Obesity: exploring neural pathophysiological pathways and improving diagnostic strategies

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Chapter 4

Association between the fat mass and obesity-associated gene risk allele, rs9939609A, and reward-related brain structures

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Obesity 2015 Oct;23:2118-22

ABSTRACT

Objective

Recently, the fat mass and obesity-associated gene (*FTO*) has been identified as a genetic risk factor for developing obesity. The underlying mechanisms remain speculative. Recently, SNPs within *FTO* have been associated with brain atrophy in frontal and occipital regions, suggesting that *FTO* might affect body weight through cerebral pathways. Behavioural studies suggested a relationship between *FTO* and the reward-related behavioural traits. We therefore investigated the relationship between the *FTO* risk-allele rs9939609A and volumes of reward-related brain structures.

Design and Methods

492 Dutch individuals (56% males, age: 70 to 82 years) participating in the PROSPER-study underwent a 3D-T1-w MRI to assess the volumes of reward-related brain structures (e.g. amygdala, nucleus accumbens) and of grey matter and white matter. Linear regression analysis was performed to test for the association of subcortical and cortical structures with rs9939609A.

Results

rs9939609A is associated with lower volumes of the nucleus accumbens (p=0.03) and trended towards lower cortical grey matter volumes (p=0.08). This association is independent of gender, age, and BMI, FDR corrected.

Conclusion

The *FTO* risk-allele is associated with lower nucleus accumbens volumes, suggesting that the higher body weight of risk-allele carriers might be due to changes within reward-related brain structures.

INTRODUCTION

For several years it has been known that alleles within *FTO* are associated with increased BMI. In 2007, Frayling et al. reported a risk allele, rs9939609A, within *FTO*, which was strongly associated with increased BMI. The per-A allele odds ratio for obesity was 1.31 (95% CI 1.23-1.39; $P = 6 \times 10^{-16}$) and for overweight 1.19 (95% CI 1.13-1.24; $P = 2 \times 10^{-17}$), compared to the T allele(1). In the following years, numerous studies confirmed the association between this and other single nucleotide polymorphisms (SNPs) within the *FTO* gene and BMI in various populations(2-4).

The *FTO* gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase(5). Cell models have shown that overexpression of FTO reduces ghrelin mRNA N6-methyladenosine and concomitantly increases ghrelin peptide levels. As such, it appears to play an important role in hypothalamic signalling of homeostatic hunger and satiation by influencing ghrelin production(6, 7). Furthermore, intronic variants within *FTO* have been shown to regulate expression of *IRX3* in the hypothalamus, a gene well known for its role in the regulation of body mass and body composition(8). These findings partially explain the earlier reported changes in eating behaviour such as impaired satiety responsiveness and increased food intake in carriers of risk alleles(9-12).

Studies investigating the association between *FTO* and feeding behaviour traits, however, have also shown an association between *FTO* risk alleles and traits such as loss of eating control, emotional control, self-regulation and symptoms of ADHD. These traits have been found to be under the influence of reward-related brain structures(13-15). This poses the question whether *FTO* also influences reward-related brain structures.

The association between BMI and brain volume has been well described, especially in regions associated with the regulation of taste, reward, and eating behaviour(16-25), such as the hippocampus(22, 24), amygdala(20, 24), thalamus(22), (orbito)frontal structures(18, 21-23), and putamen(21). Moreover, a recent study indicated that *FTO* SNPs were also associated with regional brain volume changes using a tensor-based morphometric approach(17).

The major aim of the present study was to investigate whether the *FTO* risk allele, rs9939609A, is associated with specific volume differences in the reward-system.

METHODS

Study population

Data were drawn from the nested MRI sub-study of the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER). Of the 1,095 eligible Dutch participants for PROSPER, 554 were randomly selected for MRI. Of these 554 subjects, 492 (56% males) had a suc-

cessful MRI (i.e. successful high resolution T1-w MRI scan, without excessive artefacts) and *FTO* gene analysis. Subjects undergoing an MRI exam did not differ in terms of age, gender, medical history and cardiovascular risk profile from the entire group.

PROSPER is a double-blind, randomized, placebo-controlled trial aimed at assessing the effect of pravastatin therapy on vascular events in subjects with vascular disease or at risk of vascular disease (26) in the elderly. Inclusion criteria for PROSPER were: age 70 to 82 years; total cholesterol 4.0-9.0 mmol/L; or stroke, transient ischemic attack, myocardial infarction, arterial surgery, or amputation for vascular disease >6 months before study entry; or ≥1 of the following risk factors for vascular disease: current smoker, hypertension (currently receiving drug treatment), or known diabetes mellitus or fasting blood glucose >7 mmol/L. (24).

All data including MRI were recorded at baseline of PROSPER. The mean BMI at the time of intake was 26.8 kg/m^2 (standard deviation=3.6). Systolic and diastolic blood pressure were recorded in a sitting position using a fully automatic electronic sphygmomanometer (Omron M4, Kyoto, Japan). None of the participants had been diagnosed with Minimal Cognitive Impairment (MCI) or Alzheimers Disease (AD) prior to the first visit as was established by extensive questioning of their medical history. Furthermore, subjects underwent a Mini Mental State Examination (MMSE)(27), and were excluded from PROSPER if MMSE <24 at baseline. All participants gave written informed consent.

MRI acquisition

All analyses in this study were based on 3D-T1-weighted gradient-echo MRI scans obtained at 1.5T (Philips Medical Systems, Best, The Netherlands). Acquisition parameters were: repetition time (TR) = 30 msec; echo time (TE) = 4.6 msec; flip angle = 30°; slice thickness = 1.5 mm; 120 slices; no interslice gap; field of view (FOV) = 220 x 220 mm, and a matrix size of 256 x 256.

MRI post-processing and analysis

All MRI scans were analysed using different tools of FSL (FMRIB Software Library)(28, 29). Whole brain volume, grey and white matter volumes were calculated using the FSL- SIENAX tool (Structural Image Evaluation, using Normalization, of Atrophy). SIENAX starts by extracting brain and skull images from the single whole-head input data. The brain image is then affine-registered to MNI152 space, using the skull image to determine the registration scaling. This is done in order to obtain the volumetric scaling factor, to be used as a normalization for head size. Tissue-type segmentation with partial volume estimation is performed to calculate the total volume of brain tissue, including separate estimates of grey matter and white matter volumes.

To assess local differences in cortical thickness, FSL-VBM, a voxel-based morphometry analysis was performed. First, structural images were brain-extracted using the Brain

Extraction Tool. Subsequently, tissue-type segmentation was carried out using FAST4 (FMRIB's Automated Segmentation Tool). The resulting gray matter partial volume images were aligned to MNI152 standard space using the affine registration tool FLIRT (FMRIB's Linear Image Registration Tool), followed by nonlinear registration. The resulting images were averaged to create a study-specific template, to which the native gray matter images were non-linearly re-registered. To correct for local expansion or contraction, the registered partial volume images were modulated by dividing them by the Jacobian of the warp field. The modulated segmented images were smoothed with an isotropic Gaussian kernel with a sigma of 3 mm. Finally, a voxel-wise general linear model was applied using permutation-based non-parametric testing, correcting for multiple comparisons across space (False Discovery Rate: 5%).

To determine the volume of the brain stem and the volumes of the subcortical twin structures nucleus accumbens, amygdala, caudate nucleus, hippocampus, pallidum, putamen and thalamus, FMRIB's Integrated Registration and Segmentation Tool (FIRST) was used. FIRST starts by registering all images to MNI152 templates. It then fits models for all different structures (meshes) to the images, and finally applies boundary correction for the volumetric output.

Genetic analysis

The genotyping of the *FTO* rs9939609 polymorphism [intronic nucleotide substitution T>A; reported global minor allele frequency 0.34, [http://www.ncbi.nlm.nih.gov/projects/SNP] was carried out using real-time PCR with TaqMan SNP Genotyping Assays from Applied Biosystems (Foster City, California, USA) at the Tufts University in Boston. The minor allele frequency in our cohort was 0.38, which is in accordance with various European populations reported previously(1, 3, 30). Genotyping success rate for the entire cohort was 99.1%. χ^2-testing was performed to see whether our population was within Hardy-Weinberg equilibrium for rs9939609.

Statistical analysis of baseline data

If not otherwise stated, data are presented as mean with standard error (SE). Medical history was established via a questionnaire prior to the first visit. Chi-square tests were used to test for differences in gender, history of hypertension, history of vascular disease, history of myocardial infarction (MI) or history of diabetes mellitus (DM) between groups.

Statistical analysis of relationship between genotype and reward related brain structures

For all continuous variables regression analysis was used. A linear regression model was used to assess the association of various brain tissue volume measurements with TT, TA and AA subgroups. In model 1, we corrected for age and gender. To exclude the effects

of BMI on brain structures, we additionally added BMI as a covariate (model 2). We corrected for multiple comparisons using a False Discovery Rate approach for both models. For all statistical analyses, SPSS software for windows (version 20.0.0.1; SPSS) was used.

RESULTS

Genetic testing

The *FTO* rs9939609 polymorphism's minor allele (A) frequency (MAF) was 0.38. The *FTO* TT variant was found in 185 of the 492 subjects (38%), the TA variant was found in 236 of the 492 subjects (48%), and the AA variant was found in 71 subjects (14%). Hardy-Weinberg equilibrium (HWE) testing using a χ^2-test showed that rs9939609 was in HWE (HWE = 0.09, $P = 0.760$).

Table 1 shows all demographic data for the various *FTO* genotypes. No difference in age and gender was found between groups. Subjects homozygous for the *FTO* risk allele A demonstrated a significant higher BMI, corrected for age and gender, compared with subjects with the T allele. No difference in either systolic nor diastolic blood pressure was found between groups. There was also no difference in history of hypertension, vascular disease, MI or DM between the three groups.

Table 1. Baseline characteristics

	FTO rs9939609TA polymorphism			
	TT (n=185)	**TA (n=236)**	**AA (N=71)**	**P-value**
Gender female n(%)	82 (44)	107 (45)	29 (41)	0.80
Age (years)	74.6 (0.2)	74.4 (0.2)	74.4 (0.4)	0.77
BMI (kg/m2)	26.6 (0.3)	26.6 (0.2)	27.9 (0.5)	0.015
Systolic BP (mmHg)	157.2 (1.6)	157.4 (1.3)	159.9 (2.8)	0.65
Diastolic BP (mmHg)	85.5 (0.8)	85.9 (0.7)	87.1 (1.3)	0.55
History of vascular disease n(%)	75 (40)	97 (41)	35 (49)	0.41
History of MI n(%)	23 (12)	29 (12)	7 (10)	0.83
History of hypertension n(%)	108 (58)	151 (64)	47 (66)	0.38
History of DM n(%)	31 (17)	47 (20)	7 (10)	0.14

Table 1: Abbreviations: MI= Myocardial infarction, DM= Diabetes Mellitus, BMI=Body Mass Index, BP=Blood pressure.

Relationship reward-related brain structures and FTO

Table 2 shows the volumetric data for all *FTO* mutation variants. Of all subcortical structures the nucleus accumbens demonstrated a gradual 12% decrease in volume from TT, 0.59 ml, to AA, 0.52 ml (p = 0.04 adjusted for age and sex, and FDR corrected for multiple comparisons). This association remained significant when additionally adjusting for BMI (p = 0.03). No subcortical volume changes between the *FTO* groups were found for the hippocampus, amygdala, caudate nucleus, pallidum, thalamus, and putamen.

Furthermore, we found a small (1.2%) decline in the volume of the cortical gray matter from TT to AA, which trended towards significance (p = 0.08, corrected for age, sex and BMI, and FDR corrected for multiple comparisons). To assess regional differences in cortical thickness in contrast to overall volumetric changes, VBM (voxel-based morphometry) analysis was performed; this did not reveal any significant focal cortical differences among groups. No difference between groups was found for white matter volume reduction.

Table 2. Volumes and association of different brain volumes with FTO.

	FTO rs9939609TA polymorphism			Model 1	model 2
	TT (n=185)	TA (n=236)	AA (N=71)	P-value	P-value
Grey matter (ml)	469 (4)	464 (4)	463 (7)	0.012	0.017
White matter (ml)	604 (4)	607 (5)	605 (4)	0.73	0.86
Hippocampus (ml)	4.68 (0.04)	4.58 (0.04)	4.69 (0.06)	0.48	0.37
Amygdala (ml)	2.02 (0.02)	1.97 (0.02)	2.05 (0.04)	0.90	0.75
Nucleus caudatus (ml)	3.79 (0.04)	3.74 (0.04)	3.77 (0.06)	0.55	0.58
Nuc. accumbens (ml)	0.59 (0.01)	0.55 (0.01)	0.52 (0.02)	0.004	0.003
Pallidum (ml)	1.93 (0.03)	1.94 (0.03)	1.95 (0.05)	0.81	0.88
Thalamus (ml)	8.10 (0.05)	8.04 (0.05)	8.12 (0.09)	0.62	0.53
Putamen (ml)	5.24 (0.04)	5.18 (0.04)	5.34 (0.08)	0.56	0.54

Table 2: Data are presented as Mean (SE). MODEL 1: Linear regression correcting for gender and age; MODEL 2 Linear regression correcting for gender. age and BMI.

DISCUSSION

The results of this study show that the risk allele rs9939609A of the *FTO* gene is associated with a significantly lower volume of the nucleus accumbens and trended towards

a smaller cortical grey matter volume. This association is independent of gender, age and BMI in this cohort. Although other studies have shown global grey matter volume differences with the *FTO* gene or BMI, to our knowledge, this is the first study to identify specific brain structures within the reward system that are affected by the *FTO* gene. No association between *FTO* and other subcortical brain structures was found.

Very little is known about the relationship between the *FTO* gene and the nucleus accumbens. There is, however, more known about the role that FTO plays in hypothalamic signalling. Research in chickens, mice and rats has shown expression of FTO throughout the brain with high expression in the hypothalamus (5, 31-34). The hypothalamus has an influence on the nucleus accumbens through dopaminergic projections via the ventral tegmental area.

The nucleus accumbens is a brain structure with a central role in the reward circuitry. It has a role in motivation-related behaviour, reward, and the craving of various substances including food(35, 36), and has a regulating role in dopaminergic signalling to the output-structures of the reward-system(37). The activity of the hypothalamus - nucleus accumbens pathway has recently been shown to be up-regulated by ghrelin and down-regulated by leptin, hormones known to be important for the regulation of homeostatic hunger(36). A study by Karra et al. revealed that healthy subjects with a normal weight with genotype AA at SNP rs9939609 have attenuated post-prandial ghrelin suppression (7) compared to subjects with a normal weight with genotype TT at SNP rs9939609. Furthermore, this study showed that the response to hedonic food pictures was differentially modulated between AA and TT phenotypes in the nucleus accumbens(7). In addition it was shown that knockout of *FTO* in mice impairs dopamine receptor type 2 and 3-dependent control of neuronal activity and behavioural responses(38).

Another possible mechanism through which *FTO* might have an influence on reward signalling is by regulating the expression of *IRX3*. This gene has been implicated in regulating body weight and is expressed in the hypothalamus, amygdala and caudate nucleus, all of which connect with the nucleus accumbens(8).

Our data, showing a lower nucleus accumbens volume in people with the *FTO* AA genotype, support the hypothesis that subjects with the *FTO* AA gene have attenuated regulation of dopaminergic projections to the nucleus accumbens and through that to the output-structures of the reward-system leading to the previously mentioned differences in feeding behaviour between *FTO* TT and *FTO* AA.

Our study also showed a trend line effect of the *FTO* polymorphism on the total volume of grey matter. The relationship between SNPs within the *FTO* gene and brain volume deficits has been previously reported in populations of elderly subjects (17) as well as in adolescents(39). It has been suggested that the effect of FTO on brain volume deficits probably originates in developmental stages of life, given that it is already apparent in adolescence. It was suggested that there might be an inverse relationship between FTO

and brain tissue compared to the relationship between FTO and adipose tissue(39). The findings of our study support these results, showing a trend line association between the presence of the *FTO* risk-allele and increased cortical brain atrophy (39). Since it was previously shown that BMI was not associated with lower nucleus accumbens volumes(22, 24), our finding that *FTO* is specifically associated with lower nucleus accumbens volumes indicates that the underlying cerebral process associated with BMI and brain atrophy is different.

However, our data do not support the results of a recent study by Cole et al.(40) showing that only BMI, and not *FTO*, was associated with brain atrophy. In this study younger subjects with a different SNP (rs3751812) were included(40). Still, our data unequivocally show that the association between the *FTO* polymorphism and brain volume deficits is independent of BMI.

The major limitation of this study is that we included elderly subjects with an increased cardiovascular risk profile. Though we did not find differences in cardiovascular risk profiles between the *FTO* polymorphism groups, our data may not be generalizable to a healthy population.

CONCLUSION

In conclusion, the results of our study support previous research stating that *FTO* is associated with brain volume deficits as well as studies stating a role for FTO in dopaminergic signalling within the reward-system. Further research should focus on functional imaging of the reward system and the relationship with *FTO* to determine whether the differences in reward-related brain structures are a result of altered hypothalamic signalling or an independent effect of FTO on reward-related brain structures.

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Part III

Diagnostic workup of overweight paediatric patients in clinical practice