The Fat Mass and Obesity Associated Gene, FTO, Is Also Associated with Osteoporosis Phenotypes

Yan Guo1, Hui Liu2, Tie-Lin Yang1*, Siyang M. Li3, Siyuan K. Li3, Qing Tian4, Yong-Jun Liu4, Hong-Wen Deng4,5*

1 Key Laboratory of Biomedical Information Engineering of Ministry of Education, and Institute of Molecular Genetics, School of Life Science and Technology, Xi’an Jiaotong University, Xi’an, People’s Republic of China, 2 Department of Articular Surgery, Xi’an Red Cross Hospital, Xi’an, People’s Republic of China, 3 School of Medicine, University of Missouri - Kansas City, Kansas City, Missouri, United States of America, 4 School of Public Health and Tropical Medicine, Tulane University, New Orleans, Louisiana, United States of America, 5 Center of Systematic Biomedical Research, University of Shanghai for Science and Technology, Shanghai, People’s Republic of China

Abstract

Obesity and osteoporosis are closely correlated genetically. FTO gene has been consistently identified to be associated with obesity phenotypes. A recent study reported that the mice lacking Fto could result in lower bone mineral density (BMD). Thus, we hypothesize that the FTO gene might be also important for osteoporosis phenotypes. To test for such a hypothesis, we performed an association analyses to investigate the relationship between SNPs in FTO and BMD at both hip and spine in two independent Chinese populations (818 and 809 unrelated Han subjects, respectively) and a Caucasian population (2,286 unrelated subjects). Combining the two Chinese samples, we identified 6 SNPs in FTO to be significantly associated with hip BMD after multiple testing adjustments, with the combined P values ranging from 10^{-4} to 10^{-10}. Each copy of the minor allele of each SNP was associated with increased hBMD values with the effect size (beta) of -0.025 and -0.015 in the Chinese sample 1 and 2, respectively. However, none of these 6 SNPs showed significant association signal in the Caucasian sample, by presenting some extent of ethnic difference. Our findings, together with the prior biological evidence, suggest that the FTO gene might be a new candidate for BMD variation and osteoporosis in Chinese populations.

Introduction

Osteoporosis is a major public health problem with growing prevalence. Obesity, another common disease, has been demonstrated to be closely related with osteoporosis [1,2,3]. Adipocytes and osteoblasts arise from the same progenitor, bone marrow stromal cells, and can transdifferentiate into each other [4]. With aging, the composition of bone marrow shifts to favor the presence of adipocytes, osteoclast activity increases, and osteoblast function declines, leading to osteoporosis. Moreover, both osteoporosis and obesity have high genetic predisposition and the genetic correlation between them have been established across different ethnic groups [1,3,5]. Previous candidate gene and bivariate association studies have suggested some common genes both for obesity and osteoporosis, such as RANK [6], SP7 [7], and SOX6 [8].

Recently, several independent large-scale genome-wide association studies (GWAS) consistently identified a gene FTO (fat mass and obesity associated) to be associated with obesity-related traits and obesity risk [9,10,11]. The association has been further replicated in multiple studies in different populations [12,13,14,15], resulting in much increasing attention on this gene. The FTO protein contains a double-stranded beta-helix fold homologous to those of Fe[II] and 2-oxoglutarate-dependent oxygenases, which might be involved in DNA demethylase [16]. Experimental animal studies provide direct functional evidence that FTO is a causal gene underlying obesity [17,18]. Interestingly, a recent study found that the whole body Fto knockout mice displayed immediate postnatal growth retardation with shorter body length, lower body weight, and lower bone mineral density (BMD) than control mice [19]. This study reminded us that FTO might be a common genetic factor influencing not only obesity phenotypes, but also osteoporosis phenotypes, like BMD. To test for such hypothesis, we performed an association analyses to examine the relationship between the FTO gene and BMD. Our study was performed in three sample sets from two ethnicities, including two Chinese Han populations and a Caucasian population, in order to see whether the variants identified are common or ethnicity-specific.

Results

The basic characteristics of the study subjects are presented in Table 1. The major association results for SNPs in FTO with hip
BMD are summarized in Table 2. Combining results from the two Chinese samples, 6 SNPs were identified to be significantly associated with hip BMD after multiple testing adjustments by FDR (Table 2). The most significant SNP was rs16952955, with the \( P \) values of \( 8.39 \times 10^{-4} \) and \( 4.31 \times 10^{-2} \) in the Chinese sample 1 and Chinese sample 2, respectively. After meta-analysis, the combined \( P \) value achieved a significant level of \( 1.47 \times 10^{-4} \). Besides rs16952955, there were 5 additional SNPs (rs2540766, rs2689247, rs17226942, and rs16952951) showed significant association signals with hip BMD, with the combined \( P \) values ranged from \( 4.99 \times 10^{-4} \) to \( 2.95 \times 10^{-4} \). These 6 SNPs have a consistently protective effect on BMD, since each copy of the minor allele of each SNP was associated with increased hip BMD values with the effect size (beta) of \( -0.025 \) to \( -0.015 \) in the Chinese sample 1 and 2, respectively. All of these 6 SNPs are located at the intron 8 of \( FTO \), which might have a potential role on BMD or osteoporosis.

For the spine BMD, 5 of above 6 SNPs achieved significant level \( \left( P \right) \geq 7.21 \times 10^{-3} \) across ethnicities. Our study identified 4 SNPs which were also located at the intron 8 of \( FTO \) to be nominally associated with spine BMD. For the Caucasian sample, we found another 4 SNPs which are located at the intron 8 of \( FTO \) to be nominally associated with spine BMD (rs11076017: \( P = 5.23 \times 10^{-3} \); rs1420318: \( P = 6.14 \times 10^{-4} \); rs1676942: \( P = 7.21 \times 10^{-2} \); and rs17226942: \( P = 9.23 \times 10^{-5} \)). However, when considering the Chinese samples 1 and 2 together, no SNPs remained significant after FDR adjustments. For the Caucasian sample, we found another 4 SNPs which are located at the intron 8 of \( FTO \) to be nominally associated with spine BMD (rs11076017: \( P = 5.23 \times 10^{-3} \); rs1420318: \( P = 6.14 \times 10^{-4} \); rs1676942: \( P = 7.21 \times 10^{-2} \); and rs17226942: \( P = 9.23 \times 10^{-5} \)). For the previously reported SNP rs9939609 identified by GWAS for obesity phenotypes \([9,10,11]\), we only detected nominally significant association with spine BMD in the Chinese sample 1 (\( P = 0.037 \)).

### Discussion

The \( FTO \) gene has become the hotspot for researchers since it was reported as a novel obesity-susceptibility gene by a number of GWAS and follow-up replication studies. Given the evidence that obesity and osteoporosis share some common genetic determinants, we first performed an association study examining the SNPs in \( FTO \) for association with BMD. We identified a cluster of SNPs in \( FTO \) to be significantly associated with hip BMD in Chinese populations.

The human \( FTO \) gene is expressed in many tissues including mesenteric fat, pancreas, liver and adipose tissue, with the highest concentrations found in the hypothalamus \([10,20]\). Functional studies have demonstrated the direct effect of \( FTO \) on obesity. For example, Fischer et al. have reported that the loss of \( Fto \) in mice leads to postnatal growth retardation and a significant reduction in adipose tissue and lean body mass \([18]\). Church et al. have shown that a mutation in the mouse \( Fto \) gene results in reduced fat mass, increased energy expenditure, and unchanged physical activity \([17]\). In respect to \( FTO \)'s relevance to osteoporosis, a recent study by Gao et al. has found that \( Fto \) plays an essential role in postnatal growth. The mice lacking \( Fto \) completely displayed postnatal growth retardation manifested as reduced body weight and length, lower BMD \([19]\). Taking into account of this biological evidence and our statistical findings, we suggest that \( FTO \) might have potential role on BMD or osteoporosis.

It is necessary to examine the associations in different populations from different ethnicities, since the genomic variation is greater when compared across ethnicities. Our study identified

### Table 1. Basic characteristics of the study subjects.

| Trait        | Chinese Sample 1 | Chinese Sample 2 | Caucasian Sample 1 |
|--------------|------------------|------------------|--------------------|
| Number       | 818              | 809              | 2,286              |
| Female/Male  | 412/406          | 413/396          | 1727/558           |
| Age (years)  | 28.88 (5.18)     | 40.18 (16.17)    | 51.37 (13.76)      |
| Weight (kg)  | 57.37 (9.83)     | 62.89 (10.39)    | 75.37 (17.54)      |
| Height (cm)  | 163.51 (7.62)    | 164.99 (8.62)    | 166.35 (8.47)      |
| BMI (kg/m²)  | 21.38 (2.76)     | 23.06 (3.05)     | 27.14 (5.75)       |
| Hip BMD (g/cm²) | 0.919 (0.129)   | 0.921 (0.139)    | 0.968 (0.175)      |
| Spine BMD (g/cm²) | 0.960 (0.115)   | 0.935 (0.138)    | 1.025 (0.157)      |

Note: Data are shown as mean (standard deviation, SD).

### Table 2. Significant association results for SNPs in \( FTO \) with hip BMD.

| SNP          | Position | Genic | A1/A2 | Chinese Sample 1 | Chinese Sample 2 | \( \beta_{\text{combine}} \) | Caucasian sample |
|--------------|----------|-------|-------|------------------|------------------|-----------------------------|-------------------|
|              |          |       |       | \( P \)-value | \( P \)-value | \( P \)-value                | \( P \)-value       |
| rs16952955   | Intron8  | C/A   |       | 0.146           | 0.0261           | \( 8.39 \times 10^{-4} \)  | 0.132              |
|              |          |       |       | 0.0132          | 0.0150           | \( 4.31 \times 10^{-2} \)  | 1.47 \times 10^{-4} |
| rs2540766    | Intron8  | T/C   |       | 0.144           | 0.0236           | \( 2.88 \times 10^{-3} \)  | 0.130              |
|              |          |       |       | 0.0130          | 0.0165           | \( 3.25 \times 10^{-2} \)  | 2.95 \times 10^{-4} |
| rs2540784    | Intron8  | G/C   |       | 0.146           | 0.0239           | \( 2.38 \times 10^{-3} \)  | 0.133              |
|              |          |       |       | 0.0133          | 0.0159           | \( 4.02 \times 10^{-2} \)  | 3.18 \times 10^{-4} |
| rs16952951   | Intron8  | A/G   |       | 0.144           | 0.0256           | \( 1.11 \times 10^{-3} \)  | 0.132              |
|              |          |       |       | 0.0132          | 0.0138           | \( 7.56 \times 10^{-2} \)  | 3.65 \times 10^{-4} |
| rs12447427   | Intron8  | G/A   |       | 0.145           | 0.0241           | \( 2.03 \times 10^{-3} \)  | 0.131              |
|              |          |       |       | 0.0131          | 0.0145           | \( 6.15 \times 10^{-2} \)  | 4.57 \times 10^{-4} |
| rs2689247    | Intron8  | A/G   |       | 0.147           | 0.0237           | \( 2.42 \times 10^{-3} \)  | 0.132              |
|              |          |       |       | 0.0132          | 0.0145           | \( 5.91 \times 10^{-2} \)  | 4.99 \times 10^{-4} |

Freq, frequency is shown for allele A1. Meta-analysis was conducted for Chinese samples 1 and 2 using the METAL software taking into account sample size and direction of effect \( \beta_{\text{combine}} \). We listed SNPs with \( \beta_{\text{combine}} \) remaining significant after FDR adjustment.

doi:10.1371/journal.pone.0027312.t001
Consistent association for 6 SNPs in intron 8 of FTO with hip BMD in two Chinese populations. Unfortunately, such results were not replicated in the Caucasian population, which implied some extent of ethnic difference. Such ethnic difference could be explained from two aspects. First, the allele frequencies are quite different between the Chinese and Caucasian populations. The minor alleles of these 6 significant SNPs were much common in the Chinese than in the Caucasians, which may contribute to the overall effect. Second, the difference might be age-specific. The two Chinese populations were relatively younger than the Caucasians. Although we have included age as a covariate to adjust BMD, it could not eliminate the potential confounding effect of age on BMD variations thoroughly. Since animal studies have reported that Fto plays an important role in postnatal growth [19], this might suggest that the FTO gene might be a candidate genetic marker for peak bone mass acquisition. However, it is still too early to get such conclusion. The sample size of the Chinese samples was relatively small, which might decrease the statistical power to detect genetic associations. Increasing the sample size in further studies is needed to validate our results, and we are hopeful that publication of our findings will facilitate replication analyses by other groups.

In summary, our data provide novel evidence that a cluster of SNPs in FTO are associated BMD variations in Chinese populations. Considering the prior biological findings, we suggest that FTO might be a new candidate for osteoporosis. Further studies are warranted to explore the generality of our findings and elucidate the true functional variant.

Materials and Methods

Subjects

The study was approved by the Institutional Review Board or Research Administration of Xi’an Jiaotong University, Hunan Normal University, Creighton University and University of Missouri-Kansas City. Signed informed consent documents were obtained from all study participants before entering the study.

Chinese samples. The Chinese sample 1 comprised 818 unrelated subjects, which were recruited from Southwest Chinese Han adults living in Changsha city and its neighboring areas. The Chinese sample 2 comprised 809 unrelated subjects drawing from Northwest Chinese Han adults in Xi’an City and its neighboring areas. For all the subjects, the exclusion criteria have been detailed.

Figure 1. Regional Association Plot for FTO on chromosome 16. The color scheme of a white-to-black gradient reflects lower to higher linkage disequilibrium (LD, $r^2$). The $r^2$ is calculated between the top significant SNP rs16952955 and other SNPs. The scatter graph indicates the negative logarithm of $P$ value for each SNP, which is based on the combined results in the two Chinese samples.

doi:10.1371/journal.pone.0027312.g001
in our earlier publication [21]. Briefly, subjects with chronic diseases and conditions that might potentially affect bone mass, structure, or metabolism were excluded from the study to minimize the influence of known environmental and therapeutic factors on bone variation. BMD at hip and spine was measured using Hologic 4500 W machines (Hologic Inc., Bedford, MA, USA) under the same strict protocols. The coefficient of variation (CV) values of the dual-energy X-ray absorptiometry (DXA) measurements for spine and hip BMDs were approximately 1.01% and 1.33%, respectively.

**Caucasian sample.** The Caucasian sample consisted of 2,286 unrelated adults. All of the subjects were US Caucasians of Northern European origin living in Midwestern area. The exclusion criteria were the same as with Chinese samples. BMD at hip and spine were measured using the same model Hologic 4500 W machines (Hologic Inc., Bedford, MA, USA) under the same strict protocols used in the Chinese sample. The CV values of the DXA measurements for spine and hip BMDs were approximately 1.01% and 1.33%, respectively.

**Genotyping**
Genomic DNA was extracted from peripheral blood leukocytes using standard protocols. For all the three samples, SNP genotyping was performed using Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, CA, USA), according to the Affymetrix protocol. Only samples with a minimum call rate of 95% were included. Due to efforts of repeat experiments, all samples met this criteria and the final mean call rate reached a high level of 98.93% for Caucasian sample and 98.96% for Chinese sample. SNPs that deviated from Hardy-Weinberg equilibrium (HWE, \(P<0.0001\)) and had a minor allele frequency (MAF)<0.01 were discarded in each sample set. Thus, 141 SNPs in FTO were included for subsequent association analyses. The basic characteristics of these SNPs are summarized in Table S1.

**Statistical analyses**
Before association analyses, principal component analysis implemented in EIGENSTRAT [22] was used to adjust for potential population stratification that may lead to spurious association results. The first ten principal components emerging from the EIGENSTRAT analyses, along with sex, age, height, weight and BMI, were used as covariates to adjust the raw BMD values in each sample. The residues were used for association analyses. Linear regression implemented in PLINK [23] was used to test for association under the additive inheritance model.

**Supporting Information**

**Table S1** Properties of FTO SNPs tested in this study (DOC)

**Author Contributions**
Conceived and designed the experiments: YG. Performed the experiments: T-LY HL. Analyzed the data: T-LY QT. Contributed reagents/materials/analysis tools: Y-JL H-WD SML SKL. Wrote the paper: YG.

---

**Table 3. Gender-specific association results for the six SNPs identified for hip BMD in the Chinese samples.**

| SNP   | Male Chinese Sample 1 \(P\) value | Female Chinese Sample 1 \(P\) value | Male Chinese Sample 2 \(P\) value | Female Chinese Sample 2 \(P\) value |
|-------|----------------------------------|-----------------------------------|----------------------------------|-----------------------------------|
| rs16952955 | 1.89 \(\times\) 10^{-7} | 0.164 | 0.114 | 0.252 |
| rs2540766  | 3.61 \(\times\) 10^{-4} | 0.283 | 0.092 | 0.182 |
| rs2540784  | 0.012 | 0.086 | 0.123 | 0.176 |
| rs16952951 | 2.54 \(\times\) 10^{-3} | 0.164 | 0.173 | 0.252 |
| rs12447427 | 6.02 \(\times\) 10^{-3} | 0.142 | 0.153 | 0.226 |
| rs2689247  | 7.36 \(\times\) 10^{-3} | 0.146 | 0.119 | 0.272 |

**References**
1. Toth E, Ferenc V, Meszaros S, Csopor E, Horvath G (2005) [Effects of body mass index on bone mineral density in men]. Orv Hetil 146: 1489–1493.
2. Rosen CJ, Bouxsein ML (2006) Mechanisms of disease: is osteoporosis the obesity of bone? Nat Clin Pract Rheumatol 2: 35–43.
3. Zhao LJ, Liu XJ, Liu PY, Hamilton J, Recker RR, et al. (2007) Relationship of obesity with osteoporosis. J Clin Endocrinol Metab 92: 1640–1646.
4. Gimble JM, Robinson CE, Wu X, Kelly KA (1996) The function of adipocytes in the bone marrow stroma: an update. Bone 19: 421–428.
5. Deng FY, Lei SF, Li MX, Jiang C, Dvornyk V, et al. (2006) Genetic determination and correlation of body mass index and bone mineral density at the spine and hip in Chinese Han ethnicity. Osteoporos Int 17: 119–124.
6. Zhao LJ, Guo YF, Xiong DH, Xiao P, Recker RR, et al. (2006) Is a gene important for bone resorption a candidate for obesity? An association and linkage study on the RANK (receptor activator of nuclear factor-kappaB) gene in a large Caucasian sample. Hum Genet 120: 561–570.
7. Zhao J, Bradfield JP, Li M, Zhang H, Mentch FD, et al. (2011) BMD-associated variation at the Osterix locus is correlated with childhood obesity in females. PLoS One 6: e19252.
8. Liu YZ, Pri YF, Liu JF, Yang F, Guo Y, et al. (2009) Powerful bivariate genome-wide association analyses suggest the SOX6 gene influencing both obesity and osteoporosis phenotypes in males. PLoS One 4: e6027.
9. Ding C, Meyre D, Gallina S, Durand E, Korner A, et al. (2007) Variation in FTO contributes to childhood obesity and severe adult obesity. Nat Genet 39: 724–726.
10. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, 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.
11. Scuteri A, Sanna S, Chen WM, Uda M, Alfai A, et al. (2007) Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. PLoS Genet 3: e115.
12. Chi SW, Chiu SM, Kim KS, Park BL, Kim JR, et al. (2008) Replication of genetic effects of FTO polymorphisms on BMI in a Korean population. Obesity (Silver Spring) 16: 2187–2189.
13. Chang YC, Liu PH, Lee WJ, Chang TJ, Jiang YD, et al. (2008) Common variation in the fat mass and obesity-associated (FTO) gene confers risk of obesity and modulates BMI in the Chinese population. Diabetes 57: 2245–2252.
14. Hotta K, Nakata Y, Matsuo T, Kamohara S, Kotani K, et al. (2008) Variations in the FTO gene are associated with severe obesity in the Japanese. J Hum Genet 53: 546–553.
15. Hubacek JA, Bohuslavova R, Kuthanova L, Kubinova R, Peasey A, et al. (2008) The FTO gene and obesity in a large Eastern European population sample: the HAPlIE study. Obesity (Silver Spring) 16: 2764–2766.
16. Gerken T, Girard CA, Tung YC, Webbey C, Saudack V, et al. (2007) The obesity-associated FTO gene encodes a 2-aminoacridine-dependent nucleic acid demethylase. Science 318: 1469–1472.
17. Church C, Lee S, Bagg EA, McTaggart JS, Deacon R, et al. (2009) A mouse model for the metabolic effects of the human fat mass and obesity associated FTO gene. PLoS Genet 5: e1000599.
18. Fischer J, Koch L, Emmerling C, Vierkotter J, Peters T, et al. (2009) Inactivation of the Fto gene protects from obesity. Nature 458: 894–898.
19. Gao X, Shin YH, Li M, Wang F, Tong Q, et al. (2010) The fat mass and obesity associated gene FTO functions in the brain to regulate postnatal growth in mice. PLoS One 5: e10405.
20. Stratigopoulos G, Padilla SL, LeDuc CA, Watson E, Hattersley AT, et al. (2008) Regulation of Fto/Ftm gene expression in mice and humans. Am J Physiol Regul Integr Comp Physiol 294: R1185–1196.

21. Guo Y, Tan LJ, Lei SF, Yang TL, Chen XD, et al. (2010) Genome-wide association study identifies ALDH7A1 as a novel susceptibility gene for osteoporosis. PLoS Genet 6: e1000806.

22. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, et al. (2006) Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet 38: 904–909.

23. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, et al. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81: 559–575.

24. Johnson AD, Handsaker RE, Pulit SL, Nizzari MM, O’Donnell CJ, et al. (2008) SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. Bioinformatics 24: 2938–2939.

25. Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I (2001) Controlling the false discovery rate in behavior genetics research. Behav Brain Res 125: 279–284.