Journal of Developmental Origins of Health and Disease (2013), **4**(2), 134–138.

doi:10.1017/S2040174412000748

BRIEF REPORT

Placental expression of the obesity-associated gene FTO is reduced by fetal growth restriction but not by macrosomia in rats and humans

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Genetic variants in the FTO (fat mass- and obesity-associated) gene have the highest association of all obesity-associated genes. Its placental expression was shown to relate to birth weight, suggesting that it may participate in the control of fetal weight gain. To gain more insight into the implication of FTO in fetal growth, we measured its placental expression in samples including extremes of abnormal fetal growth, such as after intrauterine growth restriction (IUGR) or macrosomia in both rats and humans. In rats, fetal growth was modulated by maternal nutritional modifications. In humans, placental villi were collected from pathological pregnancies (i.e. with IUGR or fetal macrosomia). Placental *FTO* mRNA expression was reduced by IUGR but was not significantly affected by macrosomia in either rats or humans. Our data suggest that placental FTO may participate in interactions between the *in utero* environment and the control of fetal growth under IUGR conditions by modulating epigenetic processes.

Received 3 October 2012; Revised 19 November 2012; Accepted 27 November 2012; First published online 20 December 2012

Key words: fetal growth, FTO, human, placenta, rat

Introduction

A genome-wide search for obesity and type 2 diabetes susceptibility genes identified several variants in the fat mass- and obesity-associated (FTO) gene region, which were strongly associated with human adiposity and metabolic disorders.[1] The human FTO is widely expressed in both fetal and adult tissues, with the highest concentration found specifically in the hypothalamus.[2] At this level, FTO plays a role in the control of energy intake, but other data indicate that FTO might also be important for adipogenesis and muscle function.[2,3] At a cellular level, the role of FTO is not fully understood. FTO is able to demethylate single-stranded DNA and RNA and thus might regulate gene expression, but it may also act at the translational level by affecting RNA structure and protein translation.[3,4] Reports of associations between FTO and a disturbed phenotype early in life have emerged recently. In mice, Fto was identified as one of the genes whose deletion was responsible for the fused-toe phenotype.[3] Mice homozygous for this deletion died at mid-gestation and exhibited severe malformations and growth retardation. Global loss of Fto in mice induces a high mortality rate in the immediate postnatal period and lifelong growth retardation.[4] However, mice overexpressing Fto or mice carrying a missense point mutation in the Fto gene did not display either postnatal mortality or growth retardation.[3,4] In humans, loss of function of FTO induces postnatal growth retardation and numerous malformations in the head and brain, as well as, in some patients, intrauterine growth retardation (IUGR) and cardiac and genital abnormalities.[5] Despite these observations, the implication of FTO in the body mass evolution during the perinatal life remains elusive. Recent findings indicate that *FTO* mRNA expression is highly abundant in the placenta.[6,7] An association between FTO single nucleotide polymorphisms and a trend toward increased birth weight was reported,[4] and placental FTO expression was shown to relate to birth weight in uncomplicated pregnancies.[6] The major determinant of fetal growth is the placental supply of nutrients and oxygen, which depends on its size, morphology, blood flow and transporters' abundance.[8] Placental function follows a carefully orchestrated developmental cascade and a disruption of this kinetic can lead to abnormal development of the feto-placental unit and a metabolic programming in the progeny.[9] The placental development implicates numerous genes and depends largely on the genotype and epigenotype of both the fetus and parents.[9] As FTO may control placental genes and protein expression, we hypothesized that it could be involved in several pathologies related to disturbances in fetal growth. Thus, our study aimed at assessing the gene expression of FTO in placentas including both extremes of abnormal fetal growth such as IUGR and

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macrosomia. Rat placentas from fetuses with growth abnormalities induced by maternal nutritional modifications and human placental villi samples from pathological pregnancies with fetal growth disturbances were analyzed in the present study.

Materials and methods

Animal models and tissue collection

The experiments were conducted in accordance with the European Communities Council Directive (86/609/EEC) and with accreditation (No. 04860). Animals were maintained under standard conditions as previously described.[10] Adult Wistar rats were purchased from Charles Rivers® and females were mated with a male. Embryonic day 1 (E1) was defined if spermatozoa were found in vaginal smears. Pregnant rats were fed *ad libitum* (rat chow containing 16% proteins, 3% fat and 60% carbohydrates) for controls ($n = 9$) or received, from E1 to E21, 30% of the food intake of controls (FR30 mothers; $n = 9$) for the study related to IUGR. Only control and IUGR male placentas and fetuses were collected and weighed at term (E21). Placentas were frozen in liquid N_2 and stored at −80°C. In the study related to fetal overgrowth, female offspring (F1 generation; $n = 6$) from FR30 mothers (F0 generation) were fed a high-fat diet (HFD; 23% proteins, 23% fat and 40% carbohydrates) from postnatal day 21 to the age of 3 months; then, they were mated with a male and fed the control rat chow during gestation. At E21, only male placentas and fetuses (F2 generation) were collected, weighed and frozen in liquid N_2 (Macro-F2-FR30 group). Controls ($n = 6$) were from F1 and F2 mothers fed the control diet *ad libitum*.

Human tissue collection

Placental villi samples were collected under a protocol approved by the local institutional ethics committee and informed consent was obtained from all individuals (accreditation number DC-2008-833). Placentas from normal and pathological pregnancies were collected from hospitals of Lille and Paris (France). Two cohorts of patients were used. For the study related to IUGR, the inclusion criterion used for IUGR was a pathologic flow in the uterine artery evidenced by Doppler during the first and second trimesters and a weight at birth <10th percentile for a given gestational age ($n = 8$ for IURG and $n = 11$ for controls). In the study related to macrosomia (Macro), this criterion was determined on the basis of standard growth curves for the French population.[11] Five cases from each group (C and Macro groups) without other pathologies (pre-gestational and gestational diabetes) were analyzed.

RNA extraction and quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Placental RNA extraction and cDNA conversion were performed as previously described.[12,13] Relative expression levels of RNA were quantified by TaqMan qRT-PCR. Primers for FTO and reference genes were designed using the Primer Premier software (Premier Biosoft International, Palo Alto, USA) and validated before the experiments. mRNA expression results were normalized using *Eif4A2*, *Tbp* and *Sdha* for humans and with *Hprt* and *Cyclo B* for rats using the $2^{\Delta C_t}$ method. Reference genes were chosen using GeNorm software.[14] Primers for FTO were selected in exon 3 of the gene (in both species) that encodes for the mature FTO protein. Primer sequences were 5′-AAATCTGGTGGACAGGTC AGCG-3′ and 5′-GGATCCCTGCCTTCGAGATGAG-3′ for human samples and 5′-CCTCAGAAAATGCCGTAC TTGA-3′ and 5′-GGACTCGTCATCGCTTTCATCC-3′ for rat samples.

Statistics

Data are presented as mean ± S.E.M. Samples were analyzed using ANOVA and Dunnett's test. $P < 0.05$ was considered significant.

Results

Maternal, placental and fetal parameters

In rats, maternal FR30 drastically reduced both placental and fetal weights at E21 (reduction of 29% for body weight and 22% for placental weight; $P < 0.001$). In contrast, FR30-F1 mothers fed an HFD from postnatal day 21 to the age of 3 months had E21 male fetuses (Macro-F2-FR30 group) with a significantly higher body weight (Table 1; $P < 0.05$).

In humans, the birth weight of IUGR babies was drastically reduced (−56%; $P < 0.001$; Table 1). The gestational age and some fetal parameters were also diminished, such as the height and the cranial perimeter (Table 1). Conversely, placental and birth weights were strongly increased (+55% and +42%, respectively; $P < 0.001$) in macrosomia for newborns with an approximately similar gestational age (Table 1).

Placental FTO *mRNA expression*

In rats, placental *FTO* mRNA expression was reduced (−38%; $P < 0.05$) at E21 in IUGR fetuses from FR30 mothers (Fig. 1a) but was not modified in rats with fetal overgrowth from FR30-F1 mothers (Fig. 1b).

In humans, placental villi *FTO* mRNA abundance was drastically reduced (−72%; $P < 0.01$) when IUGR was associated with a pathologic flow within the uterine arteries (Fig. 1e), but was not significantly affected despite a marked macrosomia occurrence in fetuses (Fig. 1f).

Discussion

In accordance with a previous observation,[6] we reported that human placental villi, as well as rat placentas, express large

https://doi.org/10.1017/S2040174412000748 Published online by Cambridge University Press

Table 1. *Maternal and newborns parameters*[a,b,c,d]

E21 rats	C	IUGR-FR30
Maternal weight (g)	444 ± 8	270 ± 11[e]
Fetal weight (g)	5.56 ± 0.07	3.97 ± 0.16[e]
Placental weight (mg)	592 ± 17	443 ± 19[e]
E21 rats	**C**	**Macro-F2-FR30**
Maternal weight (g)	343 ± 10	327 ± 5
Fetal weight (g)	4.94 ± 0.07	5.18 ± 0.06[f]
Placental weight (mg)	406 ± 7	402 ± 10
Human	**C**	**IUGR**
Gestational age (weeks)	38.6 ± 0.3	34.1 ± 1.1[g]
Maternal age (years)	34.1 ± 2.0	30.4 ± 1.9
Height (cm)	49.2 ± 0.6	41.9 ± 1.2[h]
Birth weight (g)	3530 ± 224	1562 ± 231[h]
Cranial perimeter (cm)	36.1 ± 0.6	30.6 ± 0.9[h]
Human	**C**	**Macro**
Gestational age (weeks)	38.6 ± 0.3	39.6 ± 0.5
BMI before gestation ($kg\,m^2$)	23.5 ± 2.5	24.3 ± 1.0
Birth weight (g)	3198 ± 106	4526 ± 191[i]
Placental weight (g)	372 ± 36	575 ± 23[i]

IUGR, intrauterine growth restriction; BMI, body mass index.
[a] Values are means ± S.E.M. ($n = 9$ mothers/group).
[b] Values are means ± S.E.M. ($n = 6$ mothers/group).
[c] Values are means ± S.E.M. ($n = 8$–11 mothers or babies/group).
[d] Values are means ± S.E.M. ($n = 5$ mothers or babies/group).
[e] $P < 0.001$, IUGR-FR30 *v.* C.
[f] $P < 0.05$, Macro-F2-FR30 *v.* C.
[g] $P < 0.01$.
[h] $P < 0.001$, IUGR *v.* C.
[i] $P < 0.001$, Macro *v.* C.

amounts of FTO. Bassols *et al.*[6] have shown that placental FTO gene expression relates to fetal growth in a large birth cohort study using placentas from uncomplicated pregnancies. We found that placental FTO expression was reduced by IUGR when the latter was associated with a pathologic flow within the maternal uterine arteries. However, as the maternal gestational age for the placental samples that we used was slightly different, we cannot exclude a putative gestational age effect on placental villi *FTO* mRNA abundance. Nevertheless, in IUGR rat fetuses from FR30 mothers, a similar significant reduction of Fto expression was also observed at term, suggesting that IUGR is indeed the factor linked to the reduced placental Fto gene expression independently of a putative gestational age effect. Under fetal overgrowth conditions, such as a drastic macrosomia in human or a slight overgrowth in rat fetuses induced by a pregestational maternal HFD, no significant modulation of placental FTO expression was observed. However, owing to the limited number of human placental tissue samples used in this study, additional studies are needed to unravel whether FTO is an important contributor of fetal weight gain as hypothesized by others.[6] The present findings suggest that FTO may be involved in processes related to IUGR. In humans, the decreased expression of FTO could be linked to maternal vascular dysfunctions. Indeed, a potential link between FTO risk alleles and human vascular pathologies continues to emerge as, for example, with acute coronary syndrome and elevated blood pressure.[2] FTO also appears to be essential for the normal development of the cardiovascular system in humans.[5] Thus, the modulation of its expression under *in utero* vascular deleterious conditions suggests that it may act as an environmental sensor at the feto-placental interface, that is, in the placenta. In rats, we demonstrated that maternal food restriction reduces Fto expression in the placenta in accordance with previous findings showing that Fto expression is modulated by nutrition in other tissues, such as in appetite-regulating nervous centers.[7,15,16] In addition, in mice at mid-gestation, we observed that maternal HFD reduces Fto expression in female placentas, and under a control diet male placentas displayed lower levels of Fto compared with female ones (unpublished observations). Taken together, our findings suggest that maternal nutrition is a controller of placental Fto gene expression and may be involved in part in IUGR etiology. Although the physiological placental functions of FTO remain to be determined, we postulate that it may act as an environmental sensor for the feto-placental unit during uncomplicated or IUGR pregnancies. Indeed, the interactions between the *in utero* environment and the fetal genome are likely to play important roles in feto-placental unit development as well as in the programming of susceptibility to common forms of obesity and metabolic disorders.[9] In this organ, placental FTO may regulate nonepigenetic and epigenetic transcription mechanisms, and could be implicated in the genesis of marks that serve as a memory of exposure to inadequate chemical/nutritional/metabolic environments conveyed via changes in DNA methylation patterns, RNA structure and protein translation at critical *in utero* periods, potentially leading to metabolic diseases in adulthood.[9,17,18] The present findings suggest that the placenta may be a relevant metabolic tissue to investigate in more detail the complex biology of FTO, which remains poorly understood. In humans, FTO risk alleles are unequivocally associated with increased food intake and obesity. Although the effect of these alleles appears modest, it was reported that they are highly common in populations with an estimated rate of 1 billion homozygous carriers in the world.[19] We suggest that, parallel to its reported functions in adults, FTO in the placenta may control both *in utero* energy balance and fetal growth during uncomplicated and IUGR pregnancies.

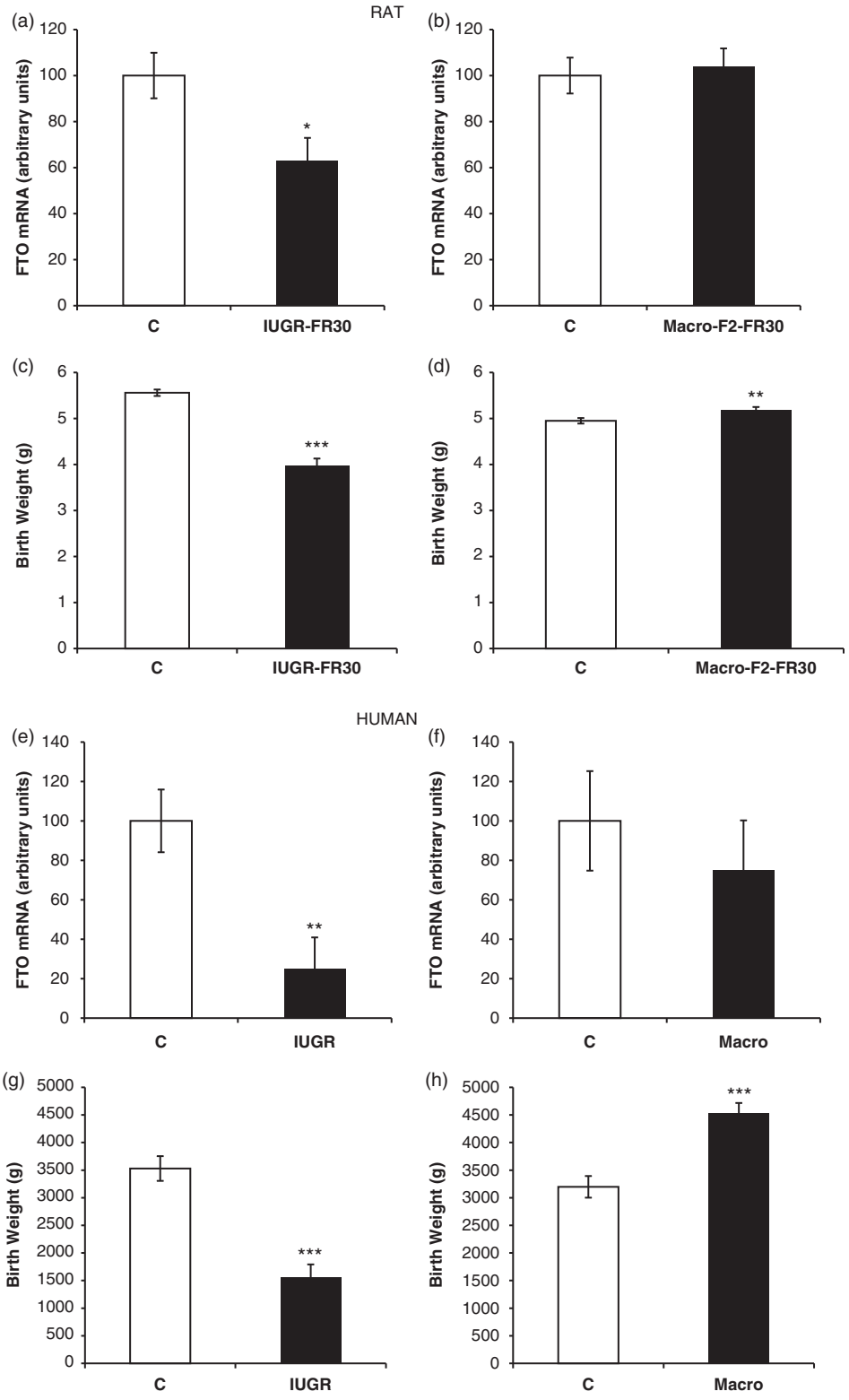

Fig. 1. Quantitative reverse transcription-polymerase chain reaction of fat mass- and obesity-associated (*FTO*) mRNA levels in rat placentas (a, b) and human placental villi (e, f) at term. Effect in rats of intrauterine growth restriction (IUGR) induced by a 70% maternal food restriction (FR30) on birth weight (c). Effect in humans of severe IUGR associated with pathologic flow in the uterine artery on the birth weight of babies (g). Effect of fetal macrosomia (Macro) in rats, induced by maternal nutritional modifications in F0 and F1 mothers, on the birth weight of F2 male offspring (d). Effect in humans of severe macrosomia without other maternal pathologies on the birth weight of babies (h). Values are means ± S.E.M. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ between groups.

https://doi.org/10.1017/S2040174412000748 Published online by Cambridge University Press

Acknowledgments

This work was carried out with the financial support of the 'ANR- Agence Nationale de la Recherche – The French National Research Agency' under the 'Programme National de Recherche en Alimentation et nutrition humaine', project 'ANR-06-PNRA-022', and the project 'PHRC 2007R/1901'.

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