Original articles

Genetic and environmental factors affecting bone mineral density in large families

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Summary

This study assessed whether relatives with low bone mineral density (BMD) could be identified in five large families using historical, biochemical, and genetic markers for osteoporosis. Fifty of 65 relatives had their bone density and bone turnover markers measured, together with an assessment of their risk factors for osteoporosis. Only 33% (5/15) of siblings, 50% (6/12) of children and 43% (10/23) of nephews and nieces had entirely normal BMD. There was no difference in life-style risk factors for osteoporosis, history of previous fractures or body mass index between normal subjects and those with osteopenia or osteoporosis. Osteopenic individuals had a significantly higher than normal osteocalcin value. Within families, there was no clear association between BMD and any of the genetic markers (vitamin D receptor gene polymorphisms, COL 1A1 and COL 1A2 polymorphisms of the collagen gene), either alone or in combination. The addition of genetic markers to the other risk factors for low BMD did not improve the prediction of BMD. In conclusion, we suggest that the presence of osteoporosis in a first degree relative should be one of the clinical indications for bone density measurement as the individuals at risk would not be picked up by other methods.

Keywords: genetics; osteoporosis; bone mineral density; risk factors

would require identification of individuals at risk. One approach would be to identify genetic markers for osteoporosis which could form the basis of a primary prevention programme. Evidence for a genetic influence on bone density comes from two types of clinical studies based on twins and small families. Studies in twins have shown that BMD is more significantly correlated in monozygotic than dizygotic twins at the spine and proximal femur.^{7–8} These studies have calculated an estimate of heritability of bone mass (the proportion of the variation in bone mass that can be explained by genetic factors after adjustment for age and years since menopause) of approximately 90% in the lumbar spine and 70% in the femoral neck. In family studies, 45–60% of the variance of bone density within a family was attributable to heredity.^{9–10}

Given the substantial genetic contribution to the determination of an individual's BMD, it would not be surprising if low bone mass were also more prevalent among close relatives of patients with osteoporosis. Several studies have shown that healthy offspring of osteoporotic mothers have a lower BMD than would be expected compared to healthy offspring of normal mothers.^{11–12} Jouanny and colleagues¹³ quantified the relative risks of children having a low BMD if their parent(s) had low BMD, after adjusting for environmental factors. A low BMD in a mother increased the relative risk of low BMD in her daughter to 5.15 and for a father-son pair, the relative risk was 3.79. If both parents were low, the relative risks of low BMD increased even further to 7.54 for a son and 34.4 for a daughter.

In addition to these clinical studies, the vitamin D receptor gene has recently been suggested as a marker for osteoporosis, with the BB genotype (absence of the restriction site on both alleles) having a lower BMD,^{14–16} although this has not been a universal observation.^{17–21} These studies have involved twins^{14–15–17} and unrelated individuals with severe osteoporosis^{18–20} but not large family groups. The vitamin D receptor gene may also influence peak bone mass in women^{22–23} and this could be a useful marker in familial osteoporosis where the problem may be one of low peak bone mass rather than accelerated age-/menopause-related bone loss.

Abnormalities in the collagen gene may also lead to osteoporosis because of the intimate relationship between collagen and bone. Osteoporosis might be regarded as the less severe end of the spectrum of osteogenesis imperfecta^{24–25}

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which is almost always due to mutations in type I collagen.²⁶

In the present study of five large families, each with an index case suffering from osteoporosis, we attempted to assess whether there was an increased number of relatives with low BMD among family members and whether these individuals could be otherwise identified using historical, biochemical, and genetic markers for osteoporosis. A secondary aim was to investigate whether these families with osteoporosis had any phenotypic or genetic evidence to suggest osteogenesis imperfecta.

Methods and subjects

SUBJECTS

Four women and a man of Caucasian origin, all with a low BMD, were studied because they had large families living in the Nottingham area. The relatives were contacted, the study was explained and an appointment for a bone density measurement was offered, followed by a clinic appointment. On that occasion, a history was taken of the relatives' life-style risk factors for osteoporosis and they were examined for the presence of hypermobility and any clinical evidence of a collagen defect. They were also asked for a history of atraumatic fractures (following a fall from standing height or less below the age of 60 years) and to fill in a dietary questionnaire and collect fasting urine samples.

MEASUREMENTS

BMD (g/cm^2) of the lumbar spine (L1–L4) and left femoral neck was measured by dual-energy X-ray absorptiometry (DEXA) using a Hologic QDR-2000 machine (Hologic Inc, Waltham, MA). The coefficient of variation for our machine during measurement on a standard phantom is 0.42%. In our centre, the coefficient of variation of lumbar spine measurements is 1.4% and 1.9% at the femoral neck. Urinary hydroxyproline was measured using a modified Organon spectrophotometric method. Osteocalcin was measured by a two-site immunoradiometric assay (Nichols Institute Diagnostics). The reference range for our population is 2.6–12.8 ng/ml. Dietary calcium intake was analysed using the Salford University 'Microdiet' system mark 8.07 (University of Salford, 1983–93) based on a prospective 3-day record.

DNA ANALYSIS

DNA was extracted from peripheral leucocytes by a modified phenol/chloroform procedure and the polymerase chain reaction (PCR) used to amplify the relevant DNA sequences. For genotyping of the vitamin D receptor alleles, the PCR products were digested by *BsmI* (New England Biolabs Inc, Beverly, MA) and genotypes determined by agarose gel electrophoresis. Genotypic polymorphisms were defined as BB (absence of restriction site on both alleles), bb (presence of restriction site on both alleles), or Bb (heterozygotes) for *BsmI* enzymes.¹⁴ Four markers for the type I collagen gene were used. COL 1A1 polymorphisms were investigated using *Msp I*, and *Rsa I* and COL 1A2

polymorphisms by means of *Eco RI* and a variable number of tandem repeats (VNTR) sequence.²⁷ Their PCR products were incubated with their respective enzymes, *Msp I* and *Rsa I* (Promega, Maddison, WI) and genotypes determined by agarose gel electrophoresis. Absence of the restriction site for *Msp I* and *Rsa I* on both alleles was defined as MM and RR, respectively, while their presence was denoted by mm and rr, Mm and Rr denoting the heterozygotes. For COL 1A2, the restriction enzyme used was *Eco RI* (Boehringer Mannheim, Germany). Genotypes were determined by agarose gel electrophoresis for *Eco I* and defined as EE (absence of restriction site on both alleles), ee (presence of restriction site on both alleles) or Ee (heterozygotes). For VNTR, a polyacrylamide gel electrophoresis was required to determine genotype which was based on the number of nucleotide repeats.

STATISTICS

Results for continuous variables are presented as means $\pm$ one standard deviation (SD). Risk factors were assessed by ANOVA and Student's *t*-test as appropriate. Differences in BMD, Z scores and bone turnover markers between the various genotypes and relationships between BMD and other variables including genetic markers were tested using ANOVA. The SPSS for Windows program (SPSS Inc, Chicago, IL) was used for these statistical tests.

The study was approved by the hospital's Ethics Committee.

Results

The five index cases had 16 siblings, 12 children and 37 nephews/nieces of whom we managed to investigate 15 siblings (94%), all the children (100%) and 23 nephews/nieces (62%). One missing sibling and 14 nephews/nieces declined to attend for a DEXA scan so that in total, we assessed 50 of 65 (77%) relatives. We were able to assess 9/20 (45%) spouses of the probands and siblings. One sibling had never married, one spouse had migrated to Australia and the other 10 declined to be assessed. Of those who were assessed, eight had normal BMD and one had osteopenia at the femoral neck (Z score). Only one parent of the probands was alive but was too frail to be assessed.

The probands were diagnosed as osteoporotic based on the World Health Organization (WHO) definitions of a T score more than 2.5 SD below the young adult mean.² However, as the study involved both male and female relatives with a wide age range (13–61 years), we used BMD Z scores (age and sex-matched) for further analysis. Individuals were considered to be osteoporotic if they had a BMD more than 2.0 SD below the age-matched mean, while osteopenic individuals were those with a BMD more than 1.0 SD, but less than 2.0 SD below the age-matched mean. Individuals were considered to be normal if they had a value for BMD within one SD of the age-matched mean. For subjects under the age of 18, we used a reference range compiled by

Table 1 Baseline characteristics of the siblings, children, nephews and nieces of the probands

	Siblings		Children		Nephews	Nieces
	Male	Female	Male	Female		
<i>n</i>	6	9	4	8	10	13
Age (years)	51.33 ± 8.31	52.11 ± 4.65	27.0 ± 5.89	30.25 ± 4.86	26.8 ± 10.3	25.77 ± 7.69
BMI	30.44 ± 8.09	26.65 ± 6.04	24.58 ± 3.45	21.03 ± 1.69*	24.54 ± 5.07	25.0 ± 5.67
Dietary Ca (mg/day)	881 ± 184	832 ± 305	1828 ± 73	859 ± 448*	900 ± 397	978 ± 314
Smoking (pack yrs)	18.75 ± 15.63	10.19 ± 11.33	7.5 ± 11.9	4.01 ± 7.03	7.86 ± 9.24	5.76 ± 8.0
Alcohol (units/wk)	18.42 ± 14.94	1.81 ± 1.70*	18.0 ± 16.08	2.22 ± 2.67*	10.5 ± 9.35	6.31 ± 6.94
Caffeine (cups/day)	5.00 ± 1.55	8.67 ± 3.94*	6.75 ± 8.3	9.5 ± 7.07	7.2 ± 5.85	5.23 ± 5.78
LS BMD (g/cm ²)	0.85 ± 0.13	0.88 ± 0.09	0.94 ± 0.24	0.96 ± 0.12	0.91 ± 0.16	0.98 ± 0.09
LS Z score	-1.80 ± 1.12	-0.75 ± 0.75*	-1.10 ± 1.97	-0.91 ± 0.90	-0.73 ± 1.36	-0.43 ± 0.77
FN BMD (g/cm ²)	0.77 ± 0.14	0.71 ± 0.11	0.76 ± 0.20	0.82 ± 0.11	0.81 ± 0.10	0.77 ± 0.08
FN Z score	-0.67 ± 1.34	-0.80 ± 0.79	-1.48 ± 2.00	-0.73 ± 0.78	-0.71 ± 1.12	-1.14 ± 0.80

All values given as mean ± 1 SD; *indicates significance at $p < 0.05$ between males and females, Student's *t*-test
 BMI=body mass index; LS=lumbar spine; FN=femoral neck.

Table 2 Number of relatives in each category of bone mineral density (at either lumbar spine or femoral neck)

	Siblings		Children		Nephews	Nieces
	Male	Female	Male	Female		
<i>n</i>	6	9	4	8	10	13
Z score						
Normal (>-1)	1 (17%)	4 (44%)	2 (50%)	4 (50%)	5 (50%)	5 (38%)
Osteopenia (-1 to -2)	2 (33%)	5 (56%)	0	3 (38%)	4 (40%)	6 (46%)
Osteoporosis (>-2)	3 (50%)	0	2 (50%)	1 (12%)	1 (10%)	2 (16%)
T score						
Normal (>-1)	1 (17%)	0	2 (50%)	4 (50%)	2 (20%)	3 (23%)
Osteopenia (-1 to -2.5)	3 (50%)	6 (67%)	0	4 (50%)	6 (60%)	10 (77%)
Osteoporosis (>-2.5)	2 (33%)	3 (33%)	2 (50%)	0	2 (20%)	0

Dr DA Bailey in Canada on children aged 7 to 18 years (H McKay, personal communication) to calculate their Z score.

None of the probands had a family history of osteoporosis. Three of the probands had a family history of hip or wrist fractures but were unsure as to whether they were low-trauma fractures. Neither the five probands nor their relatives suffered from any disease known to be associated with a low bone mass. Physical examination of all subjects was normal apart from two individuals in one family with kyphosis but no other clinical evidence of a collagen defect. The characteristics of the siblings, children and nephews/nieces are given in table 1. Male siblings drank more alcohol and less caffeine and had a significantly lower lumbar spine Z score compared to their sisters. Among the children of the probands, sons had a significantly higher body mass index (BMI), daily calcium intake and weekly alcohol consump-

tion than the daughters but there was no difference in Z scores between the two groups. As shown in table 2, only 33% (5/15) of the siblings, 50% (6/12) of the children and 43% (10/23) of the nephews and nieces had entirely normal BMD. The number of relatives with normal BMD based on the WHO definition² was only 12/50 (24%).

When we divided the subjects into groups based on their BMD, male relatives with normal BMD or osteopenia had a significantly higher alcohol intake than the females but this was not significant in those with osteoporosis. Male relatives with osteoporosis had a lower lumbar spine Z score than the females but otherwise, there was no difference in alcohol and caffeine intake, smoking, current dietary calcium or level of exercise, history of previous fractures or BMI between normal subjects and those with osteopenia or osteoporosis (table 3). Of the three relatives with a low-trauma wrist

Table 3 Characteristics of the relatives when divided into groups according to bone mineral density (at either lumbar spine or femoral neck sites)

	Normal		Osteopenia		Osteoporosis	
	Male	Female	Male	Female	Male	Female
<i>n</i>	8	13	6	14	6	3
Age (yrs)	33.63 ± 13.33	34.69 ± 12.68	32.33 ± 13.94	35.36 ± 15.12	36.83 ± 18.11	33.33 ± 5.51
BMI	28.89 ± 7.65	25.43 ± 5.37	25.47 ± 5.22	23.37 ± 5.22	23.74 ± 4.38	25.13 ± 7.30
Dietary Ca (mg/day)	976 ± 469	977 ± 350	908 ± 140	883 ± 348	1173 ± 630	704 ± 260
Smoking (pack yrs)	10.58 ± 12.7	8.85 ± 9.58	4.83 ± 5.42	4.40 ± 8.64	17.92 ± 15.03	7.33 ± 7.51
Alcohol (units/wk)	13.03 ± 14.74	3.73 ± 5.38*	17.83 ± 10.4	2.54 ± 2.41*	12.71 ± 12.77	10.67 ± 10.07
Caffeine (cups/day)	5.50 ± 6.63	7.85 ± 5.55	7.17 ± 3.49	7.29 ± 6.65	7.0 ± 5.73	6.0 ± 4.0
LS BMD (g/cm ²)	1.04 ± 0.12	1.01 ± 0.09	0.89 ± 0.05	0.90 ± 0.10	0.72 ± 0.10	0.89 ± 0.12
LS Z score	-0.08 ± 0.47	-0.27 ± 0.48	-0.94 ± 1.52	-0.87 ± 0.85	-2.7 ± 0.61	-1.34 ± 1.10*
FN BMD (g/cm ²)	0.91 ± 0.09	0.85 ± 0.09	0.76 ± 0.06	0.71 ± 0.07	0.66 ± 0.07	0.66 ± 0.01
FN Z score	0.39 ± 0.87	-0.18 ± 0.49	-1.16 ± 0.39	-1.38 ± 0.28	-2.19 ± 0.97	-2.07 ± 0.21

All values given as mean ± 1 standard deviation; *indicates significance at $p < 0.05$ between males and females, Student's *t*-test.
 BMI=body mass index; LS=lumbar spine; FN=femoral neck.

Figure 1 Serum osteocalcin by BMD category (ANOVA $p = 0.001$)

Figure 2 Serum osteocalcin by vitamin D receptor genotype (ANOVA $p = 0.66$)

fracture, two had osteoporosis and one had osteopenia (Z score).

Osteocalcin is a measure of bone formation and individuals with osteopenia had a significantly higher value ($p = 0.001$) than those who were normal (figure 1). There was no difference in hydroxyproline/creatinine ratios, a measure of bone resorption, between the three groups ($p = 0.50$). When comparing the osteocalcin levels in the different genotypes, there was a trend for the BB genotype to have higher osteocalcin values compared to bb individuals but this was not statistically significant (figure 2).

The vitamin D receptor genotype frequencies within these families were BB 11%, Bb

60% and bb 29%, which is similar to previously published results in Caucasian populations.^{14 15 17} Within families (figure 3), there was no clear association between BMD and any of the genetic markers, either alone or in combination. Analysis of the VNTR data was therefore performed after classification of subjects into homozygotes (both alleles with the same number of repeats) and heterozygotes (with different number of repeats on each allele). Table 4 gives the ANOVA p -values between each individual genetic marker and lumbar spine and femoral neck BMD and Z scores. No marker was significantly correlated with lumbar spine BMD or Z score. At the femoral neck, there were significant differences in femoral neck BMD between the different Rsa I and Eco RI genotype and also differences in femoral neck Z score between the Eco RI genotype.

BMI, dietary calcium intake and serum osteocalcin were added as co-variables to the genetic markers but at the lumbar spine and femoral neck, the addition of the vitamin D receptor and collagen gene polymorphisms did not significantly improve the prediction of BMD.

Use of the co-variables of BMI, dietary calcium intake (mg per day), and serum osteocalcin provided the best model for predicting BMD, accounting for approximately 50% of the variance at the lumbar spine and 62% at the femoral neck. The multiple regression equations are as follows:

$$y_1 = 0.89 + 0.0054(\text{BMI}) + 0.000014(\text{dietary calcium intake}) - 0.029(\text{osteocalcin})$$

where y_1 = lumbar spine BMD and

$$y_2 = 0.70 + 0.010(\text{BMI}) - 0.000053(\text{dietary calcium intake}) - 0.026(\text{osteocalcin})$$

where y_2 = femoral neck BMD

Figure 3 Example of a family tree. Key: boxes indicate men, circles women. * osteopenia; ** osteoporosis; BB/Bb/bb = vitamin D receptor polymorphism; MM/Mm/mm = Msp I polymorphism; RR/Rr/r = Rsa I polymorphism; 9/6 = number of VNTR repeats; EE/Ee/ee = Eco RI polymorphism.

Table 4 ANOVA p values between the genetic markers and lumbar spine and femoral neck BMD and Z scores

	Lumbar spine		Femoral neck	
	BMD	Z score	BMD	Z score
VDR	0.4459	0.9386	0.3003	0.7315
Msp I	0.1479	0.5858	0.1194	0.2584
Rsa I	0.4414	0.6820	0.0366*	0.0640
Eco RI	0.2216	0.1283	0.01**	0.0097**
VNTR	0.5193	0.7109	0.3016	0.1868

Where ANOVA was significant, a Scheffé range test was carried out to see which groups differed. *Significant Scheffé multiple range test between individuals with absent and heterozygote genotype; **Significant Scheffé multiple range test between individuals with absent and homozygote genotype.

VDR=vitamin D receptor; Msp I=COL1A1 marker; Rsa I=COL1A1 marker; Eco RI=COL1A2 marker; VNTR=COL1A2 marker.

Discussion

Identification of osteoporotic individuals remains difficult without recourse to bone densitometry, which is expensive and not readily available in all parts of the country. The positive predictive value of a family history of osteoporosis as an indicator of low bone mass has been shown to be 22% in men and 24% in women.²⁸ However, in our population, 56% of female and 83% of male siblings, 50% of the male and female children of osteoporotic parents, 62% of nieces and 50% of nephews

had a lower BMD than would be expected for people of their age and sex. Apart from the presence of osteoporosis in a relative, they possessed no other risk factors that may have suggested that their BMD was low. Two thirds of the premenopausal female siblings had osteopenia which has been suggested as the level at which preventative hormone replacement therapy should be initiated.²⁹ For the male siblings with osteopenia but no hypogonadism, management involves dietary and life-style modifications, although calcitonin and bisphosphonate therapy may show benefit in such men.³⁰

Low BMI, smoking and a high intake of alcohol, caffeine and low dietary calcium have all been implicated as risk factors for low bone mass³¹ but in our study, smoking, alcohol and caffeine intake was not related to the presence of osteopenia or osteoporosis. BMI and dietary calcium intake can be used in a model to predict bone mass but it would account at most for 50% of the variance in BMD. The male children drank significantly more alcohol and were heavier than the female children but this difference was unrelated to the level of BMD. In fact, the probands drank less alcohol than their relatives, but still had a lower mean BMD. Although questions about life-style risk factors may be useful when counselling patients following the discovery of low bone mass it does not predict the probability of disease. Our study thus merely reinforces the current view that risk factor assessment cannot be used to predict the occurrence of osteoporotic fractures (the end result of low bone mass).^{1, 32}

Biochemical markers have not been generally found to distinguish between those with normal or low BMD as there is considerable overlap between normal and osteoporotic populations.³³ In our study, the osteopenic individuals had a higher osteocalcin than the normal individuals suggesting an increased bone turnover but the range of values was wide and would not be helpful as a diagnostic test. Biochemical markers have also been used as a predictor of future bone loss³⁴ and the subjects in this study are currently being followed to determine whether they continue to lose bone or whether their problem was one of low peak bone mass. This would allow an assessment of whether their baseline bone markers were helpful in determining future bone loss.

Genetic polymorphisms of the vitamin D receptor and collagen are of limited value in identifying relatives with low BMD, even after correcting for body mass index, serum osteocalcin and dietary calcium intake. A recent meta-analysis found only a modest effect of vitamin D receptor on BMD, with a reduction of 2% in the BB group compared with bb genotype groups.³⁵ However, with two out of the three published twin studies showing an effect of vitamin D receptor on BMD,^{14, 15} we were interested to determine whether such an effect could be seen in groups of closely related individuals. It was therefore disappointing that there was no relationship between vitamin D receptor genotypes and BMD in these large families; in addition, a combination of risk fac-

Learning/summary points

- the prevalence of low bone mineral density in close relatives of subjects with osteoporosis is high
- life-style factors are poor predictors of bone density
- a combination of the current genetic markers are of limited value in identifying individuals with osteoporosis
- the presence of osteoporosis in a first-degree relative should be an indication for bone densitometry

tor assessment and vitamin D receptor genotyping did not improve the identification of individuals with a low BMD, even in the presence of a close relative with osteoporosis.

Over 100 mutations have been characterised in the COL 1A1 and COL 1A2 genes that encode the two α chains of type I collagen.²⁶ Almost all cases of osteogenesis imperfecta are due to mutations in type I collagen. Because the type I collagen molecule has two $\alpha 1(I)$ chains and one $\alpha 2(I)$ chain, it can be further predicted that mutations in COL 1A2 will usually lead to milder consequences than similar mutations in COL 1A1. This has been confirmed, to a small extent, by findings that two patients with severe, early osteoporosis and no phenotypic manifestations of osteogenesis imperfecta, had minor mutations in their COL 1A2 gene.^{24, 25} A larger study of 26 osteoporotic individuals with a positive family history showed that only three (12%) had collagen gene abnormalities.³⁶ One had COL 1A2 gene abnormalities which had been previously described²⁵ while the other two had abnormalities in the COL 1A1 gene. However, if only a small number of patients have mutations, detailed gene analysis on a large scale would clearly be impractical. We therefore examined established polymorphisms of the collagen gene in large families to assess whether they might be helpful in identifying individuals with low bone mineral density. Although it is theoretically a neat concept to link osteogenesis imperfecta and osteoporosis into a single disease entity, albeit with great phenotypic heterogeneity, we have not found any evidence to link low bone density with common polymorphisms of the collagen gene.

We recognise that there are several problems with this study. Firstly, not all of the available family members were studied but the proportion with a low BMD is high and the measured prevalence may be a slight overestimate. The second problem concerns the identification of the proband. We could have initially seen one of the siblings with a normal BMD (instead of our proband) and would have been falsely re-assured. It follows that an area for further research is to determine the incidence of low BMD in the community, especially in men, to see if this prevalence of suboptimal bone mass in these relatives is truly a reflection of the genetic factors predisposing

to osteoporosis or whether environmental factors are equally important.

In conclusion, we would suggest that the presence of osteoporosis in a first degree relative should be one of the clinical indications for bone density measurement although this is not current policy.³⁷ This would allow appropriate advice about hormone replacement therapy and life-style in an attempt to reduce

perimenopausal bone loss and to maximise peak bone mass. This would inevitably lead to an increase in health service workload and have substantial cost implications.

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