression rules and was demonstrated as a reliable and efficient method of calibrating BMD measurements by different DXA machines [25]. Members of the same pedigree were usually measured on the same type of machine. The measurement precision as reflected by coefficients of variation for spine BMD, hip BMD and wrist BMD was 0.9%, 1.4% and 2.3%, respectively. Weight (kg) and height (m) were measured at the same visit of the BMD measurement. The basic characteristics of study subjects in our sample are summarized in Table 1.

Table 1
Basic statistics of the study subjects

	Mean	Standard deviation	Range
Age (year)	48.2	15.6	20.0–99.8
Spine BMD (g/cm^2)	1.038	0.166	0.499–1.820
Hip BMD (g/cm^2)	0.971	0.166	0.237–1.701
Height (m)	1.69	0.10	1.37–2.04
Weight (kg)	79.0	17.6	41.3–135.4

Genotyping

For each subject, DNA was extracted from 30 ml peripheral whole blood by employing the Puregene DNA Isolation Kit (Gentra Systems, Inc., Minneapolis, MN). All the subjects were genotyped for 37, 30, 19 and 23 microsatellite markers on chromosomes 1, 4, 6 and 17, respectively. All the markers were from ABI PRISM® Linkage Mapping Sets Version 2.5 (Applied Biosystems, Forster City, CA). These markers have an average population heterozygosity of ~0.77 and are ~8.3 cM apart.

Polymerase chain reactions (PCRs) were performed on PE 9700 thermocyclers (Applied Biosystems, Foster City, CA) with cycling conditions suggested in the manual of ABI PRISM® Linkage Mapping Sets Version 2.5. Marker allele identification and sizing were performed using ABI PRISM® 3700 DNA Analyzer (Applied Biosystems, Forster City, CA) and GENESCAN™ Version 4.0 and GENOTYPER™ Version 4.0 software. A genetic database management system (GenoDB) [26] was employed to manage the genotype data. PedCheck [27] was employed for checking the Mendelian inheritance pattern at all the marker loci and for confirming the alleged relationships of family members within pedigrees. The genotyping error rate was about 0.3% after three rounds of sample replication in genotyping and analyses.

Statistical analyses

Using sequential oligogenic linkage analysis routines (SOLAR) [28], we performed multipoint linkage exclusion analyses on chromosomes 1, 4, 6 and 17, respectively. Age, sex, weight, height and age-by-sex interaction were tested for importance on BMD variation by the polygenic analysis implemented in SOLAR. This analysis estimated the proportion of BMD variance caused by these factors and determined the statistical significance of each factor. Significant factors ($P \leq 0.05$) were then adjusted as covariates for raw BMD values.

In multipoint linkage exclusion analyses, we compared a series of models with fixed QTL effect sizes of 10%, 20% and 30%, with a model allowing for no QTL effect. The likelihood ratio test was employed in comparison of the competing models, producing a test statistic asymptotically distributed as χ^2, with degrees of freedom equal to the difference in the number of independent parameters being estimated in the two models.

To estimate the statistical power of linkage exclusion analysis in our sample, we performed a simulation test using SOLAR. Simulation was based on the observed data and pedigree structure of the study sample. We assumed that the simulated population was random, and the genotype data were in Hardy–Weinberg equilibrium. Quantitative traits and genotype data were simulated for 2000 replicates of the data set. The power to exclude a region as a QTL with certain effect size was calculated as the proportion of replicates for which we obtained an LOD score ≤ −2. Thus, the exclusion power is referred to as the probability that we can correctly exclude a tested candidate chromosomal region when it is not a QTL with certain effect size. Based on the simulation results, our sample may have >78% power to exclude a QTL with effect size of 10% or higher, but only have ~10% power to exclude a QTL with effect size of 5%. Therefore, we chose 10% as the lower bound for QTL effect in our linkage exclusion analyses.

Results

In Figs. 1 and 2, we show the results of linkage exclusion analyses for spine and hip BMD on chromosomes 1, 4, 6