

# Inverse relationship between obesity and *FTO* gene expression in visceral adipose tissue in humans

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## Abstract

**Aims/hypothesis** Recently, *FTO* was identified as a candidate gene contributing to both childhood and severe adult obesity. We tested the hypothesis that mRNA expression of *FTO* and/or of the neighbouring *RPGRIP1L* in adipose tissue correlates with measures of obesity and fat distribution. We also investigated whether the *FTO* obesity risk alleles might explain variability in *FTO* and *RPGRIP1L* mRNA expression.

**Methods** In paired samples of visceral and subcutaneous adipose tissue from 55 lean and obese participants, we investigated whether *FTO* and *RPGRIP1L* mRNA expression is fat depot-specific, altered in obesity and related to measures of fat accumulation, insulin sensitivity and glucose metabolism. All participants were genotyped for the obesity-associated rs8050136 *FTO* variant.

**Results** *FTO* mRNA expression was threefold higher in subcutaneous than in visceral adipose tissue. Subcutaneous *FTO* expression correlated with visceral *FTO* expression. *FTO* gene expression in both depots correlated with age and was negatively correlated to BMI and per cent body fat. *FTO* mRNA levels were not related to measures of insulin sensitivity and glucose metabolism. *RPGRIP1L* mRNA expression was 1.6-fold higher in visceral than in subcutaneous adipose tissue, but did not correlate with anthropometric and metabolic characteristics. There was no association between rs8050136 and *FTO* or *RPGRIP1L* mRNA expression in adipose tissue.

**Conclusions/interpretation** Expression of adipose tissue *FTO* mRNA is fat depot-specific and negatively correlates with measures of obesity. However, the direction of this relationship still needs to be elucidated.

**Keywords** Adipose tissue · Fasting plasma glucose · *FTO* · Genetics of obesity · Glucose metabolism · Insulin sensitivity · Obesity · *RPGRIP1L* · Visceral fat

## Abbreviations

*FTO* fat mass and obesity associated  
*LD* linkage disequilibrium

## Introduction

Genetic factors play an important role in the development of obesity [1], but the underlying genetic causes are poorly understood [2]. Two independent studies using different approaches recently demonstrated a strong association between variants in the gene fat mass and obesity associated (*FTO*) and obesity [3, 4]. Frayling et al. [3] found the asso-

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ciation between *FTO* variants (represented by rs9939609) and obesity in a genome-wide scan for type 2 diabetes and replicated it in a total of more than 38,000 individuals. The association between SNPs in the *FTO* gene and type 2 diabetes was shown to be due to obesity [3]. In agreement with these findings, Dina et al. [4] reported a set of SNPs in the *FTO* gene (rs1421085 and rs17817449) that is consistently strongly associated with early-onset childhood and severe adult obesity. Both groups replicated the association between *FTO* and obesity in large cohorts [3, 4]. Moreover, recently Scuteri et al. [5] replicated the significant association between rs9930506 in the *FTO* gene and BMI, body weight and hip circumference in European and Hispanic Americans.

The *Fto* gene was originally described in a mouse with fused toes (*Ft* mutation) [6, 7]. Homozygous *Ft* mutation is embryonic lethal [6]; heterozygous mice have fused toes and hyperplasia of the thymus most likely due to impaired apoptosis [7]. Interestingly, obesity has not been reported in these mice. However, the *Ft* mutation consists of at least six deleted genes and no conclusions can be drawn from this model regarding the function of FTO and the relationship between the *Fto* gene and obesity [2]. Human de novo duplication on chromosome 16, which includes the *FTO* locus, has been shown to be associated with mental retardation, obesity, dysmorphic facies and severe bilateral clinodactyly of some fingers [8]. Recent bioinformatics analysis revealed that FTO shares sequence motifs with Fe<sup>2+</sup>- and 2-oxoglutarate-dependent oxygenases [9]. It has also been shown that FTO localises to the nucleus and that recombinant murine FTO catalyses the demethylation of 3-methylthymine in single-stranded DNA [9].

After the association with human obesity was discovered, the name was changed to ‘fat mass and obesity associated’ (*FTO*) [2], but the function of the *FTO* gene is still unknown. *FTO* gene is expressed in most tissues, including adipose tissue, beta cells and brain [3]. The highest *FTO* expression levels have been detected in brain [3, 9].

Functional studies are necessary to define the biological role of FTO and the major FTO-‘sensitive’ organs. We therefore tested the hypothesis that *FTO* mRNA expression in visceral and subcutaneous adipose tissue is linked to obesity in a cohort of individuals with wide ranges of obesity and body fat distribution. In addition to *FTO*, retinitis pigmentosa GTPase regulator interacting protein 1-like (*RPGRIP1L*, also known as *KIAA1005*) is also thought to be involved in the development of obesity because of its genomic position near the *FTO* gene [3, 5]. The transcription start site of *RPGRIP1L* is situated only 200 base pairs from the 5' end of *FTO* and ~61 kb from the 47 kb interval containing the BMI associations. *RPGRIP1L* is ubiquitously expressed with relatively high levels in hypothalamus and islet. We therefore also examined *RPGRIP1L* mRNA expression in

visceral and subcutaneous adipose tissue and its relation to obesity.

In addition, we investigated whether previously published obesity-associated *FTO* variants (represented by rs8050136 in our study) might explain potential variability in *FTO* and *RPGRIP1L* mRNA expression.

## Methods

**Participants and measurement of participant characteristics** Paired samples of visceral and subcutaneous adipose tissue were obtained from 55 Europid men ( $n=26$ ) and women ( $n=29$ ) who underwent open abdominal surgery for elective cholecystectomy or explorative laparotomy. Using OGTTs, we identified individuals with type 2 diabetes ( $n=19$ ) or normal glucose tolerance ( $n=36$ ). All participants had stable weight with no fluctuations of >2% of body weight for at least 3 months before surgery. Patients with malignant diseases or any acute or chronic inflammatory disease as determined by a leucocyte count  $>7,000 \times 10^9/l$ , C-reactive protein  $>50$  mg/l or clinical signs of infection were excluded from the study. Samples of visceral and subcutaneous adipose tissue were immediately frozen in liquid nitrogen after explantation. The study was approved by the ethics committee of the University of Leipzig. All participants gave written informed consent before taking part in the study.

**Assays and measures of glucose metabolism and body fat content** Basal blood samples were taken after an overnight fast. Plasma insulin was measured with an enzyme immuno-metric assay for an automated analyser (Immulite; Diagnostic Products, Los Angeles, CA, USA). Plasma leptin levels were assessed by radioimmunoassay (Linco Research, St Charles, MO, USA). OGTT was performed after an overnight fast with 75 g standardised glucose solution (Glucodex Solution 75 g; Merieux, Montreal, QC, Canada). Insulin sensitivity was assessed with the euglycaemic–hyperinsulinaemic clamp method [10]. Percentage body fat was measured by dual-energy X-ray absorptiometry. In addition, abdominal visceral and subcutaneous fat area was calculated as previously described [11, 12].

**Analysis of human *FTO* and *RPGRIP1L* mRNA expression** Human *FTO* and *RPGRIP1L* mRNA expression was measured by quantitative real-time RT-PCR in a fluorescent temperature cycler using the TaqMan assay; fluorescence was detected on an ABI PRISM 7000 sequence detector (Applied Biosystems, Darmstadt, Germany). Total RNA was isolated from paired subcutaneous and visceral adipose tissue samples using TRIzol (Life Technologies, Grand Island, NY, USA), and 1  $\mu$ g RNA was reverse transcribed

with standard reagents (Life Technologies). From each RT-PCR, 2  $\mu$ l was amplified in a 26  $\mu$ l PCR using a kit (Brilliant SYBR Green QPCR Core Reagent Kit; Stratagene, La Jolla, CA, USA) according to the manufacturer's instructions. Samples were incubated in the sequence detector for an initial denaturation at 95°C for 10 min, followed by 40 PCR cycles, each cycle consisting of 95°C for 15 s, 60°C for 1 min and 72°C for 1 min. The following primers were used: human *FTO* 5'-CTGGCCAGTGAAAGGGTCTAAT-3' (sense) and 5'-GGCAGCAAGTTCTTCCAAAGC-3' (antisense); human *RPGRIP1L* 5'-GGTATTGGCAAGCTCAGGGTAA-3' (sense) and 5'-TCAAGCTCCAAGTCATCTCTGT-3' (antisense). SYBR Green I fluorescence emissions were monitored after each cycle. Human *FTO* and *RPGRIP1L* mRNA expression was calculated relative to the mRNA expression of 18S rRNA, determined by a premixed assay on demand for human 18S rRNA (Applied Biosystems, Darmstadt, Germany). Amplification of specific transcripts was confirmed by melting curve profiles (cooling the sample to 68°C and heating slowly to 95°C with measurement of fluorescence) at the end of each PCR. The specificity of the PCR was further verified by subjecting the amplification products to agarose gel electrophoresis.

**Genotyping of the rs8050136 *FTO* variant** The SNP rs8050136 was selected for genotyping as a proxy for SNPs belonging to one linkage disequilibrium (LD) group including the previously reported rs9939609 [3]. The choice of rs8050136 was based on the better quality of genotyping compared with rs9939609. Genotyping of rs8050136 was performed using the TaqMan allelic discrimination assay (Applied Biosystems) on an ABI PRISM 7500 sequence detector (Applied Biosystems). To assess genotyping reproducibility, a random ~10% selection of the samples was re-genotyped in all SNPs with 100% concordance. Genotype data were consistent with Hardy–Weinberg equilibrium ( $p > 0.05$ ).

**Statistical analyses** Data are shown as means  $\pm$  SD unless stated otherwise. Before statistical analysis, non-normally distributed parameters were logarithmically transformed to approximate a normal distribution. Expression differences between visceral and subcutaneous adipose tissue were assessed using the paired Student's *t* test. Linear relationships were assessed by least square regression analysis. Multivariate linear relationships were assessed by a general linear model. Associations between rs8050136 and *FTO* mRNA expression levels were assessed using generalised linear regression models under the additive and dominant/recessive mode of inheritance. Statistical analysis was performed using SPSS version 12.0 (SPSS, Chicago, IL, USA). We considered *p* values  $< 0.05$  to be statistically significant.

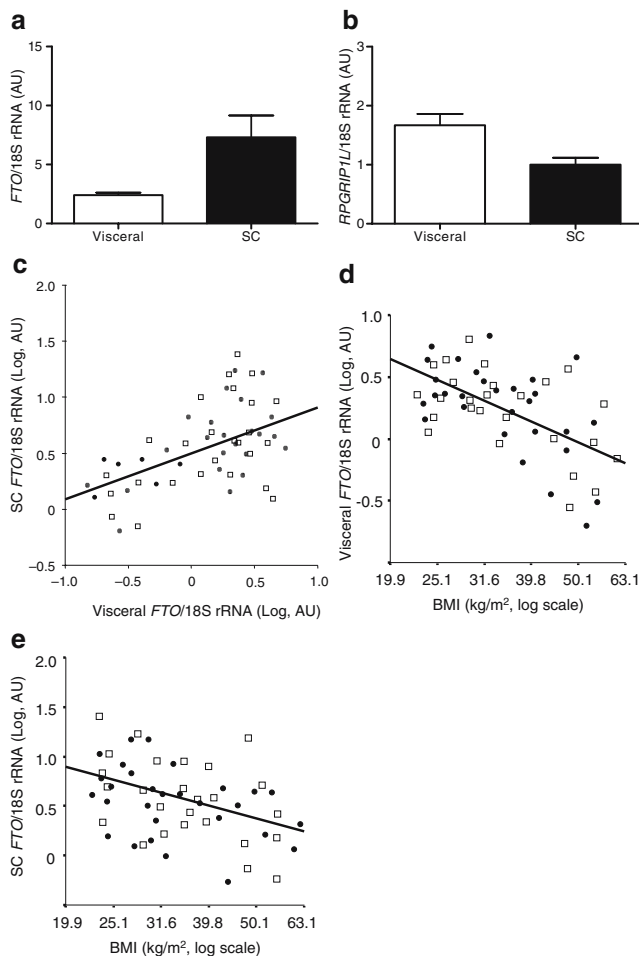
## Results

**Participant characteristics** The study population comprised 55 Europid volunteers (26 men, 29 women) and was characterised by a mean age of 61  $\pm$  15 years, mean BMI of 34.3  $\pm$  9.5 kg/m<sup>2</sup> (women: 37.7  $\pm$  10.7 kg/m<sup>2</sup>; men: 30.4  $\pm$  6.1 kg/m<sup>2</sup>,  $p < 0.05$ ), mean waist circumference of 119  $\pm$  26 cm (women: 121  $\pm$  28 cm; men: 115  $\pm$  23 cm) and mean body fat mass of 36  $\pm$  11% (women: 40.6  $\pm$  11.1%; men: 30.8  $\pm$  8.4%,  $p < 0.05$ ). Analysis of 55 paired samples of visceral and subcutaneous adipose tissue revealed threefold higher *FTO* mRNA levels in subcutaneous than in visceral adipose tissue, while *RPGRIP1L* mRNA expression was 1.6-fold higher in visceral than in subcutaneous adipose tissue (Fig. 1a,b). *FTO* and *RPGRIP1L* mRNA gene expression was not different between men and women in either fat depot. Moreover, subcutaneous and visceral *FTO* mRNA expression were significantly correlated (Fig. 1c).

**Associations of *FTO* expression** Visceral *FTO* mRNA was significantly correlated with BMI (Fig. 1d), per cent body fat, sex and age, but not with visceral fat area (Table 1). Univariate regression analyses revealed further significant correlations between subcutaneous *FTO* mRNA expression and BMI (Fig. 1e), per cent body fat, subcutaneous fat area, age, sex and leptin (Table 1). The relationship between *FTO* mRNA expression and BMI was significant independently of sex. However, in our study population the most obese participants were also the youngest and upon statistical adjustment for BMI, the age relationship disappeared (Table 2). Since the univariate correlation with BMI was much stronger, the age effect was most probably secondary to BMI. In a stepwise regression analysis age did not enter the model after BMI at a *p* level of 0.05. There was no correlation between leptin and adiponectin serum concentrations, and visceral or subcutaneous *FTO* mRNA expression beyond the associations of these adipokines with BMI or per cent body fat.

The association between *FTO* expression and BMI remained significant upon adjustment for age and sex in the visceral ( $p = 0.003$ ), but not in the subcutaneous depot ( $p = 0.08$ ; Table 2). Since no functional information is available on how *FTO* might contribute to human obesity, we also modelled the data by reverse logic, i.e. BMI as dependent variable (Table 3). Interestingly, visceral *FTO* mRNA expression remained an independent predictor of BMI even after adjustment for subcutaneous *FTO* mRNA expression.

There were no significant relationships between both visceral and subcutaneous *FTO* expression and fasting plasma glucose concentration, HbA<sub>1c</sub> and parameters of insulin sensitivity, including fasting plasma insulin concen-



**Fig. 1** *FTO* mRNA and *RPGRIP1L* mRNA expression in individuals with wide ranges of obesity, body fat distribution, insulin sensitivity and glucose tolerance. Men, white bars/symbols,  $n=26$ ; women, black bars/symbols,  $n=29$ . **a** *FTO* mRNA and **b** *RPGRIP1L* mRNA expression in visceral and subcutaneous (SC) adipose tissue. Values are means  $\pm$  SEM.  $p=0.01$ . **c** Correlation between visceral and SC *FTO* mRNA expression ( $n=55$ ).  $p=0.016$ ;  $r^2=0.11$ . **d** Correlations between visceral and **(e)** SC *FTO* mRNA expression and BMI.  $p<0.001$ ;  $r^2=0.27$  (**d**);  $p=0.018$ ;  $r^2=0.11$  (**e**). Human *FTO* and *RPGRIP1L* mRNA expression was calculated relative to the mRNA expression of 18S rRNA. Data were log transformed to achieve normal distribution. AU, arbitrary units

trations and glucose uptake during the steady state of a euglycaemic–hyperinsulinaemic clamp (Table 1).

In contrast to *FTO*, no correlation of *RPGRIP1L* mRNA expression with anthropometric and metabolic parameters was seen in either fat depot (Table 1).

*Adipose tissue FTO mRNA expression in function of rs8050136* The SNP rs8050136 was selected for genotyping as a proxy for SNPs belonging to one LD group based on HapMap Rel 21a/phaseII ([http://www.hapmap.org/cgi-perl/gbrowse/hapmap\\_B36/](http://www.hapmap.org/cgi-perl/gbrowse/hapmap_B36/)). This LD group includes rs9939609, which is a common variant previously shown to be strongly associated with obesity [3]. No significant

association of rs8050136 with visceral or subcutaneous *FTO* mRNA levels was found (Table 4). However, despite the lack of statistical power due to the small sample size, rs8050136 was still associated with obesity in our study group (data not shown).

## Discussion

Two independent studies recently revealed an association between common variants in the *FTO* gene on chromosome 16q12.2, which have been confirmed in more than 20 independent cohorts [3–5], and BMI. *FTO* is highly expressed in brain, but also detectable in most tissues, including adipose tissue and pancreatic beta cells [3, 9]. The function of the *FTO* gene product and its potential role for human obesity, however, are completely unknown at the moment. It is possible that only central mechanisms related to the control of appetite and satiety are involved. On the other hand, adipose tissue represents a primary candidate for studying the functional relevance of *FTO* in obesity. Conceivably, expression differences between lean and obese individuals could contribute to our understanding of the *FTO* gene product. Based on its genomic position near *FTO*, as well as on similar expression profiles, *RPGRIP1L* is also considered a plausible candidate gene involved in the pathophysiology of obesity [3, 5].

In this study, we provide first descriptive information on the relationship between *FTO* and *RPGRIP1L* mRNA expression in adipose tissue and simple anthropometric parameters including measures of obesity.

*FTO* mRNA expression was threefold higher in subcutaneous than in visceral fat. This difference could be due to a number of factors usually differentiating these two depots including gene transcription, insulin sensitivity and lipolysis, altered adipokine expression and others [13, 14]. Decreasing subcutaneous fat mass does not significantly improve obesity-associated metabolic abnormalities [15], whereas reduction of visceral fat mass has been shown to have significant beneficial effects on glucose metabolism and insulin sensitivity [16]. Our data indicate that fat depot differences in *FTO* expression may contribute to the specific properties of visceral adipose tissue. The observation that *FTO* expression in the two depots was only modestly correlated suggests that in a given individual up- or downregulation in one vs the other depot is controlled locally rather than in a coordinated fashion by systemic hormonal, neuronal and/or nutritional signals.

Independently of the fat depot, we found significant negative correlations between *FTO* gene expression and BMI or per cent body fat. Therefore our data suggest that *FTO* gene expression might be downregulated in response

**Table 1** Univariate linear regression between anthropometric and biochemical parameters and visceral and subcutaneous *FTO* mRNA and *RPGRIP1L* mRNA expression

Parameter	<i>FTO</i> /18S rRNA				<i>RPGRIP1L</i> /18S rRNA			
	Visceral		Subcutaneous		Visceral		Subcutaneous	
	<i>r</i>	<i>p</i> value	<i>r</i>	<i>p</i> value	<i>r</i>	<i>p</i> value	<i>r</i>	<i>p</i> value
Age	0.55	<0.001	0.32	0.03	0.04	0.79	0.05	0.76
Sex	-0.33	0.02	-0.45	0.01	-0.03	0.87	0.23	0.15
Visceral fat area	-0.08	0.64	-0.18	0.27	0.19	0.43	-0.13	0.57
Subcutaneous fat area	-0.06	0.7	-0.31	0.04	0.21	0.37	-0.10	0.67
Per cent body fat	-0.3	0.03	-0.41	0.003	0.16	0.34	-0.04	0.79
Waist circumference	-0.2	0.2	-0.28	0.06	0.35	0.06	0.03	0.87
FPG	-0.27	0.07	-0.19	0.2	0.23	0.16	-0.09	0.57
HbA <sub>1c</sub>	-0.17	0.25	-0.21	0.15	0.06	0.71	-0.29	0.07
Glucose uptake, clamp	-0.02	0.9	0.05	0.8	-0.19	0.33	-0.12	0.54
FPI	-0.06	0.7	-0.04	0.8	0.24	0.14	-0.08	0.63
Leptin	-0.29	0.04	-0.31	0.04	0.17	0.35	0.23	0.20

Participants: *n*=55  
 FPG, fasting plasma glucose; FPI, fasting plasma insulin

to fat accumulation. As indicated by the models (Table 2), this inverse relationship was significantly stronger for the visceral depot. From our correlation analysis it is impossible to extricate the direction of causality. Nevertheless, since genetic variation in *FTO* has been shown to affect the risk of obesity, it is legitimate to test the hypothesis of whether *FTO* expression predicts BMI. We identified visceral *FTO* mRNA expression as a strong predictor of BMI independently of age and sex, and even independently of *FTO* mRNA expression in the subcutaneous depot. This suggests that especially viscerally expressed *FTO* may contribute to obesity.

Obviously, the number of participants in our study was relatively small and the association between BMI and adipose tissue *FTO* mRNA expression levels will require

**Table 2** Multiple regression analysis between age, sex, BMI and *FTO* mRNA expression in visceral and subcutaneous adipose tissue

	β-Coefficient ( <i>p</i> value)	
	(log) Visceral <i>FTO</i> mRNA	(log) Subcutaneous <i>FTO</i> mRNA
<b>Model 1</b>		
Age (log)	0.49 (0.003)	0.17 (0.39)
Sex (female)	-0.08 (0.08)	-0.06 (0.36)
<b>Model 2</b>		
Age (log)	0.26 (0.1)	-0.003 (0.99)
BMI (log)	-0.66 (0.0006)	-0.52 (0.049)
<b>Model 3</b>		
Age (log)	0.27 (0.09)	0.002 (0.99)
Sex (female)	-0.02 (0.59)	-0.01 (0.89)
BMI (log)	-0.62 (0.003)	-0.51 (0.08)

Participants: *n*=55

confirmation in larger cohorts. We also cannot exclude the possibility that specific characteristics of our study population, i.e. the strong negative correlation between age and BMI, may have produced a spurious relationship between *FTO* gene expression and obesity parameters. However, the fact that the BMI association withstood all conceivable adjustment makes us confident that this finding is robust. Adipose *FTO* expression and obesity could be coregulated by a common genetic or environmental factor and not functionally linked to each other. More sophisticated study designs are necessary to dissect causal from correlative relationships with *FTO* expression in fat.

**Table 3** Multivariate linear regression analysis of visceral and subcutaneous *FTO* mRNA expression as predictor of BMI

	BMI	
	β-Coefficient	<i>p</i> value
<b>Model 1</b>		
Age (log)	-0.32	0.003
Sex (female)	0.09	0.008
SC <i>FTO</i> (log)	-0.12	0.08
<b>Model 2</b>		
Age (log)	-0.20	0.06
Sex (female)	0.07	0.02
Visceral <i>FTO</i> (log)	-0.29	0.003
<b>Model 3</b>		
Age (log)	-0.20	0.056
Sex (female)	0.07	0.03
Visceral <i>FTO</i> (log)	-0.26	0.01
SC <i>FTO</i> (log)	-0.07	0.31

Participants: *n*=55  
 SC, subcutaneous

**Table 4** *FTO* and *RPGRIP1L* mRNA expression in visceral and subcutaneous adipose tissue grouped by rs8050136 genotypes

Genotype rs8050136	n	<i>FTO</i> mRNA expression (AU)				<i>RPGRIP1L</i> mRNA expression (AU)			
		Visceral	p value	SC	p value	Visceral	p value	SC	p value
CC	13	2.63±0.39	0.72 (add)	6.44±1.41	0.90 (add)	1.80±0.64	0.74 (add)	1.11±0.33	0.42 (add)
CA	24	2.49±0.34	0.60 (dom)	8.41±3.79	0.73 (dom)	1.81±0.26	0.91 (dom)	1.00±0.18	0.64 (dom)
AA	11	2.22±0.41	0.96 (rec)	6.90±1.72	0.56 (rec)	1.73±0.27	0.69 (rec)	1.03±0.19	0.41 (rec)

Data are means±SEM

Generalised linear models were used to identify significant differences between genotypes under the additive (add), dominant (dom) and recessive (rec) models, after adjustment for age, sex and BMI

AU, arbitrary units; SC, subcutaneous

We did not find an association between *FTO* mRNA levels and either fasting plasma glucose concentrations, HbA<sub>1c</sub> or parameters of insulin sensitivity. However, we cannot rule out the possibility that our sample size was too small to detect significant relationships between *FTO* gene expression and measures of insulin sensitivity and glucose metabolism. At the least, our data suggest that adipose tissue *FTO* expression does not directly affect risk of type 2 diabetes. As such, our results are generally supportive of the findings by Frayling et al. [3], who reported that the original association between *FTO* SNPs and type 2 diabetes disappeared upon adjustment for BMI.

Although expression of *RPGRIP1L* mRNA was also fat depot-specific in our study, it does not, in contrast to *FTO*, seem to be linked to the pathophysiology of obesity as no correlations with anthropometric and metabolic characteristics were found. Our data therefore favour *FTO* rather than *RPGRIP1L* as a possible target affected by reported obesity-associated polymorphisms [3]. Recently, mutations within *RPGRIP1L* have been shown to be responsible for several syndromes such as Joubert or Meckel syndrome [17, 18]. Joubert syndrome is an autosomal recessive multi-system disease characterised by cerebellar ataxia, developmental delay, hypotonia, irregular breathing pattern, eye movement abnormalities and cerebellar vermis hypoplasia and dysplasia with accompanying brainstem defects [18]. Meckel syndrome is an autosomal recessive lethal condition characterised by central nervous system malformation, postaxial polydactyly, cystic kidney dysplasia and ductal proliferation in the portal area of the liver [17, 18]. No obesity phenotype has been reported for these syndromes caused by *RPGRIP1L* mutations.

Given the strength of the correlation between *FTO* expression and adiposity in the present study and the relatively small effect that variation in *FTO* has on BMI as reported by Frayling et al. [3], variation in the *FTO* genotype does not appear to play a major role in regulating *FTO* transcription in adipose tissue. This is supported by the fact that no association between the obesity risk polymorphism and *FTO* and/or *RPGRIP1L* mRNA expression in adipose tissue was found in our study. It is

noteworthy that, despite our study's lack of statistical power due to the small sample size, rs8050136 was still associated with obesity in this study group.

In conclusion, expression of adipose tissue *FTO* mRNA is fat depot-specific and correlates with age, while being negatively correlated with BMI and total body fat. Adipose tissue *FTO* mRNA expression is not related to rs8050136 genotype, which is in LD with the previously reported obesity-associated SNP rs9939609 [3]. However, we were unable to determine the causal direction of the association between obesity and alterations in *FTO* gene expression. Therefore further studies are necessary to elucidate whether changes in adipose tissue *FTO* gene expression are secondary to obesity or causally related.

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**Duality of interest** The authors declare that there is no duality of interest associated with this manuscript.

## References

1. Maes HH, Neale MC, Eaves LJ (1997) Genetic and environmental factors in relative body weight and human adiposity. *Behav Genet* 27:325–351
2. Groop L (2007) From fused toes in mice to human obesity. *Nat Genet* 39:706–707
3. Frayling TM, Timpson NJ, Weedon MN 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
4. Dina C, Meyre D, Gallina S et al (2007) Variation in *FTO* contributes to childhood obesity and severe adult obesity. *Nat Genet* 39:724–726
5. Scuteri A, Sanna S, Chen WM et al (2007) Genome-wide association scan shows genetic variants in the *FTO* gene are associated with obesity-related traits. *PLoS Genet* 3:e115

6. van der Hoeven F, Schimmang T, Volkmann A, Mattei MG, Kyewski B, Ruther U (1994) Programmed cell death is affected in the novel mouse mutant Fused toes (Ft). *Development* 120:2601–2607
7. Peters T, Ausmeier K, Ruther U (1999) Cloning of Fatso (Fto), a novel gene deleted by the Fused toes (Ft) mouse mutation. *Mamm Genome* 10:983–986
8. Stratakis CA, Lafferty A, Taymans SE, Gafni RI, Meck JM, Blancato J (2000) Anisomastia associated with interstitial duplication of chromosome 16, mental retardation, obesity, dysmorphic facies, and digital anomalies: molecular mapping of a new syndrome by fluorescent in situ hybridization and microsatellites to 16q13 (D16S419–D16S503). *J Clin Endocrinol Metab* 85:3396–3401
9. Gerken T, Girard CA, Tung YC et al (2007) The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. *Science* 318:1469–1472
10. DeFronzo RA, Tobin JD, Andres R (1979) Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:214–223
11. Berndt J, Klötting N, Kralisch S et al (2005) Plasma visfatin concentrations and fat depot-specific mRNA expression in humans. *Diabetes* 54:2911–2916
12. Klötting N, Graham TE, Berndt J et al (2007) Serum retinol-binding protein is more highly expressed in visceral than in subcutaneous adipose tissue and is a marker of intra-abdominal fat mass. *Cell Metab* 6:79–87
13. Wajchenberg BL (2000) Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocr Rev* 21:697–738
14. Frayn KN (2000) Visceral fat and insulin resistance—causative or correlative? *Br J Nutr* 83:S71–S77
15. Klein S, Fontana L, Young VL et al (2004) Absence of an effect of liposuction on insulin action and risk factors for coronary heart disease. *N Engl J Med* 350:2549–2557
16. Thorne A, Lonnqvist F, Apelman J, Hellers G, Arner P (2002) A pilot study of long-term effects of a novel obesity treatment: omentectomy in connection with adjustable gastric banding. *Int J Obes Relat Metab Disord* 26:193–199
17. Delous M, Baala L, Salomon R et al (2007) The ciliary gene RPGRIP1L is mutated in cerebello-oculo-renal syndrome (Joubert syndrome type B) and Meckel syndrome. *Nat Genet* 39: 875–881
18. Arts HH, Doherty D, van Beersum SEC et al (2007) Mutations in the gene encoding the basal body protein RPGRIP1L, a nephrocystin-4 interactor, cause Joubert syndrome. *Nat Genet* 39:882–888