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Associations of the *FTO* rs9939609 variant with discrete body fat depots and dietary intake in a multi-ethnic cohort

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Summary

The fat mass and obesity associated (*FTO*) gene has been implicated with obesity and dietary intake predominantly in European populations. We assessed the association between the *FTO* rs9939609 variant with body fat distribution and dietary intake in a multi-ethnic population. Aboriginal, Chinese, European and South Asian participants living in Canada ($n=706$) were assessed for body fat and inner-abdominal fat using imaging techniques, dietary intake and genotyped for the *FTO* rs9939609 variant. Linear regression was used to study the associations between the minor allele of the variant and measures of adiposity and dietary intake. Minor allele frequencies were: Aboriginals (17%), Chinese (17%), Europeans (39%) and South Asians (31%). The rs9939609 variant was associated with intake of dietary macronutrients in Aboriginals and Europeans only. In the total population, there were positive associations between the rs9939609 minor allele and greater fat mass ($0{\cdot}94\pm0{\cdot}56$ kg, $P=0{\cdot}045$), per cent body fat ($0{\cdot}7\pm0{\cdot}4$%, $P=0{\cdot}031$), relative greater subcutaneous abdominal adipose tissue ($4{\cdot}9\pm2{\cdot}8$%, $P=0{\cdot}039$) and percent daily calories from fat ($0{\cdot}4\pm0{\cdot}2$%, $P=0{\cdot}064$). Our findings suggest that the *FTO* rs9939609 minor allele may be associated with dietary intake in adults and is positively associated with regional fat deposition.

1. Introduction

The fat mass and obesity associated (*FTO*) gene is associated with body mass index (BMI), waist circumference (WC) and obesity risk (Frayling *et al.*, 2007; Willer *et al.*, 2009), and has been implicated in food and total energy intakes (Cecil *et al.*, 2008; Speakman *et al.*, 2008). These studies have been mainly in people of European origin but in the coming years, the greatest increases in obesity rates will occur in Asian countries, therefore, investigation of genetic associations in these populations is warranted. Genome-wide association studies have identified at least 50 genetic loci that are associated with obesity-related traits. The locus that was first identified as an obesity-susceptibility locus is the fat mass and obesity associated (*FTO*) gene in which genetic variation (e.g. the rs993609 SNP) has been convincingly associated with BMI, WC and obesity risk (Frayling *et al.*, 2007; Andreasen *et al.*, 2008; Bauer *et al.*, 2009; Willer *et al.*, 2009). To date, only a handful of studies have investigated associations between the rs9939609 variant with obesity in Asian populations. These studies reported the rs9939609 variant to be associated with increased BMI and/or WC in Chinese (Chang *et al.*, 2008; Li *et al.*, 2010) and South Asian people (Al-Attar *et al.*, 2008). Furthermore, this association has also been reported in North American Aboriginals (Al-Attar *et al.*, 2008; Rong *et al.*, 2009). However, not all of these studies are consistent as several others have found no associations in these populations, and none was conducted in multiple

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ethnic groups living in the same environment (Al-Attar *et al*., 2008; Li *et al*., 2008; Rong *et al*., 2009; Yajnik *et al*., 2009).

Many of the above studies have focused on anthropometric measures, but there is general agreement that BMI does not distinguish between individuals of increased weight due to excess body fat or greater muscle mass. The WC is limited in its ability to reflect total body fat and is unable to discriminate between the more metabolically active inner abdominal adipose tissue (visceral adipose tissue (VAT)) and subcutaneous abdominal adipose tissue (SAT). Our earlier research has indicated that Chinese and South Asians have a unique phenotype of increased VAT compared with Europeans of the same body size (Lear *et al*., 2007*a*, *b*). Therefore, the purpose of this investigation was to assess the association of the *FTO* rs9939609 variant with measures of body fat using precise imaging techniques in a multi-ethnic adult population and report on the effect sizes. We further assessed the association of the *FTO* variants with total and macronutrient energy intakes because studies have shown associations between homozygosity for the rs9939609 minor allele with food and total energy intakes in children (Cecil *et al*., 2008; Speakman *et al*., 2008; Wardle *et al*., 2008, 2009).

2. Materials and methods

Participants for this study were from the Multicultural Community Health Assessment Trial (M-CHAT) (Lear *et al*., 2006). Apparently healthy men and women between 30 and 65 years of age were recruited from the greater Vancouver area in Canada of either Aboriginal (reserve and non-reserve residents), Chinese (China, Hong Kong and Taiwan), European (continental Europe, Ireland and UK) or South Asian (Bangladesh, India, Nepal, Pakistan and Sri Lanka) origin. Based on self-report, participants must have indicated that all known ancestors were either of Aboriginal origin or descended from one of the three geographical areas. Due to the high prevalence of mixed ethnic origins in Aboriginal populations, Aboriginals with at least three grandparents of exclusive Aboriginal origin were recruited into the main M-CHAT study; however, only those who reported that all four grandparents were of exclusive Aboriginal origin were included in the present analyses. Of the 828 M-CHAT participants recruited, 742 provided consent for DNA collection, after excluding Aboriginal participants without four Aboriginal grandparents, the sample size available for analysis was 706 (131 Aboriginals, 202 Chinese, 184 Europeans and 189 South Asians). The study was approved by the Simon Fraser University and Children and Family Research Institute Research Ethics Boards.

(i) *Participant assessment*

Participants were assessed for diet, anthropometry, intra-abdominal fat, total body fat and provided a blood sample for DNA extraction. All data were collected on the same day, except for the blood sample that was collected within 3 weeks of the main assessment.

(ii) *Body composition assessment*

Weight was assessed using a balance-beam scale with participants in light street clothing, footwear removed and pockets emptied. Height was assessed by stadiometer and BMI was calculated as weight in kilograms divided by height in metres squared. Waist circumference was recorded in centimetres as the average of two measures taken against the skin at maximal narrowing of the waist following a normal expiration. Hip circumference was recorded in centimetres as the average of two measures taken at the point of maximal gluteal protuberance from the lateral view over undergarments.

Inner abdominal fat was assessed by computer tomography (CT) scan using a CTi Advantage scanner (General Electric, Milwaukee, WI). A cross-sectional 10 mm slice at the L4/L5 intrevertebral disc was obtained and the attenuation range of −190 to −30 Hounsfield units was used to identify adipose tissue. Computation of surface areas from the CT scans was conducted using SliceOmatic 4.2 medical imaging software (SliceOmatic v.4.2, Tomovision, Montreal). Total abdominal fat was calculated as all pixels within the attenuation range and VAT was defined as adipose tissue within the inside edge of the abdominal wall. SAT was the difference between total abdominal adipose tissue and VAT.

Total body fat was assessed by dual energy X-ray absorptiometry with a Norland XR-36 scanner (Norland Medical Systems, White Plains, NY) using Host Software Version 3.9.4 and Scanner Software 2.1.0. Where possible, participants removed all jewellery and metallic objects that could potentially affect the scan results. In some instances, participants could not remove rings from fingers or bracelets from their wrist. Per cent total body fat was calculated as total body fat divided by total body mass. Peripheral fat mass was the sum of fat mass in the arms and legs. Per cent peripheral fat mass was calculated as peripheral fat mass divided by total body mass.

(iii) *DNA extraction and genotyping*

DNA was extracted from whole blood samples using the QIAamp DNA Blood kit (Qiagen) following the manufacturer's suggested protocol. Genotyping of the *FTO* rs9939609 variant was performed by

real-time PCR using TaqMan pre-designed SNP genotyping assays (Applied Biosystems) and a 7500 Real-Time PCR System (Applied Biosystems with a call rate of 100 %). Table 1 presents data on the minor allele and genotype frequencies. The rs9939609 variant was in Hardy–Weinberg equilibrium in each ethnic group ($P > 0{\cdot}05$).

(iv) *Statistical analyses*

Continuous variables are presented as means ± SD, and categorical variables as percentages and counts. Visceral adipose tissue and SAT had non-normal distributions and were log transformed prior to analyses. Within each ethnic group additive linear regression was used to identify the association of the rs9939609 genotype with the anthropometric, body fat and dietary data adjusted for age and sex. As the associations between the *FTO* genotype and per cent daily calories from carbohydrates were in opposite directions for the Aboriginals and Europeans, we also tested for an inter-ethnic interaction for the dietary outcomes. There were 34 participants with incomplete dietary records, resulting in 672 participants included in the regression models with the dietary outcomes. To ensure that insignificant *P* values were not due to the low sample sizes within each ethnic group, we conducted an analysis combining all ethnic groups using the same linear models previously described and adding three indicator variables to account for inter-ethnic differences. Based on an effect size for the rs9939609 minor allele of 0·34 % of the variance in BMI (Willer *et al.*, 2009) and an alpha of 0·1 (one-sided), our power to detect an association is 46·3 % power (for a power of 80 %, a sample of 1813 participants is need). All analyses were conducted using *R* and the α-level was set at $<0{\cdot}05$ (one-sided) for significance.

3. Results

Table 1 outlines the minor allele and genotype frequencies, and the outcome variables. The minor allele and genotype frequencies differed among the ethnic groups ($P < 0{\cdot}001$). Europeans and South Asians had greater minor allele frequencies compared with the Aboriginals and Chinese ($P < 0{\cdot}001$ for all comparisons). There were no differences between the Europeans and South Asians, or the Aboriginals and Chinese. Dietary and anthropometric measures were significantly different across the four ethnic groups ($P < 0{\cdot}001$ for all).

In Aboriginals, the rs9939609 minor allele was associated with a $-2{\cdot}2 \pm 1{\cdot}3$ and $1{\cdot}1 \pm 0{\cdot}6$ % absolute change in per cent daily calories from carbohydrate ($P = 0{\cdot}049$) and proteins ($P = 0{\cdot}029$), respectively (Table 2). In Europeans, the rs9939609 minor allele was associated with a $9{\cdot}7 \pm 5{\cdot}7$ % relative increase in VAT ($P = 0{\cdot}047$) and a $2{\cdot}3 \pm 0{\cdot}9$ % absolute increase in percent daily calories from carbohydrates ($P = 0{\cdot}007$). There was a significant inter-ethnic interaction between ethnicity (Aboriginal versus European) and the *FTO* genotype for per cent daily calories from carbohydrates ($P = 0{\cdot}004$), there were no other significant interactions for dietary outcomes (data not shown). There were no other significant associations within the ethnic groups. When the four ethnic groups were analysed together (Table 2), there were significant associations between the rs9939609 minor allele with greater fat mass ($0{\cdot}94 \pm 0{\cdot}56$ kg, $P = 0{\cdot}045$), per cent body fat ($0{\cdot}7 \pm 0{\cdot}4$ %, $P = 0{\cdot}031$) and relative greater SAT body fat ($4{\cdot}9 \pm 2{\cdot}8$ %, $P = 0{\cdot}039$).

4. Discussion

The purpose of this study was to assess the association and report effect sizes between the rs9939609 SNP in the *FTO* gene with direct measures of body fat using imaging techniques in a multi-ethnic cohort. We found differences in minor allele frequency among the four groups such that the frequency of the minor allele was highest in the European and South Asian groups, and lowest in Aboriginal and Chinese groups. In Aboriginals and Europeans, the minor allele was associated with dietary intake and VAT (Europeans only). When the four ethnic groups were analysed together, the *FTO* rs9939609 minor allele was also associated with body fat measures and SAT.

The minor allele frequencies reported in the present study are similar to that found in a number of studies investigating similar ethnic groups (Al-Attar *et al.*, 2008; Li *et al.*, 2008; Sanghera *et al.*, 2008; Rong *et al.*, 2009; Yajnik *et al.*, 2009). Our investigation adds to this by providing direct comparisons of allele frequency in these ethnic groups recruited under the same criteria and indicates that the minor allele frequency is no different in Europeans and South Asians, and higher in Aboriginals and Chinese, with no difference between these two latter groups.

Although not significant, our per minor allele effect size on BMI was highest in Aboriginals (0·75 kg/m²) and similar to that reported by Rong *et al.* (0·8 kg/m²) (Rong *et al.*, 2009). Studies in Chinese populations have reported the *FTO* rs9939609 variant to be associated with increased obesity (Li *et al.*, 2010) with the effect sizes of the minor allele for BMI ranging from 0·37 to 0·68 kg/m² (Chang *et al.*, 2008; Tan *et al.*, 2008), which is somewhat higher than what we reported. However, not all studies are consistent as Li *et al.* did not report an association between the minor allele and BMI in 3210 Chinese men and women (Li *et al.*, 2008). For Europeans, our per minor allele effect size for BMI was 0·22 kg/m² and similar to that

Table 1. *Participant demographics, allele and genotype frequencies, dietary and anthropometric data*

	Aboriginal ($n=131$)	Chinese ($n=202$)	European ($n=184$)	South Asian ($n=189$)	*P* values for inter-ethnic comparisons
Age (years)	45·4 ± 8·1	48·0 ± 8·1	50·8 ± 9·1	45·0 ± 8·4	<0·001
Male gender (%)	66 (50%)	92 (46%)	93 (51%)	100 (53%)	0·527
FTO rs9939609 genotype					<0·001
TT	91 (70%)	140 (70%)	66 (36%)	89 (47%)	
TA	33 (25%)	53 (27%)	92 (50%)	82 (44%)	
AA	6 (5%)	7 (4%)	26 (14%)	17 (9%)	
FTO rs9939609 minor allele frequency	0·173	0·168	0·391	0·309	<0·001
Diet					
Total kilocalories	1784 ± 517	2012 ± 531	2045 ± 654	1748 ± 594	<0·001
Carbohydrate (% daily kilocalories)	50·7 ± 8·1	47·8 ± 8·1	46·8 ± 8·5	55·7 ± 8·5	<0·001
Protein (% daily kilocalories)	15·5 ± 3·7	19·3 ± 4·3	17·3 ± 4·3	16·4 ± 3·7	<0·001
Fat (% daily kilocalories)	33·1 ± 6·9	32·4 ± 6·7	33·9 ± 7·6	27·2 ± 7·5	<0·001
Body mass index (kg/m²)	29·6 ± 5·3	25·7 ± 3·5	27·8 ± 5·1	27·9 ± 5·0	<0·001
Waist circumference (cm)	96·2 ± 12·0	83·1 ± 9·2	89·7 ± 12·7	88·8 ± 12·2	<0·001
Total fat mass (kg)	29·7 ± 10·5	21·3 ± 6·8	27·0 ± 10·6	28·2 ± 10·0	<0·001
Body fat (%)	35·3 ± 9·3	31·1 ± 8·5	32·5 ± 10·0	35·9 ± 9·3	<0·001
Peripheral fat mass (kg)	11·5 ± 3·9	9·2 ± 3·0	11·9 ± 4·8	12·5 ± 4·9	<0·001
Per cent peripheral fat (%)	13·9 ± 4·1	13·5 ± 4·1	14·6 ± 5·1	16·0 ± 5·3	<0·001
Total adipose tissue (cm²)	470·2 ± 155·4	330·8 ± 117·1	411·8 ± 176·7	448·9 ± 164·7	<0·001
Subcutaneous abdominal adipose tissue (cm²)[a]	335·3 (252·3, 427·0)	222·0 (162·6, 281·4)	266·7 (198·9, 385·3)	309·4 (224·4, 391·5)	<0·001
Visceral adipose tissue (cm²)[a]	123·0 (90·4, 162·6)	99·4 (72·6, 123·6)	102·6 (79·1, 145·6)	118·9 (88·3, 162·4)	<0·001

[a] Medians and 25th and 75th percentile values presented.
Data are expressed as means and SD unless otherwise indicated.

Table 2. *Beta coefficients of the FTO rs9939609 minor allele for the dependent body composition and food intake variables*

	Aboriginal ($n=131$)	Chinese ($n=202$)	European ($n=184$)	South Asian ($n=189$)	Total Cohort ($n=706$)
Body mass index (kg/m²)	0·75 ± 0·82 ($P=0·182$)	0·24 ± 0·45 ($P=0·297$)	0·22 ± 0·56 ($P=0·346$)	0·62 ± 0·57 ($P=0·139$)	0·43 ± 0·29 ($P=0·071$)
Waist circumference (cm)	1·4 ± 1·9 ($P=0·220$)	1·1 ± 1·1 ($P=0·160$)	1·0 ± 1·3 ($P=0·216$)	0·0 ± 1·3 ($P=0·500$)	0·7 ± 0·7 ($P=0·147$)
Total fat mass (kg)	1·56 ± 1·51 ($P=0·153$)	0·71 ± 0·81 ($P=0·192$)	1·17 ± 1·10 ($P=0·145$)	0·66 ± 1·12 ($P=0·278$)	0·94 ± 0·56 ($P=0·045$)
Body fat (%)	0·5 ± 1·0 ($P=0·295$)	1·0 ± 0·7 ($P=0·094$)	1·0 ± 0·8 ($P=0·103$)	0·5 ± 0·8 ($P=0·263$)	0·7 ± 0·4 ($P=0·031$)
Peripheral fat mass (kg)	0·69 ± 0·54 ($P=0·101$)	0·18 ± 0·33 ($P=0·290$)	0·53 ± 0·46 ($P=0·127$)	0·16 ± 0·51 ($P=0·375$)	0·38 ± 0·23 ($P=0·053$)
Per cent peripheral fat (%)	0·3 ± 0·4 ($P=0·205$)	0·2 ± 0·3 ($P=0·233$)	0·4 ± 0·3 ($P=0·127$)	0·5 ± 0·4 ($P=0·443$)	0·3 ± 0·2 ($P=0·069$)
Total adipose tissue (cm²)	28·1 ± 23·9 ($P=0·120$)	16·4 ± 14·7 ($P=0·132$)	22·7 ± 19·3 ($P=0·120$)	−1·9 ± 19·2 ($P=0·460$)	13·8 ± 9·5 ($P=0·074$)
Subcutaneous abdominal adipose tissue (% increase)	5·9 ± 5·9 ($P=0·158$)	9·5 ± 5·7 ($P=0·051$)	5·9 ± 5·6 ($P=0·146$)	1·0 ± 5·2 ($P=0·427$)	4·9 ± 2·8 ($P=0·039$)
Visceral adipose tissue (% increase)	1·5 ± 7·2 ($P=0·415$)	7·1 ± 6·0 ($P=0·119$)	9·7 ± 5·7 ($P=0·047$)	−0·1 ± 5·2 (0·465)	4·3 ± 2·9 ($P=0·069$)
Total kilocalories	−54·0 ± 80·9 ($P=0·312$)	−7·3 ± 68·2 ($P=0·457$)	18·9 ± 65·3 ($P=0·387$)	10·7 ± 68·6 ($P=0·438$)	4·2 ± 35·1 ($P=0·0452$)
Carbohydrate (% daily kilocalories)	−2·2 ± 1·3 ($P=0·049$)	0·2 ± 1·1 ($P=0·440$)	2·3 ± 0·9 ($P=0·007$)	−0·9 ± 1·0 ($P=0·188$)	−0·2 ± 0·5 ($P=0·345$)
Protein (% daily kilocalories)	1·1 ± 0·6 ($P=0·029$)	−0·4 ± 0·6 (0·246)	−0·4 ± 0·5 ($P=0·223$)	0·3 ± 0·4 ($P=0·264$)	−0·0 ± 0·3 ($P=0·496$)
Fat (% daily kilocalories)	1·1 ± 1·1 ($P=0·150$)	0·4 ± 0·9 ($P=0·322$)	−1·2 ± 0·8 (0·084)	0·8 ± 0·9 ($P=0·187$)	0·1 ± 0·5 ($P=0·380$)

Adjusted for age and sex. Data are reported as per the allele change and SD with *P* values in parentheses.

reported in larger studies of Europeans (Frayling *et al*., 2007; Willer *et al*., 2009). In South Asians, while this variant is associated with risk for type 2 diabetes (Sanghera *et al*., 2008; Yajnik *et al*., 2009), no associations with BMI have been observed in these and other studies (Al-Attar *et al*., 2008; Tan *et al*., 2008). One study did report a non-significant effect size on BMI of 0·1 kg/m² (Tan *et al*., 2008), which is less than the 0·6 kg/m² we report. Although not significant, our reported effect sizes per minor allele for BMI are similar to that of previous studies apart from that in South Asians in which we report a much higher effect.

We found positive associations between the *FTO* rs9939609 variant with body fat mass, Per cent body fat and SAT when the groups were analysed together. Previous studies have found the *FTO* rs8050136 minor allele to be associated with a 1·9% and 2·5 kg increase in body fat as assessed by bioelectrical impedance and an increase in SAT volume in Europeans (Haupt *et al*., 2008). The more than twofold higher effect sizes reported in this study compared with ours may be the result of the use of bioelectrical impedance compared with the use of DEXA scanning. In another study, the rs1558902 and rs1421085 *FTO* minor alleles were found to be associated with approximately 14·5 cm² (6·5%) more SAT and 5·8 cm² (4·7%) more VAT in Japanese men and women (Hotta *et al*., 2010). These effect sizes are consistent with our findings of 4·9 and 4·3%, respectively. Together, these results suggest that *FTO* may have a role in body fat distribution. Indeed, *FTO* mRNA expression has been shown to be three times higher in SAT than in VAT from 55 lean and obese men (Kloting *et al*., 2008).

We observed an inter-ethnic interaction between Aboriginals and Europeans with respect to the association of the *FTO* rs9939609 variant with per cent daily calories from carbohydrate such that in Aboriginals, the *FTO* rs9939609 variant to be negatively associated with dietary caloric intake from carbohydrates, while the *FTO* rs9939609 variant was positively associated with dietary caloric intake from carbohydrates. It is unclear what the mechanism is for the inter-ethnic differences in dietary intake, but this may be due to the differences in the types of carbohydrate in their diets. We cannot exclude the possibility that there may be inter-ethnic differences in the reporting of dietary intake on the three-day food record. Previous studies have suggested that *FTO* is involved in energy homoeostasis and food preferences (Cecil *et al*., 2008; Tanofsky-Kraff *et al*., 2009), while others have not found this association (Bauer *et al*., 2009; Hakanen *et al*., 2009; Liu *et al*., 2010). Interestingly all but one of these studies was in children; the lone adult one found no association between a different *FTO* variant (rs1121980) and

nutrient intake in women (Bauer *et al*., 2009). At present, it is unclear why our findings are not consistent with the other study in adults, but it may be due to differences in the variants, our assessment of both men and women, and/or the assessment of dietary intake (food frequency questionnaire compared with our three-day food record). As a result, it remains unknown whether the associations reported in children do indeed extend to adults. However, studies in mice indicate that *FTO* mRNA is highly expressed in hypothalamus and regulated by fasting and refeeding (Gerken *et al*., 2007) and while the biological function of *FTO* has not been fully characterized, mice overexpressing one or two additional copies of *FTO* have a dose-dependent increase in body and fat mass and increased food intake (Church *et al*., 2010) supporting a role for *FTO* in food intake and energy homoeostasis.

(i) *Limitations*

The small sample size of our study may have limited the ability to detect certain associations, particularly within ethnic groups. However, our effect sizes for BMI are similar to that reported in previous studies giving us confidence that our analyses of associations with discrete areas of body composition using accurate imaging techniques provide novel information on effect sizes for these outcomes. We must also acknowledge the limitation of multiple comparisons; however, the majority of literature to date would indicate that the FTO variant is associated with measures of body fat and food intake. As our findings are consistent with the previous literature, we believe the likelihood of spurious findings is low. Lastly, as our study is cross-sectional, we are limited to identifying associations only and longitudinal studies investigating changes in body fat over time are needed to identify and understand more clearly the role of *FTO*.

(ii) *Conclusions*

A unique contribution of our investigation is the comparison of precise body fat imaging and dietary measures in a cohort of different ethnic groups recruited from the same environment. Our results confirm that the frequency of the minor allele of the *FTO* rs9939609 variant differs across ethnic groups and the presence of minor allele may be associated with increased body fat and SAT independent of ethnicity. In addition, our finding that the *FTO* rs9939609 variant is associated with energy intake supports a possible role for *FTO* in relation to obesity that has been observed in studies of children. Although our study is limited by a small sample size, our reported per allele effect sizes for BMI are consistent with previous studies. These results suggest that carriers of the minor rs9939609 allele may be at increased risk for excess body fat, and therefore at greater risk for cardiometabolic diseases.

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5. Declaration of Interest

None.

References

Al-Attar, S. A., Pollex, R. L., Ban, M. R., Young, T. K., Bjerregaard, P., Anand, S. S., Yusuf, S., Zinman, B., Harris, S. B., Hanley, A. J., Connelly, P. W., Huff, M. W. and Hegele, R. A. (2008). Association between the FTO rs9939609 polymorphism and the metabolic syndrome in a non-Caucasian multi-ethnic sample. *Cardiovascular Diabetology* **7**, 5.

Andreasen, C. H., Stender-Petersen, K. L., Mogensen, M. S., Torekov, S. S., Wegner, L., Andersen, G., Nielsen, A. L., Albrechtsen, A., Borch-Johnsen, K., Rasmussen, S. S., Clausen, J. O., Sandbaek, A., Lauritzen, T., Hansen, L., Jorgensen, T., Pedersen, O. and Hansen, T. (2008). Low physical activity accentuates the effect of the FTO rs9939609 polymorphism on body fat accumulation. *Diabetes* **57**, 95–101.

Bauer, F., Elbers, C. C., Adan, R. A., Loos, R. J., Onland-Moret, N. C., Grobbee, D. E., van Vliet-Ostaptchouk, J. V., Wijmenga, C. and van der Schouw, Y. T. (2009). Obesity genes identified in genome-wide association studies are associated with adiposity measures and potentially with nutrient-specific food preference. *American Journal of Clinical Nutrition* **90**, 951–959.

Cecil, J. E., Tavendale, R., Watt, P., Hetherington, M. M. and Palmer, C. N. (2008). An obesity-associated FTO gene variant and increased energy intake in children. *New England Journal of Medicine* **359**, 2558–2566.

Chang, Y. C., Liu, P. H., Lee, W. J., Chang, T. J., Jiang, Y. D., Li, H. Y., Kuo, S. S., Lee, K. C. and Chuang, L. M. (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.

Church, C., Moir, L., McMurray, F., Girard, C., Banks, G. T., Teboul, L., Wells, S., Bruning, J. C., Nolan, P. M., Ashcroft, F. M. and Cox, R. D. (2010). Overexpression of FTO leads to increased food intake and results in obesity. *Nature Genetics* **42**, 1086–1092.

Frayling, T. M., Timpson, N. J., Weedon, M. N., Zeggini, E., Freathy, R. M., Lindgren, C. M., Perry, J. R., Elliott, K. S., Lango, H., Rayner, N. W., Shields, B., Harries, L. W., Barrett, J. C., Ellard, S., Groves, C. J., Knight, B., Patch, A. M., Ness, A. R., Ebrahim, S., Lawlor, D. A., Ring, S. M., Ben-Shlomo, Y., Jarvelin, M. R., Sovio, U., Bennett, A. J., Melzer, D., Ferrucci, L., Loos, R. J., Barroso, I., Wareham, N. J., Karpe, F., Owen, K. R., Cardon, L. R., Walker, M., Hitman, G. A., Palmer,

C. N., Doney, A. S., Morris, A. D., Smith, G. D., Hattersley, A. T. and McCarthy, M. I. (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.

Gerken, T., Girard, C. A., Tung, Y. C., Webby, C. J., Saudek, V., Hewitson, K. S., Yeo, G. S., McDonough, M. A., Cunliffe, S., McNeill, L. A., Galvanovskis, J., Rorsman, P., Robins, P., Prieur, X., Coll, A. P., Ma, M., Jovanovic, Z., Farooqi, I. S., Sedgwick, B., Barroso, I., Lindahl, T., Ponting, C. P., Ashcroft, F. M., O'Rahilly, S. and Schofield, C. J. (2007). The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. *Science* **318**, 1469–1472.

Hakanen, M., Raitakari, O. T., Lehtimaki, T., Peltonen, N., Pahkala, K., Sillanmaki, L., Lagstrom, H., Viikari, J., Simell, O. and Ronnemaa, T. (2009). FTO genotype is associated with body mass index after the age of seven years but not with energy intake or leisure-time physical activity. *Journal of Clinical Endocrinology and Metabolism* **94**, 1281–1287.

Haupt, A., Thamer, C., Machann, J., Kirchhoff, K., Stefan, N., Tschritter, O., Machicao, F., Schick, F., Haring, H. U. and Fritsche, A. (2008). Impact of variation in the FTO gene on whole body fat distribution, ectopic fat, and weight loss. *Obesity* (*Silver Spring*) **16**, 1969–1972.

Hotta, K., Nakamura, M., Nakamura, T., Matsuo, T., Nakata, Y., Kamohara, S., Miyatake, N., Kotani, K., Komatsu, R., Itoh, N., Mineo, I., Wada, J., Yoneda, M., Nakajima, A., Funahashi, T., Miyazaki, S., Tokunaga, K., Kawamoto, M., Masuzaki, H., Ueno, T., Hamaguchi, K., Tanaka, K., Yamada, K., Hanafusa, T., Oikawa, S., Yoshimatsu, H., Nakao, K., Sakata, T., Matsuzawa, Y., Nakamura, Y. and Kamatani, N. (2010). Polymorphisms in NRXN3, TFAP2B, MSRA, LYPLAL1, FTO and MC4R and their effect on visceral fat area in the Japanese population. *Journal of Human Genetics* **55**, 738–742.

Kloting, N., Schleinitz, D., Ruschke, K., Berndt, J., Fasshauer, M., Tonjes, A., Schon, M. R., Kovacs, P., Stumvoll, M. and Bluher, M. (2008). Inverse relationship between obesity and FTO gene expression in visceral adipose tissue in humans. *Diabetologia* **51**, 641–647.

Lear, S. A., Birmingham, C. L., Chockalingam, A. and Humphries, K. H. (2006). Study design of the multicultural community health assessment trial (M-CHAT) – a comparison of body fat distribution in four distinct populations. *Ethnicity and Disease* **16**, 96–100.

Lear, S. A., Humphries, K. H., Kohli, S. and Birmingham, C. L. (2007*a*). The use of BMI and waist circumference as surrogates of body fat differs by ethnicity. *Obesity* **15**, 2817–2824.

Lear, S. A., Humphries, K. H., Kohli, S., Chockalingam, A. and Frohlich, J. J. (2007*b*). Visceral adipose tissue accumulation differs according to ethnic background: results of the Multicultural Community Health Assessment Trial (M-CHAT). *American Journal of Clinical Nutrition* **86**, 353–359.

Li, H., Wu, Y., Loos, R. J., Hu, F. B., Liu, Y., Wang, J., Yu, Z. and Lin, X. (2008). Variants in the fat mass- and obesity-associated (FTO) gene are not associated with obesity in a Chinese Han population. *Diabetes* **57**, 264–268.

Li, X., Song, F., Jiang, H., Zhang, M., Lin, J., Bao, W., Yao, P., Yang, X., Hao, L. and Liu, L. (2010). A genetic variation in the fat mass- and obesity-associated gene is associated with obesity and newly diagnosed type 2 diabetes in a Chinese population. *Diabetes Metabolism Research and Reviews* **26**, 128–132.

Liu, G., Zhu, H., Lagou, V., Gutin, B., Stallmann-Jorgensen, I. S., Treiber, F. A., Dong, Y. and Snieder, H. (2010). FTO variant rs9939609 is associated with body mass index and waist circumference, but not with energy intake or physical activity in European- and African–American youth. *BMC Medical Genetics* **11**, 57.

Rong, R., Hanson, R. L., Ortiz, D., Wiedrich, C., Kobes, S., Knowler, W. C., Bogardus, C. and Baier, L. J. (2009). Association analysis of variation in/near FTO, CDKAL1, SLC30A8, HHEX, EXT2, IGF2BP2, LOC387761, and CDKN2B with type 2 diabetes and related quantitative traits in Pima Indians. *Diabetes* **58**, 478–488.

Sanghera, D. K., Ortega, L., Han, S., Singh, J., Ralhan, S. K., Wander, G. S., Mehra, N. K., Mulvihill, J. J., Ferrell, R. E., Nath, S. K. and Kamboh, M. I. (2008). Impact of nine common type 2 diabetes risk polymorphisms in Asian Indian Sikhs: PPARG2 (Pro12Ala), IGF2BP2, TCF7L2 and FTO variants confer a significant risk. *BMC Medical Genetics* **9**, 59.

Speakman, J. R., Rance, K. A. and Johnstone, A. M. (2008). Polymorphisms of the FTO gene are associated with variation in energy intake, but not energy expenditure. *Obesity* **16**, 1961–1965.

Tan, J. T., Dorajoo, R., Seielstad, M., Sim, X. L., Ong, R. T., Chia, K. S., Wong, T. Y., Saw, S. M., Chew, S. K., Aung, T. and Tai, E. S. (2008). FTO variants are associated with obesity in the Chinese and Malay populations in Singapore. *Diabetes* **57**, 2851–2857.

Tanofsky-Kraff, M., Han, J. C., Anandalingam, K., Shomaker, L. B., Columbo, K. M., Wolkoff, L. E., Kozlosky, M., Elliott, C., Ranzenhofer, L. M., Roza, C. A., Yanovski, S. Z. and Yanovski, J. A. (2009). The FTO gene rs9939609 obesity-risk allele and loss of control over eating. *American Journal of Clinical Nutrition* **90**, 1483–1488.

Wardle, J., Carnell, S., Haworth, C. M., Farooqi, I. S., O'Rahilly, S. and Plomin, R. (2008). Obesity associated genetic variation in FTO is associated with diminished satiety. *Journal of Clinical Endocrinology and Metabolism* **93**, 3640–3643.

Wardle, J., Llewellyn, C., Sanderson, S. and Plomin, R. (2009). The FTO gene and measured food intake in children. *International Journal of Obesity* (*London*) **33**, 42–45.

Willer, C. J., Speliotes, E. K., Loos, R. J., Li, S., Lindgren, C. M., Heid, I. M., Berndt, S. I., Elliott, A. L., Jackson, A. U., Lamina, C., Lettre, G., Lim, N., Lyon, H. N., McCarroll, S. A., Papadakis, K., Qi, L., Randall, J. C., Roccasecca, R. M., Sanna, S., Scheet, P., Weedon, M. N., Wheeler, E., Zhao, J. H., Jacobs, L. C., Prokopenko, I., Soranzo, N., Tanaka, T., Timpson, N. J., Almgren, P., Bennett, A., Bergman, R. N., Bingham, S. A., Bonnycastle, L. L., Brown, M., Burtt, N. P., Chines, P., Coin, L., Collins, F. S., Connell, J. M., Cooper, C., Smith, G. D., Dennison, E. M., Deodhar, P., Elliott, P., Erdos, M. R., Estrada, K., Evans, D. M., Gianniny, L., Gieger, C., Gillson, C. J., Guiducci, C., Hackett, R., Hadley, D., Hall, A. S., Havulinna, A. S., Hebebrand, J., Hofman, A., Isomaa, B., Jacobs, K. B., Johnson, T., Jousilahti, P., Jovanovic, Z., Khaw, K. T., Kraft, P., Kuokkanen, M., Kuusisto, J., Laitinen, J., Lakatta, E. G., Luan, J., Luben, R. N., Mangino, M., McArdle, W. L., Meitinger, T., Mulas, A., Munroe, P. B., Narisu, N., Ness, A. R., Northstone, K., O'Rahilly, S., Purmann, C., Rees, M. G., Ridderstrale, M., Ring, S. M., Rivadeneira, F., Ruokonen, A., Sandhu, M. S., Saramies, J., Scott, L. J., Scuteri, A., Silander, K., Sims, M. A., Song, K., Stephens, J., Stevens, S., Stringham, H. M.,

Tung, Y. C., Valle, T. T., Van Duijn, C. M., Vimaleswaran, K. S., Vollenweider, P., Waeber, G., Wallace, C., Watanabe, R. M., Waterworth, D. M., Watkins, N., Witteman, J. C., Zeggini, E., Zhai, G., Zillikens, M. C., Altshuler, D., Caulfield, M. J., Chanock, S. J., Farooqi, I. S., Ferrucci, L., Guralnik, J. M., Hattersley, A. T., Hu, F. B., Jarvelin, M. R., Laakso, M., Mooser, V., Ong, K. K., Ouwehand, W. H., Salomaa, V., Samani, N. J., Spector, T. D., Tuomi, T., Tuomilehto, J., Uda, M., Uitterlinden, A. G., Wareham, N. J., Deloukas, P., Frayling, T. M., Groop, L. C., Hayes, R. B., Hunter, D. J., Mohlke, K. L., Peltonen, L., Schlessinger, D., Strachan, D. P., Wichmann, H. E., McCarthy, M. I., Boehnke, M., Barroso, I., Abecasis, G. R. and Hirschhorn, J. N. (2009). Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. *Nature Genetics* **41**, 25–34.

Yajnik, C. S., Janipalli, C. S., Bhaskar, S., Kulkarni, S. R., Freathy, R. M., Prakash, S., Mani, K. R., Weedon, M. N., Kale, S. D., Deshpande, J., Krishnaveni, G. V., Veena, S. R., Fall, C. H., McCarthy, M. I., Frayling, T. M., Hattersley, A. T. and Chandak, G. R. (2009). FTO gene variants are strongly associated with type 2 diabetes in South Asian Indians. *Diabetologia* **52**, 247–252.