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A haplotype of MATN3 is associated with vertebral fracture in Chinese postmenopausal women: Peking Vertebral Fracture (PK-VF) study

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Abstract

The Matrilin3 gene (MATN3) encodes an extracellular matrix protein, which modulates chondrocyte differentiation. The aim of this study was to test for association of MATN3 polymorphisms with bone mineral density (BMD), fracture, vertebral fracture, bone turnover or

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Conflict of interest statement

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25-hydroxyvitamin D [25(OH)D] in postmenopausal women. A community-based population of 1488 postmenopausal women was randomly selected in Beijing. The history of fracture and vertebral fracture was obtained via questionnaire and vertebral X-ray respectively. BMD of lumbar spine (2–4), femoral neck and total hip were measured by dual energy X-ray absorptiometry. Serum N-terminal procollagen of type 1 collagen (P1NP), β-isomerized type I collagen C-telopeptide breakdown products (β-CTX) and 25(OH)D were quantified. Binary logistic regression revealed that Haplotype-4 was significantly associated with vertebral fracture risk in both additive model (p=0.023, OR=1.521) and dominant model (p=0.028, OR=1.623). The significance remained after 10,000 permutation tests to correct multiple testing (p=0.042). Re-selected age matched vertebral fracture case-control groups revealed similar associations in additive model (p=0.014, OR=1.927, 95%CI=1.142–3.253) and in dominant model (p=0.011, OR=2.231, 95%CI=1.200–4.148). However, no significant association was found between MATN3 polymorphisms and serum β-CTX, P1NP, 25(OH)D levels, or BMD. In linear regression, Haplotype-2 approached marginal significance in association with femoral neck BMD T-score (p=0.050), but this would account for only 0.2% of BMD variation in our sample. This study suggests that Haplotype-4 of MATN3 is associated with vertebral fracture risk independent of BMD in Chinese postmenopausal women. Efforts should be made to replicate our finding in other, similar and ethnically diverse, populations.

Keywords

MATN3; BMD; Vertebral fracture; Bone turnover markers; 25(OH)D

Introduction

Bone mineral density (BMD), osteoporotic fracture and bone turnover markers are common osteoporosis phenotypes. Heritability of BMD is comparatively high, 60–90% in twins [1,2] and 45–70% between parents and offspring [3,4]. Due to its high heritability, stable measurement and worldwide availability, BMD is currently considered as the best surrogate phenotype of osteoporosis, and is used in population-based studies exploring the genetic etiology of osteoporosis. To ensure good representation of the general population, stratified random sampling of communities has been widely adopted across several disciplines [5,6]. In terms of osteoporosis, serum N-terminal procollagen of type 1 collagen (P1NP) and β-isomerized type I collagen C-telopeptide breakdown products (β-CTX) are two commonly used bone turnover markers, representing bone formation and resorption respectively.

MATN3, mapping to 2p24-p23, consists of 8 exons and 7 introns. It encodes matrilin-3, which is the least complex member of a group of oligomeric extracellular matrix (ECM) proteins [7]. It mainly exists in cartilage, and functions as an adapter protein, connecting matrix components as well as interacting with both collagens and noncollagenous proteins and proteoglycans [8,9]. As more studies have implicated that some genes may exert influence on both bone and cartilage, such as BMP2 [10–12], BMP4 [13,14], BMP7 [14,15] and RUNX2 [14,16], it is intriguing to investigate whether the cartilage dwelling protein, matrilin-3, is genetically related to bone.

van der Weyden et al. [17] generated a MATN3 knock-out mouse strain and observed that matrilin-3 null mice showed higher BMD at the age of 18 weeks when mice reached peak BMD. They also developed osteoarthritis (OA) with a higher incidence and severity. Moreover, single amino acid substitution of MATN3 can cause multiple epiphyseal dysplasia (MED) and spondylo-epi-metaphyseal dysplasia (SEMD) [18–20]. Many genes in which mutations result in rare bone diseases are also contributors to the variation in BMD in the general population, examples including SOST [21–23], LRP5 [24,25] and CLCLN7 [26,27]. Therefore, Huang [28] suggested that it is worthwhile to explore the role of any causative gene of monogenic bone disease in osteoporosis. In addition, Huang et al. [29] used five bioinformatics tools to analyze 13 well-replicated osteoporosis susceptibility loci. MATN3 was one of the potential osteoporosis candidate genes they identified.

Participants of our study were selected from the Peking Vertebral Fracture (PK-VF) study which is a large-scale epidemiologic study with randomly sampled community dwelling postmenopausal women in 2008. In this study, we report the association of MATN3 polymorphisms with vertebral fracture in Chinese postmenopausal women.

Material and methods

Participants

All participants were from PK-VF cohort which was constituted by randomly sampled postmenopausal women in seven districts of Beijing. One or two communities in each district were randomly selected. The community in our study was defined as a group of interacting people characterized by similar culture and lifestyle, living at a specific geographic area and sharing common venue. The populations of the seven districts in our study ranged from 350,000 to 1,920,000. The communities selected in each district had a population representing at least 5% of the total population of the district. Postmenopausal women were stratified by age and randomly sampled according to age composition of those communities. Ahead of sampling, written notices explaining the nature of the proposed study were posted on the bulletin board of each community center. Then telephone calls were made to invite the randomly selected participants to join the study. All postmenopausal women were divided into nine age groups, i.e. 40–44, 45–49, 50–54, …, 70–74, 75–79 and above 80 years old. Participants who rejected the invitation were mostly in age groups of 75–79 and above 80 years old. To make age distribution of our selected participants consistent to age composition of those communities, additional 38 phone calls were made to invite enough participants in these two age groups. In the end, a total of 2396 postmenopausal women were invited, and the PK-VF study recruited 2070 postmenopausal women. Every participant completed a questionnaire concerning items such as age, years since menopause (YSM), fracture history and medication history. Menopause was defined as the absence of menstruation for at least one year. Exclusion criteria were: 1) serious chronic liver disease; 2) chronic renal disease; 3) significant chronic lung disease; 4) rheumatoid arthritis or other connective tissue disease; 5) serious effects from cerebrovascular disease; 6) significant gastrointestinal disease; 7) metabolic or inherited bone disease; 8) corticosteroid, anticonvulsant or anti-osteoporosis therapy for more than 6 months or within the previous 12 months; 9) premature menopause (menopause before 40 years of age).

According to the exclusion criteria, 1488 participants were finally included in our study. The study was approved by the Ethic Committee of Peking Union Medical College Hospital (PUMCH). All participants signed informed consent forms before entering the study.

BMD measurement

BMD of total hip, femoral neck (FN) and lumbar spine (2–4) (LS) were measured by dual energy X-ray absorptiometry (DXA) with either Lunar DPX or Norland equipment. Calibration with manufacturer's phantom was performed every day before the measurement started. The coefficients of variation (CV) of seven hospitals were 0.75%–1.7% for LS, 0.56%–1.0% for FN and 0.39%–0.95% for total hip. Cross-calibration equations between two kinds of machines are listed as follows [30]:

$$\text{LS BMD}\left(\text{g}/\text{cm}^2\right)\text{Lunar} = 1.102 \times \text{Norland} + 0.0137$$

$$\text{FN BMD}\left(\text{g}/\text{cm}^2\right)\text{Lunar} = 1.0377 \times \text{Norland} + 0.00026$$

According to the World Health Organization definition, osteoporosis was defined as T-score≤ — 2.5SD at any site, and — 1.0>T-score> — 2.5 is considered as osteopenia. In this study, participants with T-score< — 1.0 at a certain site were included in case group of BMD categories, while control group contained participants with T-score ≥ — 1.0.

Ascertainment of osteoporotic fracture

X-rays of thoracic and lumbar spine (T4 to L5) were taken during participants' visits to the hospitals. Vertebral fractures were independently diagnosed via X-rays by two experienced radiologists, using Genant's semiquantitative technique as the criterion [31]. Fracture history of other sites such as the upper arm, the forearm, the wrist, the hand, the hip, the femur and the ankle, was obtained by self-reporting. Detailed fracture causes were obtained and classified through the questionnaire via multiple choices, such as fractures happened in routine activity without outside force, fractures happened due to mild collision and fractures happened due to severe collision. Osteoporotic fracture is defined as fractures happened in routine activities or due to mild trauma. Participants with osteoporotic fracture at either site or specific vertebral fracture were included in two case groups, namely fracture or vertebral fracture case group, respectively. A total of 971 participants who had neither fracture history nor radiological vertebral fracture, were included in control group for the aforementioned case groups.

Biochemistry

Fasting blood sample was collected from each participant. Serum concentrations of β-CTX, P1NP and 25(OH)D were determined by a fully automated Roche electrochemiluminescence system (E170, Roche Diagnostics, Switzerland) at PUMCH. The detection limit of β-CTX, P1NP and 25(OH)D was 0.01 ng/ml, 5 ng/ml and 4 ng/ml, respectively. The intraassay and interassay CV were 2.0% and 3.1% for β-CTX, 2.3% and 1.7% for P1NP and 5.7% and 6.1% for 25(OH)D.

Genotyping

Single nucleotide polymorphisms (SNP) information of MATN3 gene was obtained from Entrez Gene database (http://www.ncbi.nlm.nih.gov/gene/) and HapMap (http://hapmap.ncbi.nlm.nih.gov/). SNPs were selected according to the following criteria: 1) high heterozygosity, namely minor allele frequency (MAF) higher than 20%, in Chinese population; 2) classified as tagSNPs; 3) pairwise linkage disequilibrium (LD) exceeds the threshold of 0.8 (r^2>0.8). Finally, four tagSNPs were selected: rs10178256, rs6734005, rs11096633 and rs10856792. Each participant was genotyped for all four SNPs by TaqMan allelic discrimination assay (Applied Biosystems, USA). The whole reacting volume was 5 μl, including 2 μl (approximate 10 ng) sample DNA, 2.5 μl TaqMan Universal PCR Master Mix, 0.0875 μl TaqMan probe assay and 0.4125 μl ddH_2O. Reactions were performed on a Real-Time PCR system of ABI Prism 7900 (Applied Biosystems, USA) in a 384-well reaction plate under standard condition.

Statistics

Statistical analyses were performed using SPSS 13.0 (SPSS Inc, Chicago, 2000). LD between pairwise SNPs was measured with Lewontin's D', and calculated by the Haploview 4.1 program. Haplotypes of each participant were distinguished by Phase 2.02. Hardy-Weinberg equilibrium (HWE) was tested using the goodness-of-fit Chi-square test, which was also used to detect differences in alleles, genotypes and haplotypes between case and control groups. Genotypes and haplotypes were assigned codes of 0, 1 and 2 in the additive model, 0, 1 and 1 in the dominant model, and 0, 0 and 1 in the recessive model, according to the number of minor allele or copies of haplotype. The odds for fracture and vertebral fracture associated with genotype or haplotype were calculated, adjusting for age, BMI and YSM, using binary logistic regression. Differences in BMD T-score associated with genotype or haplotype were calculated, adjusting for age, BMI and YSM, using linear regression. Genotype and haplotype specific differences of β-CTX, P1NP (adjusted for age and YSM), and 25(OH)D (adjusted for age) were investigated with general linear model ANOVA (GLM-ANOVA). To correct for the increased possibility of false positive or false negative results caused by multiple testing, we performed 10,000 permutation tests to generate empirical p values. P<0.05 was considered statistically significant. The statistical power was calculated by Piface software 1.65 (http://www.math.uiowa.edu/~rlenth/Power/) and Quanto 1.2.4 software (http://hydra.usc.edu/GxE/).

Results

Basic characteristics of participants

Clinical profiles of the total population and participants in each case-control groups are listed in Table 1. As expected, means of age and YSM were higher in case groups than in control groups, while BMDs of LS, FN and total hip were lower in case groups. Information of four SNPs is shown in Table 2. From 5' to 3', four SNPs were arranged in the order of rs10178256, rs6734005, rs11096633 and rs10856792, with MAFs of 35.1%, 22.8%, 40.7% and 49.4%, respectively. This distribution did not differ from what is expected under HWE (P>0.05). Due to the high LD (D'≥0.90), they formed a haplotype block. Five common

haplotypes (frequency>0.10), namely, AGTA (47.4%), GACG (22.6%), GGCG (11.9%), AGTG (11.5%) and AGCG (4.8%), accounted for 98.2% of haplotype distribution (Fig. 1).

Association between MATN3 polymorphisms and BMD, fracture and vertebral fracture

Chi-square tests were performed to investigate allele, genotype and haplotype distribution differences between case and control groups. Allele frequencies of rs6734005 were significantly different between case and control groups of total hip BMD (p=0.011). In genotype analysis, rs11096633, rs6734005 and rs10178256 also showed significantly different distribution in total hip BMD case-control groups (all p<0.05) (data not shown). In haplotype analysis, frequencies of Haplotype-2 (GACG) in case-control groups of total hip BMD were significantly different (p=0.010). Moreover, Haplotypte-4 (AGTG) displayed significantly different distribution in vertebral fracture case and control groups (p=0.011). Considering of possible false positive results brought in by multiple testing, 10000 permutation tests were performed to Haplotype-4, and the significance maintained (p=0.046).

To further investigate MATN3's possible relation to fracture and vertebral fracture risk, we applied binary logistic regression to genotypes and haplotypes within case-control groups. After adjusting for confounding factors, rs10856792 and Haplotype-1 (AGTA) were found to be related to fracture risk (p=0.041 and 0.044, respectively). Haplotype-4 (AGTG) showed significant association with vertebral fracture risk in both additive model (p=0.023, OR=1.521, 95%CI=1.059–2.182) and dominant model (p=0.028, OR=1.623, 95%CI=1.052–2.503). It is noted that participants in fracture or vertebral fracture case groups were significantly older than participants in control groups. As aging is a crucial risk factor to fracture, we re-selected age matched participants of fracture and vertebral fracture categories to eliminate possible age stratification. As there were fewer participants in the case group, participants in the control group with exactly the same age as those in the case group were randomly sampled to form 1:1 age matched pairs. Only a few participants in the case groups were too old to find a match in the control group. In the end, there were 398 pairs in fracture case and control groups (mean age 66.9±8.3 years), and 124 pairs in vertebral fracture case and control groups (mean age 70.8±7.7 years). Clinical files of the re-selected case-control pairs are shown in Table 3. After adjusting for BMI and YSM, Haplotype-4's association with vertebral fracture risk remained significant in both additive model (p=0.014, OR=1.927, 95%CI=1.142–3.253) and dominant model (p=0.011, OR=2.231, 95%CI=1.200–4.148). The marginal significance of rs10856792 (p=0.041) and Haplotype-1 (p=0.044) in binary logistic regression, however, disappeared after age matching (both p>0.05) (Table 4).

As for three BMD categories, linear regression revealed Haplotype-2 approached marginal significance in dominant model (p=0.050). This haplotype, nonetheless, could only explain 0.2% of FN BMD variation in our sample (Table 5).

Association between MATN3 polymorphisms and β-CTX, P1NP and 25(OH)D

Of all 1488 postmenopausal women, 1246 participants had intact serum biochemistry results of β-CTX,P1NP and 25(OH)D. Levels of β-CTX,P1NP or 25(OH)D were not significantly different among genotype groups of any SNP or haplotype groups (Table 6).

Power calculation

According to MAF of each genotype and BMD variation, with our current sample size, we had a power higher than 80% to detect differences around 0.03, 0.02 and 0.03 g/cm^2 at LS, FN and total hip respectively. Adopting the best adjusted inheritance model (additive, dominant or recessive), the minimal detectable OR was 1.3–1.4 for any fracture, and 1.7–1.9 for any vertebral fracture.

Discussion

Our current study investigated the possible association of MATN3 polymorphisms and osteoporosis phenotypes in Chinese postmenopausal women. We found Haplotype-4 was significantly associated with vertebral fracture, and the significance maintained after 10,000 permutation tests. To avoid age confounding, we re-selected age matched case-control groups of fracture and vertebral fracture. The association of Haplotype-4 and vertebral fracture stayed significant. Since we did not detect any association between Haplotype-4 and BMD, bone turnover markers or 25(OH)D, the vertebral fracture risk caused may be independent of these factors. Though BMD is currently regarded as the most important predictor of fracture risk, several studies have demonstrated that BMD alone is not sufficient to identify the risk of fracture in a majority of women [32,33]. In regards to vertebral fracture, bone geometry, bone matrix quality and physical activities are all well-established affecting factors [34]. Considering of matrilin-3's role as an extracellular matrix protein, it is likely that MATN3 haplotypes act directly and/or indirectly on bone fragility by changing bone matrix properties. Although we did not detect association between MATN3 haplotypes and β-CTX or P1NP, we still cannot exclude the possibility that true association exists. High variation rate of bone turnover markers might have reduced the ability of detection. We also noted that the marginal significance of rs10856792 and Haplotype-1, nevertheless, disappeared after age matching. On one hand, it confirmed that age was a risk factor for fracture. On the other hand, it indicated that previous marginal significance might be brought in by sampling bias.

Though Haplotype-2 approached marginal significance in linear regression, we should interpret the associations with caution, since they only accounted for 0.2% of BMD variation in our whole sample. Our study used a large sample of 1488 participants, and each sub-category included more than 900 participants. We had a power higher than 80% to detect BMD differences of 0.02–0.03 g/cm^2 at LS, FN and total hip, which should be sufficient to detect a quantitative trait locus (QTL) of moderate effect on BMD variation. In addition, we used T-score in association analysis of MATN3 polymorphisms and BMD, which minimized the possibility of systemic error caused by two kinds of DXAs. All these advantages maximally reduced possible false negative results in the analysis.

Prior to our study, there has been no association study concerning human MATN3 polymorphisms and BMD. The relationship of MATN3 and BMD remained controversial. van der Weyden et al. [17] discovered that MATN3 knock-out mice had significantly higher total BMD, and aged MATN3 null mice were prone to develop OA. In answer to the increased BMD, they suspected it was due to enhanced mineralization caused by increased Indian hedgehog and/or decreased parathyroid hormone-related peptide expression in the

perichondrium. Contrary to the mild phenotype MATN3 null mice displayed, mutation of a single base, however, could cause MED. Many studies thus presumed that the pathogenic mechanism of MED might be mediated by a dominant negative effect [19,35,36]. Moreover, multiple growth factors, transcription factors and signal proteins of extracellular matrix, such as BMP2 [12], SOX6 [37] and PTHrp [38], have been proved to be associated with BMD. Some of the factors, such as BMPs and Wnts, are modulated by extracellular antagonist or proteins bound. Considering of matrilin-3's function of interacting with extracellular factors, it is thus tempting to speculate that MATN3's regulation of BMD is mediated by binding to related signal factors. Nicolae et al. [39], however, were not able to replicate van der Weyden's results. They claimed that it was partially attributed to genetic background and/or gender differences of mice. Our study excluded participants with rheumatoid arthritis, connective tissue diseases, metabolic or inherited bone disease and previous treatment for osteoporosis. We note that MATN3 null mice exhibited several of the same phenotypes [17] and that the exclusions may have affected the spectrum of MATN3 variation as well as BMD. However, it is difficult to estimate the extent of influence.

As for the relation of MATN3 and OA, previous studies also showed conflicting results. Stefansson et al. [40] identified an amino acid substitution (T303M, SNP5) was related to idiopathic hand OA in a large Icelandic population (relative risk=2.1). Min et al. [41] failed to replicate this result in two Dutch cohorts of patients with hand OA, but found this SNP was associated with spinal disc degeneration in one of their cohorts (OR=2.9). Moreover, they identified that SNP6 (nomenclature as described by Stefansson et al. [40]) was related to hand OA (OR=2.0). Furthermore, Pullig et al. [42] successfully repeated Stefansson et al.'s [40] results in two German cohorts rather than the results of Min et al. [41]. The partially inconsistent results may be caused by different genetic background [41] or OA diagnostic criteria [43]. While precise functions of MATN3 are still unknown, it is difficult to explain the associations observed.

With cutoff points of MAF at 20% and r^2 at 0.8, HapMap provided seven tagSNPs. Since the four tagSNPs we selected were in high LD (r^2>0.95) with the other three, they could actually represent all qualified tagSNPs of MATN3. We did not select SNP5 and SNP6 analyzed by Stefansson et al. [40] because of their unknown MAFs in Chinese population. All four tagSNPs selected in our study happened to be introns. Now that intronic SNPs may have strong LD with those within exons, the selection is justified.

In the investigation of MATN3 polymorphisms with serum biochemical markers, no significant association was detected, indicating MATN3 polymorphisms might not influence β-CTX, P1NP or 25(OH)D. To date, no conclusion of bone turnover heritability has been reached, though a few studies reported genetic associations [44,45]. Furthermore, many factors, such as circadian rhythm and season alteration, could influence serum bone turnover concentrations. Although we controlled a few confounding factors by uniform operation, to some extent, it is the large variation of bone turnover markers themselves that interfered with the detection of a true association. As for 25(OH)D, it is widely affected by a number of factors.

Our study has several strengths. First, we comprehensively investigated MATN3 polymorphisms with multiple phenotypes. Second, our sample size was large. Participants came from a population based cohort, generated by random stratified sampling of communities in Beijing, which, to a great extent, improved the sample's representation and reduced potential population stratification. Third, we selected tagSNPs of MATN3, which increased the power of our study. Fourth, we re-selected age matched case-control groups of fracture and vertebral fracture, which avoided false positive or negative results caused by age stratification.

Our study has several limitations as well. First, some of our fracture phenotypes were ascertained by self-reporting, which could probably cause inaccurate phenotype determination. Second, vertebral fracture incidence was comparatively low in our sample, which led to the small case group of vertebral fracture. Third, we did not comprehensively test risk factors of vertebral fracture, which limited our ability to explain the association observed. In addition, we selected the most informative tagSNPs with high MAF and r^2, which thereby might miss causative SNPs with low heterozygosity.

Conclusion

In conclusion, our study suggested that Haplotype-4 (AGTG) of MATN3 was significantly associated with vertebral fracture independent of BMD. However, considering of the low incidence of vertebral fracture in our population and lack of potent evidence about the SNPs' biological function, the role MATN3 plays in vertebral fracture is still indefinitive. To confirm the relation of MATN3 and vertebral fracture as well as to explore whether MATN3 embodies QTL of BMD or SNPs in LD with QTL, further verification studies in multiple ethnic groups with dense SNPs covering the whole MATN3 gene are required.

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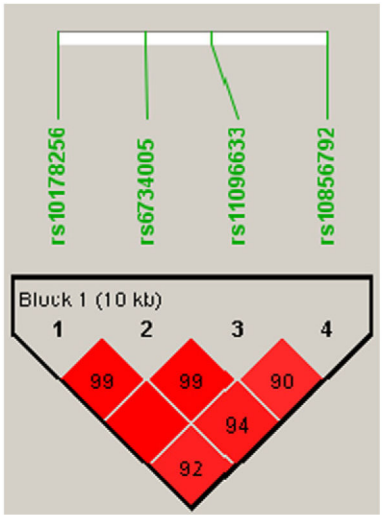
a



b

Haplotype	rs10178256	rs6734005	rs11096633	rs10856792	Frequency
1	A	G	T	A	0.474
2	G	A	C	G	0.226
3	G	G	C	G	0.119
4	A	G	T	G	0.115
5	A	G	C	G	0.048
6	A	G	C	A	0.006
7	G	G	C	A	0.005
8	G	A	C	A	0.005
9	A	A	T	G	0.002

Fig. 1.

a. LD blocks and correlation coefficients among MATN3 polymorphisms. Red squares display statistically significant association between a pair of SNPs, as measured by D'; darker shades of red indicate higher D', namely, higher LD. b. Haplotype frequencies of MATN3 polymorphisms.

Table 1

Clinical profiles of postmenopausal women in case-control groups of PK-VF study.

		N	Age (years)	BMI (kg/m^2)	YSM (years)	LS BMD (g/cm^2)	FN BMD (g/cm^2)	Total hip BMD (g/cm^2)
Total		1488	65.3±8.5	26.3±9.7	15.7±9.5	1.000±0.183	0.774±0.136	0.862±0.141
LS	Case	868	66.5±8.2	25.6±3.5	17.2±9.2	0.879±0.100	0.720±0.107	0.793±0.107
	Control	617	63.4±8.4	27.4±14.4	13.5±9.5	1.170±0.132	0.850±0.136	0.938±0.135
FN	Case	896	67.7±8.0	25.6±3.6	18.4±9.0	0.933±0.155	0.692±0.804	0.779±0.908
	Control	589	61.5±7.7	27.4±14.7	11.5±8.8	1.102±0.176	0.898±0.105	0.982±0.112
Total hip	Case	424	69.3±8.2	25.2±3.5	19.8±9.4	0.921±0.140	0.687±0.760	0.741±0.717
	Control	539	62.6±8.0	27.4±15.2	12.7±9.0	1.093±0.172	0.869±0.112	0.958±0.104
Fracture	Case	407	67.3±8.6	25.9±3.5	18.6±11.6	0.948±0.178	0.739±0.129	0.816±0.137
	Control	971	64.1±8.5	26.2±3.8	14.4±9.4	1.010±0.184	0.782±0.137	0.871±0.143
Vertebral fracture	Case	126	71.1±7.9	26.0±3.2	22.4±9.9	0.915±0.169	0.703±0.123	0.766±0.125

Values are presented as means±SD. Case groups of LS, FN and total hip included participants of osteoporosis and osteopenia at according site, while control groups included normal participants with T score higher than — 1.0 at according site. Participants had osteoporotic fracture at either site or specifically at vertebrae were included in case groups of fracture and vertebral fracture respectively. Participants who had neither fracture history nor radiologically diagnosed vertebral fracture were included in control group for both fracture and vertebral fracture case groups. BMD, bone mineral density; BMI, body mass index; YSM, years since menopause; LS, lumbar spine (2–4); FN, femoral neck.

Table 2

SNP information of MATN3 gene in postmenopausal women.

SNP ID	Location	Alleles	Genotyping call rate	HWE p value	MAF
rs10178256	intron5	G<A	98.5	1	0.351
rs6734005	intron4	A<G	98.8	0.6599	0.228
rs11096633	intron2	C<T	98.5	0.7683	0.407
rs10856792	intron1	A<G	95.5	0.1999	0.494

SNP, single nucleotide polymorphisms; HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency.

Table 3

Clinical files of age matched case-control pairs of postmenopausal women.

		N	Age (years)	BMI (kg/m^2)	YSM (years)	LS BMD (g/cm^2)	FN BMD (g/cm^2)	Total hip BMD (g/cm^2)
Fracture	Case	398	66.9±8.3	25.9±3.5	18.1±10.9	0.950±0.172	0.742±0.129	0.820±0.137
	Control	398	66.9±8.3	27.1±3.5	16.8±9.4	1.072±0.191	0.845±0.143	0.934±0.137
Vertebral fracture	Case	124	70.8±7.7	26.0±3.2	22.1±9.7	0.917±0.169	0.705±0.122	0.767±0.123
	Control	124	70.8±7.7	27.5±3.8	20.6±8.6	1.105±0.194	0.872±0.144	0.961±0.138

Values are presented as means±SD. BMD, bone mineral density; BMI, body mass index; YSM, years since menopause; LS, lumbar spine (2–4); FN, femoral neck.

Table 4

Binary logistic regression of MATN3 genotypes and haplotypes in relation to fracture and vertebral fracture risk in age matched case-control pairs.

Group	SNP ID	Additive model		Dominant model		Recessive model	
		OR(95%CI)	p value	OR(95%CI)	p value	OR(95%CI)	p value
Fracture	rs10856792	0.870(0.715–1.057)	0.161	0.800(0.585–1.194)	0.162	0.859(0.617–1.196)	0.369
	rs11096633	1.037(0.850–1.265)	0.721	1.031(0.767–1.387)	0.840	1.078(0.749–1.552)	0.685
	rs6734005	0.967(0.766–1.221)	0.779	0.892(0.669–1.188)	0.434	1.327(0.724–2.434)	0.360
	rs10178256	1.014(0.824–1.247)	0.899	0.971(0.728–1.294)	0.841	1.124(0.740–1.708)	0.583
	Haplotype-1	0.875(0.717–1.068)	0.190	0.804(0.589–1.096)	0.167	0.880(0.624–1.240)	0.464
	Haplotype-2	0.965(0.764–1.219)	0.766	0.888(0.665–1.185)	0.419	1.327(0.724–2.434)	0.360
	Haplotype-3	1.151(0.851–1.557)	0.361	1.221(0.874–1.706)	0.243	0.748(0.247–2.267)	0.608
	Haplotype-4	1.200(0.893–1.614)	0.227	1.229(0.873–1.730)	0.238	1.344(0.519–3.482)	0.542
Vertebral fracture	rs10856792	0.721(0.503–1.036)	0.077	0.685(0.383–1.226)	0.203	0.606(0.332–1.104)	0.102
	rs11096633	0.906(0.615–1.333)	0.616	1.046(0.612–1.788)	0.868	0.628(0.294–1.342)	0.230
	rs6734005	0.755(0.482–1.183)	0.220	0.636(0.376–1.074)	0.091	1.530(0.412–5.684)	0.525
	rs10178256	0.899(0.600–1.347)	0.606	0.904(0.539–1.517)	0.702	0.804(0.329–1.963)	0.631
	Haplotype-1	0.771(0.532–1.119)	0.171	0.757(0.426–1.345)	0.342	0.659(0.350–1.242)	0.197
	Haplotype-2	0.810(0.517–1.270)	0.359	0.697(0.411–1.183)	0.181	1.530(0.412–5.684)	0.525
	Haplotype-3	1.400(0.780–2.513)	0.260	1.595(0.844–3.012)	0.150	0.389(0.034–4.500)	0.450
	Haplotype-4	1.927(1.142–3.253)	**0.014**	2.231(1.200–4.148)	**0.011**	2.221(0.502–9.822)	0.293

Binary logistic regression of three models (additive, dominant and recessive), adjusted for YSM and BMI, is shown. Bold indicates $p<0.05$.

Table 5

Linear regression of MATN3 genotypes and haplotypes in relation to BMD T-scores of LS, FN and total hip.

SNP ID	Group	LS					FN					Total hip				
		N	T-score	p^a	p^b	p^c	N	T-score	p^a	p^b	p^c	N	T-score	p^a	p^b	p^c
rs10856792	AA	369	−1.129±1.438	0.406	0.461	0.533	368	−1.147±1.060	0.519	0.561	0.634	242	−0.708±1.109	0.451	0.750	0.354
	AG	722	−1.142±1.568				721	−1.096±1.057				463	−0.729±1.149			
	GG	394	−1.212±1.490				396	−1.093±1.079				258	−0.755±1.151			
rs11096633	CC	256	−1.107±1.577	0.853	0.429	0.512	258	−1.055±1.127	0.645	0.880	0.295	161	−0.621±1.201	0.554	0.144	0.443
	CT	703	−1.205±1.533				701	−1.127±1.043				459	−0.807±1.084			
	TT	526	−1.119±1.461				526	−1.108±1.059				343	−0.679±1.175			
rs6734005	AA	85	−1.120±1.575	0.758	0.776	0.845	85	−1.015±1.089	0.100	0.094	0.465	52	−0.590±1.166	0.322	0.369	0.504
	AG	518	−1.160±1.543				518	−1.077±1.107				323	−0.719±1.168			
	GG	882	−1.159±1.495				882	−1.135±1.034				588	−0.749±1.120			
rs10178256	GG	191	−1.144±1.547	0.959	0.930	0.814	192	−1.087±1.090	0.474	0.463	0.707	118	−0.607±1.164	0.861	0.762	0.412
	AG	673	−1.166±1.553				673	−1.093±1.072				434	−0.774±1.098			
	AA	621	−1.152±1.466				620	−1.130±1.046				411	−0.720±1.172			
Haplotype-1 Copies	2	335	−1.108±1.421	0.285	0.375	0.391	335	−1.124±1.059	0.655	0.525	0.939	221	−0.689±1.129	0.507	0.873	0.342
	1	741	−1.146±1.577				739	−1.111±1.062				476	−0.744±1.143			
	0	409	−1.219±1.477				411	−1.089±1.070				266	−0.741±1.141			
Haplotype-2 Copies	2	85	−1.120±1.575	0.666	0.662	0.845	85	−1.015±1.089	0.061	0.050	0.465	52	−0.590±1.166	0.322	0.368	0.504
	1	502	−1.151±1.538				502	−1.065±1.104				314	−0.712±1.175			
	0	898	−1.164±1.500				898	−1.141±1.036				597	−0.752±1.117			
Haplotype-3 Copies	2	23	−1.223±1.575	0.995	0.993	0.954	24	−1.313±0.900	0.580	0.729	0.330	15	−0.700±0.963	0.243	0.243	0.673
	1	306	−1.196±1.564				306	−1.135±1.047				201	−0.787±1.065			
	0	1156	−1.146±1.502				1155	−1.096±1.071				747	−0.716±1.161			
Haplotype-4 Copies	2	27	−1.347±1.440	0.189	0.164	0.774	27	−1.172±1.095	0.968	0.826	0.592	16	−0.688±1.479	0.900	0.676	0.373
	1	289	−1.236±1.430				289	−1.098±1.009				194	−0.796±1.109			
	0	1169	−1.134±1.537				1169	−1.109±1.076				753	−0.714±1.139			

T-scores were adjusted for age, BMI and YSM. P values were calculated under additive, dominant and recessive models. p^a, p value of the additive model; p^b, p value of the dominant model; p^c, p value of the recessive model. LS, lumbar spine (2–4); FN, femoral neck.

Table 6

GLM-ANOVA of β-CTX, P1NP and 25(OH)D in MATN3 genotype and haplotype groups.

SNP ID	Group	N	β-CTX		P1NP		25(OH)D	
			Mean±SD (ng/ml)	p value	Mean±SD (ng/ml)	p value	Mean±SD (ng/ml)	p value
rs10856792	AA	308	0.456±0.194	0.396	57.6±23.6	0.927	13.1±4.8	0.595
	AG	601	0.450±0.231		58.1±27.0		13.0±5.4	
	GG	337	0.434±0.189		58.4±32.1		13.3±5.8	
rs11096633	CC	210	0.434±0.179	0.776	57.0±21.2	0.237	13.7±6.0	0.165
	CT	593	0.452±0.232		59.6±32.5		12.9±5.3	
	TT	443	0.448±0.197		56.4±23.0		13.1±5.2	
rs6734005	AA	70	0.435±0.182	0.542	58.5±23.5	0.806	13.6±6.1	0.668
	AG	429	0.442±0.234		59.0±34.6		13.2±5.5	
	GG	747	0.452±0.200		57.4±23.3		13.0±5.2	
rs10178256	GG	155	0.423±0.173	0.408	56.6±21.3	0.763	13.6±5.7	0.421
	AG	568	0.452±0.234		59.2±32.7		13.1±5.5	
	AA	523	0.450±0.195		57.2±23.0		13.0±5.1	
Haplotype-1 copies	2	280	0.457±0.196	0.407	57.2±23.9	0.779	13.2±4.8	0.450
	1	619	0.450±0.230		58.3±26.9		12.9±5.4	
	0	347	0.435±0.187		58.2±31.7		13.3±5.8	
Haplotype-2 copies	2	70	0.435±0.182	0.497	58.5±23.5	0.868	13.6±6.1	0.824
	1	416	0.442±0.235		59.0±34.9		13.2±5.5	
	0	760	0.452±0.200		57.5±23.2		13.0±5.2	
Haplotype-3 copies	2	19	0.397±0.161	0.443	53.6±23.9	0.679	13.5±6.2	0.715
	1	261	0.454±0.208		57.7±22.9		13.3±5.4	
	0	966	0.447±0.213		58.2±28.9		13.0±5.3	
Haplotype-4 copies	2	25	0.397±0.150	0.363	58.2±21.7	0.424	11.1±4.6	0.204
	1	249	0.437±0.203		57.1±35.0		13.1±5.8	
	0	972	0.451±0.215		58.3±25.6		13.2±5.3	

β-CTX and P1NP were not normally distributed, and they underwent logarithmic or square root transformation before statistical analysis. Age and YSM were controlled as covariates for β-CTX and P1NP, and age was adjusted for 25(OH)D.