RELATION OF ALLELES OF THE COLLAGEN TYPE Iα1 GENE TO BONE DENSITY AND THE RISK OF OSTEOPOROTIC FRACTURES IN POSTMENOPAUSAL WOMEN

ANDRÉ G. UITTERLINDEN, PH.D., HUIBERT BURGER, M.D., PH.D., QIUJU HUANG, M.D., FANG YUE, M.D., FIONA E.A. MCGUIGAN, B.SC., STRUAN F.A. GRANT, PH.D., ALBERT HOFMAN, M.D., PH.D., JOHANNES P.T.M. VAN LEEUWEN, PH.D., HUIBERT A.P. POLS, M.D., PH.D., AND STUART H. RALSTON, M.D.

ABSTRACT

Background Osteoporosis is a common disorder with a strong genetic component. One way in which the genetic component could be expressed is through polymorphism of *COLIA1*, the gene for collagen type Iα1, a bone-matrix protein.

Methods We determined the *COLIA1* genotypes *SS, Ss,* and *ss* in a population-based sample of 1778 postmenopausal women using a polymerase-chain-reaction–based assay. We then related the genotypes to bone mineral density and the occurrence of osteoporotic fractures in these women.

Results As compared with the 1194 women with the *SS* genotype, the 526 women with the *Ss* genotype had 2 percent lower bone mineral density at the femoral neck ($P = 0.003$) and the lumbar spine ($P = 0.02$); the 58 women with the *ss* genotype had reductions of 4 percent at the femoral neck ($P = 0.05$) and 6 percent at the lumbar spine ($P = 0.005$). These differences increased with age ($P = 0.01$ for modification by age of the effect of *COLIA1* on femoral-neck bone density, and $P = 0.004$ for modification of the effect on lumbar-spine bone density). Women with the *Ss* and *ss* genotypes were overrepresented among the 111 women who had incident nonvertebral fractures (relative risk per copy of the *s* allele, 1.5; 95 percent confidence interval, 1.1 to 2.1).

Conclusions The *COLIA1* polymorphism is associated with reduced bone density and predisposes women to osteoporotic fractures. (N Engl J Med 1998;338:1016-21.)

©1998, Massachusetts Medical Society.

OSTEOPOROSIS is a common disorder characterized by reduced bone mineral density, deterioration of the microarchitecture of bone tissue, and increased risk of fracture.[1] Evidence from twin and family studies suggests that genetic factors have a major role in the pathogenesis of osteoporosis,[2-6] and segregation analysis has shown that bone mineral density is under polygenic control.[7] Pedigree studies of monogenetic disorders have been conducted to identify genes that could subsequently be analyzed for their contribution to the pathogenesis of osteoporosis on a population-wide basis. For example, loss-of-function mutations in the gene for the vitamin D receptor are responsible for the bone disease that characterizes 1α,25-dihydroxyvitamin D–resistant rickets.[8,9] Some, but not all, subsequent population-based and twin studies found that polymorphisms of the vitamin D receptor were associated with differences in bone density.[10,11] A man with a loss-of-function mutation of the estrogen-receptor gene had osteoporosis,[12] but intronic polymorphisms of this gene have not been consistently associated with differences in bone density in population-based studies of women.[13,14]

Similarly, girls with loss-of-function mutations in the aromatase P450 gene, who cannot synthesize estrogen, have osteoporosis,[15] but no population-based studies of this gene have been published. In more recent pedigree studies, a locus for "high peak bone density" and the locus for the osteoporosis–pseudoglioma syndrome, a recessive disorder characterized by juvenile-onset blindness and osteoporosis, were mapped to the same region on chromosome 11,[16,17] suggesting the presence of a gene with effects on bone density. Polymorphisms of other candidate genes, such as the genes for interleukin-6 and transforming growth factor β, have also been associated with differences in bone density in some populations.[18,19]

The genes encoding collagen types Iα1 and Iα2 (*COLIA1* and *COLIA2*, respectively) are also important candidates for the genetic regulation of bone density, because mutations that affect the coding regions of these genes cause osteogenesis imperfecta.[20] Although the coding regions of collagen genes are normal in the vast majority of patients with osteoporosis,[21] we described a novel guanine-to-thymidine polymorphism at the first base of a binding site for the transcription factor Sp1 in the first intron of the *COLIA1* gene that was associated with low bone density and increased occurrence of osteoporotic vertebral fracture in 299 British women.[22] To determine whether this polymorphism predicts bone density and the risk of osteoporotic fracture in other populations, we studied the relation of the *COLIA1* polymorphism to bone density and os-

From the Departments of Internal Medicine III (A.G.U., J.P.T.M.L., H.A.P.P.) and Epidemiology and Biostatistics (A.G.U., H.B., Q.H., F.Y., A.H., H.A.P.P.), Erasmus University Medical School, Rotterdam, the Netherlands; and the Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen, United Kingdom (F.E.A.M., S.F.A.G., S.H.R.). Address reprint requests to Dr. Uitterlinden at the Department of Internal Medicine III, FGG-EUR, Erasmus University Medical School, P.O. Box 1738, 3000 DR Rotterdam, the Netherlands.

Downloaded from www.nejm.org at ERASMUS UNIVERSITEIT MEDISCH BIBLIO on December 12, 2006 .
Copyright © 1998 Massachusetts Medical Society. All rights reserved.

teoporotic fracture in 1778 postmenopausal women in the Netherlands.[23]

METHODS

Study Subjects

The Rotterdam Study is a population-based cohort study of 7983 subjects who are at least 55 years old and who live in the Ommoord district of Rotterdam, the Netherlands. The study was designed to document the occurrence of disease in the elderly in relation to several potential determinants.[23] A total of 10,275 persons, of whom 9161 (89 percent) were living independently, were invited to participate in the study in 1991. Among the subjects living independently, the overall response rate was 77 percent for the home interview and 71 percent for the examination in a research center, where anthropometric characteristics and bone mineral density were measured and blood samples were taken. The Rotterdam Study was approved by the medical ethics committee of the Erasmus University Medical School, and written informed consent was obtained from each subject.

The association between the *COLIA1* genotype and osteoporosis was analyzed in a subgroup of women participating in the study. Base-line measurements of bone mineral density were available for 5931 subjects who were living independently. Of these, 1453 were excluded because they were more than 80 years of age, used a walking aid, had diabetes mellitus, or were taking diuretics, estrogen, thyroid hormone, or cytostatic drugs. From the 4478 remaining subjects, we studied a random sample of 2000 women who were 55 to 80 years old. Bone-density data or DNA samples were not available for 222 women, resulting in a final study group of 1778 women.

Measurements

Height and weight were measured at the initial examination, with the subject in a standing position without shoes. Bone mineral density (in grams per square centimeter) was determined by dual-energy x-ray absorptiometry (DPX-L densitometer, Lunar, Madison, Wis.) at the femoral neck and lumbar spine (vertebrae L2, L3, and L4), as described elsewhere.[24] Dietary intake of calcium (in milligrams per day) during the preceding year was assessed by a food-frequency questionnaire and adjusted for energy intake. Age at menopause and current use of cigarettes and alcohol were assessed by questionnaire. For 1423 women (80 percent), lateral radiographs of the spine from the fourth thoracic to the fifth lumbar vertebra were obtained at base line and examined for the presence of prevalent vertebral fractures by morphometric analysis, as previously described.[25] Incident nonvertebral fractures, including hip, wrist, and other fractures, were recorded, confirmed, and classified by a physician over a mean follow-up period of 3.8 years.

Determination of *COLIA1* Genotypes

Genomic DNA was extracted from samples of peripheral venous blood according to standard procedures, and the intronic polymorphism of the *COLIA1* gene was detected by the polymerase chain reaction (PCR) with a mismatched primer that introduces a diallelic restriction site, as previously described.[22] The test discriminates two alleles, *S* and *s*, which correspond to the presence of guanine and thymidine, respectively, as the first bases in the Sp1-binding site in the first intron of the gene for *COLIA1*. The reaction mixture of 25 μl contained 100 ng of genomic DNA, 50 mM potassium chloride, 10 mM TRIS–hydrochloric acid (pH 8.3), 1.5 mM magnesium chloride, 0.2 mM dideoxynucleotide triphosphates, 150 ng of each primer, and 0.2 unit of Super *Taq* polymerase (HT Biotechnology, Cambridge, United Kingdom). The reactions were performed in a DNA thermocycler (mode 480, Perkin–Elmer, Foster City, Calif.) with a cycling protocol of 94°C, 60°C, and 72°C for one minute each, for 35 cycles. Ten microliters of PCR product was digested with 2 units of *Msc*I restriction enzyme and 1.2 μl of a buffer, containing 150 mM TRIS–hydrochloric acid (pH 7.5), 250 mM sodium chloride, and 35 mM magnesium chloride, by incubation for 30 minutes at 37°C. The digestion products underwent electrophoresis on a 3 percent NuSieve agarose gel (FMC BioProducts, Rockland, Me.) in 44.5 mM TRIS, 44.5 mM boric acid, and 1 mM disodium EDTA for 300 volt-hours. The separation patterns were documented by Polaroid photography under ultraviolet illumination (302 nm). To confirm the accuracy of the genotyping, repeated analysis was performed on 400 randomly selected samples, including those from all *ss* homozygotes. No discrepancies were found.

Statistical Analysis

Bone mineral density and other relevant clinical variables of the three genotype groups were compared by analysis of covariance. Multiple linear regression was used to adjust bone-density values for confounding factors such as age, anthropometric variables, calcium intake, smoking, and alcohol use. A multivariate linear regression model was designed to test for the changing effects of *COLIA1* genotype on bone density with age. For this analysis, the subjects were classified into five-year age groups (55 to 59, 60 to 64, 65 to 69, 70 to 74, and 75 to 80 years), and the *COLIA1* genotypes were assigned values of 0, 1, or 2, according to the number of *s* alleles present. The analysis included an interaction term defined as age group multiplied by gene dose (the number of *s* alleles). The variance of bone density that could be explained by the *COLIA1* genotype was calculated by linear regression analysis adjusted for weight and age. The chi-square test was used to test for Hardy–Weinberg equilibrium, the distribution of alleles in women of different age groups, and the distribution of alleles in women with and without fractures. Odds ratios (with 95 percent confidence intervals) were calculated by multivariate logistic-regression analysis to estimate the relative risk of osteoporotic fracture. All statistical tests were two-sided.

RESULTS

Characteristics of the Study Subjects

Table 1 shows anthropometric, dietary, and bone-density measurements according to *COLIA1* genotype. The allelic frequencies (82 percent for the *S* allele and 18 percent for the *s* allele) and the distribution of genotypes were similar to those reported in a previous study[22] and were as predicted by the Hardy–Weinberg equation, confirming that no genotype was being selectively sampled ($P = 1.00$). The three genotype groups did not differ significantly in age, age at menopause, dietary calcium intake, smoking habits, alcohol intake, or height. There was a significant gene-dose effect on body weight (-0.97 kg per copy of the *s* allele, $P = 0.03$): body weight was lower in the *Ss* group than in the *SS* group, and lowest in the *ss* group.

Association between Genotype and Bone Mineral Density

The three genotype groups differed significantly in bone mineral density at the femoral neck and the lumbar spine. At both sites, bone mineral density was highest in the *SS* group and lowest in the *ss* group (Table 1). Bone mineral density in the *Ss* group was 2 percent less than that in the *SS* group at both sites ($P = 0.003$ for femoral neck and $P = 0.02$ for lumbar spine). In the *ss* group, bone mineral density in the femoral neck was 4 percent

Downloaded from www.nejm.org at ERASMUS UNIVERSITEIT MEDISCH BIBLIO on December 12, 2006 .
Copyright © 1998 Massachusetts Medical Society. All rights reserved.

TABLE 1. CHARACTERISTICS OF 1778 POSTMENOPAUSAL WOMEN ACCORDING TO THEIR *COLIA1* GENOTYPE.*

CHARACTERISTIC	*COLIA1* GENOTYPE			P VALUE		
	SS (N=1194)	*Ss* (N=526)	*ss* (N=58)	ANOVA	*SS* vs. *Ss*	*SS* vs. *ss*
Age (yr)	66±7	66±7	66±7	0.72	0.57	0.90
Height (cm)	162±6	162±6	162±6	0.96	0.80	0.86
Weight (kg)	69.3±10.6	68.5±10.1	66.8±8.6	0.08	0.11	0.07
Age at menopause (yr)	49±5	49±5	49±5	0.82	0.83	0.70
Dietary calcium intake (mg/day)	1103±324	1111±344	1064±268	0.61	0.67	0.38
Current smoker (%)	21	20	16	0.52	0.82	0.20
Bone mineral density (g/cm²)						
Femoral neck						
Unadjusted	0.82±0.13	0.80±0.12	0.79±0.13	0.003	0.003	0.05
Adjusted†	0.82±0.13	0.81±0.12	0.80±0.13	0.03	0.01	0.17
Lumbar spine						
Unadjusted	1.03±0.17	1.01±0.17	0.97±0.19	0.003	0.02	0.005
Adjusted†	1.03±0.17	1.01±0.17	0.98±0.19	0.02	0.07	0.02

*Plus–minus values are means ±SD. ANOVA denotes analysis of variance.

†Values are adjusted for age and weight.

TABLE 2. *COLIA1* GENE-DOSE EFFECT ON BONE DENSITY OF POSTMENOPAUSAL WOMEN ACCORDING TO AGE.*

AGE (YR)	NO. OF WOMEN	CHANGE AT FEMORAL NECK PER *s* ALLELE*	P VALUE	CHANGE AT LUMBAR SPINE PER *s* ALLELE*	P VALUE
		g/cm²		g/cm²	
55–59	422	−0.005±0.012	0.67	+0.001±0.015	0.93
60–64	463	−0.009±0.010	0.38	−0.013±0.014	0.36
65–69	376	−0.017±0.012	0.18	−0.020±0.017	0.24
70–74	307	−0.031±0.012	0.01	−0.052±0.017	0.002
75–80	210	−0.043±0.016	0.01	−0.058±0.023	0.01
Total	1778	−0.018±0.006	<0.001	−0.025±0.008	<0.001

*Plus–minus values are means ±SE.

less than that in the *SS* group (P=0.05), and in the lumbar spine it was 6 percent less than that in the *SS* group (P=0.005). Table 2 shows that these differences corresponded to significant gene-dose effects at the femoral neck and lumbar spine (P<0.001 for both sites). The effects of *COLIA1* genotype on bone density of the Ward's triangle and trochanter sites at the hip were similar (data not shown). The percentage of the variance in bone density explained by the *COLIA1* genotype was 0.3 percent at the femoral neck and 0.4 percent at the lumbar spine. When the bone-density values were adjusted for confounding variables resulting from the association between genotype and body weight, the differences between genotype groups were smaller, but they were still statistically significant.

Age-Related Changes in the Association between Genotype and Bone Mineral Density

Bone density declines with age, and genetic effects on bone density could theoretically be mediated by differences in the age-related rate of bone loss,[26] as well as by differences in peak bone density. We therefore assessed the effect of age on the relation between *COLIA1* genotype and bone density. When the study subjects were divided into five-year age categories, the differences in bone density between the genotype groups were small among the younger women (55 to 65 years old) but became larger with increasing age (Fig. 1). Among women 75 to 80 years of age, bone mineral density in the *Ss* group was 5 percent lower at the femoral neck (P=0.03) and 3 percent lower at the lumbar spine (P=0.26) than that in the *SS* group. In the *ss* group, bone mineral density was 12 percent lower at the femoral neck (P=0.04) and 20 percent lower at the lumbar spine (P=0.004) than that in the *SS* group. These numbers indicate an increased gene-dose effect with increasing age at both the femoral neck and the lumbar spine (Table 2).

When age, genotype, and bone density were considered together in a multivariate regression model, we found that age significantly modified the effect of the *COLIA1* genotype on bone density, at both the femoral neck (P=0.01 for the interaction term) and the lumbar spine (P=0.004 for the interaction term). The percentage of the variance in bone density accounted for by the *COLIA1* genotype became larger with age, reaching 2 percent for women 75 to 80 years of age. These differences were not explained by differences in the distribution of genotypes, because the allelic frequencies did not change with in-

Downloaded from www.nejm.org at ERASMUS UNIVERSITEIT MEDISCH BIBLIO on December 12, 2006 .
Copyright © 1998 Massachusetts Medical Society. All rights reserved.

creasing age and the distribution of genotypes in each age group was similar to that in the study group as a whole.

Association between Genotype and Fractures

Table 3 shows the distribution of incident nonvertebral fractures and prevalent vertebral compression fractures according to *COLIA1* genotype. Significantly more women with the *Ss* genotype had incident nonvertebral fractures or prevalent vertebral fractures than women with the *SS* genotype. Among women with the *ss* genotype, this difference increased for incident nonvertebral fractures ($P=0.02$) but not for prevalent vertebral fractures ($P=0.33$).

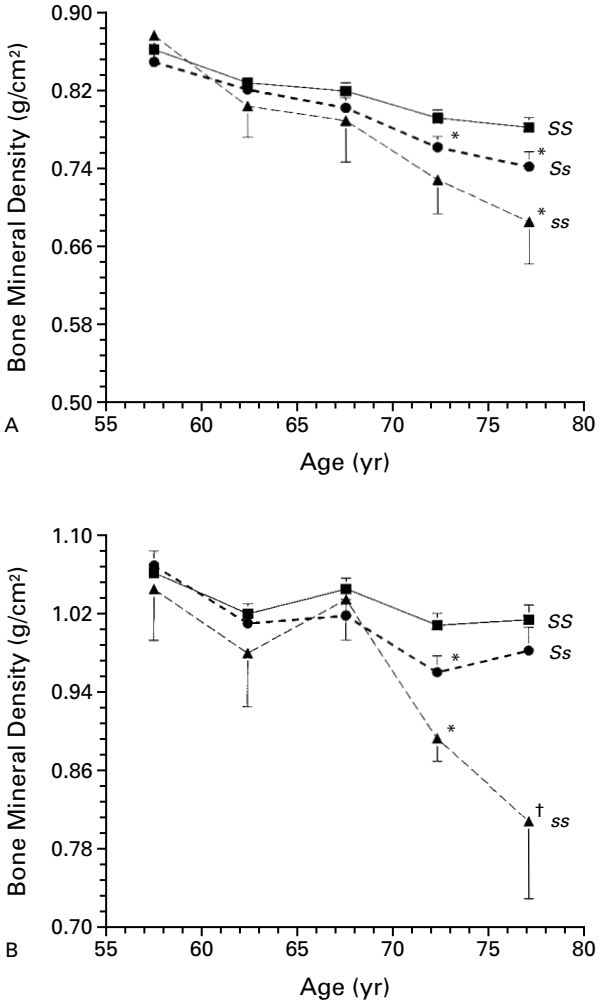
Logistic-regression analysis showed that for incident nonvertebral fractures, the women in the *Ss* group had 1.4 times the risk of the women in the *SS* group, and the women in the *ss* group had 2.8 times the risk of the women in the *SS* group. The overall gene-dose effect was an odds ratio of 1.5 per copy of the *s* allele: 3.1 for the hip (95 percent confidence interval, 1.2 to 7.6; 10 cases), 1.4 for the wrist (95 percent confidence interval, 0.8 to 2.5; 36 cases), and 1.4 for other fractures (95 percent confidence interval, 0.9 to 2.1; 65 cases). The risk of vertebral fracture was slightly increased in women in the *Ss* group but not in those in the *ss* group. The relative risk of fracture did not change after adjustment for potential confounding factors such as age, weight, and bone density in the regression analysis (Table 3).

DISCUSSION

Genetic factors have an important role in determining bone density,[2-6,27] and bone density is an important determinant of the risk of osteoporotic fracture.[28,29] The genes for collagen types Iα1 and Iα2 (*COLIA1* and *COLIA2*) are important candidates for regulators of bone density and risk of fracture, because point mutations that affect their coding regions result in osteogenesis imperfecta.[20] Although similar mutations in the coding regions of the collagen genes have not been found in patients with osteoporosis,[21] the polymorphism that we described, which affects a binding site for the transcription factor Sp1 in a regulatory region of the *COLIA1* gene, was associated with differences in bone density and risk of fracture in 299 British women.[22] The data presented here confirm and extend these findings to a population-based cohort of 1778 postmenopausal women from the Netherlands. We found a consistent association between this *COLIA1* polymorphism and differences in bone density at the spine and hip, with evidence of gene-dose effects at both sites: bone-density values were highest in women with the *SS* genotype, intermediate in those with the *Ss* genotype, and lowest in those with the rare *ss* genotype.

These differences in bone density were accompanied by differences in body weight, and the difference between genotypes in bone density was reduced in magnitude after correction for weight. This is not unexpected in view of the close association between weight and bone density[30,31] and suggests that the association between *COLIA1* genotype and bone density may be mediated in part by a genetic effect of *COLIA1* on body weight.

The genotype-specific differences in bone density



Genotype	55–59 yr	60–64 yr	65–69 yr	70–74 yr	75–80 yr
SS	301	296	250	205	142
Ss	107	151	116	91	61
ss	14	16	10	11	7

Figure 1. Mean (±SE) Age-Related Differences in Bone Mineral Density of the Femoral Neck (Panel A) and Lumbar Spine (Panel B) in Postmenopausal Women According to *COLIA1* Genotype.

The numbers of women with each genotype in each age group are shown below the figure. The asterisks denote $P\leq 0.05$, and the dagger $P<0.01$ for the comparison with the *SS* genotype.

Downloaded from www.nejm.org at ERASMUS UNIVERSITEIT MEDISCH BIBLIO on December 12, 2006 .
Copyright © 1998 Massachusetts Medical Society. All rights reserved.

TABLE 3. NUMBER OF POSTMENOPAUSAL WOMEN WITH FRACTURES AND ODDS RATIOS FOR FRACTURES ACCORDING TO *COLIA1* GENOTYPE.

GENOTYPE	INCIDENT NONVERTEBRAL FRACTURES	ODDS RATIO (95% CI)*		PREVALENT VERTEBRAL FRACTURES	ODDS RATIO (95% CI)*	
		AGE-ADJUSTED	MULTIVARIATE		AGE-ADJUSTED	MULTIVARIATE
	no. with fracture/total no. (%)			no. with fracture/total no. (%)		
SS	64/1194 (5.4)	1.0	1.0	44/949 (4.6)	1.0	1.0
Ss	39/526 (7.4)	1.4 (0.9–2.1)	1.4 (0.9–2.0)	28/420 (6.7)	1.4 (0.8–2.3)	1.2 (0.8–2.1)
ss	8/58 (13.8)	2.8 (1.3–6.3)	2.6 (1.2–5.9)	2/44 (4.5)	1.0 (0.2–4.6)	0.8 (0.2–3.8)
Per copy of the *s* allele	—	1.5 (1.1–2.1)	1.5 (1.1–2.0)	—	1.2 (0.8–1.9)	1.1 (0.7–1.7)

*Odds ratios have been adjusted for age, weight, and bone density. For incident nonvertebral fractures, adjustment was also made for femoral-neck bone density, and for prevalent vertebral fractures, for lumbar-spine bone density. CI denotes confidence interval.

were small in the study group as a whole but increased with age. The allelic frequencies did not change with age, an observation that argues against selection bias due to mortality-related effects of the polymorphism as an explanation for this effect. This raises the possibility that the *COLIA1* polymorphism may be a marker for accelerated bone loss in older women, rather than a marker for lower peak bone density.

The overrepresentation of the *Ss* and *ss* genotypes among women with osteoporotic fractures was not unexpected, in view of the well-established relation between bone density and risk of fracture,[28,29] which was also found in this study. The relative risk of fracture, however, was about two to three times as great as that predicted by the differences between genotypes in bone density, and it remained significant after adjustment for weight and bone density. This raises the possibility that the *COLIA1* polymorphism may act as a marker for differences in bone quality, such as bone structure or matrix composition, as well as bone quantity. The number of fractures was small, however.

The mechanisms by which the different *COLIA1* alleles affect bone density and fracture risk are not known. Oligonucleotides corresponding to the *s* allele bind the Sp1 protein with greater affinity than those corresponding to the *S* allele,[32] and subjects who are heterozygous for the polymorphism have three times as many transcripts derived from the *s* allele as from the *S* allele (unpublished data). This raises the possibility that carriage of the *s* allele may be associated with a disturbance in the relative abundance of *COLIA1* and *COLIA2* messenger RNAs, as occurs in osteogenesis imperfecta.[33]

Studies of the genetic basis of osteoporosis are important to identify genes that act as regulators of bone density, bone quality, and the risk of fracture. Genetic markers for osteoporosis could be useful clinically for identifying subjects at risk of osteoporosis and for targeting preventive treatment. The association we describe between *COLIA1* genotype and osteoporotic fracture in postmenopausal women, coupled with the divergence of bone-density values with increasing age between different genotype groups, raises the possibility that genotyping at the polymorphic *COLIA1* Sp1 site may provide information on susceptibility to osteoporotic fracture that could complement information gained from bone-density measurements alone.

Supported by grants from the Dutch Praeventiefonds (002824890), the Nestor stimulation program for geriatric research in the Netherlands (Ministry of Health and Ministry of Education), the Municipality of Rotterdam, the Netherlands Organization for Scientific Research, the European Commission, the Chief Scientist's Office of the Scottish Home and Health Department, the Wellcome Trust, and the Grampian Osteoporosis Trust.

REFERENCES

1. Kanis JA, Melton LJ III, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. J Bone Miner Res 1994;9:1137-41.
2. Smith DM, Nance WE, Kang KW, Christian JC, Johnston CC Jr. Genetic factors in determining bone mass. J Clin Invest 1973;52:2800-8.
3. Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN, Eberl S. Genetic determinants of bone mass in adults: a twin study. J Clin Invest 1987;80:706-10.
4. Evans RA, Marel GM, Lancaster EK, Kos S, Evans M, Wong SYP. Bone mass is low in relatives of osteoporotic patients. Ann Intern Med 1988;109: 870-3.
5. Seeman E, Hopper JL, Bach LA, et al. Reduced bone mass in daughters of women with osteoporosis. N Engl J Med 1989;320:554-8.
6. Soroko SB, Barrett-Connor E, Edelstein SL, Kritz-Silverstein D. Family history of osteoporosis and bone mineral density at the axial skeleton: the Rancho Bernardo Study. J Bone Miner Res 1994;9:761-9.
7. Gueguen R, Jouanny P, Guillemin F, Kuntz C, Pourel J, Siest G. Segregation analysis and variance components analysis of bone mineral density in healthy families. J Bone Miner Res 1995;12:2017-22.
8. Hughes MR, Malloy PJ, Kieback DG, et al. Point mutations in the human vitamin D receptor gene associated with hypocalcemic rickets. Science 1988;242:1702-5.
9. Malloy PJ, Weisman Y, Feldman D. Hereditary 1α,25-dihydroxyvitamin D-resistant rickets resulting from a mutation in the vitamin D receptor deoxyribonucleic acid-binding domain. J Clin Endocrinol Metab 1994;78: 313-6.

Downloaded from www.nejm.org at ERASMUS UNIVERSITEIT MEDISCH BIBLIO on December 12, 2006 .
Copyright © 1998 Massachusetts Medical Society. All rights reserved.

10. Morrison NA, Qi JC, Tokita A, et al. Prediction of bone density from vitamin D receptor alleles. Nature 1994;367:284-7.
11. Cooper GS, Umbach DM. Are vitamin D receptor polymorphisms associated with bone mineral density? A meta-analysis. J Bone Miner Res 1996;11:1841-9.
12. Smith EP, Boyd J, Frank GR, et al. Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. N Engl J Med 1994;331: 1056-61.
13. Kobayashi S, Inoue S, Hosoi T, Ouchi Y, Shiraki M, Orimo H. Association of bone mineral density with polymorphism of the estrogen receptor gene. J Bone Miner Res 1996;11:306-11.
14. Han KO, Moon IG, Kang YS, Chung HY, Min HK, Han IK. Nonassociation of estrogen receptor genotypes with bone mineral density and estrogen responsiveness to hormone replacement therapy in Korean postmenopausal women. J Clin Endocrinol Metab 1997;82:991-5.
15. Morishima A, Grumbach MM, Simpson ER, Fisher C, Qin K. Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. J Clin Endocrinol Metab 1995;80: 3689-98.
16. Gong Y, Vikkula M, Boon L, et al. Osteoporosis-pseudoglioma syndrome, a disorder affecting skeletal strength and vision, is assigned to chromosome region 11q12-13. Am J Hum Genet 1996;59:146-51.
17. Johnson ML, Gong G, Kimberling W, Recker SM, Kimmel DB, Recker RB. Linkage of a gene causing high bone mass to human chromosome 11 (11q12-13). Am J Hum Genet 1997;60:1326-32.
18. Murray RE, McGuigan F, Grant SFA, Reid DM, Ralston SH. Polymorphisms of the interleukin-6 gene are associated with bone mineral density. Bone 1997;21:89-92.
19. Langdahl BL, Knudsen JY, Jensen HK, Gregersen N, Eriksen EF. A sequence variation: 713-8delC in the transforming growth factor-beta 1 gene has higher prevalence in osteoporotic women than in normal women and is associated with very low bone mass in osteoporotic women and increased bone turnover in both osteoporotic and normal women. Bone 1997;3:289-94.
20. Byers PH, Steiner RD. Osteogenesis imperfecta. Annu Rev Med 1992; 43:269-82.
21. Spotila LD, Colige A, Sereda L, et al. Mutation analysis of coding sequences for type I procollagen in individuals with low bone density. J Bone Miner Res 1994;9:923-32.
22. Grant SFA, Reid DM, Blake G, Herd R, Fogelman I, Ralston SH. Reduced bone density and osteoporosis associated with a polymorphic Sp1 binding site in the collagen type I α1 gene. Nat Genet 1996;14:203-5.
23. Hofman A, Grobbee DE, de Jong PTVM, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol 1991;7:403-22.
24. Burger H, van Daele PLA, Algra D, et al. The association between age and bone mineral density in men and women aged 55 years and over: the Rotterdam Study. Bone Miner 1994;25:1-13.
25. Burger H, Van Daele PLA, Grashuis K, et al. Vertebral deformities and functional impairment in men and women. J Bone Miner Res 1997;12: 152-7.
26. Kelly PJ, Nguyen T, Hopper J, Pocock N, Sambrook P, Eisman J. Changes in axial bone density with age: a twin study. J Bone Miner Res 1993;8:11-7.
27. Jouanny P, Guillemin F, Kuntz C, Jeandel C, Pourel J. Environmental and genetic factors affecting bone mass: similarity of bone density among members of healthy families. Arthritis Rheum 1995;38:61-7.
28. Hui SL, Slemenda CW, Johnston CC Jr. Baseline measurement of bone mass predicts fracture in white women. Ann Intern Med 1989;111: 355-61.
29. Cummings SR, Black DM, Nevitt MC, et al. Bone density at various sites for prediction of hip fractures. Lancet 1993;341:72-5.
30. Krall EA, Dawson-Hughes B. Heritable and life-style determinants of bone mineral density. J Bone Miner Res 1993;8:1-9.
31. Seeman E, Hopper JL, Young NR, Formica C, Goss P, Tsalamandris C. Do genetic factors explain associations between muscle strength, lean mass, and bone density? A twin study. Am J Physiol 1996;270:E320-E327.
32. Grant SFA. Studies on the genetic susceptibility to osteoporosis: analysis of cis-acting sequences in the collagen I α 1 gene. (Ph.D. thesis. Aberdeen, Scotland: University of Aberdeen, 1996.)
33. Willing MC, Deschenes SP, Scott DA, et al. Osteogenesis imperfecta type I: molecular heterogeneity for COL1A1 null alleles of type I collagen. Am J Hum Genet 1994;55:638-47.

ELECTRONIC ACCESS TO THE *JOURNAL'S* CUMULATIVE INDEX

At the *Journal*'s site on the World Wide Web (http://www.nejm.org) you can search an index of all articles published since January 1990. You can search by author, subject, title, type of article, or date. The results will include the citations for the articles plus links to the abstracts of articles published since 1993. Single articles and past issues of the *Journal* can also be ordered for a fee through the Internet (http://www.nejm.org/customer/).

Downloaded from www.nejm.org at ERASMUS UNIVERSITEIT MEDISCH BIBLIO on December 12, 2006 .
Copyright © 1998 Massachusetts Medical Society. All rights reserved.