Published in final edited form as:

Osteoporos Int. 2011 March ; 22(3): 911–921. doi:10.1007/s00198-010-1364-5.

Polymorphisms in the 5' flank of *COL1A1* gene and osteoporosis: meta-analysis of published studies

H. Jin,

Rheumatic Disease Unit, Molecular Medicine Centre, Institute of Genetics and Molecular Medicine, Western General Hospital, University of Edinburgh, Edinburgh EH4 2XU, UK

E. Evangelou,

Clinical and Molecular Epidemiology Unit, Department of Hygiene and Epidemiology, University of Ioannina School of Medicine, Ioannina, Greece

J. P. A. Ioannidis, and

Clinical and Molecular Epidemiology Unit, Department of Hygiene and Epidemiology, University of Ioannina School of Medicine, Ioannina, Greece. Biomedical Research Institute, Foundation for Research and Technology-Hellas, Ioannina, Greece. Center for Genetic Epidemiology and Modeling, ICRHPS, and Tufts Clinical and Translational Science Institute, Tufts University School of Medicine, Boston, USA

S. H. Ralston

Rheumatic Disease Unit, Molecular Medicine Centre, Institute of Genetics and Molecular Medicine, Western General Hospital, University of Edinburgh, Edinburgh EH4 2XU, UK

S. H. Ralston: stuart.ralston@ed.ac.uk

Abstract

Summary—A meta-analysis of studies was conducted involving 24,511 participants with 7,864 fractures in which polymorphisms in the 5' flank of *COL1A1* (rs1107946, rs2412298, and rs1800012) were related to osteoporosis phenotypes. Polymorphisms of all three sites were associated with BMD, and rs1800012 was associated with fracture but effect sizes were modest.

Introduction and hypothesis—Polymorphisms in the 5' flank of *COL1A1* gene have been implicated as genetic markers for susceptibility to osteoporosis, but previous studies have yielded conflicting results.

Methods—We conducted a meta-analysis of 32 studies including 24,511 participants and 7,864 fractures in which alleles at the -1997G/T (rs1107946), -1663in/delT (rs2412298), and Sp1 binding site polymorphisms (rs1800012) of *COL1A1* had been related to bone mineral density (BMD) or fracture.

Results—For the Sp1 polymorphism, BMD values in TT homozygotes were 0.13 units [95% CI, 0.03 to 0.24] lower at the spine ($p=0.01$) and 0.16 units [0.10 to 0.23] lower at the hip ($p = 1 \times 10^{-6}$) than GG homozygotes. Clinical fractures were 1.31-fold [1.04–1.65] increased in TT

Correspondence to: S. H. Ralston, stuart.ralston@ed.ac.uk.

Conflicts of interest SHR holds patents on the use of *COL1A1* genotyping as a diagnostic test for susceptibility to osteoporosis.

homozygotes ($p=0.02$) and vertebral fractures were 1.34-fold [1.01–1.77] increased ($p=0.04$). We also observed associations between spine BMD and allelic variants at the -1997G/T ($p=0.05$) and the -1663indelT ($p=0.009$) sites. We found no association between alleles at the -1997G/T or -1663indelT sites and fracture but power was limited.

Conclusions—The *COL1A1* Sp1 polymorphism is associated with a modest reduction in BMD and an increased risk of fracture, although we cannot fully exclude the possibility that the results may have been influenced by publication bias. Further studies are required to fully evaluate the contribution of the -1997G/T and -1663in/delT sites to these phenotypes and to determine if they interact with the Sp1 polymorphism to regulate susceptibility to osteoporosis.

Keywords

BMD; COL1A1; Fracture; Genetic; Meta-analysis; Osteoporosis; Polymorphism

Introduction

Osteoporosis is a common disease characterized by low bone mass, micro-architectural deterioration of bone tissue and enhanced bone fragility which leads to an increased incidence of fracture. It is now well established that genetic factors play a major role in regulating bone mineral density (BMD) [1] other determinants of fracture risk [2] and fracture itself [3]. Recently, genome-wide association studies (GWAS) have been successful in identifying several common variants that are significantly associated with BMD and with fracture risk [4–7]. Candidate gene association studies have also been used to identify genetic variants that are associated with BMD and fractures [8–12]. An important limitation of many candidate gene studies has been the fact that the samples sizes were limited. This has resulted in the publication of many false negative results due to lack of statistical power and also many false positive results which could not be replicated in subsequent studies [13, 14]. Candidate gene studies can sometimes yield useful information however, since the GWAS techniques currently available do not capture all variants within candidate genes, especially rare variants [15]. In this regard, polymorphisms of the *COL1A1* gene which have previously been associated with osteoporosis in several studies [8, 10, 16] are poorly captured by the single-nucleotide polymorphism (SNP) used in the recent GWAS studies of osteoporosis [13].

Over recent years, meta-analysis has been employed to validate associations between genetic variants and phenotype in complex diseases such as osteoporosis, and has been applied to candidate gene studies [16–18], linkage studies [19], and GWAS studies [4]. Five meta-analyses of the *COL1A1* gene have been conducted so far in relation to BMD or fracture but all were limited to studies of the Sp1 binding site polymorphism within intron 1 of *COL1A1* (rs1800012) [8, 16, 20–22]. Since these studies were published, two further polymorphisms have been identified in the 5' flank of the *COL1A1* gene at positions -1997G/T (rs1107946) and -1663ins/delT (rs2412298) which have been associated with BMD [23]. Moreover, several further studies of the Sp1 polymorphism have been published that were not included in previous meta-analyses [24–32]. In view of this, the aim of the present meta-analysis was to re-evaluate the effect of the Sp1 binding site polymorphism in

relation to BMD and osteoporotic fractures and for the first time to conduct a meta-analysis of the -1997G/T and -1663ins/delT polymorphism in relation to BMD and fracture.

Methods

Retrieval of studies and data extraction

Association studies in which any of the three *COL1A1* polymorphisms have been studied in relation to BMD and/or fractures were identified by electronic searches of MEDLINE between 1996 and 2009, using several search terms including “collagen”, “*COL1A1*”, “polymorphism”, “genetics”, “BMD”, “fracture”, “Sp1” (rs1800012), “-1997G/T” (rs1107946), and “-1663 ins/delT” (rs2412298). The studies were included in the meta-analysis provided that the study outcomes included BMD or fracture and complete genotype data were provided either in the paper or by communication with the corresponding author, when data or important details thereof were not available in the published paper ($N=4$). We recorded the unadjusted mean BMD and standard deviation for each genotype at lumbar spine and femoral neck, and the number of individuals in each genotype group with or without fracture in each study.

Statistical analysis

Data were analyzed using Review Manager (version 5) and Stata 10. Both random and fixed effects were considered for the analysis. In fixed effect models it is assumed that the true effect of the genetic risk is the same in each study. The random effects model incorporates the between-study heterogeneity and allows the risk allele effects for each study to vary around some overall average effect [33]. In the absence of heterogeneity, the fixed and the random effects coincide. Between-study heterogeneity was assessed by the Q statistic (Cochrane's Q) which is considered significant for $p<0.1$. The heterogeneity was quantified by the I^2 metric and its 95% confidence intervals were calculated [34]. Values for I^2 can range from 0% to 100% and it is usually considered small, moderate large, and very large for values of 1–24%, 25–49%, 50–74%, and >75%, respectively [30]. For the BMD analysis, we calculated the standardized mean difference in BMD values in different genotype groups (equivalent to BMD Z-score values) based on the actual BMD values reported in the different studies. For the analysis of fractures, we computed the natural logarithms of the odds ratios from individual studies in order to compute the summary effect sizes. In order to identify any potential small-study effect (whether small studies yield more spectacular results), we performed the Egger test for BMD [35] and Harbord test for fractures [36]. Moreover, we applied the Ioannidis–Trikalinos test [37] to examine whether there was an excess of single studies with nominally significant results. All of these tests probe the possibility of biases in the accumulated evidence.

Thresholds for statistical significance

Throughout the manuscript, we present unadjusted p values and odds ratios, but since we performed multiple analyses of related phenotypes and SNP we estimated the adjusted significance threshold based on the following calculations. Since the three SNP studied are in strong linkage disequilibrium with each other (D' value 0.90 [24]), we estimated that this constituted 1.1 independent tests. Similarly, we analyzed BMD at two sites (hip and spine),

which are known to be correlated ($r=0.6$ [24]) which equates to 1.6 independent tests. Combining these data with the analysis of fracture amounts to 3.76 independent tests giving an adjusted threshold for significance of $p=0.013$.

Results

Studies included in the meta-analysis

Sixty studies were identified in the initial search. We excluded studies which simply recorded the prevalence of *COL1A1* alleles in different populations ($n=1$); studies of children (age<15-years old, $n=5$); studies on diseases other than osteoporosis ($n=1$); and studies where there was no information on either BMD or fractures ($n=16$). We also identified studies in which subsets of populations had been published upon more than one time ($n=5$) and selected only one study for analysis so that duplicate samples were not included. Following these exclusions, 32 eligible studies with a total of 25,411 subjects were selected for the analysis as summarized in Table 1. The minor allele frequencies were similar in the control subjects from the different populations. For the Sp1 polymorphism the frequency of the T allele (mean $\pm$ SD) was 0.19 ± 0.05 with a range of 0.07 in the study of Selezneva et al. [31] to 0.32 in the study of Braga et al. [38]. For the -1663 indelT polymorphism, corresponding values were 0.20 ± 0.01 with a range of 0.20 in the study of Husted et al. [29] to 0.22 in the study of Bustamante et al. [27]. For the -1997G/T polymorphism, values were 0.20 ± 0.01 with a range of 0.13 in the study of Bustamante et al. [27] to 0.38 in the study of Yamada et al. [25]. Of all the studies listed, prospective genotyping was performed so far as we are aware, only for the GENOMOS study [16].

Association between Sp1 polymorphism and BMD

For the Sp1 polymorphism (rs1800012), 26 studies were identified with a total of 24,511 participants (6,584 men and 17,927 women) for which hip BMD had been measured and 23,757 participants (5,843 men and 17,914 women) for which spine BMD has been measured. Random and fixed effect model estimates from analyses including all subjects are presented in Table 2. At the lumbar spine, individuals with the TT genotype had BMD values 0.13 units [95% CI, 0.03 to 0.24] lower than GG homozygotes under a random effects model ($p = 0.01$). At the femoral neck TT homozygotes had BMD values 0.16 units [95% CI, 0.10 to 0.23] lower than GG homozygotes under a random effects model ($p = 1 \times 10^{-6}$). Nominally, significant results were also obtained under a recessive model (TT vs. GG +GT) at lumbar spine ($p=0.03$) and femoral neck ($p = 3 \times 10^{-6}$).

Gender specific analysis showed that females who were homozygous for the T/T genotype had lumbar spine BMD values 0.13 units [95% CI, 0.03 to 0.22] lower than G/G homozygotes ($p=0.007$). Similarly, BMD values at the femoral neck in TT homozygotes were 0.18 units [95% CI, 0.10 to 0.25] lower than GG homozygotes under a random effects model ($p<0.001$; Fig. 1). There were no significant differences in BMD values for males at the lumbar spine or femoral neck (not shown). All of the effect sizes were very similar in the fixed effects model, because the between-study heterogeneity was modest with I^2 values ranging from 16% to 50% for lumbar spine BMD and 0% to 30% for femoral neck BMD (Table 2).

Association between Sp1 polymorphism and fractures

Fracture data was analyzed in 20 studies including 13,870 females and 5,056 males. Within these studies, there were 7,864 clinical fractures and 2,531 vertebral fractures. The results are summarized in Table 3. The GG genotype was associated with reduced risk for all fractures under a random effects model with an odds ratio of 0.89 [95% CI, 0.82 to 0.97], ($p=0.01$), whereas TT homozygotes had an increased risk for all fractures: 1.31 [95% CI, 1.04 to 1.65] ($p=0.02$) and vertebral fracture: 1.34 [95% CI, 1.01 to 1.77] ($p=0.04$). The I^2 estimates ranged from 43% to 53% for all fractures and 19% to 62% for vertebral fractures (Table 3).

Gender specific analysis revealed that female TT homozygotes had a 1.35-fold [95% CI, 1.02 to 1.79] increased risk for clinical fractures at any site compared with the GG and GT genotypes ($p=0.04$) (Fig. 2a). For vertebral fracture, female TT homozygotes had a 1.50-fold [95% CI, 1.04 to 2.17] ($p=0.03$) increased risk compared with GT and GG genotypes (Fig. 2b). No association with fracture was observed for males (not shown).

Allele based analysis showed that Sp1 T allele was associated with a 1.13-fold increased risk for all fractures [95% CI, 1.04 to 1.23] ($p=0.003$). Gender specific analysis showed no significant association with fracture in males. In females, the Sp1 T allele was associated with a 1.18-fold increased risk for all fractures [95% CI, 1.06 to 1.30] ($p=0.002$) and a 1.19-fold increased risk for vertebral fracture [95% CI, 1.01 to 1.40] ($p=0.03$).

Association between promoter polymorphisms, BMD, and fractures

There were fewer eligible studies for analysis of associations between the promoter polymorphisms and osteoporosis-related phenotypes. For the -1997G/T polymorphism (rs1107946) we identified five eligible studies which together included 8,257 women and 5,706 men. There was no significant association between this polymorphism and BMD overall with the exception of the comparison between GG homozygotes and carriers of the T allele where the association was borderline significant under a fixed effects model (Table 4). Gender specific analysis showed a significant association between the -1997G/T polymorphism in females as depicted in Fig. 3. Accordingly, BMD values were 0.06 units [95% CI, 0.01 to 0.11] lower in female -1997 G/G homozygotes as compared with G/T heterozygotes at the lumbar spine ($p=0.02$; Fig. 3a). A similar difference was observed at the femoral neck (Fig. 4b), although it did not reach statistical significance ($p=0.09$). Similar results were obtained under a recessive model at lumbar spine ($p=0.02$). There was no association with BMD in males although power was limited in view of the small number of males studied for this polymorphism.

For the -1663ins/delT polymorphism (rs2412298) there were only three eligible studies which together included 3,999 women and 184 men. As shown in Table 4, under a fixed effects model there was a significant association at the lumbar spine where BMD values in -1663 delT/delT homozygotes were 0.16 units [95% CI, 0.02 to 0.31] lower than insT/insT homozygotes ($p=0.03$). Corresponding values under a recessive model were 0.19 [0.05 to 0.33] ($p=0.006$). The significance was also present in females, but not in males at either site. However, the significance disappeared under a random effects model due to modest

heterogeneity ($I^2=43\%$). There was no significant association between either -1997G/T or -1663ins/delT and fracture (data not shown) but fracture data were only available in three published studies.

Evaluation of bias

For all analyses, both Egger and Harbord tests revealed no evidence of publication bias and examples of funnel plots for lumbar spine BMD and femoral neck BMD in the whole study population are shown in Fig. 4. Furthermore, each of the meta-analyses shown in Figs. 1, 2, and 3 had between zero and five single studies with nominally significant results, and thus there was no suggestion that there were too many single studies with significant results.

Discussion

This study extends the observations made in previous meta-analyses of the *COL1A1* Sp1 polymorphism [8, 16, 20–22] and provides the only meta-analysis of the -1997G/T and -1663ins/delT polymorphisms. We confirmed the association previously reported between the Sp1 polymorphism, BMD, and osteoporotic fractures and found that this was strongest with vertebral fracture. Under a recessive model of inheritance, the effect size was 0.08 units at lumbar spine and 0.15 units at femoral neck, which is very similar to the results previously reported by Mann et al. [20] who reported that spine BMD values in TT homozygotes were 0.09 units lower than in GG homozygotes and reported that femoral neck BMD values in TT homozygotes were 0.19 units lower than in GG homozygotes. The reasons which underlie the smaller effect size at the lumbar spine as compared with the femoral neck are unclear but could possibly reflect the fact that spine BMD values in older subjects are confounded by coexisting problems such as osteoarthritis, degenerative disk disease, and aortic calcification. All of these factors would be expected to reduce power to detect genotype-related difference in BMD at this site. Although recent GWAS studies have detected a greater number of significant hits at the spine than the hip [4] it should be noted that most of the populations included in these studies were younger than those included in this meta-analysis. Our study included some cohorts of young people, but most of the study populations had an average age above 60 years and many subjects were aged greater than 70 years where DEXA examination of the spine can give misleading results due to the factors mentioned above.

However, in this study we found no difference in BMD at femoral neck between GG homozygotes and G/T heterozygotes which was in agreement with what was reported in the GENOMOS meta-analysis [16] but not with the results of a previous meta-analysis [20]. The modest increase in risk for fractures at any site found in this study was driven mainly by the vertebral fracture and most of the significant associations were observed in females. This could indicate that genetic variation at the *COL1A1* locus influences susceptibility to osteoporosis in a gender specific manner as has been demonstrated in linkage studies of mice [39], and in human linkage studies [40]. However, another perhaps more likely possibility is that the lack of significant associations in men was due to reduced power given that only 26% of the study population were male.

We also observed a significant association between the -1997G/T polymorphism and BMD in females but we found no significant associations between the -1663ins/delT polymorphisms and BMD and neither polymorphism was associated with fracture. However this could be due to the fact that we had reduced power to evaluate these outcomes as the promoter polymorphisms have been much less widely studied than the Sp1 polymorphism.

We and others have previously presented evidence to suggest that the three polymorphisms studied here interact with each other to regulate *COL1A1* gene transcription by modifying gene expression and transcription factor binding [8, 41]. There is also evidence to suggest that allelic variants at the Sp1 binding site polymorphism adversely affect bone quality [8, 41] which is consistent with the data presented here in which the observed increase in vertebral fracture risk for Sp1 female TT homozygote (50%) was greater than the risk predicted by the modest genotype-specific reduction in spine BMD (13%). Some previous studies have shown positive associations between the alleles at the Sp1 polymorphism and hip fracture [30, 42, 43], and in one study, a rare haplotype defined by all three polymorphisms was strongly associated with hip fracture, although these were very highly selected patients and the sample size was small [44]. It would be of interest to perform a meta-analysis of *COL1A1* haplotypes in relation to BMD and osteoporotic fracture, but only three studies had been performed where haplotype data were available. More studies on the relationship between *COL1A1* haplotypes and osteoporosis-associated phenotypes including fracture would therefore be of great interest.

We made a meticulous effort to identify all relevant data; but publication bias, population stratification within individual studies, and other reporting biases are a threat for the validity of significant associations emerging in the literature and we cannot completely exclude the possibility that one or more of these biases may have been operative in this study. The tests that assess the existence of small-study effects were also not significant indicating that there is no evidence that the smaller studies had biased the results or that there were too many single studies with nominally significant results. While bias still cannot be fully excluded, the validity of the Sp1 associations reported here is corroborated by the concordant results of a prospective meta-analysis which should be immune to reporting biases [16]. The associations we report here are relatively weak and explain a very small fraction of the heritability of BMD and fracture risk.

The association with BMD reported here falls short of the accepted threshold for genome-wide significance although the *p* values for association with fracture and the effect size reported here are in keeping with those previously reported by recent GWAS studies [4]. Using a modest prior for the credibility of these associations [45, 46], the available data suggest that the Sp1 G/T associations are very likely to be genuine, while the other two polymorphisms still lack strong evidence. Further research will be required to fully define the role that variants in the *COL1A1* gene play as genetic determinant of osteoporosis. Evaluation of the role that the polymorphisms described here play in osteoporosis has been impaired by the fact that they are not efficiently tagged by the markers used in the GWAS performed so far in the osteoporosis field [13]. For example the best r^2 value between the Sp1 polymorphism and the 6 SNP analyzed from the *COL1A1* region in the GWAS of

Richards and colleagues [5] was 5% with the rs2586471 in the 3' flank of the gene (Jin and Ralston, unpublished data)

It is possible however that emerging initiatives such as the 1000 Genomes Project (www.1000genomes.org) will allow us evaluate the role of the *COL1A1* alleles described here in populations that have already undergone GWAS. Even if the results of this were to be positive however, our findings indicate that at best, the SNP we studied in *COL1A1* account for only a small proportion of the genetic risk of osteoporosis.

Acknowledgments

This study was supported in part by grants from the European Commission (GENOMOS; QLK6-CT-2002-02629) and from the Arthritis Research Campaign (15389).

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Fig. 1. Meta-analysis of Sp1 polymorphism and association with BMD in females. *Panel a* Sp1 GG homozygotes versus TT homozygotes for lumbar spine BMD. *Panel b* Sp1 GG homozygotes versus TT homozygotes for femoral neck BMD. Each study is shown as the point estimate of the standardized mean difference with 95% confidence intervals as analyzed using a random effects model. The diamond shows the overall effect. Where the diamond lies to the right of the vertical line this indicates a higher BMD value in the GG genotype compared with the TT genotype group. The p values shown have not been corrected for multiple testing

Fig. 2.

Meta-analysis for Sp1 polymorphism and association with fracture in females. Odds ratio (OR) for fracture is reported with 95% confidence intervals as analyzed using a random effects model for **a** all fractures or **b** vertebral fracture. The diamond shows the overall risk and where it lies towards the right of the vertical line, this indicates an increased risk of fracture associated with the genotype. The *p* values shown have not been corrected for multiple testing

Fig. 3.

Association between -1997 G/T polymorphism and BMD in females. Comparisons are shown in *panel a* for lumbar spine BMD and *panel b* for femoral neck BMD. Each study is shown as the point estimate of the standardized mean difference with 95% confidence intervals as analyzed using a random effects model. *Diamonds which lie to the left of the vertical line* indicates a reduced BMD in the -1997 GG genotype compared with G/T and TT genotype groups. The *p* values shown have not been corrected for multiple testing

Fig. 4.

Funnel plots of spine and hip BMD in the whole study population. **a** Funnel plot of lumbar spine BMD in whole study population. **b** Funnel plot of femoral neck BMD in whole study population. Standardized mean difference is plotted on the horizontal axis and its standard error on the vertical axis

Table 1

Studies included in the meta-analysis

Study	Location	Mean age (range)	Study design (n)	Gender/number	Fracture type	SNP studied
Aerenssens [42]	Belgium	76.0 (69–87)	Case-control, (135/239)	F/374	hip	Sp1
Alvarez-Hernandez [47]	Spain	64.0 (50–86)	Population-based	M/156	vert, osteoporotic	Sp1
Ashford [48]	UK	79.9 (>75)	Population-based	F/314	vert	Sp1
Bernad [49]	Spain	59.5 (51–67)	Clinic referrals	F/319	vert, wrist	Sp1
Braga [38]	Italy	63.5 (63–64)	Clinic referrals	F/715	any	Sp1
Braga [50]	Italy	58.4 (50–60)	Population-based	M/253	–	Sp1
Bustamante [27]	Spain	54.7 (46–63)	Population-based	F/719	–	Sp1, -1997, -1663
Efstathiadou [51]	Greece	54.2 (47–61)	Clinic referrals	F/154	–	Sp1
Garnero [52]	France	38.9 (34–46)	Population-based	F/220	–	Sp1
Gerhem [28]	Sweden	75.2 (75)	Population-based	F/964	Any fracture	Sp1
Grant [53]	UK	61.3 (50–70)	Case-control	F/299	vert	Sp1
Harris [54]	USA	70.2 (>65)	Intervention	M/108, F/135	–	Sp1
Heegaard [55]	Denmark	50.9 (45–54)	Population-based	F/133	vert	Sp1
Husted [29]	Denmark	60.1 (45–73)	Case-control, (291/283)	M/120, F/454	vert	Sp1, -1997, -1663
Hustmyer [56]	USA	33.9 (21–49)	Case-control, (56/78)	M/4, F/130	vert	Sp1
Keen [57]	UK	54.3 (45–64)	Case-control, (55/130)	F/185	osteoporotic	Sp1
Liden [58]	Sweden	67.7 (58–77)	Case-control, (64/72)	F/136	vert	Sp1
McGuigan [59]	UK	70.8 (69–75)	Case-control, (93/88)	F/181	vert	Sp1
McGuigan [60]	UK	64.2 (56–77)	Clinic referrals	M/156, F/185	–	Sp1
Mezquita-Raya [61]	Spain	60.9 (53–70)	Case-control, (43/101)	F/144	vert	Sp1
Nguyen [30]	Australia	70.0 (63–77)	Population-based	F/677	hip	Sp1
Peris [62]	Spain	48.3 (31–71)	Case-control, (35/60)	M/95	–	Sp1
Roux [63]	France	63.1 (45–90)	Case-control, (110/107)	F/217	–	Sp1
Ralston [16]	GENOMOS	63.7 (20–80)	multi-center	M/4,776, F/7,759	any, vert, non-vert	Sp1
Seleznova [31]	Russia	N/A (50–70)	Clinic referrals	F/124	osteoporotic	Sp1, -1997, -1663
Sowers [64]	USA	37.3 (28–48)	Population-based	F/259	–	Sp1
Stewart [24]	UK	54.8 (45–54)	Population-based	F/3,270	–	Sp1, -1997, -1663
Valimaki [43]	Finland	89.0 (85–98)	Population-based	F/601	hip	Sp1

Study	Location	Mean age (range)	Study design (n)	Gender/number	Fracture type	SNP studied
van Pottelbergh [65]	Belgium	75.0 (71–86)	Population-based	M/352	–	Sp1
Weichtova [32]	Czech Republic	60.5 (45–70)	Case-control, (183/178)	F/361	wrist	Sp1
Yamada [25]	Japan	59.2 (45–65)	Population-based	M/1,126, F/1,100	–	Sp1, -1997
Yazdamanah [26]	Netherlands	68.0 (60–74)	Population-based	M/2,452, F/3,374	vert, non-vert	Sp1, -1997

Details of the studies included are shown

Values for age are mean (range) in years

Number of cases and controls are indicated in the study design column

Vert vertebral fracture, non-vert non vertebral fracture, M male, F female

Table 2
Meta-analysis of Sp1 polymorphism in relation to spine and hip BMD in the whole study population

Studies/N	Comparison	Fixed effect model		Random effects model		
		Lumbar spine BMD	P	Lumbar spine BMD	P	I ² (95% CI)
26/22,764	GG vs. GT	0.03 (0, 0.06)	0.04	0.03 (0, 0.07)	0.05	19 (0–44)
26/16,446	GG vs. TT	0.10 (0.04, 0.17)	0.002	0.13 (0.03, 0.24)	0.01	50 (30–65)
26/23,757	GG vs. GT+TT	0.04 (0.01, 0.06)	0.006	0.04 (0.01, 0.08)	0.01	22 (0–47)
26/23,757	GG+GT vs. TT	0.08 (0.02, 0.14)	0.01	0.08 (0.01, 0.16)	0.03	16 (0–42)
		Femoral neck BMD		Femoral neck BMD		
26/23,522	GG vs. GT	0.01 (–0.01, 0.04)	0.30	0.01 (–0.02, 0.05)	0.42	23 (0–47)
26/17,048	GG vs. TT	0.16 (0.10, 0.23)	1×10 ^{–6}	0.16 (0.10, 0.23)	1×10 ^{–6}	0 (0–35)
26/24,511	GG vs. GT+TT	0.03 (0, 0.06)	0.02	0.03 (0, 0.07)	0.09	30 (0–51)
26/24,511	GG+GT vs. TT	0.15 (0.09, 0.22)	3×10 ^{–6}	0.15 (0.09, 0.22)	3×10 ^{–6}	0 (0–35)

The number of eligible studies and total number of participants is indicated for each comparison

Values are standardized mean difference (95% CI)

Positive values denote higher BMD values in the first listed genotype group of the comparison

The *p* values shown have not been corrected for multiple testing

I² Heterogeneity

Table 3
Meta-analysis of the Sp1 polymorphism in relation to fracture in the whole study population

Studies/N	Comparison	Fixed effect model		Random effects model	
		All fractures	P	All fractures	P
20/28,352	Sp1 GG vs. GT+TT	0.94 (0.89, 0.99)	0.03	0.89 (0.82, 0.97)	0.01
17/24,218	Sp1 GT vs. GG+TT	1.05 (0.99, 1.12)	0.13	1.10 (0.99, 1.22)	0.08
17/27,444	Sp1 TT vs. GG+GT	1.20 (1.04, 1.39)	0.01	1.31 (1.04, 1.65)	0.02
		Vertebral fracture		Vertebral fracture	
10/17,029	Sp1 GG vs. GT+TT	0.94 (0.86, 1.04)	0.23	0.93 (0.79, 1.09)	0.38
8/16,646	Sp1 GT vs. GG+TT	1.04 (0.94, 1.15)	0.46	1.08 (0.90, 1.29)	0.42
8/16,646	Sp1 TT vs. GG+GT	1.38 (1.10, 1.74)	0.006	1.34 (1.01, 1.77)	0.04

The number of eligible studies and total number of participants is indicated for each comparison

Values are odds ratio (95% CI)

Values greater than 1.0 denote an increased risk of fracture for the first listed genotype group of the comparison compared with the other groups

The *p* values shown have not been corrected for multiple testing

*I*² Heterogeneity

Table 4
Meta-analysis of promoter polymorphisms in relation to BMD in the whole study population

	Studies/V	Comparison	Fixed effect model		Random effects model		
			Lumbar spine BMD	P	Lumbar spine BMD	P	I ² (95%CI)
-1997G/T	5/11,232	GG vs. GT	-0.04 (-0.08,-0.00)	0.06	-0.04 (-0.09, 0.01)	0.16	28 (0-68)
	5/8,480	GG vs. TT	-0.05 (-0.15,0.04)	0.30	-0.03 (-0.17, 0.10)	0.62	37 (0-72)
	5/11,750	GG vs. GT+TT	-0.04 (-0.08,-0.00)	0.05	-0.04 (-0.09, 0.01)	0.14	29 (0-68)
	5/11,750	TT vs. GG+GT	0.05 (-0.05,0.14)	0.33	0.03 (-0.08, 0.15)	0.55	24 (0-65)
-1663in/delT	3/3,975	insT/insT vs. insT/delT	0.03 (-0.04,0.10)	0.36	0.03 (-0.04, 0.10)	0.36	0 (0-85)
	3/2,927	insT/insT vs. delT/delT	0.16 (0.02, 0.31)	0.03	0.23 (-0.04, 0.49)	0.09	43 (0-81)
	3/4,183	Dominant ^a	-0.06 (-0.12,0.01)	0.09	-0.06 (-0.12, 0.01)	0.09	0 (0-85)
	3/4,183	Recessive ^b	-0.19 (-0.33,-0.05)	0.006	-0.30 (-0.63, 0.03)	0.07	41(0-80)
-1997G/T	5/11,143	GG vs. GT	-0.04 (-0.08, 0.01)	0.09	-0.03 (-0.09, 0.02)	0.23	28 (0-68)
	5/8,409	GG vs. TT	-0.01 (-0.11,0.08)	0.77	-0.01 (-0.13, 0.11)	0.91	25 (0-66)
	5/11,657	GG vs. GT+TT	-0.04 (-0.08, 0.01)	0.09	-0.03 (-0.08, 0.03)	0.32	36 (0-72)
	5/11,657	TT vs. GG+GT	-0.01 (-0.10, 0.08)	0.88	-0.01 (-0.12, 0.10)	0.87	20 (0-62)
-1663in/delT	3/3,892	insT/insT vs. insT/delT	0.03 (-0.04,0.09)	0.46	0.03 (-0.04,0.09)	0.46	0 (0-85)
	3/2,862	insT/insT vs. delT/delT	0.13 (-0.01,0.28)	0.07	0.13 (-0.04, 0.30)	0.12	5 (0-85)
	3/4,085	Dominant ^a	-0.04 (-0.10,0.03)	0.26	-0.04 (-0.10,0.03)	0.26	0 (0-85)
	3/4,085	Recessive ^b	-0.12 (-0.27,0.02)	0.09	-0.12 (-0.29, 0.04)	0.14	6 (0-85)

Values are standardized mean difference (95% CI)

Positive values denote higher BMD values in the first listed genotype group of the comparison

The *p* values shown have not been corrected for multiple testing

I²: heterogeneity

^a Dominant model: insT/delT+delT/delT vs. insT/insT

^b Recessive model: delT/delT vs. insT/insT+insT/delT