Birth weight is associated with placental fat mass- and obesity-associated gene expression and promoter methylation in a Chinese population

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Abstract

Objective: To explore the relationship between birth weight and fat mass- and obesity-associated (FTO) gene expression and promoter methylation status in the Chinese population.

Methods: Seventy-five neonates and their mothers were recruited from Yuying Children’s Hospital of Wenzhou Medical University. Subjects were divided into three groups by birth weight: low (<3500 g, n = 20), medium (3500–3999 g, n = 30) and high (≥4000 g, n = 25). Placental FTO transcript levels and promoter methylation were determined by quantitative PCR and Sequenom MassARRAY®.

Results: Placental FTO mRNA expression was significantly increased in the high- and medium-weight groups compared to the low-weight group (p = 0.023). Methylation rates of CpG11 sites were significantly decreased in high-birth weight newborns (p = 0.018). Multiple linear regressions showed placental FTO mRNA, maternal pre-pregnancy body mass index (BMI) and CpG11 methylation rate were independently associated with increased fetal birth weight. Additionally, FTO mRNA expression was negatively associated with CpG6.7.8.9 methylation in mothers that underwent C-section.

Conclusions: High placental FTO expression is associated with increased birth weight in Chinese neonates, and FTO promoter methylation level at a specific CpG site is negatively associated with birth weight. Further work is needed to determine the functionality of this CpG site in placentas.

Introduction

The fat mass- and obesity-associated (FTO) gene is located on the long arm of chromosome 16 and encodes a Fe(II)- and 2-oxoglutarate-dependent 3-methylthymine demethylase, which catalyzes demethylation of single-stranded DNA [1] and RNA [2]. The FTO gene is widely expressed in fetal and adult tissues, with high expression levels in adipose tissue and the hypothalamus [3]. Increasing evidence suggests that FTO participates in regulating food intake [4–6], energy expenditure [7] and lipolysis [8,9]. Consequently, FTO is associated with human adiposity and metabolic disorders [10].

The FTO gene is also involved in processes related to growth regulation. Several studies have reported the effects of FTO on embryonic development and fetal growth [11]. Fischer et al. observed that FTO loss in mice leads to postnatal growth retardation and a significantly reduced body weight at 6 weeks after birth [8]. Compared to wild-type (FTO+/+) mice, global germline FTO knockout (FTO+/−, FTO−/−) mice showed higher perinatal lethality and growth restriction [12]. In humans, the FTO gene is associated with increased fetal weight and length in Caucasians [9]. A 2013 study revealed that both rat and human placental FTO expression is reduced in intrauterine growth restriction (IUGR) fetuses compared to normal pregnancies, but not in macrosomia [13]. These studies strongly suggest that FTO gene expression is closely correlated to fetal growth and development.

There is evidence that several common FTO gene single nucleotide polymorphisms (SNPs), such as rs8050136, rs9939609 and rs9930506, are significantly associated with obesity in European populations [10,14–17]. However, a cohort study of Chinese Han (n = 3210) indicated that none of these FTO SNPs are associated with obesity and obesity-related traits. Furthermore, the allele frequencies of these specific SNPs in the Chinese Han population are substantially lower than in European populations [18]. These studies
suggest that there are differences in allele frequencies and linkage disequilibrium structure in FTO gene among various ethnicities. However, it remains unknown whether a racial difference exists in the effects of FTO on fetal growth.

It is well-known that DNA methylation is an important epigenetic determinant that regulates gene expression. Generally, methylation of CpG dinucleotides in CpG islands is believed to inhibit promoter activity, while demethylation induces promoter activity [19]. Two studies reported the CpG methylation rate in the first FTO intron is increased in type-2 diabetics versus unaffected individuals; however, there was no significant relationship between altered CpG methylation and FTO mRNA expression [20,21].

The present study investigated the association between placental FTO gene expression and fetal birth weight in the Chinese population, and assessed whether FTO gene expression is associated with promoter methylation changes. We also explored whether there are FTO gene methylation variations in placentas from newborns with different birth weights, and evaluated whether such methylation changes are associated with fetal birth weight.

Methods

Subjects

Subjects were recruited between April 2011 and March 2012 at the Obstetrics Department of Yuying Children’s Hospital of Wenzhou Medical University, China. The Wenzhou Medical University Ethics Committee had approved the project. Informed written consent was obtained from all subjects (mothers). Samples were collected from females whose infants were singleton, full-term (≥37 weeks), viable without known genetic disorders and from normal pregnancies. Normal pregnancies were defined as having a lack of hypertension, hepatitis, heart disease, psychological disorders, gestational diabetes and no fetal congenital malformations [22]. Excluded were potential participants with established diabetes, post-term pregnancy, pregnancy complications (such as diabetes, hypertension, cardiovascular disease, etc.), preeclampsia, uteroplacental insufficiency and newborn congenital malformations. Neonates were immediately weighed after birth, and their development was monitored. Basic data on the mothers and neonates were collected by self-report questionnaires [23].

Sample collection

A chorionic villous biopsy (~1 g) was excised and frozen in tubes containing diethyl pyrocarbonate (DEPC) immediately after delivery of the placenta. Tissue specimens were preserved in RNAlater® solution (Ambion, Austin, TX) at 4 °C overnight and then stored frozen at −80 °C.

Isolation of total RNA and real-time PCR

Total RNA was extracted from tissue samples using TRIzol® reagent (Life Technologies Corp., Carlsbad, CA) and cDNA was prepared according to ReverTra Ace® qPCR RT kit (Toyobo, Osaka, Japan) instructions. The reference gene was GAPDH. Target genes were quantified by SYBR® green dye method (Life Technologies Corp., Carlsbad, CA). Primers used for amplification of FTO were forward primer 5′-GAACCTGTTGAAGAGAAGTAGT-3′ and reverse primer 5′-TCAGCAGTAATGTTCCGGCAAT-3′. Primers used for amplification of GAPDH were forward primer 5′-AGAAGCTGCTGGGC TCATTG-3′ and reverse primer 5′-AGGGGCCATCACCAG TCTTC-3′ [24]. Real-time PCR (RT-PCR) conditions were: 95 °C for 2 min and 72 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 30 s. Fluorescence signals were acquired during the extension step at 60 °C. Target gene expression was calculated by the 2−ΔΔCT method [25].

DNA methylation

The FTO gene sequence was obtained from GenBank and the position of the FTO promoter was predicted by using the Promoter 2.0 Prediction Server (http://www.cbs.dtu.dk/services/Promoter). The MethPrimer primer design website (http://www.urogene.org/methprimer/) was used to predict the CpG island positions [26]. Genomic DNA was extracted using the Cell and Tissue DNA purification kit (BioTek, Beijing, China). Two-hundred nanograms of genomic DNA from each sample was bisulfite-treated using the EZ-96 DNA methylation™ kit (Zymo Research Corp., Irvine, CA) according to manufacturer instructions. After treating with sodium bisulfite, unmethylated cytosines are converted to uracils in the genomic DNA. The Sequenom MassARRAY® platform (CapitalBio, Beijing, China) was used to quantify FTO gene promoter methylation. This platform is composed of matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry and combined with RNA base-specific cleavage [27]. For FTO island 1, forward primer was 5′-AGGAAGAGAGAGTGTAGTGGGGGCTTTAGTAATTT-3′ and the reverse primer was 5′-CAGATAATCCAGACTCATATAGGAAGAGGCTTTAACAAAAATTT-CCAAAATC-3′. For FTO island 2, the forward primer was 5′-AGGAAGAGAGAGTGTAGTGGGGGCTTTAGTAATTT-3′ and the reverse primer was 5′-CAGATAATCCAGACTCATATAGGAAGAGGCTTTAACAAAAATTT-CCAAAATC-3′. All primers for methylation measurement were designed using Epidesigner software (Sequenom, San Diego, CA). PCR was performed to amplify the methylation regions of the two candidate FTO islands in a thermocycler (Eppendorf Mastercycler® Gradient, Hamburg, Germany). Mass spectra of PCR products were obtained via MassARRAY® Compact MALDI-TOF (Sequenom, San Diego, CA). FTO gene methylation ratios were generated using EpiTYPER® software version 1.0 (Sequenom, San Diego, CA) [23].

Statistical analysis

Statistical analyses were performed using SPSS 14.0 software (SPSS/IBM, Chicago, IL). Means were compared by one-way analysis of variance (ANOVA) and. Medians were compared by the Mann–Whitney rank-sum test. Linear correlation and multiple linear regression models were performed to determine which factors were significantly and independently associated with birth weight. The level of statistical significance was set at p < 0.05.
The analytic region of FTO promoter region maintained very low CpG island 1 was 290 bp (0.122 ± 0.054, n = 75; Figure 1A). Placental FTO expression was positively correlated with newborn birth weight (r = 0.310, p = 0.010). But CpG 6.7.8.9 methylation was not (r = −0.112, p = 0.359).

Placental FTO expression and CpG site methylation are related to newborn birth weight

Pearson's correlation results indicated that newborn birth weight was associated with the mothers' pre-pregnancy BMI, FTO expression level and CpG11 methylation rate. Thus, in multiple linear regression models, the dependent factor was birth weight and independent factors included FTO expression level, CpG11 methylation rate and pre-pregnancy BMI. After multivariate adjustment, the FTO expression level was persistently and positively associated with birth weight (b = 152.8, p = 0.007). As the CpG11 methylation rate was reduced by 1%, birth weight increased by 63.9 g (b = −63.9, p = 0.034). A higher pre-pregnancy BMI was associated with increased newborn birth weight (b = 60.8, p = 0.006, Supplementary Table 1).

Results

Baseline data

Seventy-five mothers and their neonates were recruited into our study. Maternal age ranged from 19- to 39-years-old, maternal pre-pregnancy body mass index (BMI) ranged from 16.42 to 28.55 kg/m² and gestational age ranged from 37 to 41 weeks. Newborn birth weight ranged from 2840 to 4920 g. For comparison, newborns were divided into three groups: low birth weight (<3500 g, n = 20), medium birth weight (3500–3999 g, n = 30) and high birth weight (≥4000 g, n = 25). There was no significant difference among the three groups in baseline data except for maternal weight before pregnancy (F = 3.707, p = 0.030) and maternal pre-pregnancy BMI (F = 4.436, p = 0.015, Table 1). Pre-pregnancy BMI was positively correlated to newborn birth weight (r = 0.367, p = 0.002; Pearson's correlation).

FTO mRNA expression is correlated with newborn birth weight

Pearson's correlation showed that placental FTO mRNA expression was positively correlated with newborn birth weight (r = 0.324, p = 0.005, n = 75; Figure 1A). Placental FTO mRNA expression was significantly increased in the medium- (0.164 ± 0.063) and high-birth weight groups (0.174 ± 0.069) versus that of the low-birth weight group (0.122 ± 0.054, F = 3.971, p = 0.023; Figure 1B).

FTO promoter region maintained very low methylation status

The analytic region of FTO sequences was from −2000 to 417 bp (total 2417 bp), and included the promoter (−2000 to −1 bp), exon 1 (1–267 bp) and part of intron 1 (268–417 bp). There were two CpG islands in this region. The length of CpG island 1 was 290 bp (−180 to 110 bp) and that of CpG island 2 was 238 bp (115–352 bp). These two islands contained a total of 46 CpG sites (23 sites in each island; Supplementary Figure 1). Sequenom MassARRAY® analysis detected that CpG islands in the FTO promoter were hypomethylated. The overwhelming majority of CpGs’ (40/46) methylation levels were less than 10%, and the respective average methylation rates of CpG islands 1 and 2 in the FTO promoter were 5.36 ± 0.93% and 3.79 ± 0.78% (n = 75 subjects), respectively. When methylation rates of different birth-weight groups were compared, there was no significant difference in average methylation rate in either island (p = 0.444 and 0.236; Figure 2A), but the average methylation rate of CpG island 1 was higher than that of CpG island 2 in each group (all p values <0.001; Figure 2B). For individual CpG site methylation status, the methylation rates of the CpG6.7.8.9 and CpG11 sites in CpG island 1 differed, medium- and high-birth weight groups compared to low-birth weight group (p = 0.034 and 0.018; Figure 2C). Other CpG sites’ methylation statuses are shown in Figure 2, panels C and D. Furthermore, Spearman correlation results showed that CpG11 methylation was negatively correlated with newborn birth weight (r = −0.310, p = 0.010), but CpG 6.7.8.9 methylation was not (r = −0.112, p = 0.359).
FTO promoter methylation status is correlated with mRNA expression in mothers that underwent C-section

FTO expression levels were not correlated to the average methylation rates of the promoter or individual CpG sites \( (r_s = -0.005 \text{ to } 0.178, \ p = 0.323-0.969) \). However, in 48 mothers that delivered by C-section, placental FTO expression was negatively correlated with methylation status of CpG6.7.8.9 in CpG island 1 (Pearson’s correlation: \( r = -0.399, \ p = 0.006 \)). A linear regression model was used to examine the association between placental FTO expression and CpG6.7.8.9 methylation rate in mothers that underwent C-section.
C-section, after maternal age, gestational weeks, newborn birth weight and baby gender were adjusted for. The final regression model suggested that the CpG6.7.8.9 methylation rate was independently associated with placental FTO mRNA expression \( (b = -0.013, p = 0.011, R^2 = 11.7\%) \).

**Discussion**

This study explored FTO mRNA expression in placentas from Chinese mothers and verified the association between FTO expression level and fetal birth weight in this population. It was previously reported that European and Chinese populations possess different allele frequencies and linkage disequilibrium structures in the FTO gene. However, FTO played a consistent role in promoting fetal intrauterine growth in the Chinese population, which was in agreement with the findings of Caucasian populations [9,18]. We presume that upregulated FTO expression can reduce cellular lipolytic activity and energy expenditure in fetal tissues, thereby increasing adipocyte size and adipose tissue weight gain. On one hand, high lipolytic activity is an important protective factor against excess weight gain, and increased lipolysis limits lipid accumulation within fat cells and adipocyte enlargement [4]. Indeed, Fisher et al. observed a significant reduction in white adipose tissue mass and adipocyte size in FTO-deficient mice [8]. Meanwhile, mice that overexpress FTO showed significantly increased fat mass [5]. An explanation could be that a higher FTO level inhibits adipocyte lipolytic activity, which is indicated by slowed lipidolysis, increased fat mass and increased newborn body weight. On the other hand, FTO might regulate energy expenditure. Fisher et al. [8] and McMurray et al. [12] observed that mice with adult-onset global FTO loss exhibit increased oxygen consumption and decreased carbon dioxide output. The respiratory-exchange-ratio of FTO-deficient mice was significantly lower than that in wild-type mice [8,12]. This may indicate that a reduced FTO level could reduce body weight by increasing energy expenditure. Thus, upregulated FTO expression could increase fetal intrauterine weight gain by reducing cellular lipolytic activity and energy expenditure in fetal adipose cells and tissues. However, the specific mechanism needs to be explored in further studies.

To assess the methylation status of the FTO promoter and whether it was involved in regulating mRNA expression, methylation levels of CpG islands in the FTO gene promoter were measured by Sequenom MassARRAY® analysis. The average methylation level detected in CpG sites in the placental FTO promoter and exon 1 region was approximately 5%. This methylation level is within the range of the total methylation level of human genome-wide DNA (3–6%), while it is significantly lower than the levels in human peripheral blood (30.2–53.1%). The reported CpGs were located in the second half of the FTO intron 1, exon 2 and intron 2 (beginning 53 kbp from the transcriptional start site) [20,21]. Therefore, it could be considered that the difference in CpG methylation status between our findings with placental FTO and others’ findings came from analyzing different regions in the DNA chain and from tissue-specific DNA methylation patterns. Methylation of CpG dinucleotides in the 5’ promoter regions of genes is generally considered indicative of transcription silencing [19]. Thus, we speculate that the hypomethylated status of CpG sites in the placental FTO promoter serve to maintain FTO transcriptional and protein levels. However, further study is necessary to elucidate the specific biological implications of this observation.

This was a hospital-based cross-sectional study. All subjects were patients from Yuying Children’s Hospital of Wenzhou Medical University, a large comprehensive hospital that serves patients from all 11 counties in the Wenzhou region. Thus, our sample effectively represents the population from the Wenzhou region of China. Moreover, all of our participants were recruited within an entire year, providing birth populations from every season. The sample size of this study was limited; nonetheless, it was large enough to provide sufficient statistical power to analyze our selected parameters. We also used a multiple linear regression model to adjust for potential FTO expression-related confounding factors, and we still were able to reliably define the relationship between newborn birth weight and FTO expression and FTO CpG site methylation.

In conclusion, placental FTO gene expression and maternal pre-pregnancy BMI are positively associated with fetal birth weight. The FTO promoter manifests hypomethylation status, and specific CpG sites are negatively associated with newborn birth weight in this Chinese population.

**Declaration of interest**

All authors declare no conflicts of interest, financial or otherwise.

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**References**

1. Gerken T, Girard CA, Tung YCL, et al. The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. Science 2007;318:1469–72.
2. Jia G, Yang CG, Yang S, et al. Oxidative demethylation of 3-methylthymine and 3-methyluracil in single-stranded DNA and RNA by mouse and human FTO. FEBS Lett 2008;582:3313–19.
3. Frayling TM, Ong K. Piecing together the FTO jigsaw. Genome Biol 2011;12:104.
4. Dahlman I, Dicker A, Jiao H, et al. A common haplotype in the G-protein-coupled receptor gene GPR74 is associated with leanness and increased lipolysis. Am J Hum Genet 2007;80:1115–24.
5. Church C, Moir L, McMurray F, et al. Overexpression of Pto leads to increased food intake and results in obesity. Nat Genet 2010;42:1086–92.
6. Labayen I, Ruiz JR, Ortega FB, et al. Association between the FTO rs9939609 polymorphism and leptin in European adolescents: a possible link with energy balance control. The HELENA study. Int J Obes (Lond) 2011;35:66–71.
7. Lopez-Bermejo A, Petry CJ, Diaz M, et al. The association between the FTO gene and fat mass in humans develops by the postnatal age of two weeks. J Clin Endocrinol Metab 2008;93:1501–5.
8. Fischer J, Koch L, Emmerling C, et al. Inactivation of the Fto gene protects from obesity. Nature 2009;458:894–8.
9. Bassols J, Prats-Puig A, Vazquez-Ruiz M, et al. Placental FTO expression relates to fetal growth. Int J Obes (Lond) 2010;34:1365–70.
10. Frayling TM, Timpson NJ, Weedon MN, et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 2007;316:889–94.

Z.-W. Liu et al.
11. Sebert SP, Hyatt MA, Chan LL, et al. Influence of prenatal nutrition and obesity on tissue specific fat mass and obesity-associated (FTO) gene expression. Reproduction 2010;139:265–74.
12. McMurray F, Church CD, Larder R, et al. Adult onset global loss of the fto gene alters body composition and metabolism in the mouse. PLoS Genet 2013;9:e1003166.
13. Mayeur S, Cisse O, Gabory A, et al. Placental expression of the obesity-associated gene FTO is reduced by fetal growth restriction but not by macrosomia in rats and humans. J Dev Orig Health Dis 2013;4:134–8.
14. Webby GL, Prater KN, Ryckman KK, et al. Candidate gene study for smoking, alcohol use, and body weight in a sample of pregnant women. J Matern Fetal Neonatal Med 2014 [Epub ahead of print].
15. Dina C, Meyre D, Gallina S, et al. Variation in FTO contributes to childhood obesity and severe adult obesity. Nat Genet 2007;39:724–6.
16. Peeters A, Beckers S, Verrijken A, et al. Variants in the FTO gene are associated with common obesity in the Belgian population. Mol Genet Metab 2008;93:481–4.
17. Seal N, Weaver M, Best LG. Correlates of the FTO gene variant (rs9939609) and growth of American Indian infants. Genet Test Mol Biomarkers 2011;15:633–8.
18. Li H, Wu Y, Loos RJ, et al. Variants in the fat mass- and obesity-associated (FTO) gene are not associated with obesity in a Chinese Han population. Diabetes 2008;57:264–8.
19. Bird A. DNA methylation patterns and epigenetic memory. Genes Dev 2002;16:6–21.
20. Bell CG, Finer S, Lindgren CM, et al. Integrated genetic and epigenetic analysis identifies haplotype-specific methylation in the FTO type 2 diabetes and obesity susceptibility locus. PLoS One 2010;5:e14040.
21. Toperoff G, Aran D, Kark JD, et al. Genome-wide survey reveals predisposing diabetes type 2-related DNA methylation variations in human peripheral blood. Hum Mol Genet 2012;21:371–83.
22. Mazaki-Tovi S, Romero R, Kim SK, et al. Could alterations in maternal plasma visfatin concentration participate in the phenotype definition of preeclampsia and SGA? J Matern Fetal Neonatal Med 2010;23:857–68.
23. Xu X, Yang X, Liu Z, et al. Placental leptin gene methylation and macrosomia during normal pregnancy. Mol Med Rep 2014;9:1013–18.
24. Zhou D, Liao H, Wei Y, et al. The role of estrogen in regulating FTO gene expression and cell proliferation in human endometrial carcinoma cell. Prog Obstet Gynecol 2011;20:600–9.
25. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(−ΔΔC(T)) method. Methods 2001;25:402–8.
26. Li LC, Dahiya R. MethPrimer: designing primers for methylation PCRs. Bioinformatics 2002;18:1427–31.
27. Andraweera PH, Dekker GA, Dissanayake VH, et al. Vascular endothelial growth factor family gene polymorphisms in pre-eclampsia in Sinhalese women in Sri-Lanka. J Matern Fetal Neonatal Med 2013;26:532–6.