EXTENDED REPORT

Collagen type I α1 Sp1 polymorphism, osteoporosis, and intervertebral disc degeneration in older men and women

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Objectives: To examine whether collagen type I α1 (COLIA1) Sp1 polymorphism is associated with osteoporosis and/or intervertebral disc degeneration in older people.
Methods: COLIA1 genotype was determined in 966 men and women (⩾65 years) of the Longitudinal Aging Study Amsterdam. The guanine (G) to thymidine (T) polymorphism in the first intron of the COLIA1 gene was detected by PCR and *Msc*I digestion. In the total sample, quantitative ultrasound (QUS) measurements, serum osteocalcin (OC), and urine deoxypyridinoline (DPD/Cr_{urine}) were assessed. A follow up of fractures was done every three months. In a subsample, total body bone mineral content (n = 485) and bone mineral density (BMD) of the hip and lumbar spine (n = 512) were measured by dual energy *x* ray absorptiometry (DXA). Prevalent vertebral deformities and intervertebral disc degeneration were identified on radiographs (n = 517).
Results: People with the TT genotype had a higher risk of disc degeneration than those with the GG and GT genotypes (OR = 3.6; 95% CI 1.3 to 10). For men, higher levels of OC were found in those with the T allele than in those without it (GG *v* (GT+TT) 1.96 (0.06) nmol/l *v* 2.19 (0.09) nmol/l). COLIA1 polymorphism was not significantly associated with other measures of osteoporosis in either men or women.
Conclusion: COLIA1 Sp1 polymorphism may be a genetic risk factor related to intervertebral disc degeneration in older people. Previously reported associations between the COLIAI Sp1 genotype and lower BMD or QUS values, higher levels of DPD/Cr, and an increased fracture risk in either men or women could not be confirmed.

Osteoporosis and osteoarthritis (OA) are common skeletal disorders that cause pain, physical limitations, and disability in later life.[1 2] Over the past two decades, clinical and epidemiological studies observed that osteoporosis is inversely related to spinal degeneration diseases or intervertebral disc degeneration.[3–6] The inverse relationship between osteoporosis and intervertebral disc degeneration may give insight into the pathogenesis of both disorders.

Until now, there is no clear explanation for this inverse relationship. Because both osteoporosis[7 8] and intervertebral disc degeneration[9 10] are largely genetically determined, it has been suggested that the inverse relationship may be attributed to a shared set of genetic factors underlying both disorders. Recent studies found that polymorphisms which have previously been shown to be associated with osteoporosis, such as the vitamin D receptor gene,[11 12] the oestrogen receptor gene,[13] and transforming growth factor β1 gene,[14] were also associated with disc degeneration.[15–17] Thus genes that play a part in the development of osteoporosis may also be considered potential candidate genes for the occurrence of disc degeneration.

A likely candidate gene to be involved in disc degeneration and osteoporosis is the collagen type I α1 gene (COLIA1). This gene encodes a part of type I collagen, which is the major protein in bone. Mutations in this gene cause the connective tissue disorders Ehlers-Danlos syndrome, characterised by cutis laxa, hypertension of the joints and low bone mass, and osteogenesis imperfecta, which is associated with a very low bone mass and an increased fracture risk.[18] In 1996, Grant *et al* described a guanine (G) to thymidine (T) polymorphism affecting a binding site for the transcription factor Sp1 in the first intron of COLIA1.[19] This study showed that the T allele was more prevalent in patients with osteoporosis than in controls. Additional studies showed that the GT and TT genotypes were associated with lower bone mineral density (BMD) values,[19–23] increased bone loss,[24] lower quantitative ultrasound (QUS) values,[25] higher levels of bone turnover,[23] and an increased fracture risk.[19 21 23 26 27] However, other studies failed to show an association between the COLIA1 Sp1 binding site polymorphism and osteoporosis in postmenopausal women.[28–32] Thus, the association between the COLIA1 gene and osteoporosis is still not definitely established.

Until now, no previous study has examined the association between COLIA1 and intervertebral disc degeneration, but two studies have been published on the association between COLIA1 and osteoarthritis of the hip and knee.[33 34] In a large case-control study, in which 371 probands who had undergone total joint replacement of the hip and/or knee were compared with 369 unaffected spouses, Loughlin *et al* did not find an association between COLIA1 gene and hip OA. [33] A similar negative result was found in a smaller study including 75 female patients who underwent total hip replacement and 239 controls.[34] However, the association of COLIA1 Sp1 polymorphism with intervertebral disc degeneration may differ from the association with hip and/or knee OA. Although the pathogenesis of disc degeneration resembles OA in the peripheral joints, disc degeneration may be a separate clinical entity influenced by different risk factors.

Abbreviations: ANOVA, analysis of variance; BMC, bone mineral content; BMD, bone mineral density; BUA, broadband ultrasound attenuation; CI, confidence interval; COLIA1, collagen type I α1; DPD, deoxypyridinoline; DXA, dual energy *x* ray absorptiometry; G, guanine; JSN, joint space narrowing; LASA, Longitudinal Aging Study Amsterdam; OA, osteoarthritis; OR, odds ratio; PCR, polymerase chain reaction; QUS, quantitative ultrasound; RR, relative risk; SOS, speed of sound; T, thymidine; VUMC, VU University Medical Centre

Moreover, disc degeneration may be more strongly influenced by genetic factors than OA of the knee or hip.[35]

To know whether COLIA1 is involved in both osteoporosis and disc degeneration, it is important to examine the association of COLIA1 with both disorders in the same group of people. To our knowledge, this has not been done before. In addition, the contradictory results for the association between COLIA1 and osteoporosis need further study.

The Longitudinal Aging Study Amsterdam (LASA) is a large prospective study among community dwelling elderly men and women in the Netherlands. In this study a comprehensive variety of measures of osteoporosis, and disc degeneration were determined. The study aimed at examining whether the COLIA1 Sp1 polymorphism is associated with osteoporosis and/or intervertebral disc degeneration in older men and women.

METHODS

Study sample

The LASA is a continuing cohort study on predictors and consequences of changes in autonomy and wellbeing in the aging population in the Netherlands.[36] The sampling and data collection procedures have been described in detail elsewhere.[37 38] Briefly, a sample of older men and women (aged 55–85), stratified by age, sex, and urbanisation, was drawn from the population registers of 11 municipalities in areas in the west (Amsterdam and its vicinity), north east (Zwolle and vicinity), and south (Oss and vicinity) of the Netherlands. Data collection took place in 1992–93, in 1995–96, and in 1998–99.

Figure 1 shows the recruitment of the subjects. Of the 3805 older people who were initially approached, 3107 (81.7%) took part in the baseline examination in 1992–93. Non-response was related to age ($p<0.001$), the oldest people being less likely to participate. In 1995–96, 2302 (87.2%) of the 2639 eligible respondents completed a main interview. Loss to follow up between the first and second cycle was mainly due to death: 417/3107 (13.4%) respondents died.[38] The present study on COLIA1 Sp1 polymorphism, osteoporosis, and disc degeneration was performed within a subsample of the LASA sample, which consisted of respondents who participated in the medical interview of the second data collection cycle and who were born in 1930 or before (aged 65 years and older as of the first of January, 1996). Of the 1720 eligible respondents, 1509 (87.7%) took part in the medical interview. After this interview at home, respondents were invited to the VU University Medical Centre (VUMC) (respondents living in Amsterdam and vicinity) or a healthcare centre near their homes (respondents living in Zwolle or Oss and vicinity), where BMD and QUS measurements were performed, radiographs of the lumbar and thoracic spine were assessed, and blood and urine samples were obtained. Blood samples were collected in 1321 of the 1509 respondents. Adequate buffy coats for DNA isolation were obtained in 966 subjects (471 men and 495 women) of the 1509 respondents (64.0%). Of these, fasting levels of serum osteocalcin (OC) were available in 964, urinary deoxypyridinoline (DPD/Cr_{urine}) in 934, and QUS measurements in 951 respondents. Information on incident fractures between the first cycle of data collection in 1992–93 until the

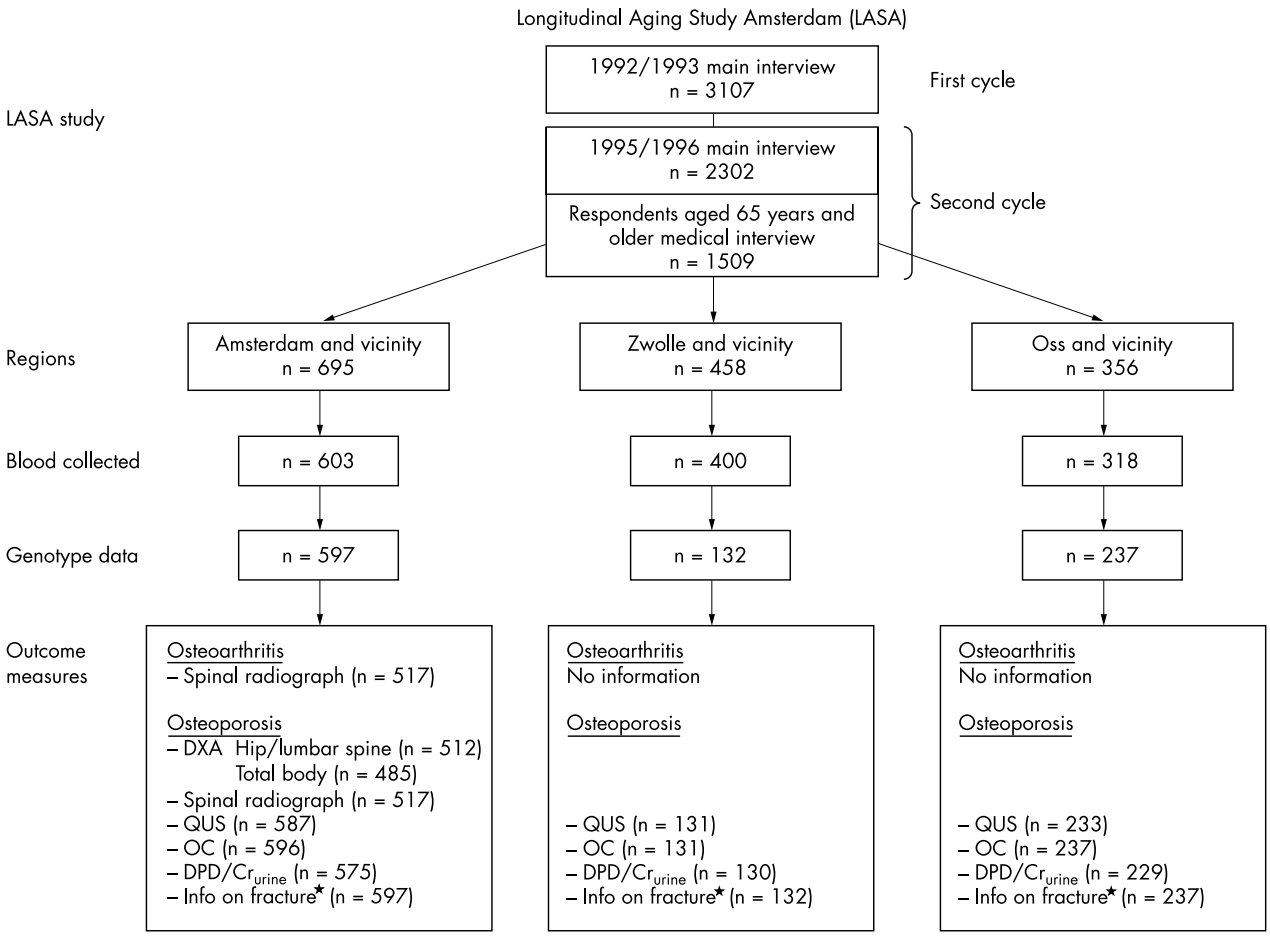

Figure 1 Recruitment of participants. *Fractures that occurred between 1992–93 and between 1995–96 were assessed retrospectively in 1995–96, whereas fractures that occurred between 1995–96 and between 1998–99 were assessed prospectively.

third data collection in 1998–99 was obtained for all the 966 respondents. In a subsample, including respondents who were living in the west of the Netherlands, BMD of the hip and lumbar spine (n = 512), total bone mineral content (BMC) (n = 485), prevalent vertebral deformities, and disc degeneration of the thoracic and lumbar spine (n = 517) were assessed. Except incident fractures, measures were cross sectionally determined during the second measurement cycle in 1995–96. Of the 1720 respondents who were eligible, the respondents who did not have QUS measurements (n = 412) were more often female, were older, had a lower level of education, were more often cognitively impaired, and had lower physical performance scores ($p<0.05$). The same was true for subjects who did not have BMD measurements or spine *x* rays, except that there were no differences in sex.

All interviews were conducted by specially trained and intensively supervised interviewers (main interview) and nurses (medical interview) and were tape recorded in order to monitor the quality of the data. Informed consent was obtained from all respondents. The study was approved by the Medical Ethics Committee of the VUMC and conducted according to the principles of the Helsinki declaration.

Measurements

COLIA1 genotyping

Buffy coats were obtained from EDTA-blood during the examination in 1995–96 and stored at −80℃ until DNA isolation and COLIA1 genotyping in 1999. At the endocrine laboratory of the VUMC, the G to T polymorphism in the Sp1 binding site in the COLIA1 gene was detected by a polymerase chain reaction (PCR) based method, described by Uitterlinden *et al*.[21] Briefly, DNA was extracted from buffy coats by standard phenol extraction methods. As described by Grant *et al*, PCR was performed with mismatch primers,[19] which introduce a restriction site for *Msc*I if the polymorphism is present. After digestion with *Msc*I and gel electrophoresis, the alleles were defined as G or T according to the absence or presence of the restriction site, respectively. The genotypes were named GG, GT, or TT, which corresponds with SS, Ss, and ss, respectively, the designation previously used.

Assessment of disc degeneration

Lateral radiographs of the thoracic and lumbar spine (T4–L5) were made at the end of 1995 or in 1996 in each respondent according to the protocol of the European Vertebral Osteoporosis Study.[39] The thoracic film was centred at T7 and the lumbar film at L2. The *x* ray tube to film distance was 115 cm. All radiographs were assessed by an experienced clinician and a researcher for disc degeneration on the four point Kellgren scale.[9] The assessments of the presence of osteophytes and articular joint space narrowing (JSN) were combined into one score, ranging from 1 (no or very small osteophytes, no JSN) to 4 (large osteophytes, pronounced JSN). Severe disc degeneration was defined as a Kellgren score of 4, which corresponds with the upper quartile of the population. In a random sample of 50 radiographs, the intraobserver agreement of the score was estimated using a weighted κ score, as described by Landis and Koch.[40] In this sample, the weighted κ score for the Kellgren score (1–4) was 0.63.

Measures of osteoporosis

Bone mineral density

Total body BMC and BMD of the hip (total hip, femoral neck, trochanter) and lumbar spine (L1–4) were measured by dual energy *x* ray absorptiometry (DXA, Hologic, QDR 2000, Hologic Inc, Waltham, Massachusetts, USA; software version V5.67A). For hip density, the right hip was scanned. In people with single hip joint replacement, the other hip was scanned (n = 23). Respondents with both hips replaced were excluded (n = 13).

Quantitative ultrasound measurements

QUS data were obtained using the CUBA Clinical instrument (McCue Ultrasonics, Winchester, UK). Broadband ultrasound attenuation (BUA) (dB/MHz) and speed of sound (SOS) (m/s) were measured twice in both the right and left calcaneus. Mean BUA and SOS values were calculated from these four measurements.[41]

Bone markers

Fasting morning serum levels of intact OC and overnight urinary excretion of DPD were determined at the Endocrine Laboratory of the VUMC. Serum OC was measured by an immunoradiometric assay (Biosource Diagnostics, Fleuris, Belgium). DPD was determined by a competitive immunoassay on the automated ACS 180 System (Chiron Diagnostics, Emeryville, USA). The values were corrected for creatinine concentration (Cr) in the same urine sample.

Prevalent vertebral deformities

Two observers (see ''Assessment of disc degeneration'') also assessed the radiographs for the presence and degree of vertebral deformities using a semiquantitative method[42] (mild deformity: 20–25% reduction in anterior, central, or posterior vertebral height; moderate deformity: >25–30% reduction in vertebral height; severe deformity: >30% reduction in vertebral height), as described elsewhere.[43] Weighted κ scores for the presence of deformity (yes/no) and severity of deformities were 0.80 and 0.75, respectively.[43] In this study, reduction of the anterior, central, or posterior vertebral height of >25% was defined as a vertebral deformity.

Ascertainment of fractures

Fractures that occurred between the first LASA examination in 1992–93 and the second examination in 1995–96, were retrospectively assessed in 1995–96. Data on fractures that occurred between the second examination and the third examination in 1998–99 were prospectively collected with a calendar. Eighty two per cent of all reported fractures were verified by a doctor or by radiographs. To have sufficient power, all fractures were used in the analysis. Duration of follow up was calculated as the time from the first examination to the first occurrence of a fracture. Fractures caused by an (motor vehicle) accident (n = 10) and fractures of the head, fingers, and toes (n = 15) were excluded.

Potential confounders

Baseline information on age and sex was derived from the municipal registries. During the first and second data examination, body weight, body height, current smoking (yes/no), alcohol use (number of drinks a week), physical activity, lifetime exercise, mobility, and age at menopause (years) were assessed in a face to face interview. Body weight was measured without clothes and without shoes using a calibrated bathroom scale. Height was measured with a stadiometer. Physical activity was assessed with a validated questionnaire for the elderly, covering household activities, sports and leisure activities during the previous two weeks.[44 45] Walking outside, bicycling, sporting activities, doing light and heavy household activities were summed to give a physical activity score (range 0–5). Level of mobility was assessed with three physical performance tests,[46] which included the time needed to walk three metres back and forth along a rope (walking test), time needed to stand up and sit down five times with arms folded (chair stands), and time

needed to put on a cardigan and take it off (cardigan test). For each test, a score of 1 to 4 points was assigned corresponding to the quartile of the time needed. The more time that was needed, the lower the score. Participants who could not perform a test obtained a score of zero points. The scores of the three tests were summed to obtain a physical performance score (range 0–12). Lifetime exercise was assessed retrospectively by asking the respondents whether they had exercised earlier in life, including exercise during work. Lifetime exercise was categorised into five groups (0, light to moderate exercise during whole life; 1, heavy to very heavy exercise during one period in life; 2, heavy to very heavy exercise during two periods in life; 3, heavy to very heavy exercise during three periods in life; 4, heavy to very heavy exercise during whole life). Heavy lifetime exercise was defined as a score of ⩾3.

Data analysis

Hardy-Weinberg equilibrium was calculated using the program available from Professor J Ott (Rockefeller University, New York, USA; ott@linkage.rockefeller.edu). Because of the substantial differences in BMD, QUS, and bone markers between men and women, all analyses with osteoporosis measures as outcome were stratified by sex. One way analysis of variance (ANOVA) was used to examine differences in normal continuous variables between the three genotype groups, whereas the Kruskal-Wallis test was used to examine differences in skewed continuous variables. The χ^2 test was used to test for differences in categorical variables. Analysis of covariance was performed to adjust the associations between COLIA1 Sp1 polymorphism and the continuous outcome measures BMD, BUA, SOS, OC, and DPD/Cr_{urine} for potential confounders. The distributions of OC and DPD/Cr_{urine} were normalised by transformation to their natural logarithm to improve the plots of the residual analyses. To examine the association between COLIA1 Sp1 polymorphism and disc degeneration, and vertebral deformities, respectively, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using logistic regression analysis. The relative risk (RR) (95% CI) of any fracture was assessed with Cox regression analysis. In all models, potential confounders were included to control for confounding and to enhance precision. Because data on fractures were collected since the baseline, models with any fracture as outcome were adjusted for potential confounders determined at baseline, whereas models with the other outcome measures were adjusted for potential confounders measured during the second data examination. For each association we evaluated which of the potential confounders changed the size of the effect measure. Those which changed the strength of the association substantially (>10%) were included as confounders. Because age is a very well known risk factor for osteoporosis and disc degeneration, all analyses were adjusted for age. Body height was included in all models with BMD as dependent variable because it has been shown to influence DXA measurements.[47 48]

RESULTS

The frequencies of the GG, GT, and TT genotypes were 66.5, 29.5, and 4.0% in men, and 70.1, 26.5, and 3.4% in women. Allele frequencies in men were 81.2% G and 18.8% T, whereas in women this was 83.3% G and 16.7% T. The distribution of the allele frequency was as predicted by the Hardy-Weinberg equilibrium, both in men ($p = 0.47$) and in women ($p = 0.29$).

Respondent characteristics

Table 1 shows the respondent characteristics of the second data examination in 1995–96 for the total sample. No significant differences were found in the characteristics between the three genotype groups in either men or women. Within the subsample, the heterozygote GT women reported a higher age at menopause than women with the GG or TT genotype. Moreover, the median number of alcohol consumptions per week was higher among men with the TT genotype than among men with the GG and GT genotypes. Differences in characteristics of the total population at baseline in 1992–93 were similar to the examination in 1995–96.

Association between COLIA1 Sp1 genotype and disc degeneration

The percentage of people with severe disc degeneration, defined as a Kellgren score of 4, was higher in the TT genotype group than in the GG or GT genotype group. Logistic regression analysis, adjusted for age, sex, body

Table 1 Characteristics of 966 respondents* (471 men, 495 women) according to COLIA1 genotype, stratified by sex

	Men				Women			
Characteristics	GG	GT	TT	p Value	GG	GT	TT	p Value
No (%)	313 (66.5)	139 (29.5)	19 (4.0)		347 (70.1)	131 (26.5)	17 (3.4)	
Age (years)	75.8 (6.4)	75.3 (6.8)	73.6 (6.0)	0.33	75.7 (6.6)	75.8 (6.7)	75.1 (5.4)	0.90
Height (cm)	172 (15.9)	172 (17.0)	172 (5.7)	0.86	158 (16.9)	159 (6.3)	163 (6.9)	0.28
Body weight (kg)	77.5 (11.6)	78.4 (12.6)	76.8 (10.5)	0.68	71.5 (13.0)	69.6 (10.7)	71.2 (14.3)	0.36
Current smoker (%)	27.2	25.2	31.6	0.81†	12.4	16.0	11.8	0.57†
Alcohol use (drinks/week)‡	6 [1–21]‡	7 [2–21]‡	12 [3–21]‡	0.06§	1 [0–6]‡	1 [0–6]‡	1 [0–3]‡	0.68§
Physical activity (total score 1–5)‡	3 [3–4]‡	3 [2–4]‡	3 [2–4]‡	0.76§	3 [2–4]‡	3 [2–4]‡	3 [2–4]‡	0.83
Heavy lifetime exercise (%)	37.8	37.0	42.1	0.91†	36.9	41.1	29.4	0.55†
Physical performance	7.0 (2.8)	6.9 (2.7)	7.3 (2.3)	0.80	6.6 (2.8)	6.7 (2.9)	7.4 (2.9)	0.48
Age at menopause (years)	NA	NA	NA	NA	48.7 (5.5)	49.3 (5.0)	47.6 (4.7)	0.31
QUS measurements								
BUA (dB/MHz)	80.2 (1.0)	81.0 (1.5)	77.6 (4.2)	0.74	61.6 (0.9)	59.4 (1.4)	61.7 (3.9)	0.74
SOS (m/s)	1626 (2.5)	1629 (3.7)	1614 (10.0)	0.37	1597 (2.1)	1597 (3.4)	1601 (9.5)	0.87
Biochemical bone markers								
DPD/Cr urine (nmol/mmol)	5.09 (0.17)¶**	4.92 (0.26)¶**	5.18 (0.67)¶**	0.56	6.22 (0.13)¶**	5.94 (0.22)¶**	6.42 (0.61)¶**	0.38
Osteocalcin (nmol/l)	1.96 (0.06)¶**	2.21 (0.09)¶**	2.01 (0.25)¶**	0.02	2.33 (0.06)¶**	2.24 (0.09)¶**	2.69 (0.27)¶**	0.52

Values are means (SD), unless otherwise indicated.
*Within the subsample consisting of subjects living in the western part of the Netherlands, differences in characteristics showed a similar pattern, except that women with the GT genotype reported a higher age at menopause ($p = 0.03$); †differences in frequencies were examined with χ^2 test; ‡median [interquartile range]; §differences in skewed parameters were examined with the Kruskal-Wallis test; ¶differences in means were examined after ln transformation; **values were adjusted for age and body weight.
NA, not applicable

Table 2 Associations between COLIA1 genotype and disc degeneration in men and women

		Disc degeneration (n = 493)*	
Genotype	No (%)	Crude OR (95% CI)	Adjusted† OR (95% CI)
GG	82/346 (24)	1.0	1.0
GT	28/130 (22)	0.9 (0.5 to 1.4)	0.9 (0.5 to 1.5)
TT	8/17 (47)	2.9 (1.1 to 7.7)	3.6 (1.3 to 10)

*For 493 of the 517 subjects, data were available on age, sex, body weight, lifetime exercise, and femoral neck BMD; †adjusted for age, sex, body weight, lifetime exercise, and femoral neck BMD.

weight, lifetime exercise, and femoral neck BMD, showed that the risk of disc degeneration for people with the TT genotype was more than three times higher than for people with the GG genotype (table 2). When disc degeneration was defined as a Kellgren score of 3 or greater, the adjusted relative risk was about twice as high in people with TT genotype than in people with the GG genotype (GT *v* GG: RR = 1.1 (95% CI 0.7 to 1.6); TT *v* GG: RR = 2.3 (95% CI 0.8 to 6.4), but this was borderline significant (p = 0.07)).

Association between COLIA1 Sp1 genotype and osteoporosis

Association between COLIA1 Sp1 genotype and BMD

Univariate and multivariable analyses, adjusted for age, height, weight, Kellgren score, age at menopause (only in women), and alcohol use (only in men) did not show significant differences in BMD and BMC with COLIA1 genotype at any skeletal site (table 3). In the univariate analyses, lumbar spine BMD tended to be higher in the male TT group (p = 0.06). However, after adjustment for disc degeneration (Kellgren score), this association did not remain significant.

Association between COLIA1 Sp1 genotype, QUS, and bone markers

No significant differences in QUS values were found between the three genotype groups in either men, or women (table 1). Men with the GT and TT genotypes had significantly higher serum OC than men with the GG genotype (GG *v* (GT+TT) was 1.96 (0.06) nmol/l *v* 2.19 (0.09) nmol/l) (p = 0.02), whereas urinary levels of DPD/Cr did not differ between the three genotypes. In women no differences in DPD/Cr_{urine} or OC were found between the genotypes.

Association between COLIA1 Sp1 genotype and fracture risk

No significant associations of COLIA1 Sp1 polymorphism with vertebral deformities or with any non-vertebral fracture (data not shown) were seen. To increase statistical power, we also performed the analyses by pooling the data for the GT and TT group (table 4). However, these analyses also did not show any statistically significant differences with fracture risk.

DISCUSSION

In this study, we showed that people with an Sp1 polymorphism of the COLIA1 gene had an increased risk of intervertebral disc degeneration. In addition, we examined the association between COLIA1 Sp1 polymorphism and osteoporosis. Although, our results indicate that male carriers of the T allele had higher levels of serum OC, the COLIA1 Sp1 polymorphism did not seem to be related to any other indicator of male osteoporosis. In women, none of the

Table 3 Means (SEM) of hip and lumbar spine BMD and total body BMC according to COLIA1 Sp1 genotype, stratified by sex, in the subsample with DXA measurements

	Men (n = 255)*			ANOVA	Women (n = 252)†			ANOVA
Skeletal site	GG (n = 173)	GT (n = 70)	TT (n = 12)	p value	GG (n = 186)	GT (n = 61)	TT (n = 5)	p value
Total hip (g/cm^2)	0.91 (0.01)	0.92 (0.02)	0.90 (0.04)	0.94	0.79 (0.01)	0.78 (0.01)	0.74 (0.05)	0.50
Femoral neck (g/cm^2)	0.74 (0.01)	0.74 (0.01)	0.74 (0.03)	0.99	0.66 (0.01)	0.67 (0.01)	0.69 (0.04)	0.81
Trochanter (g/cm^2)	0.72 (0.01)	0.72 (0.01)	0.70 (0.03)	0.83	0.61 (0.01)	0.60 (0.01)	0.55 (0.04)	0.25
Lumbar spine (g/cm^2)	1.03 (0.01)	1.04 (0.02)	1.10 (0.05)	0.25	0.91 (0.01)	0.91 (0.02)	0.96 (0.07)	0.67
Total body BMC (g)	2460 (23.0)	2491 (35.9)	2520 (85.8)	0.57	1834 (18.9)	1793 (33.3)	1781 (126.8)	0.55

All values are presented as means (SEM); in men, values were adjusted for age, height, body weight, Kellgren score, and alcohol use; in women values were adjusted for age, height, body weight, Kellgren score, and age at menopause.
*For 255 of the 256 men with DXA measurements, data were available on age, body weight, and alcohol use; †for 252 of the 256 women with DXA measurements, data were available on age, body weight, and age at menopause.

Table 4 Odds ratios (ORs) of vertebral deformities (1995–96) and relative risks (RRs) of incident non-vertebral fractures (1992–99) by COLIA1 genotype (GG *v* GT+TT) in men and women

	Vertebral deformity (n = 502)			Any non-vertebral fracture (n = 937)		
Genotype	No (%)	Crude OR (95% CI)	Adjusted* OR (95% CI)	No (%)	Crude RR (95% CI)	Adjusted† RR (95% CI)
Men	n = 256‡			n = 457¶		
GG	26/173 (15)	1.0	1.0	22/301 (7)	1.0	1.0
(GT+TT)	16/83 (19)	1.4 (0.7 to 2.7)	1.5 (0.7 to 3.0)	6/156 (4)	0.5 (0.2 to 1.3)	0.5 (0.2 to 1.3)
Women	n = 246§			n = 480**		
GG	36/180 (20)	1.0	1.0	39/335 (12)	1.0	1.0
(GT+TT)	12/66 (18)	0.9 (0.4 to 1.8)	0.9 (0.4 to1.8)	18/145 (12)	1.1 (0.6 to 1.9)	1.1 (0.6 to 1.9)

*Adjusted for age, body weight and lumbar spine BMD; †adjusted for age and body weight; ‡for 256 of the 258 men with radiographs, data were available on age, body weight and lumbar spine BMD; §for 246 of the 259 women with radiographs, data were available on age, body weight, and lumbar spine BMD; ¶for 457 of the 464 men with fracture follow up, data were available on age and body weight; **for 480 of the 492 women with fracture follow up, data were available on age and body weight.

outcome measures of osteoporosis was found to be associated with this studied polymorphism.

Although several recent studies have focused on the association of the COLIA1 Sp1 polymorphism with osteoporosis, until now not much attention has been given to the relation of this genotype with disc degeneration. As far as we know this is the first study to have demonstrated an association between the COLIA1 Sp1 polymorphism and an increased risk of disc degeneration. Although the number of respondents with disc degeneration in this sample is relatively small, and therefore we cannot exclude the possibility that our results are due to chance, this is an interesting observation. When the COLIA1 Sp1 polymorphism can be identified as a genetic risk factor for disc degeneration in other studies, it may be useful for both the prediction of future disc degeneration and for the elucidation of biological mechanisms underlying this disease.

The association between COLIA1 Sp1 polymorphism and disc degeneration has not been studied before. However, two previous studies examined the association between COLIA1 Sp1 polymorphism and OA of the hip and knee.[33 34] In these studies, no association between COLIA1 Sp1 polymorphism and OA was found in either men[33] or women.[33 34] There are several explanations for the discrepancy between the studies. First, Loughlin *et al*[33] and Aerssens *et al*[34] ascertained patients with OA who underwent total hip[33 34] or knee replacement[33] in a hospital, whereas we assessed patients with radiographic disc degeneration. Possibly, COLIA1 Sp1 polymorphism is mainly involved in disc degeneration. This hypothesis is in line with the finding that genetic factors play a larger part in the pathogenesis of disc degeneration than in OA of the peripheral joints.[35] However, the possibility cannot be excluded that the finding of a significant association in our study sample is due to a type 1 error. A study with a large number of cases is needed to examine further the association between COLIA1 Sp1 polymorphism and disc degeneration.

The mechanism by which the COLIA1 polymorphism may be associated with disc degeneration is not known. Mann *et al* found that presence of the T allele in the COLIA1 Sp1 binding site has functional effects on the collagen gene regulation that leads to a higher COLIA1 mRNA expression level, an increased COLIA1 protein expression level, and increased COLIA1/COLIA2 protein ratios.[49] An abnormal ratio of collagen is associated with impaired bone structure. Type 1 collagen is a constituent of bone and superficial layers of osteophytes.[50] How the intronic polymorphism of the Sp1 binding site of COLIA1 may affect the transcription of collagen 1 has to be resolved. There may be interaction with other genes or environmental factors. Moreover, how defects in collagen 1 might influence the development of disc degeneration is also unclear and needs further investigation.

The lack of an association between COLIA1 genotype and BMD is in line with the findings of several other recently published data.[28 31 32] In contrast, the results of the other Dutch, community based study, the Rotterdam Study, showed that women with the T allele had significantly lower BMD values than those with the GG genotype.[21] In that study differences were, however, rather small and mainly present in the oldest women, indicating that there was effect modification by age. Because in our study BMD measurements were only performed in a relatively small subsample, it was not possible to stratify for age, and possible differences between the genotypes might have remained undetected.

Because several studies have observed that the association between the COLIA1 genotype and fracture risk persisted after adjustment for BMD,[21 23] Uitterlinden *et al*[21] and Langdahl *et al*[26] speculated that the COLIA1 Sp1 polymorphism may affect the mechanical strength of the bone. This was very recently supported by the study of Kann *et al*,[25] which showed a negative association between COLIA1 Sp1 polymorphism and ultrasound transmission velocity in the calcaneus in postmenopausal women. However, in agreement with the study of Ashford *et al*,[32] we did not find an association between the ultrasound parameters and COLIA1 Sp1 polymorphism.

In our study we observed increased serum levels of OC in men with a T allele. We are not aware of other studies that have examined the association between COLIA1 and OC in men. Therefore, further studies are needed to confirm this finding. In accordance with other studies,[24 25 30] no genotype differences in serum of OC or excretion of DPD/Cr_{urine} were found in women.[23 29]

In this study we could not confirm the associations previously demonstrated between COLIA1 and an increased risk of non-vertebral fractures,[21 23 26 27] which is in line with the findings of Liden *et al*,[28] and Aerssens *et al*.[29] Moreover, we did not observe an association between the T allele and prevalent vertebral deformities, which confirms the results of Uitterlinden *et al*[21] and Ashford *et al*[32]

Our study has several limitations. Firstly, the respondents of this study are a selective group of relatively healthy older men and women, because the frailest respondents of the LASA study could not visit the hospital or healthcare centre. If these non-responders were more often carriers of a T allele, underestimation of the associations might have occurred. Secondly, although the sample size was relatively large compared with most studies, the power to detect significant differences was still limited for most outcome measures. Moreover, we cannot exclude the possibility that type 1 errors might have occurred. Another potential limitation of this study is the way in which disc degeneration was defined. Because we used the Kellgren score, a composite score for assessing the grade of osteophytes and JSN, we could not distinguish between the different features of disc degeneration. Moreover, because the Kellgren score is a semiquantitative measure and the grades of osteophytes and JSN were in some cases difficult to assess owing to imaging artefacts or bad radiographic quality, non-differential misclassification might have occurred. This may have resulted in an underestimation of the observed associations.

In conclusion, the results of this large community based study suggest that the COLIA1 Sp1 polymorphism may be a possible genetic risk factor related to disc degeneration in older people. In contrast, we could not confirm the association previously reported between the COLIAI Sp1 genotype and lower BMD or QUS values, higher levels of DPD/Cr, and an increased fracture risk. Thus, identification of the COLIA1 Sp1 polymorphism may be beneficial, in particular, for the prediction of disc degeneration in older men and women.

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