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ORIGINAL ARTICLE

Association between fat-mass-and-obesity-associated (*FTO*) gene and hip fracture susceptibility

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Summary

Objective Common variants in the fat-mass-and-obesity-associated (*FTO*) gene are related to body mass index (BMI), which is a predictor of hip fracture risk. This study sought to examine the association between variants in the *FTO* gene and hip fracture risk.

Design and participants This is a prospective study including 934 postmenopausal women aged 60 years and above living in Dubbo, Australia (Dubbo Osteoporosis Epidemiology Study), followed up between 1989 and 2007.

Measurements Six single nucleotide polymorphisms (SNPs) (rs1421085, rs1558902, rs1121980, rs17817449, rs9939609 and rs9930506) of the *FTO* gene were genotyped using Taqman assay. Bone mineral density at the lumbar spine and femoral neck was measured by DXA (GE-Lunar) at baseline. Incidence of hip fractures during the follow-up was ascertained by reviewing X-ray reports. We used Cox's models to estimate the association between the genetic variants and hip fracture risk. We also utilized Bayes factor to evaluate the association.

Results One hundred and two women (11%) had sustained a hip fracture. The incidence of hip fracture was greater in women homozygous for the minor allele of all SNPs. Women homozygous for the minor allele (AA) of rs1121980 had significantly higher risk of hip fracture (hazard ratio, 2·06; 95% CI 1·17–3·62) than women homozygous for the major allele (TT). The observed data favoured the hypothesis of *FTO* gene and fracture association over the hypothesis of nonassociation by a factor of nine.

Conclusion Common variations in the *FTO* gene are associated with hip fracture risk in women and that *FTO* gene may help improve the predictive value of hip fracture risk.

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Introduction

Hip fracture is a major public health problem because it is a serious health problem in one's lifetime. Among women aged 50 years or older, the residual lifetime risk of hip fracture is approximately 10%,[1] which is equivalent to the risk of breast cancer. Hip fracture is associated with increased risks of subsequent fractures[2] and premature mortality[3] and incurs significant healthcare cost.[4] Moreover, up to 24% women will die within the first 3 months after experiencing a hip fracture.[5] Survivors after the fracture usually suffer from chronic pain, increased dependence and reduced quality of life.[6] Therefore, a major effort in osteoporosis research is to identify risk factors for hip fracture, so that predictive models can be developed for assessing the susceptibility to the fracture for each person.

Previous studies have identified a range of risk factors for hip fracture, including advancing age, fall, history of fracture, low bone mineral density (BMD), low body mass index (BMI) and genetic factors.[7,8] Evidence from twin and family studies suggested that up to 50% of the liability to hip fracture is attributable to genetic factors,[9] although the heritability appears to be greater in premenopausal women than in postmenopausal women. However, until now, it is not clear which genes are causally linked to hip fracture risk. Using candidate gene association approach, it was shown that variation in the collagen I alpha 1 gene (*COLIA1*)[10] and apolipoprotein E (*APOE*) gene was associated with hip fracture,[11] although the latter was not observed in the Rotterdam Study.[12] Genomewide association studies (GWAS) have identified 62 loci that are associated with BMD, and among the loci, only eight SNPs were associated with fracture risk at the genomewide significance level.[13] However, it is remained to be established whether the eight SNPs are associated with hip fracture risk.

Bone mineral density (BMD) and body mass index (BMI) are two independent risk factors for hip fracture. Each standard deviation decrease in BMD and BMI is associated with 2-fold and 1·9-fold increase in the risk of hip fracture, respectively.[14,15] Moreover, BMD and BMI are under strong genetic influence. Twin studies suggested that between 47% and 90% of variance in BMI is attributable to genetic factors.[16] Genetic factors could account for up to 70% of variance in BMD.[17,18] We have also

previously shown that fat mass and BMD have a shared genetic component.[17]

A recent GWAS studies have suggested that genetic variants in the fat-mass-and-obesity-associated gene (*FTO*) were associated with variation in BMI.[19,20] Given the evidence of pleiotropic effect,[17] we hypothesized that variants in the *FTO* gene are associated with BMD and hip fracture risk. The present study sought to test the hypothesis by examining the association between common variants in the *FTO* gene and risk of hip fracture.

Materials and methods

Study design

The study was based on the on-going Dubbo Osteoporosis Epidemiology Study (DOES), which study design and protocol have been described elsewhere.[21] Briefly, the study was designed as a population-based longitudinal study, in which people aged 60 + (as at 1989) living in Dubbo were invited to participate in the investigation. Dubbo is a city located approximately 400 km north-west of Sydney, with a population of 32 000 people. The city was selected as a setting for the study because it has a stable and representative population. Moreover, the city population is served by two hospitals which we have access to fracture data, and this would allow the total ascertainment of fracture incidence in the city. The study's protocol and procedures were approved by the St Vincent's Hospital Ethics Committee, and informed consent was obtained from each participant at the study entry.

Participants were invited to visit the local clinic and interviewed by a nurse co-ordinator. Each participant had multiple visits which had taken place for every 2 years. The present analysis excluded individuals with the following symptoms or diseases: Paget's disease or hyperparathyroidism, Cushing's syndrome, complete or partial gastrectomy, chronic malabsorptive syndromes and jejuno-ileal bypass, chronic renal disease, chronic liver disease or prolonged immobilization. Furthermore, exclusion was also applied to individuals who had recovered from strokes or other serious illnesses such as metastatic malignancies, or were currently on medications deemed to affect bone metabolism, such as corticosteroids (in excess of 7·5 mg prednisolone/day or 1600 μg/day inhaled beclomethasone or equivalents), T_4, anticonvulsants or bone-active agents such as calcitonins, bisphosphonates, fluorides and calcitriol, in the past 5 year for greater than 6 months.

Measurements

At baseline (1989), each participant was administered a standardized questionnaire to solicit information on general health (history of diseases, use of medications and surgical treatment), anthropometric data, lifestyle factors and fracture history. Height (cm) was measured by a wall-mounted stadiometer without shoes to the nearest 0·1 cm. Weight (kg) was measured with light clothing and without shoes by an electronic scale to the nearest 0·1 kg. Body mass index (BMI, kg/m^2) was calculated as the ratio of weight (kg) and squared height (m^2).

Bone mineral density (BMD, g/cm^2) at the femoral neck and lumbar spine was measured by dual energy X-ray absorptiometry using a LUNAR DPX densitometer (GE-LUNAR Corp, Madison, WI, USA). The coefficient of variation of this measurement in our institution in normal subjects for BMD is 1·5% for the lumbar spine and 1·3% for the femoral neck.[22] The radiation dose of this method is <0·1 μGγ. Quality control was performed regularly using phantom to ensure the reliability of the densitometer. In this study, femoral neck BMD was used to assess the relationship between genetic variants and fracture, because it is less susceptible to age-related degenerative changes than the lumbar spine.

Ascertainment of hip fracture

Hip fractures occurring during the follow-up period (1989–2007) were identified by reviewing radiologist' reports from the only two centres providing X-ray services for the entire Dubbo population. Circumstances surrounding the fracture were then obtained from participants on the interview that was close to the fracture event. Fractures were only included in the analysis if the report of fracture was definite on the interview and had occurred with minimal trauma (i.e. with a force equivalent to a fall from standing height or less). Fractures due to major trauma such as motor vehicle accident or pathological fractures were excluded from the analysis.

Genotyping

Blood samples were collected and stored at −80 °C. DNA was extracted from leucocytes using either QIAamp® DNA Mini Blood Kit (Qiagen Pty Ltd., Valencia, CA, USA) or phenol/chloroform. Six single nucleotide polymorphisms (SNPs: rs1421085, rs1558902, rs1121980, rs17817449, rs9939609 and rs9930506) in the first intron of *FTO* gene were genotyped using a predesign of Taqman SNP Genotyping Assay (Applied Biosystem, Foster City, CA, USA). The allelic discrimination was determined by ABI 7900 Sequence Detection System and SDS software (example is shown in Fig. S1). The SNPs were selected because they have been shown to be significantly associated with BMI variation in previous GWAS.[19,20,23,24] Successful call rate of the genotyping was greater than 90% of the available DNA samples. To validate the accuracy of genotyping, 10% of samples were randomly selected, and the accuracy was 100%.

Data analysis

The distribution of genotypes for all studied SNPs was tested for the deviation from the Hardy–Weinberg equilibrium (HWE) law by the likelihood chi-squared test. The association between each SNP and hip fracture risk was analysed by the Cox's proportional hazards model, with time to the fracture being the outcome. Because hip fracture is known to be associated with age, BMI, BMD and a history of fracture, these factors were treated as covariates in the Cox's regression analysis. Based on the Cox's regression analysis results, we

calculated hazard ratio (HR) and 95% confidence interval (CI) for each genotype. The appropriateness of the proportionality assumption was tested using the scaled Schoenfeld residuals and plots of expected survival curves[25] which tests for each variable in the Cox's model. We estimated the *FTO* gene-associated population attributable risk fraction (PAR) as a function of the prevalence of a risk genotype and its relative risk of hip fracture.

As a secondary analysis, we also examined the association between each SNP and BMI and BMD using the analysis of variance (ANOVA), with age being the covariate. We did not adjust for multiple comparisons in the analyses, because all SNPs were in tight linkage disequilibrium (i.e. nonindependence) and our primary purpose was to estimate effects size rather than *P*-value. All statistical analyses were performed using the Statistical Analysis System (SAS), version 9.1 (SAS Institute, Inc, Cary, NC, USA).

Evaluation of evidence

The strength of evidence was considered next. Because *P*-value is not a good metric of evidence, the Bayes factor was used as a metric for evaluating the evidence of association. Two competing hypotheses were considered concerning the association between *FTO* genetic variants and hip fracture risk: the null hypothesis of no effect (denoted by H_0), and the alternative hypothesis that there is an effect (H_1). Denoting the observed data (i.e. relative risk and 95% confidence interval) as D, the Bayes factor (BF) is operationally defined as the ratio of the probability of obtaining the observed data D given that H_1 is true over the probability of obtaining the observed data D under H_0: $BF = \frac{P(D|H_1)}{P(D|H_0)}$. Thus, BF provides an objective measure of evidence for a hypothesis. Thus, if $BF > 1$, the weight of evidence for association is greater than that for no association; if $BF < 1$, the weight of evidence for no association is greater than that for an association. A BF of between 3 and 10 considered 'substantial' evidence, whereas a $BF > 10$ is 'strong evidence'.[26,27]

In clinical research, any finding is uncertain because it can be a false discovery. We assessed this possibility by computing the Bayesian false discovery probability (BFDP) as described by Wakefield.[27] Briefly, BFDP is a function of three parameters: (i) prior probability of association, (ii) the cost of a false discovery relative to the cost of true discovery and (iii) Bayes factor. In this analysis, we set the prior probability of association to range from 0·05 to 0·80, and the upper hazards ratio of three. The cost of false discovery to true discovery was set at three. Using the observed confidence interval from the Cox's proportional hazards model, we determined the BFDP for each of the prior probability. A finding with BFDP < 0·2 is commonly considered 'noteworthy' finding.[28]

Results

Characteristics of participants

The study included 934 postmenopausal women, whose baseline demographic characteristics are shown in Table 1. All participants were Caucasians**.** The average age of participants was 69 years, with range being from 57 to 96 years. The participants had been followed for a median of 9 years (interquartile range 4–17 years). During the follow-up period, 102 individuals (11%) had sustained at least one hip fracture. Women with hip fracture were significantly older and had lower weight, lower BMI and lower BMD at both skeletal sites than those without hip fracture. Women with hip fracture also had shorter stature and lower levels of physical activity than those without fracture ($P < 0·001$ for each variable). However, no significant differences in levels of alcohol consumption and smoking status were found between hip fracture and fracture-free individuals.

Approximately 19% of women ($n = 246$) were classified as 'obese' (BMI ≥ 30 kg/m^2), and another 40% ($n = 512$) was 'overweight' (BMI between 25 and 29 kg/m^2). Greater BMI was associated with lower risk of hip fracture. The incidence rate of fracture among obese women was 3·2% as compared to 16% among women with BMI lower than 24 kg/m^2 ($P < 0·0001$).

About 23% of women ($n = 291$) had osteoporosis (i.e., BMD T-scores $\leq -2·5$). Almost two-thirds of hip fracture occurred in women with osteoporosis. The incidence of hip fracture among osteoporotic women was approximately 23%, 6·8-fold greater than the risk among nonosteoporotic women (3·3%).

FTO *gene and hip fracture risk*

For all SNPs, the distribution of genotypes was consistent with the Hardy–Weinberg equilibrium law. The relative frequency of the minor homozygous genotypes ranges from 17% to 19%. Linkage disequilibrium (LD) analysis showed that the six SNPs were physically correlated, with the coefficient of pairwise LD (r^2) ranging from 0·84 to 0·97 (Table 2).

The distribution of hip fracture and nonfracture cases stratified by genotype for all SNPs is presented in Table 3. Women who were homozygous for the minor allele of each SNP had a greater risk of hip fracture (as reflected by the incidence rate) than those homozygous for the major allele (Fig. 1). For

Table 1. Baseline characteristics of participants classified by fracture status

Characteristics	No fracture ($n = 832$)	Hip fracture ($n = 102$)
Age (years)	68·2 (6·3)	75·7 (7·5)**
Weight (kg)	68·3 (12·8)	58·3 (11·2)**
Height (cm)	160·5 (6·0)	157·4 (7·4)**
BMI (kg/m^2)	26·5 (4·9)	23·4 (3·9)**
FNBMD (g/cm^2)	0·83 (0·13)	0·66 (0·11)**
LSBMD (g/cm^2)	1·09 (0·19)	0·94 (0·20)**
Physical activity, METs	30·9 (2·9)	29·7 (3·5)**
Dietary Ca intake, mg/day	715 (400)	659 (357)
Alcohol users, No. (%)	372 (44·7)	33 (32·4)
Smoking, No. (%)	254 (30·5)	29 (28·4)

Values are mean (SD), or otherwise stated. FNBMD, femoral neck bone mineral density; LSBMD, lumbar spine bone mineral density; METs, metabolic equivalent tasks. **$P < 0·001$.

instance, after adjusting for covariates, the hazard ratio (HR) of hip fracture among women homozygous for the minor allele (AA) for rs1421085 was 1·82 (95% CI 1·04–3·20) relative to those with TT genotype. A similar effect size was also observed for rs1558902, rs1121980, rs17817449 and rs9930506, but not for rs9939609. Schoenfeld residual test for each SNP showed no significant deviation from the zero slope (data not shown). Approximately 17% of hip fractures were attributable to the SNP rs1121980. None of the SNPs was significantly associated with BMI or BMD (Table 4).

Assessment of false discovery

We next evaluated the strength of evidence for association via Bayes factor and Bayesian false discovery probability (Table 5). Bayes factor analysis showed that the weight of data supported the hypothesis of association was highest for rs1121980, followed by rs1421085 and rs9930506. For example, for the SNP rs1121980, the data favoured the hypothesis of association (relative to the hypothesis of no association) by a factor of 9·4. As expected, the Bayesian false discovery probability was highly dependent on the prior of probability of association. If the prior probability of association is believed to be 0·5, then the associations between rs1121980 and rs1421085 and fracture risk are deemed to be 'noteworthy'. However, if the prior probability is believed to be 0·1 or 0·05, then none of the associations are considered noteworthy.

Discussion

Hip fracture is a multifactorial event, in the sense that its susceptibility is determined by genetic and nongenetic factors. Several nongenetic factors, including fall, low BMD and low BMI, have been identified as major risk factors for fracture. These nongenetic factors explained a modest proportion of hip fracture cases in the general population, and a large number of unexplained cases are assumed due to genetic factors. However, the identification of specific genes that are possibly related to hip fracture is much more difficult, because in part there is no obvious Mendelian pattern of inheritance in hip fracture. In this study, using a candidate gene approach, we have demonstrated that polymorphic variation at the *FTO* gene was associated with hip fracture risk and that the association was independent of established risk factors such as BMD and BMI.

Table 3. Association between *FTO* gene variants and hip fracture risk

		Fracture status and HR		
SNP	Genotype	No fracture	Hip fracture*	HR (95% CI)†
rs1421085	TT	262	29 (10·0)	1·00
	TC	363	43 (10·6)	1·00 (0·61–1·64)
	CC	129	24 (15·7)	**1·82 (1·04–3·20)**
	P-value (trend)			0·06
rs1558902	TT	281	31 (9·9)	1·00
	TA	396	46 (10·4)	1·21 (0·75–1·94)
	AA	124	23 (15·7)	**2·05 (1·16–3·60)**
	P-value (trend)			**0·02**
rs1121980	GG	253	25 (9·0)	1·00
	GA	355	43 (10·8)	1·16 (0·70–1·92)
	AA	136	26 (16·1)	**2·06 (1·17–3·62)**
	P-value (trend)			**0·02**
rs17817449	TT	286	27 (8·6)	1·00
	TG	381	43 (10·1)	1·31 (0·80–2·16)
	GG	123	21 (14·6)	**2·17 (1·20–3·96)**
	P-value (trend)			**0·01**
rs9939609	TT	260	27 (9·4)	1·00
	TA	321	37 (10·3)	1·37 (0·82–2·30)
	AA	118	15 (11·3)	1·45 (0·74–2·82)
	P-value (trend)			0·21
rs9930506	AA	241	22 (8·4)	1·00
	AG	364	47 (11·4)	1·31 (0·79–2·20)
	GG	134	23 (14·7)	**2·19 (1·20–4·01)**
	P-value (trend)			0·01

*Values are actual number of fracture and incidence rate (%, in bracket). †Values are hazard ratio derived from the Cox's proportional hazards model with adjustment for age (+1 year), femoral neck BMD (+1 SD), BMI (+5 kg/m^2) and prior fracture (yes/no). Bold values indicate *P* values less than 0.05.

This finding, to the best of our knowledge, is the first demonstration of association between the *FTO* gene and hip fracture risk. The magnitude of association observed in this study is comparable with the association between hip fracture risk and polymorphisms of the apolipoprotein E gene[11] and the *COLIA1* gene.[10] Given the fact that approximately 19% of women are carriers of the risk genotype (AA of the SNP rs1121980), it can be estimated that approximately 17% of hip fracture cases are attributable to this variant in the *FTO* gene. More importantly, the effect of the *FTO* gene on hip fracture

Table 2. Pairwise linkage disequilibrium among single nucleotide polymorphisms (SNPs) in the *FTO* gene

	rs1558902	rs1121980	rs17817449	rs9939609	rs9930506
rs1421085	0·98 (0·96)	0·96 (0·93)	0·97 (0·94)	0·85 (0·83)	0·90 (0·88)
rs1558902		1·00 (0·97)	0·99 (0·97)	0·87 (0·86)	0·94 (0·91)
rs1121980			0·99 (0·95)	0·88 (0·84)	0·94 (0·94)
rs17817449				0·89 (0·89)	0·98 (0·93)
rs9939609					0·88 (0·84)

Values are pairwise linkage disequilibrium coefficient for six SNPs in the *FTO* gene, expressed as D' (r^2).

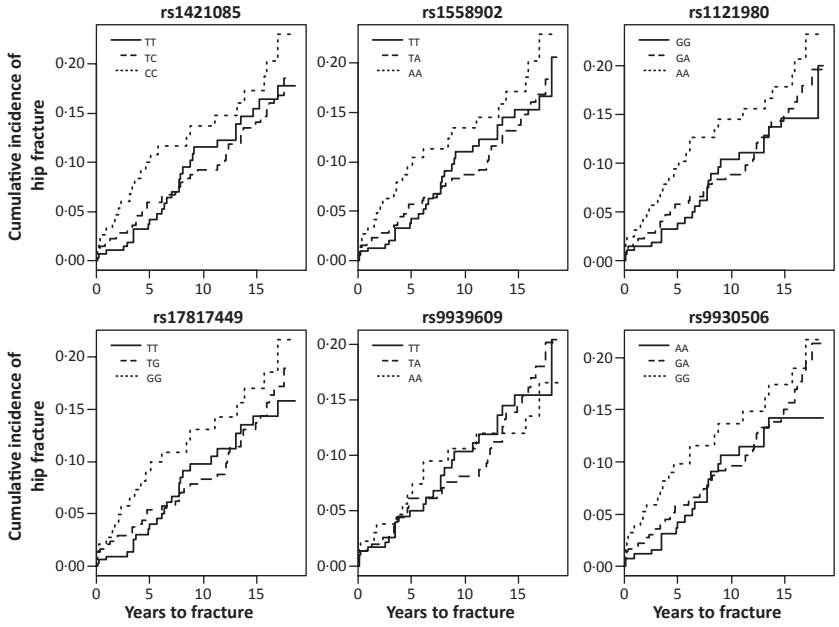

Fig. 1 Cumulative probability of hip fracture stratified by genotype of the *FTO* gene.

Table 4. Association between *FTO* gene variants and BMI and BMD at femoral neck

		Mean (SD)	
SNP	Genotype(%)	BMI	FNBMD
rs1421085	TT (34·2)	26·5 (5·1)	0·80 (0·13)
	TC (48·1)	25·9 (4·7)	0·80 (0·14)
	CC (17·7)	25·8 (4·6)	0·80 (0·14)
	P-value	0·10	0·61
rs1558902	TT (34·5)	26·2 (4·7)	0·79 (0·13)
	TA (49·0)	25·9 (4·7)	0·81 (0·13)
	AA (16·5)	25·6 (4·4)	0·78 (0·13)
	P-value	0·31	0·08
rs1121980	GG (33·3)	26·4 (5·0)	0·79 (0·13)
	GA (47·7)	25·9 (4·8)	0·81 (0·14)
	AA (19·0)	25·7 (4·6)	0·79 (0·14)
	P-value	0·14	0·58
rs17817449	TT (35·3)	26·4 (4·9)	0·79 (0·13)
	TG (48·3)	26·0 (4·8)	0·81 (0·14)
	GG (16·4)	25·8 (4·5)	0·79 (0·13)
	P-value	0·27	0·13
rs9939609	TT (36·0)	26·4 (5·0)	0·80 (0·13)
	TA (46·3)	26·0 (4·7)	0·81 (0·13)
	AA (17·6)	25·5 (4·4)	0·79 (0·13)
	P-value	0·05	0·12
rs9930506	AA (31·7)	26·3 (5·0)	0·79 (0·13)
	AG (49·5)	25·8 (4·8)	0·81 (0·14)
	GG (18·8)	25·7 (4·4)	0·80 (0·14)
	P-value	0·20	0·24

FNBMD, femoral neck bone mineral density.

risk was independent of nongenetic factors, suggesting that genotypes of the gene can contribute to the improvement in hip fracture risk prediction.

Table 5. Approximate Bayes factor and Bayesian false discovery probability for the association between *FTO* genetic variants and hip fracture risk

		Prior probability of association					
SNP	Approximate Bayes Factor	0·8	0·7	0·6	0·5	0·1	0·05
rs1421085	3·91	0·060	0·100	0·146	0·204	0·698	0·830
rs1558902	1·03	0·047	0·078	0·116	0·364	0·639	0·789
rs1121980	9·43	0·026	0·043	0·066	0·096	0·488	0·668
rs17817449	1·95	0·114	0·181	0·255	0·340	0·822	0·907
rs9939609	0·65	0·279	0·399	0·508	0·607	0·933	0·967
rs9930506	3·85	0·061	0·101	0·148	0·207	0·701	0·832

The analysis was based on the prior probability from 0·05 to 0·80, and the cost of false discovery was three times more costly than that of true discovery.

The assessment of fracture risk has been gradually moving from a univariate and group-based approach to a multivariate and individualized approach. In the latter, a personal risk of fracture is estimated from his/her risk profile with multiple risk factors, including genes. However, existing fracture prediction algorithms, including FRAX[29] and the Garvan Fracture Risk Calculator,[30] do not use any genetic variant in their estimation of fracture risk. This is true because until now it is not clear which genes are causally related to hip fracture risk. Recent genomewide association studies (GWAS) have identified 56 loci that were associated with BMD, and 13 of these loci were associated with fracture[31] at the genomewide significance level. However, there is no evidence that any of the loci is associated with hip fracture. The present study, together with previous studies,[10,11,32] has so far identified five possible genes, namely the *COLIA1* gene, Apolipoprotein E gene, oestrogen receptor, vitamin D receptor and the *FTO* gene that are independently associated with hip fracture. Their independent

effects suggest that these genetic variants can help improve the accuracy of prediction over and above that of clinical risk factors alone. Indeed, we have shown that a profile of 50 genetic variants, each with odds ratio ranging from 1·02 to 1·15, could improve the accuracy of fracture prediction beyond that obtained by existing clinical risk factors.[33] Thus, genetic profiling when integrated in existing risk assessment models could inform a more accurate prediction of fracture risk in an individual.[13]

The biological mechanism of the association between the *FTO* gene and hip fracture risk is unknown. In human, expression of the *FTO* gene was found in various tissues such as adipose tissue and beta cells,[34] but no evidence for the expression in bone. In animal model, *FTO*-knockout mice showed a significant reduction in adipose tissue and body lean mass,[35] and reduced lean mass is associated with weaken femur bone strength[36] predisposing to increased risk of hip fracture. The *FTO* gene could influence fracture risk via its effect on structural traits that are not measured by BMI or BMI, but this remains a speculation because there is so far no evidence for such a modulated association. The present finding was based on an association analysis, which does not necessarily mean that the *FTO* gene is causally linked to the susceptibility to fracture. However, it is likely that polymorphisms in this gene influence risk factors associated with hip fracture liability such as muscle strength.[37] Moreover, *FTO* RNA expression is dependent on age and sex and could influence metabolisms of fat and glucose.[38] The age-associated decline in *FTO* RNA expression could play role in the determination of muscle tissues and hence influences bone strength.

Could the observed association between *FTO* variants and hip fracture be a false finding? While as a norm it is impossible to completely rule out the chance of a false finding (because it is dependent on the prior probability of association which is subjective and cannot be reliably quantified), it is possible to have an objective assessment of the data for competing hypotheses. We have demonstrated that the weight of evidence from the data favour the hypothesis of association over the hypothesis of no association by a factor of nine. Thus, although an independent validation is necessary, it seems clear that the observed association is trustworthy.

It is interesting to note that we did not find any significant association between any *FTO* polymorphism and BMI. This lack of association was also noted in previous studies.[39] In fact, if any, our finding was opposite to what was initially reported.[19] In our study, we found there was no association between rs9939609 and BMI or body weight. It is uncertain why there was such a stark discrepancy between the present data and previous studies. However, it should be noted that our study population consisted of postmenopausal women, whereas previous studies were mainly based on younger populations.[19,40] Thus, age could be a factor that modulates the relationship between *FTO* gene and BMI, as a meta-analysis in Scandinavian populations showed that the effect of *FTO* gene on BMI is likely to occur before adulthood.[41]

The present finding must be interpreted within the context of potential strengths and limitations. The study sample size was reasonably large with long duration of follow-up, which ensures the reliability of association otherwise not possible in studies with small sample sizes. The incidence of hip fracture was ascertained by X-ray reports which ensure the accuracy of data. However, the present finding was based on an association analysis, which does not necessarily show that the *FTO* gene is causally involved in the susceptibility to hip fracture. Any genetic association can be biased by unmeasured confounders that cannot be addressed by any single study. Therefore, it is important that the findings from this study are replicated in independent cohorts, before the gene can be used for assessing the risk of hip fracture in an individual. We note that a previous study found a significant association between *FTO* polymorphisms and BMD in a Chinese population, but the association was not present in Caucasian population.[42] The data were based on a sample of Caucasian women, whose lifestyles and environmental living conditions are relatively homogeneous; thus, the results may not apply to other populations or to men. The number of hip fractures was low which could result in a rather moderate uncertainty of the estimate of association between the gene and hip fracture risk. Moreover, there are likely many other yet-to-identify genes that are associated with hip fracture risk but were not considered in the present study.

In conclusion, the present study has shown that genetic variation within the *FTO* gene is associated with hip fracture risk. The data also showed that approximately 17% of the variability in hip fracture risk is attributable to a polymorphism in the *FTO* gene. Given the independent effect of the *FTO* gene polymorphism on hip fracture risk, it is possible that an integration of this genetic information into existing predictive models could enhance its accuracy and predictive value for an individual. The association between the *FTO* gene and hip fracture risk generates new opportunities for delineating the mechanism of association.

Disclosure summary

All authors have no conflicts of interest to disclose.

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References

1 Nguyen, N.D., Ahlborg, H.G., Center, J.R. *et al.* (2007) Residual lifetime risk of fractures in women and men. *Journal of Bone and Mineral Research*, **22**, 781–788.

2 Center, J.R., Bliuc, D., Nguyen, T.V. *et al.* (2007) Risk of subsequent fracture after low-trauma fracture in men and women. *JAMA*, **297**, 387–394.

3 Center, J.R., Nguyen, T.V., Schneider, D. *et al.* (1999) Mortality after all major types of osteoporotic fracture in men and women: an observational study. *Lancet*, **353**, 878–882.

4 Randell, A., Sambrook, P.N., Nguyen, T.V. *et al.* (1995) Direct clinical and welfare costs of osteoporotic fractures in elderly men and women. *Osteoporosis International*, **5**, 427–432.

5 Hindmarsh, D.M., Hayen, A., Finch, C.F. *et al.* (2009) Relative survival after hospitalisation for hip fracture in older people in New South Wales, Australia. *Osteoporosis International*, **20**, 221–229.

6 Randell, A.G., Nguyen, T.V., Bhalerao, N. *et al.* (2000) Deterioration in quality of life following hip fracture: a prospective study. *Osteoporosis International*, **11**, 460–466.

7 Lauritzen, J.B. (1996) Hip fractures: incidence, risk factors, energy absorption, and prevention. *Bone*, **18**, 65S–75S.

8 Ralston, S.H. (2002) Genetic control of susceptibility to osteoporosis. *Journal of Clinical Endocrinology and Metabolism*, **87**, 2460–2466.

9 Michaelsson, K., Melhus, H., Ferm, H. *et al.* (2005) Genetic liability to fractures in the elderly. *Archives of Internal Medicine*, **165**, 1825–1830.

10 Nguyen, T.V., Esteban, L.M., White, C.P. *et al.* (2005) Contribution of the collagen I alpha1 and vitamin D receptor genes to the risk of hip fracture in elderly women. *Journal of Clinical Endocrinology and Metabolism*, **90**, 6575–6579.

11 Cauley, J.A., Zmuda, J.M., Yaffe, K. *et al.* (1999) Apolipoprotein E polymorphism: a new genetic marker of hip fracture risk–The Study of Osteoporotic Fractures. *Journal of Bone and Mineral Research*, **14**, 1175–1181.

12 Schoofs, M.W., van der Klift, M., Hofman, A. *et al.* (2004) ApoE gene polymorphisms, BMD, and fracture risk in elderly men and women: the Rotterdam study. *Journal of Bone and Mineral Research*, **19**, 1490–1496.

13 Nguyen, T.V. & Eisman, J.A. (2013) Genetic profiling and individualized assessment of fracture risk. *Nature reviews. Endocrinology*, **9**, 153–161.

14 De Laet, C., Kanis, J.A., Oden, A. *et al.* (2005) Body mass index as a predictor of fracture risk: a meta-analysis. *Osteoporosis International*, **16**, 1330–1338.

15 Cummings, S.R., Black, D.M., Nevitt, M.C. *et al.* (1993) Bone density at various sites for prediction of hip fractures. The Study of Osteoporotic Fractures Research Group. *Lancet*, **341**, 72–75.

16 Elks, C.E., den Hoed, M., Zhao, J.H. *et al.* (2012) Variability in the heritability of body mass index: a systematic review and meta-regression. *Frontiers in Endocrinology*, **3**, 29.

17 Nguyen, T.V., Howard, G.M., Kelly, P.J. *et al.* (1998) Bone mass, lean mass, and fat mass: same genes or same environments? *American Journal of Epidemiology*, **147**, 3–16.

18 Pocock, N.A., Eisman, J.A., Hopper, J.L. *et al.* (1987) Genetic determinants of bone mass in adults. A twin study. *The Journal of Clinical Investigation*, **80**, 706–710.

19 Frayling, T.M., Timpson, N.J., Weedon, M.N. *et al.* (2007) A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science*, **316**, 889–894.

20 Scuteri, A., Sanna, S., Chen, W.M. *et al.* (2007) Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. *PLoS Genetics*, **3**, e115.

21 Simons, L.A., McCallum, J., Simons, J. *et al.* (1990) The Dubbo study: an Australian prospective community study of the health of elderly. *Australian and New Zealand Journal of Medicine*, **20**, 783–789.

22 Nguyen, T.V., Sambrook, P.N. & Eisman, J.A. (1997) Sources of variability in bone mineral density measurements: implications for study design and analysis of bone loss. *Journal of Bone and Mineral Research*, **12**, 124–135.

23 Dina, C., Meyre, D., Gallina, S. *et al.* (2007) Variation in FTO contributes to childhood obesity and severe adult obesity. *Nature Genetics*, **39**, 724–726.

24 Thorleifsson, G., Walters, G.B., Gudbjartsson, D.F. *et al.* (2009) Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. *Nature Genetics*, **41**, 18–24.

25 Grambsch, P.M. & Therneau, T.M. (1994) Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika*, **81**, 515–526.

26 Wacholder, S., Chanock, S., Garcia-Closas, M. *et al.* (2004) Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. *Journal of the National Cancer Institute*, **96**, 434–442.

27 Wakefield, J. (2007) A Bayesian measure of the probability of false discovery in genetic epidemiology studies. *American Journal of Human Genetics*, **81**, 208–227.

28 Goodman, S.N. (1999) Toward evidence-based medical statistics. 2: the Bayes factor. *Annals of Internal Medicine*, **130**, 1005–1013.

29 Kanis, J.A., Johnell, O., Oden, A. *et al.* (2008) FRAX and the assessment of fracture probability in men and women from the UK. *Osteoporosis International*, **19**, 385–397.

30 Nguyen, N.D., Frost, S.A., Center, J.R. *et al.* (2007) Development of a nomogram for individualizing hip fracture risk in men and women. *Osteoporosis International*, **18**, 1109–1117.

31 Estrada, K., Styrkarsdottir, U., Evangelou, E. *et al.* (2012) Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture. *Nature Genetics*, **44**, 491–501.

32 Aerssens, J., Dequeker, J., Peeters, J. *et al.* (2000) Polymorphisms of the VDR, ER and COLIA1 genes and osteoporotic hip fracture in elderly postmenopausal women. *Osteoporosis International*, **11**, 583–591.

33 Tran, B.N., Nguyen, N.D., Nguyen, V.X. *et al.* (2011) Genetic profiling and individualized prognosis of fracture. *Journal of Bone and Mineral Research*, **26**, 414–419.

34 Kloting, N., Schleinitz, D., Ruschke, K. *et al.* (2008) Inverse relationship between obesity and FTO gene expression in visceral adipose tissue in humans. *Diabetologia*, **51**, 641–647.

35 Fischer, J., Koch, L., Emmerling, C. *et al.* (2009) Inactivation of the Fto gene protects from obesity. *Nature*, **458**, 894–898.

36 Travison, T.G., Araujo, A.B., Esche, G.R. *et al.* (2008) Lean mass and not fat mass is associated with male proximal femur strength. *Journal of Bone and Mineral Research*, **23**, 189–198.

37 Bravard, A., Lefai, E., Meugnier, E. *et al.* (2011) FTO is increased in muscle during type 2 diabetes, and its overexpression in myotubes alters insulin signaling, enhances lipogenesis and ROS production, and induces mitochondrial dysfunction. *Diabetes*, **60**, 258–268.

38 Grunnet, L.G., Nilsson, E., Ling, C. *et al.* (2009) Regulation and function of FTO mRNA expression in human skeletal muscle and subcutaneous adipose tissue. *Diabetes*, **58**, 2402–2408.

39 Dlouha, D., Suchanek, P., Lanska, V. *et al.* (2011) Body mass index change in females after short-time life style intervention is not dependent on the FTO polymorphisms. *Physiological Research*, **60**, 199–202.

40 Rutters, F., Nieuwenhuizen, A.G., Bouwman, F. *et al.* (2011) Associations between a single nucleotide polymorphism of the FTO Gene (rs9939609) and obesity-related characteristics over

time during puberty in a Dutch children cohort. *Journal of Clinical Endocrinology and Metabolism*, **96**, E939–E942.

41 Hertel, J.K., Johansson, S., Sonestedt, E. *et al.* (2011) FTO, type 2 diabetes, and weight gain throughout adult life: a meta-analysis of 41,504 subjects from the Scandinavian HUNT, MDC, and MPP studies. *Diabetes*, **60**, 1637–1644.

42 Guo, Y., Liu, H., Yang, T.L. *et al.* (2011) The fat mass and obesity associated gene, FTO, is also associated with osteoporosis phenotypes. *PLoS ONE*, **6**, e27312.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Example of Taqman SNP Genotyping results of major homozygote (in blue), minor homozygote (in red) and heteozygote (in green) of rs17817449.