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Original Article

Association between Fat Mass and Obesity-Related Transcript Polymorphisms and Osteoporosis Phenotypes

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Background: Common variants in the fat mass and obesity-related transcript (*FTO*) gene are related to body mass index and obesity, suggesting its potential association with bone mineral density (BMD) and fracture risk. This study sought to define the association between *FTO* gene variants and the following phenotypes: (1) BMD; (2) bone loss; and (3) fracture risk. **Methods:** This analysis was based on the Dubbo Osteoporosis Epidemiology Study that included 1,277 postmenopausal women aged ≥60 years living in Dubbo, Australia. BMD at the femoral neck and lumbar spine was measured biennially by dual energy X-ray absorptiometry (GE Lunar). Fractures were radiologically ascertained. Six single nucleotide polymorphisms (SNPs; rs1421085, rs1558902, rs1121980, rs17817449, rs9939609, and rs9930506) of the *FTO* gene were genotyped using TaqMan assay. **Results:** Women homozygous for the minor allele (GG) of rs9930506 had a significantly higher risk of hip fracture (adjusted hazard ratio, 1.93; 95% confidence interval, 1.15–3.23) than those homozygous for the major allele (AA) after adjusting for potential confounding effects. Similar associations were also observed for the minor allele of rs1121980. However, there was no significant association between the *FTO* SNPs and BMD or the rate of bone loss. **Conclusions:** Common variations in the *FTO* gene are associated with a hip fracture risk in women, and the association is not mediated through BMD or bone loss.

Key Words: Alpha-ketoglutarate-dependent dioxygenase *FTO* · Genetic variation · Phenotype · Polymorphism, single nucleotide · Osteoporosis

INTRODUCTION

The fat mass and obesity-related transcript (*FTO*) gene encodes the nuclear protein, Fe(II)/2-oxoglutarate dependent methylase, a type of RNA demethylase.[1] In 2007, a type 2 diabetes genome-wide association study (GWAS) identified *FTO* single nucleotide polymorphisms (SNPs) were associated with body mass index (BMI) and obesity.[2] Other studies conducted independently on Asian,[3,4] African,[5,6] and European [7,8] populations have also shown the correlations between SNPs at intron 1 of *FTO* with waist-to-hip ratio, obesity, type 2 diabetes, body weight, and

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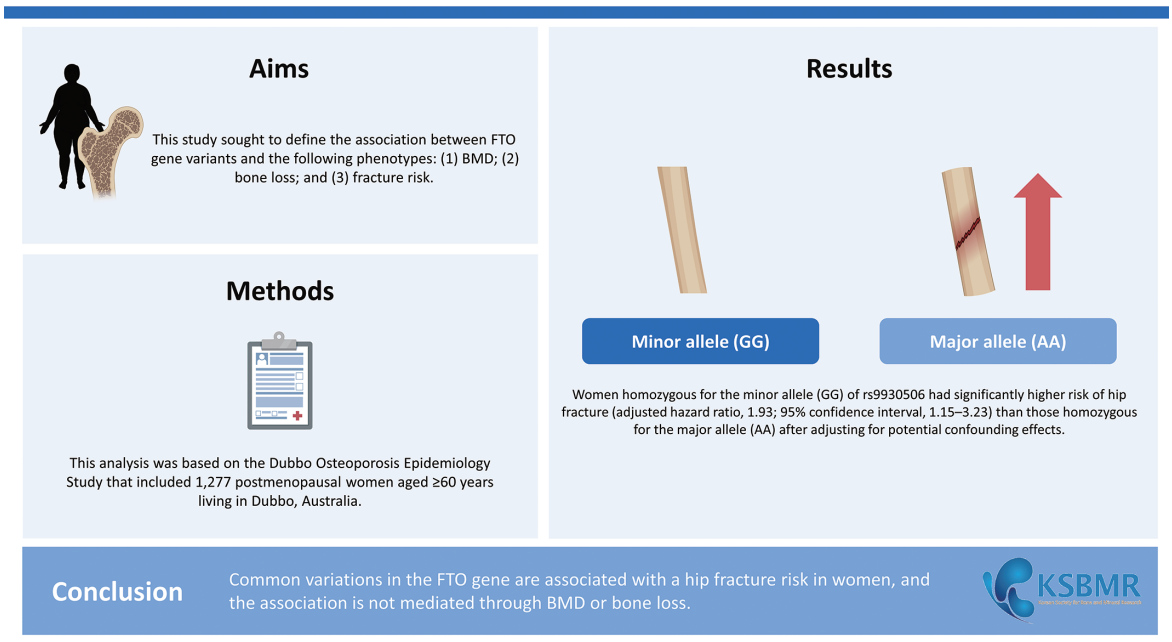
Graphical Abstract



other BMI-related phenotypes. Among hundreds to thousands of *FTO* SNPs studied in these populations, the *FTO* variants rs9939609, rs1421085, rs17817449, and rs1121980 have been consistently shown to be significantly associated with body weight and related phenotypes in various ethnicities.[3,4,6-8]

BMI and obesity are strongly associated with several osteoporosis phenotypes, including bone mineral density (BMD) and fracture risk.[9,10] Each unit increase in BMI (kg/cm^2) has been found to be associated with approximately 0.0082 g/cm^2 increase in BMD amongst American adults.[11] Greater BMI has been found to be associated with a lower risk of fracture, independently of BMD.[12,13] The relationship between BMI and fracture risk is complex, and dynamic due to the association between BMI and BMD.[12]

Although several GWASes on BMD and other osteoporosis phenotypes have been conducted,[14] there was no signal for the *FTO* gene. We hypothesise *FTO* genotypes are associated with different osteoporosis phenotypes. Specifically, the present study sought to test that hypothesis by pursuing the following specific aims: to quantify the association between *FTO* polymorphisms with (1) BMD; (2) bone loss; and (3) fracture in postmenopausal women. By determining the association of *FTO* polymorphisms with osteoporosis phenotypes, we can utilize *FTO* as a genetic factor to predict the development of osteoporosis, including fracture prediction.

METHODS

1. Study design

This study was based on the Dubbo Osteoporosis Epidemiology Study (DOES) with the protocols and study structure described elsewhere.[15] Briefly, DOES was designed as a prospective, population-based investigation that was operational from 1989 to 2020. Approximately 4,000 men and women aged 60 years and older have been recruited and followed up for almost 30 years. The study was approved by the St. Vincent's Campus Research Ethics Committee and written informed consent was acquired from each participant. As this study was primarily focused on post-menopausal women, we excluded men and any participants without *FTO* genotype data (Fig. 1).

2. Measurements

A nurse co-ordinator used a structured questionnaire to obtain baseline measurements at 1989 and subsequent

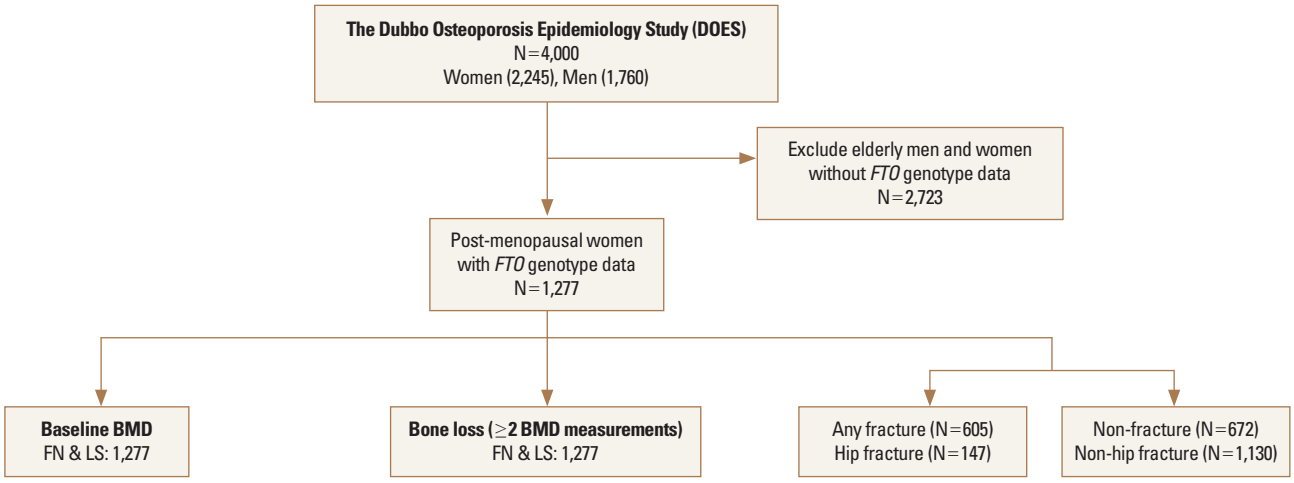


Fig. 1. Flow chart of recruitment and follow up. *FTO*, fat mass and obesity-related transcript; BMD, bone mineral density; FN, femoral neck; LS, lumbar spine.

visits at approximately 2 to 3 yearly intervals. The structured questionnaire included age, weight, height, smoking and alcohol intake, physical activity, calcium intake, medication, number of falls in the last year, and fracture history. Weight (kg) and height (cm), without shoes and in light clothing, was measured on an electronic scale (to the nearest 0.1 kg) and by a wall-mounted stadiometer (to the nearest 0.1 cm). BMI (kg/m^2) was calculated as the ratio of weight (kg) and squared height (m^2).

BMD (g/cm^2) at the femoral neck and lumbar spine was measured at baseline and biennially by a dual energy X-ray absorptiometry (DXA) instrument using a LUNAR DPX densitometer (GE-Lunar, Madison, WI, USA). The coefficient of variations of BMD measurement of the femoral neck and lumbar spine was 1.5% and 1.3%, respectively.[16] All measurements were conducted by the same technicians, following the same standard operating procedure.

3. Assessment of outcomes

All fractures were ascertained from the 2 or 3 radiology centres servicing the Dubbo area, and circumstances surrounding fracture were ascertained by personal interview following the fracture event. Only low-trauma fractures caused by a fall from a standing height or less were included in the study. Fractures caused by high trauma, such as motor vehicle accidents, pathological fractures from bone diseases other than osteoporosis (e.g., hyperparathyroidism, Paget's disease), or fractures of the skull and digits were excluded. Vertebral fractures were determined either through clinical diagnosis or detected incidentally on X-ray. Deaths were ascertained from funeral lists, obituary reports, and Dubbo media reports, and verified from the New South Wales Bureau of Births, Deaths, and Marriages.

4. Genotyping

The genotype data for this study was drawn from a previous investigation, which describes the genotyping protocol used.[17] Briefly, blood samples were collected and stored at -80°C. Extraction of DNA was done either by using QIAamp DNA Mini Blood Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions, or phenol/chloroform. Six SNPs (rs1421085, rs1558902, rs1121980, rs17817449, rs9939609, and rs9930506) in the first intron of the *FTO* gene were genotyped using a predesign of Taqman SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA). The ABI 7900 Sequence Detection System (SDS) software was used to compute the allelic discrimination. From the participants of DOES, the DNA of 1,277 women and 758 men were able to be genotyped. The number of fractures was too small to conduct a sensitive analysis for elderly men, therefore, we primarily focused on post-menopausal women with *FTO* genotype data.

5. Data analysis

The first analysis included participants with BMD measured at baseline. We performed a multiple-adjusted linear regression to quantify the association between *FTO* genotypes and baseline BMD at the femoral neck using the AA

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genotype as the reference group. The model was adjusted for predefined covariates of age and BMI. A predefined sensitivity analysis was also considered for lumbar spine BMD. To control for potential confounding effects of metabolic diseases, an exploratory analysis was conducted to further adjust for the presence of diabetes mellitus.

For the second aim, the rate of change in BMD was calculated for each participant with at least 2 BMD measurements using a linear mixed-effects regression. We also conducted a linear mixed-effects regression, adjusted for the predefined covariates to examine the association between *FTO* genotypes and bone loss. A linear mixed-effects regression has been shown to be statistically superior to a conventional linear regression in analysis of repeated measurement.[18,19] A linear mixed-effects regression is capable of accounting for not only a regression-toward-the-mean phenomenon, but also missing data which are very common in any analysis with repeated measures.[18] Moreover, a linear mixed-effects regression is also able to account for variation between and within subjects.[19]

For our third aim, the Cox's proportional hazards regression was used to quantify the association between *FTO* genotypes and fracture risk, adjusted for age, BMD, and BMI. The follow-up time for participants with a fracture was calculated between the study entry and the date of fracture. For participants without a fracture, their follow-up was computed as a time interval between the study entry and date of death or the study end (March 5, 2018), whichever came first. The assumption of proportional hazards was graphically checked using the scaled Schoenfeld residuals.[20]

We computed the E-value [21] as a predefined sensitivity analysis to assess the robustness of our findings in the potential of residual confounding effects. Technically, the E-value quantifies possibilities that an uncontrolled confounder would make the reported association statistically non-significant. No adjustments were made for multiple comparisons. The analyses were performed using R language [22] with a *P*-value of less than 0.05 considered statistically significant.

Table 1. Baseline characteristics by fat mass and obesity-related transcript genotypes (rs9930506) in women

	AA (N=349)	GA (N=546)	GG (N=209)
Age (yr)	69±7	69±7	70±6
Height (cm)	160±6	160±6	160±6
Weight (kg)	67±13	67±13	66±12
Body mass index (kg/m^2)	26±5	26±5	26±4
Type-2 diabetes	16 (4.61)	26 (4.81)	9 (4.31)
Bisphosphonates use	31 (8.93)	59 (10.9)	17 (8.13)

The data is presented as mean±standard deviation or N (%). The differences between the genotypes were examined using analysis of variance for a continuous variable and chi-squared test for a categorical variable.

RESULTS

1. *FTO* genotypes and baseline BMD

The current study involved 1,277 women with an average age of 69 years (±6.6) whose *FTO* genotype data were available (Fig. 1). The distribution of *FTO* genotypes is consistent with the Hardy-Weinberg principle as the proportion of the major (32%–38%), heterozygous (44%–49%) and minor (16%–20%) allele are similar in the *FTO* SNP rs9930506 (Table 1) and other *FTO* SNPs-rs1421085, rs1558902, rs1121980, rs17817449, and rs9939609 (Supplementary Table 1). Given the great similarities between the *FTO* SNPs and their close proximity to each other at intron 1 of the *FTO* gene, we present the results of rs9930506 in the main paper and leave those of other *FTO* genotypes in the appendix.

Table 2 illustrates the association between *FTO* genotypes rs9930506 and baseline BMD. We found *FTO* genotypes were not associated with BMD after accounting for potential confounding effects of age and BMI (Table 2). Similarly, there was no association between BMD and the other *FTO*

Table 2. Association of fat mass and obesity-related transcript genotypes (rs9930506) and bone mineral density in women

	AA[a] (N=349)	GA (N=546)	GG (N=209)	GA vs. AA	GG vs. AA	GA vs. GG
Femoral neck BMD (g/cm^2)	0.79±0.13	0.81±0.14	0.80±0.14	0.017 (0.002, 0.033)	0.013 (-0.006, 0.033)	-0.004 (-0.022, 0.014)
Lumbar spine BMD (g/cm^2)	1.05±0.19	1.05±0.20	1.05±0.21	-0.001 (-0.025, 0.024)	0.005 (-0.026, 0.037)	0.006 (-0.024, 0.035)

Bone mineral density (BMD) values are presented as mean±standard deviation. The association between fat mass and obesity-related transcript genotype and BMD is presented as mean difference (95% confidence interval) derived from a multivariable-adjusted linear regression, adjusted for age and body mass index.
[a]Reference genotype: AA.

SNPs – rs1421085, rs1558902, rs1121980, rs17817449, and rs9939609 (Supplementary Table 2-6).

2. *FTO* genotypes and longitudinal BMD change

In our study population of post-menopausal women, the overall rates of BMD change were -0.004 g/cm^2 per year at the femoral neck and 0.002 g/cm^2 per year at the lumbar spine. In the *FTO* SNP rs9930506, the annual rate of bone change was similar across the *FTO* genotypes at the femoral neck (-0.004 g/cm^2) and lumbar spine (0.002 g/cm^2) (Table 3). The analysis did not find a significant difference in the rate of BMD change over time between the *FTO* genotypes after taking the potential confounding effect of age and BMI into account (Table 3, Supplementary Table 2-6).

3. *FTO* genotypes and fracture risk

During a median follow-up of 14 years (interquartile range, 10–22 years), 605 women sustained at least one incident fracture, yielding a fracture rate of 40/1,000 person-years (95% confidence interval [CI], 37–44). We also found 147 women with a hip fracture rate of 8/1,000 person-years (95% CI, 6-9). We found *FTO* genotypes were not independently associated with the risk of any fracture (Table 4, Supplementary Table 4, 5). However, women with the minor allele (GG) were associated with a significantly greater risk of hip fracture (hazard ratio, 1.82; 95% CI, 1.10–3.03). The association remained significant after adjusting for potential confounding effects of age, BMI, and BMD. Interestingly, we found the E-value for the *FTO*-hip fracture association was as high as 3.25 (95% CI, 1.57–5.85), indicating that the finding would have become non-significant only if there was a very strong residual confounding effect associated with almost a 3-fold risk of hip fracture existing in the study population. Similarly, the minor allele of *FTO* SNPs rs1121980 was also associated with a greater risk of hip fracture (Supplementary Table 4). The association between the minor allele of rs1421085 (P=0.09), rs1558902 (P=0.06), and rs17817449 (P=0.05) with hip fracture only achieved marginally statisti-

Table 3. Differences in the rate of bone mineral density change at the femoral neck and lumbar spine between fat mass and obesity-related transcript genotypes (rs9930506) in women

	AA[a]	GA	GG	GA vs. AA	GG vs. AA	GA vs. GG
Femoral neck BMD (g/cm^2)	-0.004±0.004	-0.004±0.003	-0.004±0.003	<-0.001 (-0.001, 0.001)	<-0.001 (-0.001, 0.001)	<0.001 (-0.001, 0.001)
Lumbar spine BMD (g/cm^2)	0.002±0.006	0.002±0.007	0.002±0.006	<0.001 (-0.001, 0.001)	0.001 (-0.001, 0.003)	0.001 (-0.001, 0.003)

The rate of bone mineral density (BMD) change is presented as mean±standard deviation. The association between fat mass and obesity-related transcript genotype and bone loss is presented as mean difference (95% confidence interval) derived from a multivariable-adjusted linear mixed-effects regression, adjusted for age and body mass index.
[a]Reference genotype: AA.

Table 4. Association between fat mass and obesity-related transcript genotypes (rs9930506) and fracture risk in women

Any fracture					
Genotype	Number[a]	Age-adjusted		Multivariable adjusted[b]	
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
AA (N=357)	165 (46.2)	Reference	-	Reference	-
GA (N=556)	266 (47.8)	1.08 (0.88–1.31)	0.47	1.12 (0.92–1.37)	0.26
GG (N=213)	96 (45.1)	1.01 (0.78–1.30)	0.96	1.04 (0.81–1.34)	0.76
Hip fracture					
Genotype	Number[a]	Age-adjusted		Multivariable adjusted[b]	
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
AA (N=357)	30 (8.4)	Reference	-	Reference	-
GA (N=556)	65 (11.7)	1.38 (0.89–2.13)	0.15	1.40 (0.91–2.17)	0.10
GG (N=213)	30 (14.1)	**1.82 (1.10–3.03)**	**0.02**	**1.92 (1.15–3.20)**	**0.01**

P<0.05 is statically significant. Bold values indicate statistical significance.
[a]The data is presented as N (%) and indicates the number of patients with a fracture.
[b]Adjusted for age, femoral neck bone mineral density and body mass index.
HR, hazard ratio; CI, confidence interval.

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cally significant results (Supplementary Table 2, 3, and 5). The exploratory analysis further adjusted for the presence of metabolic diseases provided consistent results (Supplementary Table 7), confirming the robustness of our primary findings.

DISCUSSION

Common variants in the *FTO* gene are related to BMI and obesity, suggesting its potential contribution to the key osteoporosis phenotypes, such as BMD and fracture risk. Using data from a well-established, prospective cohort study, we have presented polymorphic variation at the *FTO* gene associated with hip fracture risk, independent of age, BMD, and BMI. However, our analysis did not find a significant association between the *FTO* gene and either BMD or bone loss, suggesting that the association between the *FTO* gene and hip fracture is unlikely mediated via BMD or bone loss.

Our finding is consistent with a previous study that could not find an association between the *FTO* gene and BMD in Caucasians.[10] They however found the *FTO* gene was significantly associated with BMD in Chinese individuals. Studies have shown weight-related health risks were greater in Asians than Caucasians, Africans, and Hispanics [23,24] with Asians usually exhibiting a higher body fat percentage than Caucasians.[25] Given the close correlation of *FTO* with body weight and obesity, it is possible that *FTO* polymorphisms exhibit a greater effect on populations strongly affected by weight-related health risks, such as Asians.

Our finding of the relationship between *FTO* and fracture risk supports previous Findings.[17] That polymorphic variation at the *FTO* gene was associated with hip fracture risk in postmenopausal women, even after accounting for potential confounding effects. Although the findings suggest an increased risk of hip fracture virtually associated with all *FTO* SNPs, only rs1121980 and rs9930506 were found to be significantly associated with hip fracture after accounting for potential confounding effects. Indeed, the *FTO* SNPs rs1421085, rs1558902, and rs17817449 and were associated with a 1.5-fold increased risk of hip fracture, though the associations only achieved marginally statistically significant ($P \approx 0.10$) results. Taken together, our finding suggests that neither BMD nor bone loss mediates the association between *FTO* and an increased risk of hip fracture among post-menopausal women.

The biological mechanism underlying the association between *FTO* polymorphic variation and increased hip fracture is unknown. In human and animal models, expression of the *FTO* gene plays a role in the determination of whole-body lean mass, fat mass, and pancreatic β-cells.[26,27] Lean mass is known to be associated with reduced bone fracture. A stronger influence is observed with lean mass as opposed to fat mass which has a positive association with hip strength.[28] Taken together, these findings suggest that the association between *FTO* variants and hip fractures maybe mediated through lean mass. However, this hypothesis needs to be verified in future studies.

The present study's findings must be interpreted within the context of potential strengths and weaknesses. To our knowledge, this is the first study that examines the association between *FTO* polymorphic variations and bone loss. The study was based on a well-characterized cohort, a reasonably large sample size with a long duration of follow-up, which allows accurate assessment of bone loss at the individual level and sufficient statistical power for analysing fracture risk. Additionally, BMD was measured using the DXA, considered the robust and standard method, whereas fractures were ascertained radiologically, minimizing the risk of measurement biases. The linear mixed-effects regression we used in this study is considered much more robust than the conventional linear regression.

However, the study is an observational investigation, and it is not possible to make any causal inference concerning the association between *FTO* genotypes and bone phenotypes. The finding might have been affected by residual confounding effects that were not measured in this study. However, given the E-value of 3.25, it is unlikely that the association between *FTO* gene and hip fracture was only confounded by unmeasured confounders associated with at least 3-fold risk of fracture. Such a very strong residual confounding effect is considered uncommon in reality, indicating our findings are statistically robust. Finally, the study population was limited to Caucasians, and extrapolation to non-Caucasian populations requires careful consideration.

In conclusion, the present study has demonstrated that polymorphic variation at the *FTO* gene is associated with hip fracture risk, but this association is unlikely mediated through the low BMD of bone loss. Further research is need-

ed to examine the candidate genes of associated causal variants with osteoporosis phenotypes, as GWAS alone cannot comprehensively explain the biological mechanism of the identified genetic variants.[29]

DECLARATIONS

Acknowledgments

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Ethics approval and consent to participate

The study was approved by the St Vincent's Campus Research Ethics Committee. Written informed consent was acquired from each participant and their legal guardians. All procedures were performed in accordance with the Declaration of Helsinki.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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Supplementary Table 1. Baseline characteristics stratified by *FTO* genotype (rs1421085, rs1558902, rs1121980, rs17817449, and rs9939609)

rs1421085	CC (N=202)	CT (N=549)	TT (N=392)
Age (yr)	69±6	70±7	70±7
Height (cm)	160±6	160±6	160±6
Weight (kg)	66±12	66±12	68±13
BMI (kg/m^2)	26±5	26±5	26±5
Diabetes	11 (5.45)	25 (4.60)	16 (4.12)
Bisphosphonates use	18 (8.91)	47 (8.66)	39 (10.1)
rs1558902	AA (N=201)	AT (N=593)	TT (N=417)
Age (yr)	69±6	69±7	70±7
Height (cm)	160±6	160±6	160±6
Weight (kg)	65±12	67±12	66±12
BMI (kg/m^2)	26±4	26±5	26±5
Diabetes	8 (3.98)	30 (5.10)	17 (4.12)
Bisphosphonates use	16 (7.96)	55 (9.35)	44 (10.7)
rs1121980	AA (N=214)	AG (N=535)	GG (N=374)
Age (yr)	70±7	69±7	70±7
Height (cm)	160±6	160±6	160±6
Weight (kg)	66±13	68±13	68±13
BMI (kg/m^2)	26±5	26±5	26±4
Diabetes	7 (3.27)	21 (3.97)	18 (4.85)
Bisphosphonates use	17 (7.94)	50 (9.45)	40 (10.8)
rs17817449	GG (N=193)	GT (N=571)	TT (N=415)
Age (yr)	69±6	69±7	69±7
Height (cm)	160±6	160±6	160±6
Weight (kg)	66±12	67±13	68±13
BMI (kg/m^2)	26±4	26±5	26±5
Diabetes	9 (4.66)	28 (4.96)	18 (4.38)
Bisphosphonates use	17 (8.81)	53 (9.38)	43 (10.5)
rs9939609	AA (N=183)	AT (N=480)	TT (N=373)
Age (yr)	69±6	69±7	69±7
Height (cm)	160±6	161±6	160±6
Weight (kg)	65±11	67±13	68±12
BMI (kg/m^2)	26±4	26±5	26±5
Diabetes	8 (4.37)	22 (4.64)	14 (3.77)
Bisphosphonates use	19 (10.4)	46 (9.70)	41 (11.1)

The data is presented as mean±standard deviation or N (%). The differences between the genotypes were examined using analysis of variance for a continuous variable and chi-squared test for a categorical variable.

Supplementary Table 2. Association between fat mass and obesity-related transcript genotypes of rs1421085 and bone mineral density, bone loss and fracture

I. BMD[a]						
	CC (N=202)	TC (N=549)	TT[b] (N=392)	TC vs. TT	CC vs. TT	TC vs. CC
FN BMD (g/cm^2)	0.80±0.14	0.80±0.14	0.80±0.13	0.007 (-0.007, 0.022)	0.001 (-0.018, 0.021)	-0.006 (-0.025, 0.012)
LS BMD (g/cm^2)	1.05±0.21	1.04±0.20	1.05±0.19	-0.006 (-0.030, 0.018)	0.002 (-0.030, 0.034)	0.008 (-0.022, 0.038)
II. Rate of BMD change[a]						
	CC	TC	TT[b]	TC vs. TT	CC vs. TT	TC vs. CC
FN BMD (g/cm^2/year)	-0.004±0.003	-0.004±0.004	-0.004±0.004	<0.001 (<-0.001, 0.001)	<-0.001 (-0.001, 0.001)	<-0.001 (-0.001, 0.001)
LS BMD (g/cm^2/year)	0.002±0.007	0.002±0.007	0.002±0.007	<0.001 (-0.001, 0.001)	<0.001 (-0.001, 0.002)	<0.001 (-0.002, 0.002)

III. Fractures						
	Variables	Number[c]	Age adjusted		Multivariable adjusted[d]	
			HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Any fracture	CC (N=206)	98 (47.6)	1.00 (0.78–1.28)	0.97	1.01 (0.79–1.29)	0.94
	TC (N=559)	268 (47.9)	1.03 (0.85–1.23)	0.78	1.04 (0.86–1.25)	0.70
	TT[b] (N=399)	190 (47.6)	Reference	-	Reference	-
	Variables	Number[c]	Age adjusted		Multivariable adjusted[d]	
			HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Hip fracture	CC (N=206)	32 (15.5)	1.46 (0.92–2.30)	0.10	1.47 (0.93–2.31)	0.09
	TC (N=559)	58 (10.4)	0.87 (0.59–1.28)	0.47	0.87 (0.59–1.29)	0.48
	TT[b] (N=399)	46 (11.5)	Reference	-	Reference	-

[a]Values for BMD and rate of bone change are presented as mean±standard deviation. The association between fat mass and obesity-related transcript genotype and bone mineral density (BMD) and bone loss are presented as mean difference (95% confidence interval [CI]) derived from a multivariable linear regression (I. BMD) or mixed-effects regression (II. bone loss), adjusted for age and body mass index (BMI).
[b]Reference genotype: TT.
[c]The data is presented as N (%) and indicates the number of patients with a fracture.
[d]Adjusted for age, femoral neck BMD and BMI.
FN, femoral neck; LS, lumbar spine; HR, hazard ratio.

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Supplementary Table 3. Association between fat mass and obesity-related transcript genotypes of rs1558902 and bone mineral density, bone loss and fracture

I. BMD[a]						
	AA (N=201)	TA (N=593)	TT[b] (N=417)	TA vs. TT	AA vs. TT	TA vs. AA
FN BMD (g/cm^2)	0.79±0.13	0.81±0.13	0.79±0.13	0.014 (-0.001, 0.028)	-0.002 (-0.022, 0.017)	-0.016 (-0.034, 0.002)
LS BMD (g/cm^2)	1.04±0.21	1.04±0.19	1.05±0.19	-0.007 (-0.030, 0.016)	- 0.005 (-0.036, 0.026)	0.002 (-0.027, 0.032)
II. Rate of BMD change[a]						
	AA	TA	TT[b]	TA vs. TT	AA vs. TT	TA vs. AA
FN BMD (g/cm^2/year)	-0.004±0.007	-0.004±0.004	-0.004±0.004	<0.001 (-0.001, 0.001)	<-0.001 (-0.001, 0.001)	<-0.001 (-0.001, 0.001)
LS BMD (g/cm^2/year)	0.002±7.26	0.002±0.007	0.002±0.006	<0.001 (-0.001, 0.002)	<0.001 (-0.001, 0.002)	<-0.001 (-0.002, 0.001)

III. Fractures

	Variables	Number[c]	Age adjusted		Multivariable adjusted[d]	
			HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Any fracture	AA (N=204)	98 (47.6)	1.02 (0.80–1.31)	0.85	1.03 (0.81–1.32)	0.80
	TA (N=603)	268 (47.9)	1.03 (0.86–1.23)	0.78	1.07 (0.89–1.28)	0.48
	TT (N=426)	201 (47.2)	Reference	-	Reference	-
	Variables	Number[c]	Age adjusted		Multivariable adjusted[d]	
			HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Hip fracture	AA (N=204)	32 (15.7)	1.47 (0.94–2.31)	0.09	1.54 (0.98–2.42)	0.06
	TA (N=603)	65 (10.8)	0.93 (0.64–1.36)	0.72	1.00 (0.69–1.46)	1.00
	TT (N=426)	48 (11.3)	Reference	-	Reference	-

[a]Values for BMD and rate of bone change are presented as mean±standard deviation. The association between fat mass and obesity-related transcript genotype and bone mineral density (BMD) and bone loss are presented as mean difference (95% confidence interval [CI]) derived from a multivariable linear regression (I. BMD) or mixed-effects regression (II. bone loss), adjusted for age and body mass index (BMI).
[b]Reference genotype: TT.
[c]The data is presented as N (%) and indicates the number of patients with a fracture.
[d]Adjusted for age, femoral neck BMD and BMI.
FN, femoral neck; LS, lumbar spine; HR, hazard ratio.

Supplementary Table 4. Association between fat mass and obesity-related transcript genotypes of rs1121980 and bone mineral density, bone loss and fracture

I. BMD[a]						
	AA (N=214)	GA (N=535)	GG[b] (N=374)	GA vs. GG	AA vs. GG	GA vs. AA
FN BMD (g/cm^2)	0.79±0.14	0.80±0.14	0.79±0.13	0.012 (-0.003, 0.027)	0.006 (-0.013, 0.026)	- 0.005 (-0.024, 0.013)
LS BMD (g/cm^2)	1.05±0.22	1.04±0.19	1.06±0.19	- 0.015 (-0.040, 0.010)	<0.001 (-0.031, 0.032)	0.015 (-0.014, 0.045)
II. Rate of BMD change[a]						
	AA	GA	GG[b]	GA vs. GG	AA vs. GG	GA vs. AA
FN BMD (g/cm^2/year)	-0.004±0.003	-0.004±0.004	-0.004±0.004	<-0.001 (-0.001, 0.001)	<-0.001 (-0.002, 0.001)	<-0.001 (-0.001, 0.001)
LS BMD (g/cm^2/year)	0.002±0.007	0.002±0.007	0.002±0.006	<0.001 (-0.001, 0.002)	<0.001 (-0.001, 0.002)	<-0.001 (-0.002, 0.002)

III. Fractures						
	Variables	Number[c]	Age adjusted		Multivariable adjusted[d]	
			HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Any fracture	AA (N=217)	102 (47.0)	0.97 (0.76–1.24)	0.82	1.00 (0.78–1.27)	0.97
	GA (N=545)	262 (48.1)	1.03 (0.85–1.24)	0.78	1.06 (0.87–1.28)	0.58
	GG (N=381)	181 (47.5)	Reference	-	Reference	-
	Variables	Number[c]	Age adjusted		Multivariable adjusted[d]	
			HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Hip fracture	AA (N=217)	35 (16.1)	**1.68 (1.06–2.65)**	**0.02**	**1.76 (1.11–2.79)**	**0.01**
	GA (N=545)	57 (10.5)	1.03 (0.68–1.56)	0.89	1.03 (0.68–1.56)	0.88
	GG (N=381)	38 (10.0)	Reference	-	Reference	-

P<0.05 is statically significant. Bold values indicate statistical significance.
[a]Values for BMD and rate of bone change are presented as mean±standard deviation. The association between fat mass and obesity-related transcript genotype and bone mineral density (BMD) and bone loss are presented as mean difference (95% confidence interval [CI]) derived from a multivariable linear regression (I. BMD) or mixed-effects regression (II. bone loss), adjusted for age and body mass index (BMI).
[b]Reference genotype: GG.
[c]The data is presented as N (%) and indicates the number of patients with a fracture.
[d]Adjusted for age, femoral neck BMD and BMI.
FN, femoral neck; LS, lumbar spine; HR, hazard ratio.

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Supplementary Table 5. Association between fat mass and obesity-related transcript genotypes of rs17817449 and bone mineral density, bone loss and fracture

I. BMD[a]						
	GG (N=193)	TG (N=571)	TT[b] (N=415)	TG vs. TT	GG vs. TT	TG vs. GG
FN BMD (g/cm^2)	0.79±0.14	0.80±0.14	0.79±0.13	0.015 (<-0.001, 0.029)	<0.001 (-0.020, 0.019)	-0.015 (-0.034, 0.004)
LS BMD (g/cm^2)	1.04±0.20	1.05±0.20	1.05±0.19	-0.005 (-0.028, 0.018)	-0.007 (-0.039, 0.024)	-0.002 (-0.032, 0.028)
II. Rate of BMD change[a]						
	GG	TG	TT[b]	TG vs. TT	GG vs. TT	TG vs. GG
FN BMD (g/cm^2/year)	-0.004 (0.003)	-0.004 (0.003)	-0.004 (3.69)	<0.001 (-0.001, 0.001)	<-0.001 (-0.001, 0.001)	<-0.001 (-0.001, 0.001)
LS BMD (g/cm^2/year)	0.002 (0.007)	0.002 (0.007)	0.002 (0.006)	<0.001 (-0.001, 0.001)	<0.001 (-0.001, 0.002)	<0.001 (-0.001, 0.002)

III. Fractures						
	Variables	Number[c]	Age adjusted		Multivariable adjusted[d]	
			HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Any fracture	GG (N=196)	91 (46.4)	0.99 (0.77–1.27)	0.92	0.99 (0.77–1.28)	0.96
	TG (N=581)	272 (46.8)	1.02 (0.85–1.23)	0.80	1.07 (0.89–1.28)	0.49
	TT (N=424)	196 (46.2)	Reference	-	Reference	-
	Variables	Number[c]	Age adjusted		Multivariable adjusted[d]	
			HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Hip fracture	GG (N=196)	29 (14.8)	1.59 (0.95–2.45)	0.08	1.59 (0.99–2.55)	0.05
	TG (N=581)	56 (9.6)	0.92 (0.62–1.37)	0.69	0.98 (0.66–1.47)	0.93
	TT (N=424)	43 (10.1)	Reference	-	Reference	-

[a]Values for BMD and rate of bone change are presented as mean±standard deviation. The association between fat mass and obesity-related transcript genotype and bone mineral density (BMD) and bone loss are presented as mean difference (95% confidence interval [CI]) derived from a multivariable linear regression (I. BMD) or mixed-effects regression (II. bone loss), adjusted for age and body mass index (BMI).
[b]Reference genotype: TT.
[c]The data is presented as N (%) and indicates the number of patients with a fracture.
[d]Adjusted for age, femoral neck BMD and BMI.
FN, femoral neck; LS, lumbar spine; HR, hazard ratio.

Supplementary Table 6. Association between fat mass and obesity-related transcript genotypes of rs9939609 and bone mineral density, bone loss and fracture

I. BMD[a]						
	AA (N=183)	TA (N=480)	TT[b] (N=373)	TA vs. TT	AA vs. TT	TA vs. AA
FN BMD (g/cm^2)	0.79±0.13	0.81±0.13	0.80±0.13	9.76 (-5.70, 25.23)	-6.71 (-26.94, 13.53)	-16.47 (-35.91, 2.96)
LS BMD (g/cm^2)	1.05±0.20	1.05±0.20	1.06±0.19	-14.74 (-39.67, 10.19)	-6.73 (-39.35, 25.89)	8.01 (-23.33, 39.35)
II. Rate of BMD change[a]						
	AA	TA	TT[b]	TA vs. TT	AA vs. TT	TA vs. AA
FN BMD (g/cm^2/year)	-0.004 (0.003)	-0.004 (0.003)	-0.004 (0.004)	<-0.001 (-0.001, 0.001)	<0.001 (-0.001, 0.001)	<0.001 (-0.001, 0.001)
LS BMD (g/cm^2/year)	0.002 (0.008)	0.002 (0.007)	0.002 (0.006)	<0.001 (-0.001, 0.001)	<0.001 (-0.001, 0.002)	<0.001 (-0.001, 0.002)
III. Fractures						
	Variables	Number[c]	Age adjusted		Multivariable adjusted[d]	
			HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Any fracture	AA (N=187)	88 (47.1)	1.02 (0.79–1.33)	0.83	1.01 (0.78–1.31)	0.94
	TA (N=487)	232 (47.6)	1.10 (0.91–1.35)	0.33	1.13 (0.92–1.38)	0.24
	TT (N=381)	171 (44.9)	Reference	-	Reference	-
	Variables	Number[c]	Age adjusted		Multivariable adjusted[d]	
			HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Hip fracture	AA (N=187)	23 (12.3)	1.19 (0.71–1.99)	0.51	1.20 (0.71–2.00)	0.05
	TA (N=487)	51 (10.5)	1.02 (0.68–1.55)	0.91	1.03 (0.68–1.57)	0.88
	TT (N=381)	40 (10.5)	Reference	-	Reference	-

[a]Values for BMD and rate of bone change are presented as mean±standard deviation. The association between fat mass and obesity-related transcript genotype and bone mineral density (BMD) and bone loss are presented as mean difference (95% confidence interval [CI]) derived from a multivariable linear regression (I. BMD) or mixed-effects regression (II. bone loss), adjusted for age and body mass index (BMI).
[b]Reference genotype: TT.
[c]The data is presented as N (%) and indicates the number of patients with a fracture.
[d]Adjusted for age, femoral neck BMD and BMI.
FN, femoral neck; LS, lumbar spine; HR, hazard ratio.

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Supplementary Table 7. Association between fat mass and obesity-related transcript gene variants and fracture risk

rs1421085	CC	CT	TT
Any fracture	1.00 (0.78–1.28)	1.03 (0.85–1.24)	-
Hip fracture	1.42 (0.90–2.24)	0.84 (0.57–1.25)	-
rs1558902	AA	AT	TT
Any fracture	1.02 (0.80–1.31)	1.06 (0.88–1.27)	-
Hip fracture	1.51 (0.96–2.36)	0.97 (0.67–1.42)	-
rs1121980	AA	AG	GG
Any fracture	0.99 (0.78–1.27)	1.05 (0.87–1.27)	-
Hip fracture	**1.74 (1.10–2.76)**	1.02 (0.67–1.54)	-
rs17817449	GG	GT	TT
Any fracture	0.99 (0.77–1.27)	1.06 (0.88–1.28)	-
Hip fracture	1.55 (0.97–2.50)	0.96 (0.64–1.44)	-
rs9939609	AA	AT	TT
Any fracture	1.00 (0.77–1.30)	1.12 (0.92–1.37)	-
Hip fracture	1.17 (0.70–1.97)	1.01 (0.66–1.54)	-
rs9930506	GG	GA	AA
Any fracture	1.04 (0.81–1.34)	1.12 (0.92–1.36)	-
Hip fracture	**1.90 (1.14–3.16)**	1.39 (0.89–2.15)	-

Values are hazard ratio derived from the Cox's proportional hazards model with adjustment for age, femoral neck bone mineral density, body mass index, and diabetes mellitus. $P<0.05$ is statically significant. Bold values indicate statistical significance.