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Quantitative Trait Loci for BMD Identified by Autosome-Wide Linkage Scan to Chromosomes 7q and 21q in Men from the Amish Family Osteoporosis Study

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ABSTRACT: Using autosome-wide linkage analysis in 964 Amish, strong evidence was found for the presence of genes affecting hip and spine BMD in men on chromosomes 7q31 and 21q22 (LOD = 4.15 and 3.36, respectively).

Introduction: BMD is highly heritable, with genetic factors accounting for 60–88% of variation. The goal of this study was to localize genes contributing to BMD variation.
Materials and Methods: The Amish Family Osteoporosis Study was designed to identify genes affecting bone health. The Amish are a genetically closed population with a homogeneous lifestyle. BMD was measured at the spine, hip, and radius using DXA in 964 participants (mean age, 50.2 ± 16.3 [SD] years; range, 18–99 years) from large multigenerational families. Genotyping of 731 highly polymorphic microsatellite markers (average spacing of 5.4 cM) and autosome-wide multipoint linkage analysis were performed.
Results: In the overall study population, no strong evidence for linkage was detected to any chromosomal region (peak LOD: 2.11 for radius BMD on chromosome 3q26). In a subgroup analysis of men ($n = 371$), strong evidence was detected for a quantitative trait locus (QTL) influencing BMD variation on chromosome 7q31 at the total hip (LOD = 4.15) and femoral neck (LOD = 3.09) and for a second QTL influencing spine BMD at 21q22 (LOD = 3.36). Suggestive evidence of linkage was found in men for a QTL at 12q24 affecting total hip BMD (LOD = 2.60) and at 18p11 for femoral neck (LOD = 2.07), and in women ($n = 593$) at 1p36 for femoral neck BMD (LOD = 2.02) and at 1q21 for spine BMD (LOD = 2.11). In age subgroup analyses, suggestive evidence for linkage was found for those <50 years of age ($n = 521$) on chromosomes 11q22 and 14q23 (LODs = 2.11 and 2.16, respectively) and for those >50 years of age ($n = 443$) on 3p25.2 (LOD = 2.32).
Conclusions: These results strongly suggest the presence of genes affecting hip and spine BMD in men on chromosomes 7q31 and 21q22. Modest evidence was found for genes affecting BMD in women on chromosomes 1p36 and 1q21 and in men at 12q24, replicating results from other populations.
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Key words: osteoporosis, linkage scan, genetics, sex-specific genes, Amish Family Osteoporosis Study

INTRODUCTION

Osteoporosis is a major public health problem in the United States, causing >1.5 million fractures annually that are associated with significant morbidity and increased mortality.[1] The etiology of osteoporosis is complex with multiple factors contributing to the acquisition of peak bone mass, subsequent bone loss, and ultimate bone strength.[2] Factors that influence risk of fracture include genes, hormonal influences, lifestyle and environmental exposures, and susceptibility to falls.[3] Low BMD is one of the strongest predictors for osteoporotic fracture.[4] BMD is estimated to be highly heritable, with genes accounting for 60–88% of total variation in BMD.[5–12]

Genome-wide linkage scans to search for quantitative trait loci (QTLs) that regulate BMD have been carried out in at least 10 different populations.[13–24] Numerous QTLs have been reported with varying support for linkage, although relatively few of these linkages have been replicated. Evidence for replication of linkage across populations seems to be strongest for a QTL influencing hip BMD on chromosome 1p36.[18,21] More modest evidence for replication of BMD linkages has also been reported from human and/or mouse studies at human chromosomes 4q32,[17,18] 12q24,[13,25] 13q14,[13,26] 16p13,[14,26] and spine BMD on chromosome 1q21.[15,16,27]

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TABLE 1. CHARACTERISTICS OF THE AFOS POPULATION

	Probands		*First-degree relatives*		*Non–first-degree relatives*	
	Males	*Females*	*Males*	*Females*	*Males*	*Females*
Total	18	38	140	225	224	343
Mean age (years)	61.7 ± 14.4	69.0 ± 14.0	52.4 ± 17.0	53.7 ± 16.5	48.1 ± 15.4	47.0 ± 14.7
Mean BMI (kg/m^2)	26.7 ± 4.3	25.2 ± 5.2	26.6 ± 4.7	27.8 ± 6.0	26.3 ± 3.9	28.4 ± 6.0
Total hip BMD	0.83 ± 0.15	0.67 ± 0.17	0.95 ± 0.12	0.86 ± 0.15	1.02 ± 0.11	0.94 ± 0.13
T score	–1.32 ± 1.01	–2.12 ± 1.42	–0.57 ± 0.81	–0.66 ± 1.25	–0.06 ± 0.79	–0.04 ± 1.09
Femoral neck BMD	0.70 ± 0.15	0.62 ± 0.16	0.81 ± 0.13	0.77 ± 0.15	0.89 ± 0.12	0.85 ± 0.14
T score	–1.83 ± 1.32	–2.26 ± 1.46	–0.93 ± 0.98	–0.74 ± 1.35	–0.27 ± 0.91	0.01 ± 1.22
Trochanter BMD	0.65 ± 0.12	0.53 ± 0.13	0.74 ± 0.10	0.67 ± 0.01	0.80 ± 0.09	0.72 ± 0.11
T score	–0.97 ± 0.92	–1.75 ± 1.37	–0.33 ± 0.79	–0.38 ± 1.19	0.16 ± 0.73	0.19 ± 1.07
Spine BMD	0.76 ± 0.09	0.69 ± 0.13	0.92 ± 0.14	0.87 ± 0.16	0.97 ± 0.13	0.95 ± 0.14
T score	–3.04 ± 0.80	–3.23 ± 1.17	–1.54 ± 1.30	–1.53 ± 1.94	–1.07 ± 1.18	–0.86 ± 1.16
Total radius BMD	0.61 ± 0.06	0.44 ± 0.06	0.65 ± 0.06	0.55 ± 0.07	0.68 ± 0.05	0.58 ± 0.06
T score	–1.29 ± 1.13	–2.45 ± 1.23	–0.54 ± 1.20	–0.22 ± 1.47	0.08 ± 0.91	0.26 ± 1.18

Values are mean ± SD.

TABLE 2. NUMBERS OF RELATIVE PAIRS IN THE AMISH FAMILY OSTEOPOROSIS STUDY

Relative pair	*Total sample*	*Males*	*Females*	*Age ≤50 years*	*Age >50 years*
Parent–offspring	651	115	242	25	87
Siblings	1555	274	598	783	532
Grandparent–grandchild	90	12	34	0	3
Avuncular	2208	304	885	214	287
First cousins	2234	260	924	1488	262
Other*	2353	298	953	953	145

* Other relative pair types include complex relationship pairs closer than first cousins (e.g., 1first/second cousins and double first cousins) and pairs more distal than first cousin (e.g., first cousin once removed, second cousin, second cousins once removed, third cousins, etc.).

The difficulty in replicating linkages across study populations has been ascribed to a number of factors, including (1) differences in genetic determinants of BMD within and between populations (genetic heterogeneity); (2) differences in ascertainment, phenotypic characterization, and analytic strategies; (3) differences in environmental influences on BMD between populations that may interact with genetic determinants; (4) lack of power to replicate QTLs of modest effect size; and (5) false positives in the original study. The Old Order Amish (OOA) of Lancaster, PA, are a closed genetically homogeneous white founder population with large sibships, well-documented genealogies, and a relatively homogeneous lifestyle. These characteristics make the OOA attractive for gene mapping studies. In this study, we performed an autosome-wide linkage scan for BMD in 964 men and women from 48 large multigenerational families participating in the Amish Family Osteoporosis Study (AFOS). The density of the pedigree structures and large sizes of Amish families led to a very large number of relative pairs informative for linkage analysis and provided us the opportunity to perform additional linkage analyses on subgroups defined by sex and age (≤50 and >50 years of age) for the purpose of localizing QTLs whose effects might be more prominent in a given sex or age group.

MATERIALS AND METHODS

Subjects

Study subjects were families enrolled in the AFOS, a study started in 1997 with the goal of identifying the genetic determinants of osteoporosis.[28,29] Individuals believed to be at risk for osteoporosis by virtue of their fracture history and/or prior BMD measurements were recruited into the study as index cases. These individuals were recruited by word-of-mouth, a community-wide mailing, advertisements in an Amish newspaper, and referral from local physicians. BMD was measured by DXA at the Amish Research Clinic in Strasburg, PA. Individuals with a T score of –2.5 or less in either the hip or spine were designated as probands. We invited spouses and all first-degree relatives of the proband who were ≥20 years of age to participate in the study. Any spouse or first-degree relative who also had a T score of –2.5 or lower at the spine or hip was also designated as a proband, and their spouse and first-degree relatives were recruited. The protocol was approved by the Institutional Review Board of the University of Maryland. Informed consent, including permission to contact relatives, was obtained before participation. This report includes data from 964 AFOS participants (371 men and 593 women, 18–99 years of age) recruited between March 1997 and February 2002. Initial recruitment consisted of large multigenera-

tional families that could be connected into a single 14-generation pedigree by including additional ancestors; however, computational constraints precluded analysis of the single unbroken pedigree. Thus, we merged several families by selectively adding in ancestors so that the final analysis set included 48 pedigrees, ranging in size from 2 to 125 phenotyped individuals, with 9 families including >30 phenotyped individuals and 4 families including >100 phenotyped individuals.[30,31] Excluded from the analyses presented in this study are 13 individuals with very low BMD who were screened for mutations in collagen formation genes (*COL1A1* and *COL1A2*) and were subsequently diagnosed with osteogenesis imperfecta on the basis of having a *COL1A* mutation. No other individuals in AFOS subjects carried this mutation.

Phenotypes

Physical examination included weight in standard Amish clothing without shoes and height using a stadiometer. BMD was measured by DXA, using a Hologic 4500W (Bedford, MA, USA), at the lumbar spine (L_1–L_4), hip (total and femoral neck), and forearm by a registered nurse certified in bone densitometry. The CV, determined annually by three sequential measures on 1 day for each of 15 individuals, was 0.90% for total hip and 0.71% for the spine (L_1–L_4).

Genotypes

DNA was isolated from leukocytes using Qiagen Maxipreps (Santa Clarita, CA, USA) for PCR and semiautomated genotyping. A genome scan was performed at the Marshfield Medical Research Foundation (Marshfield, WI, USA) of all 22 autosomes, using 731 highly polymorphic microsatellite markers (Marshfield marker sets 51 and 11), with average spacing of 5.4 cM. For genotyping quality control, we used PedCheck[32] to identify genotypes causing Mendel errors and SimWalk2[33] to identify genotypes causing double recombinants. These genotypes were removed for analysis. We estimated genetic distances between markers using Crimap[34] rather than relying on published values from Marshfield.

Statistical analyses

The primary aim of these analyses was to identify QTLs that contribute to variation in BMD of the hip, spine, and forearm in the total AFOS sample ($n = 964$ individuals). We further hypothesized that QTLs might exist that have preferential effects on BMD in men or women or in younger or older age groups. Thus, secondary analyses were performed restricted to men only ($n = 371$), women only ($n = 593$), and subjects ≤50 years old ($n = 521$) and >50 years old ($n = 443$).

Multipoint genome scans were performed using a variance components method that has been extended for use on multigenerational pedigrees as implemented in SOLAR.[35] Briefly, maximum likelihood methods were used to estimate the genetic variance attributable to the region around a specific genetic marker (σ^2_m) by specifying the expected genetic covariances between arbitrary relatives as a function of the identity-by-descent (IBD) relationships at a given marker locus assumed to be tightly linked to a locus influencing the quantitative trait. Sex, age, sex × age, age^2, and sex × age^2 were included as covariates. We compared the likelihood of the restricted model of no linkage, where the variance caused by the marker, σ^2_m, is constrained to 0, with the likelihood of the unconstrained model, where the variance σ^2_m is estimated, to maximize the likelihood. True multipoint IBD probabilities were computed using the Markov chain Monte Carlo algorithm implemented in Loki.[36] To assess the significance of the multipoint LOD scores for all traits, we generated empirical null distributions of nominal LOD scores for each phenotype by simulating 5,000 unlinked markers and evaluating evidence for linkage to each marker. All LOD scores given in the text are empirically adjusted LOD scores.

We estimated the power of our sample to detect QTL effects that accounted for 5–30% of the phenotypic variation in BMD traits. These results, obtained by simulation, revealed that we would have 80% power at an LOD > 3 to detect a QTL that accounted for 21% of the phenotypic variation.

RESULTS

The mean age of the study population was 50.2 ± 16.3 (SD) years (range: 18–99 years), and mean body mass index was 26.5 ± 4.2 kg/m^2 for men and 28.0 ± 6.0 kg/m^2 for women. Mean Z scores at the hip were 0.02 ± 0.86 and 0.27 ± 1.31 for men and women, respectively, suggesting that BMD in this sample of Amish differs little from that of non-Amish whites (Table 1). Mean Z scores at the spine were −0.98 ± 1.27 and −0.32 ± 1.25 for men and women; at the radius, mean Z scores were 0.27 ± 1.04 and 0.85 ± 1.04, respectively. As expected, mean T scores for BMD at all three sites were lowest in the osteoporotic probands, intermediate in their first-degree relatives, and highest in non–first-degree relatives of the probands (Table 1). Table 2 shows the number and types of relative pairs included in the sample on which the linkage analyses were based. In the total sample, there were 6738 relative pairs that were first cousins or closer. For males and females, there were 965 and 2683 relative pairs, respectively. Among subjects ≤50 years of age, there were 2510 relative pairs, and for those >50 years of age, there were 1305 relative pairs.

No strong evidence for linkage was detected in the genome-wide linkage analysis of the study population as a whole. The highest LOD score was 2.11 for radius BMD, occurring on chromosome 3q27 near Marshfield marker GATA22F11. Maximum LOD scores for each chromosome and phenotype are shown in Table 3 and in the plots shown in Figs. 1 (for total hip BMD) and 2 (for lumbar spine BMD).

We hypothesized that some QTLs influencing BMD might be more easily detectable in sex- or age-specific subgroups; thus, we performed secondary linkage analyses in AFOS subjects stratified by sex and age. These analyses revealed several strong and suggestive linkages with BMD phenotypes as shown in Table 4. For men, strong evidence for linkage was observed on chromosome 7q31 for hip

Table 3. Maximum LOD Score by Chromosome From Multipoint Linkage Analysis Conducted in the Entire AFOS Cohort (n = 964)

	Total hip		*Femoral neck*		*Trochanter*		*Lumbar spine*		*Radius*	
Chromosome	*LOD*	*Position (cM*)*	*LOD*	*Position (cM*)*	*LOD*	*Position (cM*)*	*LOD*	*Position (cM*)*	*LOD*	*Position (cM*)*
1	1.21	184 (AGAT119, ATA73A08)	0.82	185 (AGAT119, ATA73A08)	0.34	211 (ATA4E02, AAT200)	0.49	43 (GATA27E01, TTTA063)	0.81	181 (GATA43A04, AGAT119)
2	0.44	18 (GATA116B01, GAAT1A5)	0.7	110 (GATA69E12, GATA70F12)	0.22	54 (GATA8F07, AGAT117)	0.82	88 (GATA130A05, GATA66D01)	1.17	94 (GATA130A05, GATA66D01)
3	1.37	138 (GATA68D03, GATA84B12)	0.73	108 (AAC023, GGAT2G03)	0.99	140 (GATA84B12, ATC4D07)	1.08	45 (GGAA4B09, GATA73D01)	**2.11**	210 (GATA92B06, GATA22F11)
4	0.77	152 (GATA150B10, GATA11E09)	0.97	149 (GATA150B10, GATA11E09)	0.92	150 (GATA150B10, GATA11E09)	1.28	39 (GATA70E01, ATA27C07)	1.30	110 (ATA2A03, GAAT1F09)
5	1.63	153 (GATA51D11, GATA2H09)	1.64	44 (GATA135G01, GATA134B03)	1.35	41 (ATAG078, GATA135G01)	1.62	49 (GATA12A08, GATA7C06)	1.15	89 (TAGA010, GATA52A12)
6	1.76	4 (UT6540, ATA109H09)	1.65	4 (UT6540, ATA109H09)	0.99	5 (UT6540, ATA109H09)	1.04	36 (AAT256, ATC4D09)	0.84	34 (ATTT030, AAT256)
7	0.60	110 (GATA73D10, GATA87D11)	1.03	113 (GATA73D10, GATA87D11)	0.70	115 (GATA3F01, ATA78C09)	0.26	156 (GATA145G10, GATA43C11)	1.13	188 (GATA189C06, TATG002)
8	0.61	22 (GATA25C10, ATT070)	0.50	23 (ATT070, TATC012)	0.77	131 (GATA6B02, AAAT121)	1.01	9 (ATT023, ATAA009)	0.95	13 (ATT023, ATAA009)
9	0.86	151 (SNP446030, ATA63D01)	0.53	149 (TCTA017, SNP446030)	0.75	151 (SNP446030, ATA63D01)	0.50	24 (GATA187D09, SNP9558)	0.45	149 (TCTA017, SNP446030)
10	0.19	192 (ATGT006)	0.02	189 (GGAA23C05, ATGT006)	0.28	192 (ATGT006)	0.24	42 (ATAG055, GATA70E11)	0.47	124 (GATA115E01, GGAA2F11)
11	0.61	99 (GATA30G01, TCTA025)	0.31	45 (GATA73B08, ATA25D12)	0.40	117 (GATA35, GATA28D01)	1.13	75 (GATA46A12, AAT268)	0.68	111 (GATA35, GATA28D01)

TABLE 3. CONTINUED

Chromosome	Total hip LOD	Total hip Position (cM*)	Femoral neck LOD	Femoral neck Position (cM*)	Trochanter LOD	Trochanter Position (cM*)	Lumbar spine LOD	Lumbar spine Position (cM*)	Radius LOD	Radius Position (cM*)
12	1.35	160 (ATA080, GATA32F05)	1.60	150 (GATA4H01, GATA5H03)	0.51	155 (GATA5H03, ATA080)	0.40	161 (GATA32F05, AAT253)	0.39	117 (GATA85A04, PAH)
13	0.39	34 (GATA86H01, GATA6B07)	0.91	24 (AGAT110, GATA86H01)	0.45	35 (GATA6B07, GATA11C08)	0.04	35 (GATA6B07, GATA11C08)	0.15	55 (GATA73A05, GATA64F08)
14	1.13	56 (ATA19H08, AGAT131)	1.49	55 (GGAA30H04, ATA19H08)	0.78	117 (GATA136B01, ATT198Z)	0.59	19 (TCTA023, AGAT116)	0.88	69 (ATA069, GATA169E06)
15	0.74	94 (GATA63B12, ATA28G05)	0.93	95 (ATA28G05, TTAT027)	0.73	94 (GATA63B12, ATA28G05)	0.19	43 (GATA63A03, GATA153F11)	0.23	0 (AATA036, GATA88H02)
16	0.67	14 (ATA67B07, ATA41E04)	0.92	72 (ATA55A11, GATA151C03)	0.80	3 (ATA67B07, ATA41E04)	0.73	106 (TCTA026, MFD466)	1.78	22 (ATA41E04, GATA5H07)
17	0.44	61 (GATA169F02, GATA25A04)	0.58	57 (GGAA9D03, GATA169F02)	0.42	61 (GATA169F02, GATA25A04)	0.55	127 (GATA28D11, TTCA006)	0.65	147 (AAT095)
18	0.28	0 (CTG008, GATA166D05)	0.31	0 (CTG008, GATA166D05)	0.16	0 (CTG008, GATA166D05)	0.36	53 (GATA64H04, GATA183H03Z)	0.01	122 (AGAT138)
19	0.20	4 (GATA146H09, GATA21G05)	0.19	68 (UT7544, AAT257)	0.24	1 (GATA44F10, GATA146H09)	0.15	38 (GATA66B04, GGAA21A04)	0.11	61 (GATA156F11, AAT260)
20	0.20	41 (GATA129B03, AGAT139)	0.32	102 (GATA45B10, UT254)	0.13	104 (UT1772)	0.15	60 (GATA65E01, GATA42A03)	0.43	41 (GATA129B03, AGAT139)
21	0.07	21 (UT1355z, GATA188F04)	0.20	56 (TATC057, GGAA3C07)	0.58	21 (UT1355z, GATA188F04)	1.25	23 (UT1355z, GATA188F04)	0.17	10 (UT1355z, GATA188F04)
22	0	0 (GATA198B05, AGAT120)	0	0 (GATA198B05, AGAT120)	0.01	0 (GATA198B05, AGAT120)	0.10	70 (GATA030, TCTA015)	0.06	25 (ATTT019, AGAT055Z)

* Positions are in centimorgans on our Amish map.

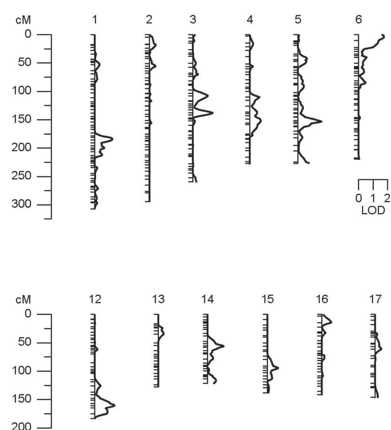

FIG. 1. Multipoint plots for entire study population for chromosomes 1–22 for total hip BMD phenotype.

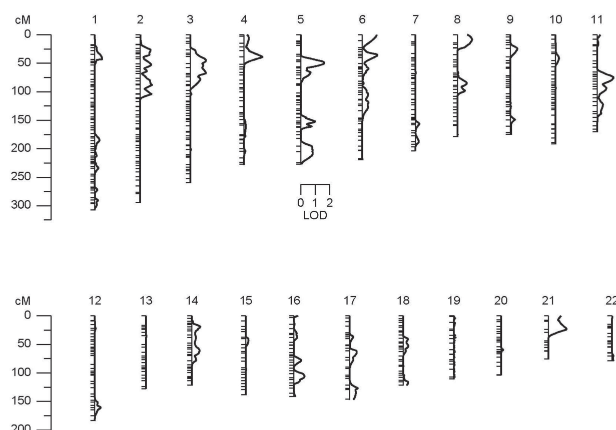

FIG. 2. Multipoint plots for entire study population for chromosomes 1–22 for spine BMD phenotype.

TABLE 4. MAXIMUM LOD SCORES OBTAINED FROM SUBGROUP ANALYSIS BY SEX (n = 371 MEN, 593 WOMEN) AND AGE (n = 521 ≤ 50 YEARS, n = 443 > 50 YEARS)

Subgroup	*Chromosome band*	*BMD site*	*LOD*	*1 – LOD support (Max LOD) (cM)**	*Closest markers*
Men	7q31	Total hip	4.15	142–148 (145)	AGAT133 GATA148F05
	7q31	Femoral neck	3.09	131–149 (145)	GATA141H10 TTTA001
	12q24	Total hip	2.60	153–165 (157)	GATA5H03 ATA080
	18p11	Femoral neck	2.07	34–44 (41)	AGAT127 GATA11A06
	21q22	Spine	3.36	17–32 (27)	GATA188F04 ATA27F01
Women	1p36	Femoral neck	2.02	28–63 (34)	GATA27E01 TTTA063
	1q21	Spine	2.11	170–186 (176)	GATA13C08 GATA43A0
Age ≤0 years	11q22	Radius	2.11	102–116 (111)	GATA35 GATA28D01
	14q23	Femoral neck	2.16	45–72 (57)	ATA19H08 AGAT131
Age >50 years	3p25	Spine	2.32	35–70 (43)	GGAA4B09 GATA73D01

* Positions are in centimorgans on our Amish map.

BMD (LODs = 4.15 and 3.09 for total hip and femoral neck BMD, respectively) and on chromosome 21q22 for spine BMD (LOD = 3.36; Fig. 3). For women, more modest evidence for linkage was observed on chromosome 1p36 for total hip BMD (LOD = 2.02), and on 1q21 for spine BMD (LOD = 2.11). For men and women ≤50 years of age, suggestive linkages were as follows: radius BMD on chromosome 11 (LOD = 2.11) and femoral neck on 14q22 (LOD = 2.16). For men and women >50 years of age, suggestive evidence for linkage was found to spine BMD on chromosome 3p25 (LOD = 2.32). Table 5 shows LOD scores from previously published studies at loci near those found in AFOS.

All multipoint plots for subgroup analyses can be found on our website.[38]

DISCUSSION

We conducted an autosome-wide linkage scan for BMD in the hip, spine, and radius in large multigenerational families of OOA, a closed genetically homogeneous white population who arose from a relatively small number of founders who migrated to the United States in the 1700s. We hypothesized that these founders would have carried into the population a subset of common osteoporosis susceptibility gene variants that (1) might be easier to detect because of decreased genetic heterogeneity and (2) would be relevant to the general European and U.S. white populations and perhaps other populations as well. Indeed, our autosome-wide scan of type 2 diabetes in the Amish[39] identified a region on chromosome 1q21-q24 that is also linked to dia-

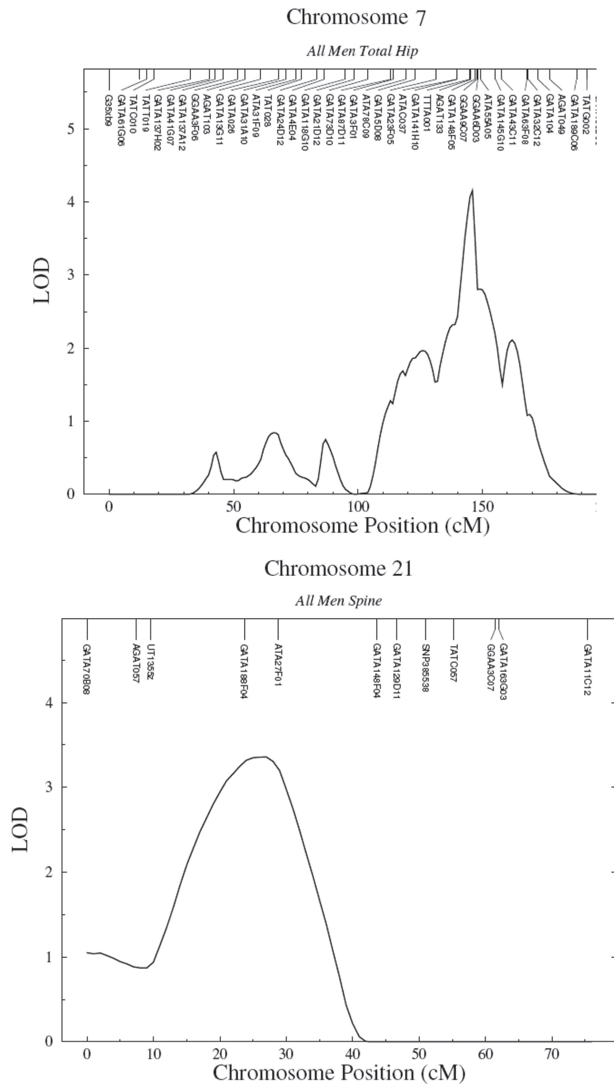

FIG. 3. Multipoint plot for men on chromosomes 7 (total hip) and 21 (spine).

betes in several other European and white populations, as well as in Pima Indians and Chinese.

Our previous studies indicate that the BMD of Amish women is slightly higher than that in non-Amish white women, with a corresponding lower incidence of hip fracture in Amish women, perhaps attributable to their high levels of physical activity.[28] As in other populations, we found BMD traits to be significantly heritable in the Amish (h^2 = 63–87%),[9] a finding that led us to perform this autosome-wide linkage analysis. Similar to most other published studies, we found no strong evidence of linkage with any BMD phenotype using the entire sample.[14,16,17,19–21,40] However, further subgroup analyses revealed strong evidence of linkage for QTLs for total hip and femoral hip BMD on chromosome 7q31 for men (LODs = 4.15 and 3.09, respectively). Also in men, strong evidence of linkage of QTLs for spine BMD was found on 21q22 (LOD = 3.36), and suggestive evidence of linkage was found at 12q24 (LOD = 2.60) for total hip and on 18p11 (LOD = 2.07) for femoral neck BMD.

In women, suggestive evidence of linkage was found for total hip BMD at 1p36 (LOD = 2.02) and for the spine at 1q21 (LOD = 2.11). In addition, in individuals ≤50 years of age, we detected suggestive evidence of linkage for QTLs influencing femoral neck BMD on 14q22 (LOD = 2.16) and radius BMD on 11q22 (LOD = 2.11). In individuals >50 years of age, we detected suggestive evidence of linkage for a QTL influencing spine BMD on 3p25 (LOD = 2.32). Although these subgroup analyses should be interpreted cautiously, these findings are consistent with the view that multiple loci contribute to BMD and that these loci may contribute variably in men and women as well and at different ages.

For complex traits like BMD, in which multiple loci contribute modest to moderate variation in the trait, high LOD scores of genome-wide significance are difficult to obtain. Thus, replication of modest linkage signals to the same chromosomal region in genome scans of other populations has become an important criterion for defining true positive signals from potentially false-positive signals.[41] Our linkages and overlapping linkages of other published studies are summarized in Table 5. Our strongest linkage signal (LOD = 4.15) was on chromosome 7q31 for total hip BMD in men. To our knowledge, no previous linkage has been reported for BMD phenotypes in this region. However, studies of hip structural phenotypes revealed linkage to femoral head width (LOD = 5.0, at D7S1804, chromosome 7q32.3) in premenopausal sister pairs,[37] ~8 Mb telomeric to our 1 – LOD support interval (Table 5). Although femoral head width is not precisely the same phenotype as hip BMD, it is possible that the same locus is responsible for linkage to both traits. The 1 – LOD support interval for our 7q31 linkage spans a 6-cM (~10 Mb) region containing 68 genes. Candidate genes in this region include *LEP* (leptin), *WNT2*, and *CAV*. *WNT2* is expressed in bone marrow mesenchymal cells and may be involved in signaling pathways shown to be important in osteoblast differentiation and bone formation.[42] *CAV* (caveolin 1) is a caveolar protein involved in signaling of tyrosine kinases, including BMP receptors 1a and 2.[43]

Our second strongest linkage (LOD = 3.36) was for spine BMD in men at 21q22. This linkage peak is at the same marker as a linkage peak from the Framingham study[20] for hip BMD (LOD = 2.39 at D21S2055); however, because the phenotypes of the two linkages are different, it is unclear whether our findings are confirmatory to those in the Framingham population. The 1 – LOD support interval for our 21q22 linkage peak near D21S055 (40.1 Mb) spans a 15-cM region containing 375 genes. Candidate genes in this region include *COL6A1*, *COL6A2*, and *CBS* (cystathionine β synthase, deficiency of which causes homocystinuria, a known risk factor for low BMD).

We observed several other linkage signals with LOD scores between 2.00 and 3.00. Several seem to replicate findings of others. Our linkage of total hip BMD to a QTL on chromosome 1p36 at D1S1597 (LOD = 2.02) in women is within the linkage peak found for femoral neck BMD in European (Greek, Italian, Middle Eastern) men and

TABLE 5. MAXIMUM LOD SCORES FROM PUBLISHED STUDIES AT LOCI NEAR THOSE FOUND IN AFOS

Chrom location	*Distance (cM)**	*BMD site*	*Peak LOD in AFOS*	*AFOS population*	*Flanking Marshfield markers*	*Supporting data*			*Candidate genes*
						Population	*Distance*	*Reference*	
1p36	36	FN	2.02	F	GATA27E01, TTTA063	M, F	36	18	*ALPL, EGFL3*
						F < 50	17	21	*MTHFR*
1q21	176	LS	2.87	F	GATA13C08, GATA43AO	Sisters	169	16	*IL-6R, S100A1–4*
						F < 50		14	*IL6R*
3p25	43	LS	2.32	>50	GGAA4B09, GATA73D01				*CASR*
		FN	0.35	>50		M	55	4	
7q31	146	TH	4.15	M	AGAT133, GATA148F05	Sisters†	136	37	*WNT2*
11q22	111	Rad	2.11	≤50	GATA35, GATA28D01				*MMP1, FRP*
		LS	1.03	≤50		F	111	20	
12q24	157	TH	2.60	M	GATA5H03, ATA080				
		LS	1.84	M		M, F	169	17	
						M,F	137	22	
14q22	57	TH	2.24	≤50	ATA19H08, AGAT131				*BMP4*
		LS	0			M,W	56	20	
18p11	39	FN	2.07	M	ATAT127, GATA11A06				
		LS	0.97	M		M,F	11	19	
						F > 50	48	14	
21q22	27	LS	3.36	M	GATA188F04, ATA27F01				*COL6A1.2*
		TH	0.03	M		M,F	27	20	

* Positions are in centimorgans on our Amish map.
† Phenotype was femoral head width.
FN, femoral neck; TH, total hip; LS, lumbar spine; Fh wid., femoral head width; Rad, radius; M, male; F, female.

women (LOD = 3.53 in the chromosomal location 1p36.2–36.3),[18] and is within the observed linkage for femoral neck BMD in female twins ≤50 years old from the United Kingdom (LOD = 2.38).[21] Our linkage on 1q21 (LOD = 2.11) for lumbar spine BMD in women is close to a linkage found in white premenopausal women reported by at least two other groups.[14,16] Linkages to BMD have also been reported previously in regions near 11q22 and 12q24,[17,20,22] and 3p25 and 18p11.[4,14,19]

There are also some chromosomal regions for which very strong evidence for linkage has been reported in other populations (e.g., QTLs on chromosome 4p in Mexican Americans [LOD = 4.33][13] and on chromosome 10q21 in a European cohort of men [LOD = 4.42])[14] for which we have little or no evidence for linkage. These findings are consistent with genetic heterogeneity or type 2 error.

Our autosome-wide linkage scan suggests that BMD in the Amish is polygenic as has been found in other populations, with several loci contributing to its variation. In addition, some of the genes that contribute to BMD variation seem to differ between sexes and in young and old. The most compelling statistical evidence for linkage appeared on chromosomes 7q21, 12q24, and 21q22, with six other regions providing more modest evidence for linkage. Several of these regions have been replicated in other populations, suggesting that they may be true positives that are relevant across populations. Positional cloning through linkage disequilibrium mapping and positional candidate gene analysis will ultimately be required to definitively identify genes, their variants, and their functional consequences that contribute to BMD and osteoporotic fracture.

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