Association of the Collagen Type 1 (COL1A 1) Sp1 Binding Site Polymorphism to Femoral Neck Bone Mineral Density and Wrist Fracture in 1044 Elderly Swedish Women

P. Gerdhem,[1] H. Brändström,[2] F. Stiger,[2] K. Obrant,[1] H. Melhus,[2] Ö. Ljunggren,[2] A. Kindmark,[2] K. Åkesson[1]

[1]Department of Orthopedics, Lund University, Malmö University Hospital, Malmö, Sweden
[2]Department of Medical Sciences, Uppsala, Sweden

Abstract. Identification of risk factors for osteoporosis has been essential for understanding the development of osteoporosis and related fragility fractures. A polymorphism of the binding site for the transcription factor Sp1 of the collagen I alpha 1 gene (COLIA1) has shown an association to bone mass and fracture, but the findings have not been consistent, which may be related to population differences. The Sp1 polymorphism was determined in 1044 women, all 75 years old, participating in the population-based Osteoporosis Prospective Risk Assessment study in Malmö (OPRA). Bone mineral density, heel ultrasound and all previous fractures were registered. BMD was 2.7% lower in the femoral neck in women carrying at least one copy of the ''s'' allele ($P = 0.027$). There was no difference in bone mass at any other site, weight, BMI or age at menopause. Women with a prevalent wrist fracture (n = 181) had an increased presence of the ''s'' allele. The odds ratio for prevalent wrist fracture was 2.73 (95% CI 1.1–6.8) for the ss homozygotes and 1.4 (95% CI 1.0–2.0) for the Ss heterozygotes when compared with the SS homozygotes. In conclusion, in this large and homogenous cohort of 75-year-old Swedish women, there was an association among the Sp1 COLIA1 polymorphism, bone mass, and fracture. The presence of at least one copy of the ''s'' allele was associated with lower femoral neck BMD and previous wrist fracture and in addition, it was related to an increased risk for wrist fracture.

Key words: Collagen — Genotype — Bone mineral density — Ultrasound — Fracture

Genetic factors play a central role for the development and maintenance of bone mass. Genetic factors also play a role in the pathogenesis of osteoporosis, with environmental factors acting as more or less pronounced modifiers. Influences on bone are believed to be polygenic in nature, and several potential candidate genes have been implicated in various regulatory steps of bone metabolism. Bone collagen provides the matrix for bone mineralization, but it also provides the tensile strength necessary for bone elasticity. Two genes regulate bone collagen production, the collagen Iα1 (COLIA1) gene located on chromosome 17, and the collagen Iα2 gene located on chromosome 7. Mutations in the COLIA1 gene give rise to osteogenesis imperfecta, with severe osteoporosis [1].

Several polymorphisms in the COLIA1 gene have been identified, and have been subject to association studies in different populations with varying results [2, 3]. A polymorphism at the binding site for the transcription factor Sp1 on the COLIA1 gene, with a single nucleotide exchange of G to T, has been identified [4]. The polymorphism is located within the first intron in a region involved in the regulation of collagen transcription [5]. Indications of functional effects attributable to the Sp1 polymorphism has been described, while other polymorphisms at the COLIA1 locus appear nonfunctional in relation to osteoporosis [2, 3]. The ''s'' allele of the Sp1 polymorphism has been linked to an increased transcription of mRNA and a relative increase in the amount of α1(I) protein to α2(I) collagen with an effect on bone strength [2]. Overrepresentation of the ''s'' allele in women with osteoporosis and osteoporotic fracture has been suggested but the findings are inconsistent, where some have found an association [6–8] while others have not [9–11]. In a recent meta-analysis, including 16 studies and a total of 4,965 individuals of various ages, the association was significant between the presence of the ''s'' allele and low BMD, reduced BMI, and fragility fractures [2]. A possible explanation for the inconsistency may be related to the age and geography. The aim of this study therefore was to evaluate whether an association of the Sp1 polymorphism exists among bone mass, bone quality and fracture in elderly Swedish women.

Correspondence to: K. Åkesson; E-mail: kristina.akesson@orto.mas.lu.see

Subjects and Methods

Subjects

The Malmö OPRA-study cohort consists of 1044 women, all 75 years old. In this population-based study, aimed at identifying risk factors for fracture, 1604 women were randomly selected from the city files and invited by letter; 1044 agreed to participate giving an overall response rate of 65%. All were of Caucasian background, with 12 having immigrated from other European countries. Of the 560 women who did not participate in the investigation, 13 died shortly thereafter 139 could not come due to illness, 376 women were not interested or could not attend due to reasons other than illness and 32 were not reached despite repeated letters and phone calls.

Of the 1044 women, DXA of the spine was examined in 974 and hip (neck and trochanter) in 951 women. Quantitative ultrasound of the calcaneus (QUSc) was performed in 854 women. The participants were not examined for bone mass with any or both devices because of QUSc instrument failure; and for DXA, high body weight, a disability that prevented the patient from assuming the supine position for the time required, or prior surgery interfering with the measurement. A comprehensive questionnaire was used for health, illness and function, as well as for previous fracture.

Venous blood samples were obtained from 969 women, and DNA was extractable and usable for the COLIA1 genotyping in 964 of them. These subjects were included in the present analysis provided they had a valid bone density measurement from at least one site (spine 904, hip 884). Lund University ethics committee approved the study and informed consent was obtained from all participants.

Genotyping

Genomic DNA from each individual was extracted from 3 ml of whole blood using a Qia Amp Blood kit (Qiagen GmbH, Promega, Madison, USA). The genotype of each individual was determined by solid phase minisequencing. Briefly, a 152-bp fragment of the collagen 1A1 gene was amplified with a biotinylated forward primer (TCCAATCAGCCGCTCCCA) and a reverse primer (GGGAGGGCAGGCTCGTG). PCR reactions were run on a Gene Amp PCR system-9700 robot using Ampli-Taq Gold® kits and standard reagents (Perkin Elmer Co, Norwalk CT, USA.). The amplification profile consisted of denaturation at 96°C for 10 min, followed by 36 cycles with denaturation at 96°C for 30 sec, annealing at 62°C for 30 sec and elongation at 72°C for 1.5 min, and final extension at 72°C for 7 min. The PCR products were captured in streptavidin-coated microtiterplate-wells and rendered single stranded. The polymorphic nucleotide was detected in the captured DNA strand by single-base extension of the primer GTCCAGCCCTCATCCCGCCC with ^{3}H labeled nucleotides, the primer anneals immediately adjacent to the polymorphic site. The genotype of the individual was defined by the ratio between incorporated ^{3}H-labelled nucleotides.

Bone Density Measurements

BMD measurements were made with a LUNAR DPX-L scan (Lunar Corporation, Madison, WI, USA) of the hip (neck and trochanter) region and lumbar spine (vertebrae L2-L4) (g/cm^2). By double measurements in 14 healthy individuals, the precision of the DXA measurements in our laboratory has previously been determined to be 0.5% (lumbar spine), 1.6% (femoral neck), and 2.2% (trochanter) [12]. The stability of the DPX-L equipment was checked every morning using a phantom. The long-term precision of the apparatus during a 3–4 year follow-up was calculated by daily measurements of a special referee phantom to 0.48% during the two measurements [13].

Ultrasound Measurement of the Calcaneus

Quantitative ultrasound of the right calcaneus was performed with a Lunar Achilles® (Lunar Corporation, Madison, WI, USA) of the right calcaneus (if previous injury or fracture, the left calcaneus was used). The result was given as speed of sound (SOS; m/s), broadband ultrasound attenuation (BUA; dB/MHz), and stiffness. The precision of the ultrasound equipment in our laboratory, as assessed by double measurements in 14 healthy persons, after repositioning, has previously been determined to be 1.5% [14]. The long-term stability of the apparatus was controlled by daily calibration with two phantoms provided by the manufacturer.

Statistics

The values are presented as mean ± one standard deviation, unless otherwise stated. The genotype groups are reported separately or dichotomized in the presence or absence of the "s" allele, since presence of "s" has been implicated as the unfavorable allele. According to the power analysis and with the observed sample size, the study had 80% power to detect a 0.025 g/cm^2 BMD difference or 1.54 in odds ratio at the 5% significance level for SS versus presence of at least one copy of "s." The study was not powered to detect differences in incident hip fractures at this age group. For differences of the continuous variables between genotype groups, ANOVA or t-test, as appropriate, was used to compared the means. For categorical variables the Chi-square test was used. Multivariate linear regression was applied to analyze the effect of genotype on bone density and BMI; age adjustment was not applicable. A multiple logistic regression model was constructed to test the relative risk of fracture, assigning odds ratios with 95% confidence intervals. Values are considered significant for P-values < 0.05. Statistical analysis was carried out using the STATISTICA[TM] statistical program.

Results

The women were all of the same age (75.01–75.99 yrs) and therefore age adjustment was not applicable. Six hundred seventy-five (70%) were homozygous for SS, 263 (27%) were heterozygous carrying Ss, and 26 (3%) were homozygous for ss. The COLIA1 Sp1 genotypes were in Hardy-Weinberg equilibrium. The characteristics of the three groups are shown in Table 1. Body weight or height did not differ between the genotype groups. Age at menopause was similar, as was previous and current hormone use (total n = 17). Other potentially confounding factors, such as use of bisphosphonates (n = 31), glucocorticosteroids (n = 38), calcium (n = 46), vitamin D (n = 30) or smoking (n = 131) did not differ between the genotype groups (P = 0.20–0.89).

Bone Mineral Density

Since the ss group was small and a gene-dose effect was apparent at the hip, presence of the "s" allele is also reported. BMD was significantly lower in the hip-femoral neck in women carrying one copy of the s allele, but no difference was evident in the trochanter or spine, even if the trend was similar. BMD was 2.7% lower in the

Table 1. Genotype frequency and characteristics according to genotype group

	SS	Ss	ss	Ss/ss		ANOVA SS vs "s"
No. of women	675	263	26	289		
	70%	27%	3%	ANOVA by genotype P-value[a]	30%	
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD		Mean $\pm$ SD	P-value[b]
Age (yrs)	75.2 $\pm$ 0.1	75.2 $\pm$ 0.1	75.2 $\pm$ 0.1	0.56	75.2 $\pm$ 0.1	0.31
Body height (cm)	161 $\pm$ 6	161 $\pm$ 6	162 $\pm$ 4	0.71	161 $\pm$ 6	0.90
Body weight (kg)	68 $\pm$ 11	67 $\pm$ 11	69 $\pm$ 12	0.41	67.2 $\pm$ 11.2	0.77
BMI (kg/m^2)	26.3 $\pm$ 4.2	26.1 $\pm$ 4.0	26.5 $\pm$ 4.3	0.62	26.2 $\pm$ 4.0	0.42
Age at menopause (yrs)	49 $\pm$ 5.0	49 $\pm$ 4.8	48 $\pm$ 5.3	0.59	49 $\pm$ 4.8	0.68
Femoral neck BMD (g/cm^2)	0.754 $\pm$ 0.127	0.735 $\pm$ 0.125	0.721 $\pm$ 0.128	0.08	0.734 $\pm$ 0.125	0.03[c]
Trochanteric BMD (g/cm^2)	0.706 $\pm$ 0.143	0.696 $\pm$ 0.135	0.685 $\pm$ 0.126	0.52	0.695 $\pm$ 0.134	0.28
Lumbar spine BMD (g/cm^2)	0.998 $\pm$ 0.195	0.974 $\pm$ 0.188	1.018 $\pm$ 0.180	0.22	0.978 $\pm$ 0.187	0.17
Heel BUA (dB/MHz)	99.1 $\pm$ 9.9	99.2 $\pm$ 10.8	99.2 $\pm$ 8.2	0.98	99.2 $\pm$ 10.6	0.85
Heel SOS (m/s)	1502 $\pm$ 28	1503 $\pm$ 28	1499 $\pm$ 23	0.76	1502 $\pm$ 27	0.94
Heel stiffness	66.9 $\pm$ 13.1	66.9 $\pm$ 13.6	65.8 $\pm$ 11.2	0.92	66.8 $\pm$ 13.4	0.85

The total number of genotyped women n = 964. Spinal BMD was available in 904, hip in 884 and ultrasound in 854 women
[a]ANOVA-by genotype
[b]ANOVA-comparison SS vs presence of "s"
[c]P significant $P < 0.05$

Table 2. Women never having fractured or having had any type of fracture during their lifetime categorized according to genotype.

	SS n (%)	Ss n (%)	ss n (%)	Total n	Chi^2 by genotype (P-value)	Ss/ss n (%)	Chi^2 SS vs "s" (P-value[a])
No. of women/genotype group	675	263	26	964		289	
No. fracture at any time	390 (57.8)	143 (54.4)	11 (42.3)	544		154 (53.3)	
Fracture during life-time	285 (42.2)	120 (45.6)	15 (57.7)	420	0.22[b]	135 (46.7)	0.18[b]
Subgroups of women with:							
Any fracture after age 50 yrs	237 (35.1)	99 (37.6)	13 (50.0)	349	0.26[b]	112 (38.8)	0.28[b]
wrist fracture	117 (17.3)	55 (20.9)	9 (34.6)	181	0.047[c*]	64 (22.1)	0.07[c]
hip fracture	30 (4.4)	16 (6.1)	2 (7.7)	48	0.32[c]	18 (6.2)	0.24[c]

Women with fracture are further subgrouped into women with fracture after age 50 (any type) and into women with fractures of wrist or hip. The percentage values are calculated from the number of women per genotype group
[a]P-value for Chi^2-test (presence of "s" vs SS)
[b]Comparison between the never and any groups
[c]Comparison of group of women without any fractures (n = 544)
[d]P-value < 0.05

femoral neck in those carrying at least one copy of "s" ($P = 0.027$). The corresponding T-score values for the genotype groups were between -1.88 and -2.15 in the femoral neck and -1.68 and -1.88 in the spine. Thirty-one percent of the women had osteoporosis with a T-score below -2.5 at the hip and 33% at the spine. Bone quality as measured by ultrasound of the heel was similar among the different genotype groups.

Using stepwise regression analysis including body weight in the model, the genotype contributed significantly to the variance in BMD ($r^2 = 0.0046$, $P = 0.02$) although body weight was the major contributor ($r^2 = 0.22$, $P = 0.001$). The contribution of BMI was less pronounced than for body weight.

All Types of Fractures

Of the 420 women reporting at least one fracture during their lifetime, 104 had sustained more than 2 fractures during their lifetime; 349 women reported fractures after the age of 50, fractures of potentially osteoporotic origin. In the small group of women homozygous for ss (n = 26), the relative number having sustained any type of fracture was greater, particularly compared to the SS homozygotes, but the difference between the groups did not reach significance, regardless of recording lifetime fractures or fractures since age 50 (Table 2). Also, when combining Ss heterozygotes and ss homozygotes, the association to fracture remained insignificant.

Table 3. Comparison of women sustaining a wrist fracture (n = 181) during their life to women who had never suffered any fracture during their lifetime (n = 544)

	Wrist fracture Mean ± SD	No fracture at any time Mean ± SD	P-value
Body height (cm)	161 ± 5.4	160 ± 5.6	0.033
Body weight (kg)	66 ± 10.4	68 ± 11.5	0.041
Femoral neck BMD (g/cm^2)	0.720 ± 0.113	0.773 ± 0.125	< 0.001
Femoral neck T-score	−2.2 ± 0.9	−1.7 ± 1.0	< 0.001
Trochanteric BMD (g/cm^2)	0.672 ± 0.119	0.729 ± 0.137	< 0.001
Trochanteric T-score	−1.1 ± 1.1	−0.6 ± 1.2	< 0.001
Lumbar spine BMD (g/cm^2)	0.944 ± 0.165	1.016 ± 0.200	< 0.001
Lumbar spine T-score	−2.1 ± 1.4	−1.5 ± 1.7	< 0.001
Heel BUA (dB/MHz)	96.5 ± 9.2	101.3 ± 9.8	< 0.001
Heel SOS (m/s)	1494 ± 25	1508 ± 29	< 0.001
Heel stiffness	62.6 ± 11.9	69.9 ± 13.1	< 0.001

Wrist Fracture

In the cohort, 181 (18.7%) women had suffered a fracture of the distal radius, thus representing a distinct phenotype. These women were compared with the 544 (56.4%) women who had never sustained any type of fracture during their lifetime, the completely fracture free group. The wrist fracture and never fracture group significantly differed in all bone mass variables (Table 3). BMD was lower at all measured sites by 6.9–7.8% (P < 0.0001), corresponding to a T-score difference of −0.44 to −0.60 SD. The difference in ultrasound parameters was more varied ranging between 1.0 and 10.4% (P < 0.0001).

The genotype distribution differed between those with previous wrist fracture and those never having fractured, with overrepresentation of the "s" allele in the fracture group (Table 2). The association to fracture was most pronounced among women with the ss genotype (P = 0.024).

Logistic regression analysis showed that BMD of the hip or spine were independent predictors for wrist fracture (Table 4). The risk related to genotype showed that women with the ss genotype had 2.7 times the risk, and women with the Ss genotype 1.3 times the risk over women in the SS genotype group, each additional copy of "s" contributing to a gene-dose effect. The odds ratios for homozygot ss genotype as an independent predictor for wrist fracture did not change after adjusting for trochanteric BMD (OR 3.18 (CI 1.22−8.30, P = 0.018)), spine BMD (OR 2.75 (CI 1.01−7.47, P = 0.047)) or ultrasound SOS (OR 3.15 (CI 1.18−8.39, P = 0.021)), with or without inclusion of weight in the model. Femoral neck BMD changed the odds ratio, and genotype was no longer independently predictive (OR 2.56 (CI 0.94−6.98, P = 0.064)). This independent association of femoral neck BMD may be one of the reasons for the s allele not having an independent effect on fracture, when femoral neck BMD is entered into the model, and suggesting that the effect of

the "s" allele could be mediated through femoral neck BMD.

Discussion

In this large and well-characterized cohort of elderly Swedish women we found women homozygous for SS to have a significantly higher BMD at the femoral neck compared to women carrying at least one copy of the "s" allele. However, the BMD effect of the COLIA1 Sp1 polymorphism was not consistently found at other measuring sites. We found no association at the trochanter and lumbar spine, in spite of a similar trend. Furthermore, we found no association between genotype and heel ultrasound measurements. An association between carrying one copy of the "s" allele and lower BMD at the hip has been described [6, 7, 15, 16], while others have found no association [11, 17–21]. Similar discrepancies are described for the lumbar spine and association to the COLIA1 Sp1 polymorphism. One study is specifically addressing elderly women above the age of 75 and no association between COLIA1 genotype and bone mass was found, however, the sample only represents a response rate of 17% [9].

Nevertheless, from the studies included in the meta-analysis, a pronounced effect of the Sp1 allelic variation and bone mass, but also vertebral fracture was seen [2, 22]. A possible explanation may be related to the age of the studied populations, which generally has been below 75 years or with rather limited number of subjects above the age of 75. In accordance with a potential age-related effect, the more pronounced associations to lumbar spine BMD have been seen in perimenopausal or recently postmenopausal women [6, 7, 15]. However, in the study by Uitterlinden et al. [7] including 210/1778 women age 70–80, the age effect on both hip and spine BMD was more marked with increasing age. Additionally, geographic or ethnic influence may play a role, even if most reports include populations of Caucasian back-

Table 4. Odds ratio of having sustained a wrist fracture according to bone density and COL1A1 genotype

	Odds ratio	CI (95%)[b]
Femoral neck T-score[a]	0.6	0.6–0.8
Trochanteric T-score[a]	0.7	0.6–0.8
Lumbar spine T-score[a]	0.8	0.7–0.9
SS homozygotes	1.0	
Ss heterozygotes	1.0	0.9–1.9
ss homozygotes	2.7	1.1–6.8
Presence of "s" allele	1.4	1.0–2.0

[a] Odds ratio calculated per unit T-score (each T-score equals one standard deviation change from peak bone mass for young adult women)
[b] 95% Confidence interval

ground in northern Europe, but the COLIA1 Sp1 polymorphism is not associated with bone mass or fracture in Finnish women, for example [23]. In this respect our cohort is homogenous by origin and with a comparatively high response rate (65% compared to e.g., UK studies of 14%). Nevertheless, the non-attendees are most likely in a poorer state of health than those attending, allowing for a possible bias.

Bone quality assessed by ultrasound (US) of the heel and a potential association to collagen polymorphisms has been studied to a lesser extent. Despite the theoretical attractiveness of collagen as a major contributing factor to bone quality and the postulated assessment of bone quality by QUS, we found no apparent association among these elderly women and COLIA1 Sp1 genotype distribution. The reason for the almost identical QUS properties is unclear, but similar to the findings of Ashford et al. [9] who found no association between ColIA1 and QUS at several sites, including the calcaneus, in slightly older women.

Body weight is a well-known independent determinant for bone mass. From the meta-analysis, an independent association of the Sp1 polymorphism to BMI and body weight was reported [2]. In these elderly women there was no association between the COLIA1 Sp1 polymorphism and body weight, height, BMI, menopausal age or HRT. However, when looking separately at women with prevalent wrist fracture there was a small, but significant difference in weight.

Prevalent vertebral fractures have been associated with carrying at least one copy of the s allele [4, 8, 15] but also with nonvertebral fractures [6], whereas other investigators found a less clear relationship [7], and in a case control study of 138 Swedish women, no association was found [21]. Because of the cohort size, a fairly large number of women with wrist fracture was identified and regarded as a specific phenotype. They were compared with those women within the cohort who had never sustained any fracture during their lifetime. This approach has the advantage of identifying both cases and controls within the same cohort, with the same age,

selection criteria, and inclusion for both groups as compared to control findings after the case identification. As expected, all bone mass variables differed between the wrist fracture and nonfracture groups. In addition, genotype and the "s" allele were associated with fracture, with women homozygous for ss having an increased odds ratio for sustained fracture. In this respect our data are similar to findings in a different population of wrist fracture patients [24].

In these 75-year-old women, polymorphism at the COLIA1 Sp1 site was not associated with previous fracture in general, i.e., all types of previous nonvertebral or vertebral fractures without specification. This lack of association was not related to when the fracture had occurred—during the entire lifetime or after the age of 50, the latter of which could have been an indicator of osteoporotic fragility fractures.

Despite having evaluated a large cohort with a genotype distribution similar to that of previously studied populations, the ss homozygotes are relatively few in numbers. This taken together with standard deviations of between 10 and 18% for bone mass measurements at this age, it is difficult to identify potential associations between genotype and bone mass and requires even larger cohorts. In the lumbar spine particularly where degenerative or other changes can affect the measured values, possible associations may be masked in older age groups. In this study, prevalent fractures have been identified through recall, which may over or under estimate the true fracture incidence, but most women at this age clearly remember fractures of the wrist or hip.

In summary, in these elderly women reaching their fracture prone years, particularly with an increasing risk of hip fracture, the presence of the "s" allele is associated with lower bone mass at the femoral neck, whereas no such linkage was obvious to other sites, including heel ultrasound or unspecific fractures. Bone mass in women with distal radius fractures differed from women who had never suffered any type of fracture, with a significant contribution from the "s" allele. In addition, the ss genotype was more prevalent in women who had sustained wrist fractures, with an odds ratio of 2.7. These findings suggest an influence of the COLIA1 Sp1 polymorphism from the presence of the "s" allele on bone mass and fracture in elderly Swedish women.

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