

Interaction between the obesity-risk gene *FTO* and the dopamine D2 receptor gene *ANKK1/TaqIA* on insulin sensitivity

Martin Heni^{1,2,3} · Stephanie Kullmann^{2,3} · Emma Ahlqvist⁴ · Robert Wagner^{1,2,3} · Fausto Machicao^{2,3} · Harald Staiger^{2,3,5,6} · Hans-Ulrich Häring^{1,2,3,6} · Peter Almgren⁴ · Leif C. Groop^{4,7} · Dana M. Small^{8,9} · Andreas Fritsche^{1,2,3} · Hubert Preissl^{1,2,3,5,6,10}

Received: 23 May 2016 / Accepted: 11 August 2016 / Published online: 6 September 2016
© Springer-Verlag Berlin Heidelberg 2016

Abstract

Aims/hypothesis Variations in *FTO* are the strongest common genetic determinants of adiposity, and may partly act by influencing dopaminergic signalling in the brain leading to altered reward processing that promotes increased food intake. Therefore, we investigated the impact of such an interaction on body composition, and peripheral and brain insulin sensitivity. **Methods** Participants from the Tübingen Family study ($n=2245$) and the Malmö Diet and Cancer study ($n=2921$) were genotyped for *FTO* SNP rs8050136 and *ANKK1* SNP rs1800497. Insulin sensitivity in the caudate nucleus, an important reward area in the brain, was assessed by fMRI in 45 participants combined with intranasal insulin administration. **Results** We found evidence of an interaction between variations in *FTO* and an *ANKK1* polymorphism that associates with dopamine (D2) receptor density. In cases of reduced D2 receptor availability, as indicated by the *ANKK1* polymorphism, *FTO* variation was associated with increased body fat and waist circumference and reduced peripheral insulin sensitivity. Similarly, altered central insulin sensitivity

was observed in the caudate nucleus in individuals with the *FTO* obesity-risk allele and diminished D2 receptors.

Conclusions/interpretation The effects of variations in *FTO* are dependent on dopamine D2 receptor density (determined by the *ANKK1* polymorphism). Carriers of both risk alleles might, therefore, be at increased risk of obesity and diabetes.

Keywords *ANKK1* · Body fat · Dopamine · *FTO* · Insulin sensitivity · *TaqIA*

Abbreviations

BOLD	Blood oxygenation level-dependent
D2/D3	Dopamine receptor type 2/3
fALFF	Fractional amplitude of low-frequency fluctuation
HWE	Hardy–Weinberg equilibrium
LFF	Low-frequency fluctuations
MDC CA	Malmö Diet and Cancer study cardiovascular arm

✉ Stephanie Kullmann
stephanie.kullmann@med.uni-tuebingen.de

¹ Department of Internal Medicine, Division of Endocrinology, Diabetology, Angiology, Nephrology and Clinical Chemistry, Eberhard Karls University Tübingen, Tübingen, Germany

² Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Center Munich at the University of Tübingen, Otfried-Müller-Straße 47, 72076 Tübingen, Germany

³ German Center for Diabetes Research (DZD e.V.), Tübingen, Germany

⁴ Lund University Diabetes Centre, Department of Clinical Sciences Malmö, Lund University, Malmö, Sweden

⁵ Institute of Pharmaceutical Sciences, Department of Pharmacy and Biochemistry, Eberhard Karls Universität Tübingen, Tübingen, Germany

⁶ Interfaculty Centre for Pharmacogenomics and Pharma Research, Eberhard Karls University Tübingen, Tübingen, Germany

⁷ Finnish Institute for Molecular Medicine, University of Helsinki, Helsinki, Finland

⁸ Department of Psychiatry, Yale University School of Medicine, New Haven, CT, USA

⁹ The John B. Pierce Laboratory, New Haven, CT, USA

¹⁰ Institute for Diabetes and Obesity, Helmholtz Diabetes Center, Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Neuherberg, Germany

p_{add}	p for additive inheritance model
p_{dom}	p for dominant inheritance model
TUEF	Tübingen Family study

Introduction

The prevalence of obesity and diabetes is increasing dramatically worldwide. In addition to behavioural factors, such as changes in eating habits and lack of physical activity, hereditary factors strongly determine body weight [1]. Variations in the fat mass and obesity-associated gene (*FTO*) are currently the strongest known genetic factors that predispose humans to non-monogenic obesity [2–4]. Carriers of the risk allele are more liable to have a higher BMI due to increased energy intake [5, 6] and decreased satiety [7]. Underlying mechanisms may include altered postprandial responses in reward- and appetite-regulating brain areas [8, 9], and impaired postprandial suppression of the hunger hormone ghrelin [9]. In addition, *FTO* polymorphisms are a common genetic determinant for type 2 diabetes, particularly via their effects on adiposity [2, 10].

Interestingly, recent data indicate that *FTO* variations determine mitochondrial activity via a network that involves expressional changes in *IRX3* and *IRX5* [11, 12]. In addition to associations with adipocyte function [12], *FTO* polymorphisms influence the expression of *IRX3* in the human brain [11]. *FTO* variations have also been shown to coincide with altered regulation of a molecular network including *RPGRIP1L* in neuronal cells that ultimately leads to altered cellular function and potentially promotes adiposity [13].

Recent work in rodent models suggests that variations in *Fto* may be linked to increased food intake and obesity through an influence on dopamine signalling [14]. Dopamine neurons express *Fto*. Conditional loss of *Fto* in dopamine neurons specifically impairs dopamine receptor type 2/3 (D2/D3)-mediated neuronal activation. Accordingly, *Fto*-deficient mice show altered D2/D3 receptor-dependent regulation of locomotor activity and reward sensitivity. These findings also highlight the possibility that *Fto* influences food intake by altering D2 receptor-dependent feeding behaviour.

In terms of metabolism, dopamine signalling is crucial for the regulation of whole-body insulin sensitivity [15]. Deep brain stimulation of dopaminergic areas in the brain modulates glucose metabolism and body weight in rodents [16, 17]. Accordingly, the dopamine receptor agonist bromocriptine improves insulin sensitivity and induces weight loss in humans [18], and is therefore approved for the treatment of type 2 diabetes in some countries [18]. On a cellular level, there is clear evidence for an interaction between insulin and dopamine signalling [19–21] in the regulation of reward and food intake [22, 23].

Therefore, one possible hypothesis is that variations in *FTO* affect dopaminergic regulation of insulin sensitivity, thereby influencing body weight. Indeed, the *FTO* risk allele is linked to central insulin resistance [24]. Furthermore, there is evidence that central insulin regulates peripheral insulin sensitivity [25–29]. However, the well-known associations of *FTO* variants with whole-body insulin sensitivity [30–32] and diabetes risk [2, 10] are mainly driven by associations with BMI [2, 10, 31].

Alterations in dopamine signalling via D2 receptors are linked to common genetic variations. The *TaqIA* restriction fragment length polymorphism (rs1800497) is located in the ankyrin repeat and protein kinase domain-containing protein (*ANKK1*) gene approximately 9 kb downstream of the D2 receptor gene [33]. In candidate gene studies, carriers of the risk allele (T allele) show diminished D2 receptor density [34, 35], reduced D2 receptor binding potential [36] and an increased risk of substance abuse [37]. With regard to food intake, carriers of the *ANKK1* risk allele have reduced responses to palatable and energy-dense food in brain regions involved in reward processing [38]. While the polymorphism is not a major determinant of body weight per se [39], carriers of the risk allele have difficulty losing or maintaining body weight [39, 40]. Specifically, an attenuated response to food in the striatum of risk-allele carriers was a predictor of further weight gain [41]. A recent study in humans identified an interaction between *FTO* and *ANKK1* polymorphisms that determines midbrain activity during reward learning [42].

Since there is evidence that both *FTO* and *ANKK1* confer risk for obesity by their influence on D2 receptors, we investigated whether common variations in these two genes interact to increase the risk for high body weight, unfavourable body fat distribution, altered glucose metabolism and decreased insulin sensitivity of the whole body, as well as of the brain, specifically the striatum (including the putamen, pallidum and caudate nucleus). Such an interaction would be relevant given that approximately 30% of all populations carry at least one risk allele of both variants (mean minor allele frequency of *FTO* and *ANKK1* in various populations is approximately 33% according to 1000 genomes data [www.1000genomes.org; accessed 20 May 2015]). Moreover, the presence of an interaction would highlight the importance of the role of *FTO* in dopamine signalling in humans.

Methods

Participants of the Tübingen Family cohort The Tübingen Family (TUEF) study is an ongoing project that includes European volunteers from southern Germany who have an elevated risk of type 2 diabetes but do not have manifest diabetes [28]. Subjects are recruited by personal contact as

well as via local advertisements, for example in newspapers. All participants are metabolically characterised by a 75 g OGTT (see details below). Less than 5% of the cohort are related. Exclusion criteria include major medical and psychiatric diseases, as well as treatment with medication for weight loss or blood glucose control. Informed written consent is obtained from all participants, and the local ethics committee have approved the protocol.

We included 2245 TUEF study participants with a BMI <40 kg/m² in the current analyses. Based on ADA criteria for glucose tolerance, 1588 had normal glucose tolerance, 224 had impaired fasting glucose, 189 had impaired glucose tolerance, 124 displayed both impaired fasting glucose and impaired glucose tolerance, and 120 had type 2 diabetes. The clinical characteristics of the TUEF study population are presented in Table 1. For all participants, total body fat and lean body mass were measured by bioelectrical impedance (BIA-101; RJL Systems, Detroit, MI, USA).

Participants of the MDC CA reinvestigation cohort The Malmö Diet and Cancer (MDC) study is a population-based cohort collected in Malmö, southern Sweden. Between 1991 and 1996, 30,000 individuals born between 1923 and 1950 were recruited to the study, from which, 6103 non-related individuals were randomly selected to take part in the MDC CA (cardiovascular arm) study. All selected individuals who were alive and had not emigrated from Sweden ($n=4924$) were invited to a re-examination starting in 2007. Of these, 3734 accepted and underwent a standard 75 g OGTT. All participants provided written informed consent, and the study was approved by the Lund University ethics review committee.

Genotyping For the TUEF cohort, DNA from whole blood was isolated with a commercial DNA isolation kit (NucleoSpin, Macherey – Nagel, Düren, Germany).

Table 1 Clinical characteristics of the TUEF and MDC study populations

	TUEF cohort	MDC cohort
Sex (female/male)	1416/829	1411/974
Age (years)	40.9 ± 13.9	72.4 ± 5.6
BMI (kg/m ²)	27.7 ± 5.3	26.6 ± 4.1
Percentage body fat (%)	31 ± 11	25 ± 7 (subgroup of $n=1279$)
Waist circumference (cm)	92 ± 14	92 ± 12
Fasting glucose (mmol/l)	5.2 ± 0.7	5.9 ± 0.7
Glucose, 120 min OGTT (mmol/l)	6.6 ± 2.7	7.2 ± 2.8
HbA _{1c} (%)	5.4 ± 0.5	–
HbA _{1c} (mmol/mol)	35.5 ± 5.3	–

Data are presented as means ± SD

Genotyping for *FTO* SNP rs8050136 and *ANKK1* SNP rs1800497 was performed by mass spectrometry (Sequenom, Hamburg, Germany). The genotypes validated by bidirectional sequencing in 50 participants were 100% identical. Minor allele frequencies were 43% for the *FTO* A risk allele and 19% for the *ANKK1* T risk allele. Both SNPs were in Hardy–Weinberg equilibrium (HWE; *FTO* SNP $p=0.7$, *ANKK1* SNP $p=0.2$).

The MDC cohort was genotyped at the Broad genotyping facility (Cambridge, MA, USA) using the Illumina OmniExpressExome Beadchip v1.0 B array (Illumina, San Diego, CA, USA). Genotypes were called using the Gencall algorithm in Genomestudio (Illumina) with refinement of rare variants with missing calls undertaken using zCall [43]. Standard quality control filters were applied. In brief, SNPs were excluded if they were monomorphic, had a low call rate (<99%) or deviated from HWE ($p < 10^{-4}$). Samples were excluded if they were duplicates, unexpected first/second relatives, sex mismatched, heterozygosity outliers (>3 SD from mean), ancestral outliers or had a low call rate (<95%). No principal component adjustment was used since the population was homogeneous and participants were recruited at a single centre. Both SNPs were in HWE (*FTO* SNP $p=0.6$, *ANKK1* SNP $p=0.8$).

OGTT A 75 g OGTT was performed as previously described [44]. In the TUEF participants, glucose, insulin and C-peptide were measured five times during the experiment (0, 30, 60, 90 and 120 min). For the MDC cohort, blood samples were obtained at 0, 30 and 120 min.

Hyperinsulinaemic–euglycaemic clamp In a subgroup of 499 participants, insulin sensitivity was assessed using a hyperinsulinaemic–euglycaemic glucose clamp as previously described [44]. The clamp-derived insulin sensitivity index was calculated by dividing the mean glucose infusion rate during steady state by the plasma insulin concentrations during this time [44].

Analytical procedures In the TUEF study, plasma glucose was measured by the glucose-oxidase method (Yellow Springs Instruments, Yellow Springs, OH, USA). Insulin and C-peptide concentrations were measured using commercial chemiluminescence assays for ADVIA Centaur (Siemens Medical Solutions, Fernwald, Germany). In the MDC study, plasma glucose was measured using a glucose dehydrogenase method (Hemocue, Ängelholm, Sweden) and serum insulin was measured using ELISA (Dako, Glostrup, Denmark).

fMRI Forty-five healthy volunteers (19 women, 26 men; mean age 26.4 ± 3.4 years) underwent fMRI to assess regional insulin action in the brain. They were retrospectively

genotyped for the two SNPs of interest. Eleven individuals were homozygous for both non-risk major alleles (*FTO* rs8050136 and *ANKK1* rs1800497), 20 were homozygous major allele carriers of the *ANKK1* SNP and homozygous minor allele carriers of the *FTO* SNP, five were homozygous minor allele carriers of the *ANKK1* SNP and homozygous for the *FTO* SNP major allele, and nine were homozygous for the minor alleles of both polymorphisms.

Experiments were conducted after an overnight fast of at least 10 h and commenced at 07:00 hours with a ‘resting state’ fMRI measurement under basal conditions. Following the basal measurement, 160 U of human insulin was administered via intranasal spray as previously described [28, 45]. After 30 min, a second ‘resting state’ fMRI measurement was performed.

fMRI data acquisition Whole-brain fMRI data was obtained with a 3.0 T scanner (Siemens Tim Trio, Erlangen, Germany). Functional data were collected by echo-planar imaging sequence: repetition time 2 s, echo time 30 ms, field of view 210 mm², matrix 64 × 64, flip angle 90°, voxel size 3.3 × 3.3 × 3.6 mm³, slice thickness 3.6 mm, 1 mm gap and the images were acquired in ascending order. Each brain volume comprised 26 axial slices and each functional run contained 176 image volumes, resulting in a total scan time of 6.04 min. In addition, high-resolution T1 weighted anatomical images (MPRage: 192 slices, matrix: 256 × 224, 1 × 1 × 1 mm³) of the brain were obtained.

Fractional amplitude of low-frequency fluctuation

Low-frequency (0.01–0.08 Hz) fluctuations (LFF) of the blood oxygenation level-dependent (BOLD) signal in resting state fMRI data are believed to reflect intrinsic neural activity. We carried out the fractional amplitude of LFF (fALFF) analysis on the preprocessed functional data using REST (<http://resting-fmri.sourceforge.net>), as previously described [45]. The fALFF maps of the basal fMRI measurement were subtracted from the fALFF maps of the 30 min fMRI measurement for each participant. We took a region-of-interest approach by using the WFU PickAtlas tool (<http://fmri.wfubmc.edu/software/PickAtlas>, accessed 13 February 2015) to extract mean fALFF values of the striatum, a critical component of the dopaminergic reward system. Areas of the striatum include the caudate nucleus, putamen and pallidum. The extracted fALFF values were then further analysed in a linear regression model.

Calculations Insulin sensitivity during the OGTT was estimated as $10,000/(G_0 \times I_0 \times G_{\text{mean}} \times I_{\text{mean}})^{1/2}$, where G = glucose (in mmol/l) and I = insulin (in pmol/l). AUC was calculated according to the trapezoid method.

Statistical analysis Data that were not normally distributed were log_e-transformed to approximate a normal distribution prior to analysis. ANCOVA was used to identify interactions between SNPs. Associations were analysed by multiple linear regression analysis. A *p* value <0.05 was considered to be statistically significant; a *p* value <0.1 was considered to indicate a trend. The statistical software package JMP (SAS Institute, Cary, NC, USA) was used for statistical analysis.

Results

Associations with body composition and metabolic traits in the TUEF cohort

Interactions between the *FTO* SNP rs8050136 and *ANKK1* SNP rs1800497 on body composition and metabolic traits were investigated by ANCOVA for both polymorphisms. There were interactions between the two SNPs for percentage body fat, waist circumference and waist-to-hip ratio, as well as for the insulin sensitivity index and fasting insulin in models adjusted for sex and age (all *p* < 0.05, Table 2). No significant interactions were detected for lean body mass, fasting and post-load glycaemia, or insulin secretion (assessed as insulin_{30min} and AUC_{C-peptide}/AUC_{glucose}). Repeating the analyses in unadjusted models revealed comparable results (all *p* < 0.05), except for waist-to-hip ratio and the insulin sensitivity index which only showed trends towards significance (*p* = 0.1 and *p* = 0.08, respectively).

The group was then stratified according to *ANKK1* rs1800497 genotype in homozygous carriers of the C allele vs those carrying at least one copy of the T risk allele (see Table 2). Associations of *FTO* rs8050136 were then tested for both *ANKK1* groups separately. The *FTO* SNP was associated with body weight and BMI independent of the *ANKK1* SNP; however, the effect sizes were markedly larger in carriers of the *ANKK1* T allele than in CC allele carriers (Table 2). While the *FTO* SNP was not associated with body fat content or waist-to-hip ratio in homozygous carriers of the *ANKK1* C allele (*p* for additive inheritance model [*p*_{add}] 0.1, β 0.017 ± 0.012, Fig. 1a; *p*_{add} 0.5, β -0.003 ± 0.004, respectively), associations were found in participants harbouring the T allele (*p*_{add} 0.0006, β 0.056 ± 0.016, Fig. 1a; *p*_{add} 0.03, β 0.017 ± 0.008, respectively). Furthermore, it was only against the *ANKK1* T allele background that the *FTO* obesity-risk allele carriers had a lower insulin sensitivity index and higher fasting insulin than the *FTO* non-risk allele carriers (*p*_{add} 0.0012, β -0.121 ± 0.037, Fig. 1b; *p*_{add} 0.0002, β 0.126 ± 0.034, respectively). This association was not present in the *ANKK1* SNP CC carriers (Table 2, Fig. 1b).

Associations with body composition and metabolic traits in the MDC cohort

In the MDC cohort, interactions between

Table 2 Associations between *FTO* rs8050136 and metabolic traits after stratification for *ANKK1* rs1800497 in the TUEF cohort

	CC				XT				
	CC <i>n</i> = 486	XA <i>n</i> = 1010	CC <i>n</i> = 246	XA <i>n</i> = 502	<i>p</i> _{add} / <i>p</i> _{dom}	$\beta \pm SE$	<i>p</i> _{add} / <i>p</i> _{dom}	<i>p</i> _{add} for interaction	Interaction (<i>ANKK1</i> × <i>FTO</i>) <i>p</i> _{dom} for interaction
<i>FTO</i> rs8050136									
Height (cm)	171 ± 9	172 ± 9	172 ± 9	171 ± 9	0.5/0.4	0.0008 ± 0.0014	0.1/0.3	0.1	0.2
Weight (kg)	79 ± 16	80 ± 16	80 ± 16	83 ± 17	0.0005/0.0081	0.0254 ± 0.0073	0.0016/0.0005	0.4	0.2
BMI (kg/m ²)	27.0 ± 5.1	27.8 ± 5.4	26.9 ± 5.0	28.4 ± 5.4	0.0005/0.0161	0.0240 ± 0.0068	<0.0001/<0.0001	0.1	0.0609
Percentage body fat (%)	30 ± 11	31 ± 11	29 ± 11	32 ± 11	0.1/0.4	0.0172 ± 0.0115	0.0006/0.0004	0.0711	0.0231
Lean body mass (kg)	55 ± 14	55 ± 12	57 ± 13	56 ± 12	0.0017/0.0023	0.0177 ± 0.0056	0.5/0.2	0.3	0.3
Waist circumference (cm)	91 ± 14	92 ± 14	91 ± 14	94 ± 14	0.0230/0.1	0.0119 ± 0.0053	0.0002/0.0004	0.0422	0.0417
Waist-to-hip ratio	0.90 ± 0.46	0.87 ± 0.09	0.87 ± 0.11	0.88 ± 0.10	0.5/0.1	-0.0025 ± 0.0039	0.0342/0.08	0.0131	0.0082
Fasting glucose (mmol/l)	5.2 ± 0.7	5.2 ± 0.6	5.2 ± 0.9	5.2 ± 0.7	0.9/0.8	-0.0007 ± 0.0041	0.6/0.4	0.8	0.6
Glucose 120 min OGTT (mmol/l)	6.5 ± 2.1	6.7 ± 3.3	6.4 ± 2.4	6.5 ± 2.1	0.9/0.9	-0.0009 ± 0.0108	0.1/0.07	0.2	0.1
OGTT-derived insulin sensitivity index (AU)	14.7 ± 9.5	13.9 ± 9.4	15.8 ± 10.6	13.3 ± 8.9	0.4/0.3	-0.0228 ± 0.0248	0.0012/0.0009	0.0287	0.0300
Fasting insulin (pmol/l)	66.6 ± 49.3	68.2 ± 49.3	64.2 ± 50.9	74.9 ± 59.9	0.5/0.5	0.0143 ± 0.0225	0.0002/0.0008	0.0165	0.0159
Insulin 30 min OGTT (pmol/l)	538.8 ± 416.3	554.1 ± 383.1	512.6 ± 369.1	558.0 ± 411.1	0.0739/0.1	0.0434 ± 0.0242	0.0519/0.07	0.5	0.6
AUC _{C-peptide} /AUC _{glucose} (× 10 ⁻³)	296 ± 92	298 ± 99	290 ± 103	305 ± 108	0.4/1.0	0.0104 ± 0.0127	0.0836/0.2	0.4	0.2
Clamp-derived insulin sensitivity (AU) ^a	0.089 ± 0.061	0.085 ± 0.053	0.107 ± 0.068	0.079 ± 0.038	0.6/0.7	-0.0221 ± 0.0435	0.1/0.0361	0.4	0.1

Data presented are unadjusted means ± SD

Genotypes were compared by multiple linear regression analysis with adjustments for sex and age

^a Available in a subgroup of 499 participants

AU, arbitrary units

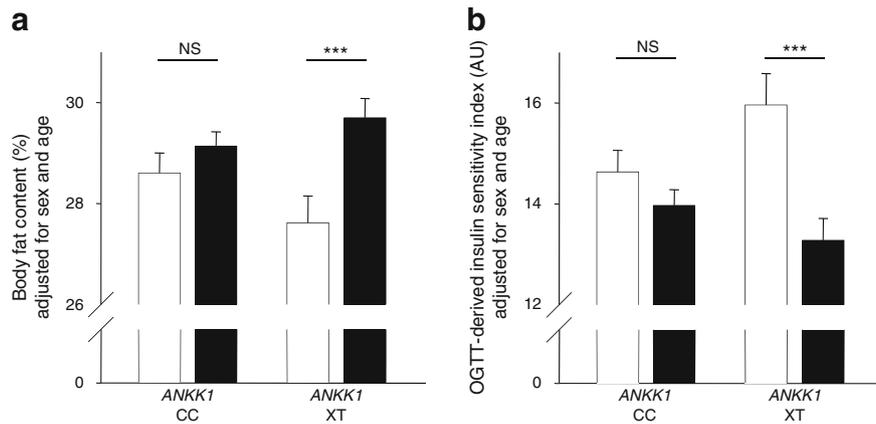


Fig. 1 The obesity-risk polymorphism in *FTO* and the D2 receptor polymorphism in *ANKK1* interact to affect (a) body fat content and (b) peripheral insulin sensitivity in the TUEF cohort. Participants were stratified according to their *ANKK1* rs1800497 genotype in CC and XT carriers. *FTO* rs8050136 genotype is indicated by bar colour: white bars, *FTO* CC allele carriers; black bars, *FTO* XA obesity-risk allele carriers. With regard to body fat content, no association with the *FTO* SNP was found in *ANKK1* CC carriers, while carriers of the *FTO* non-risk allele

had lower body fat than obesity-risk allele carriers under the *ANKK1* XT background. Accordingly, the *FTO* SNP was not associated with OGTT-derived peripheral insulin sensitivity index in *ANKK1* CC carriers, while peripheral insulin sensitivity was better in *FTO* non-risk than in *FTO* obesity-risk allele carriers under the *ANKK1* XT background. Data are sex- and age-adjusted means \pm SEM. AU, arbitrary units; NS, non-significant ($p > 0.05$), *** $p < 0.001$

the *ANKK1* and *FTO* polymorphisms were detected for waist circumference and waist-to-hip ratio (Table 3).

Corresponding with the results from the TUEF cohort, waist circumference was associated with the *FTO* polymorphism in participants with the *ANKK1* T allele ($p_{\text{add}} 0.025$, $\beta 0.019 \pm 0.008$), while no such association was detected in *ANKK1* CC allele carriers ($p_{\text{add}} 0.2$, $\beta -0.005 \pm 0.006$). Also the insulin sensitivity index and fasting insulin levels were only associated with the *FTO* SNP in *ANKK1* T allele carriers ($p_{\text{add}} 0.02$, $\beta -0.084 \pm 0.005$; $p_{\text{add}} 0.04$, $\beta 0.078 \pm 0.04$, respectively), but not in participants with the *ANKK1* CC allele ($p_{\text{add}} 0.7$, $\beta 0.027 \pm 0.04$; $p_{\text{add}} 0.9$, $\beta -0.001 \pm 0.03$, respectively; see Table 3).

Associations with insulin action in the caudate nucleus

Since *FTO* rs8050136 is known to be linked to brain insulin sensitivity [24], we investigated how the two polymorphisms relate to brain insulin action in the striatum, a major dopaminergic area in the brain in which the *ANKK1* SNP is known to be important [41], following intranasal insulin administration.

To increase statistical power in this small group, we only tested dominant inheritance models. As for peripheral insulin sensitivity, we detected an interaction between the *FTO* rs8050136 and *ANKK1* rs1800497 SNPs on insulin response in the caudate nucleus (ANCOVA p for dominant inheritance model [p_{dom}] 0.0060). While the reaction to insulin did not differ between *FTO* genotypes in carriers of the *ANKK1* CC alleles, it differed in the *ANKK1* T allele carriers. Therefore, insulin reduced activity in the caudate nucleus in *FTO* non-risk allele carriers, but increased activity in obesity-risk allele carriers (Fig. 2).

Discussion

In this study, we have demonstrated an interaction between variations in *FTO* and the *ANKK1/TaqIA* polymorphism that affects body composition, as well as peripheral and central insulin sensitivity. While the *FTO* SNP was not associated with body fat distribution or insulin sensitivity in persons carrying the *ANKK1* non-risk allele, it was associated with both variables in participants carrying the *ANKK1/TaqIA* polymorphism.

These findings, that were replicated in the independent MDC cohort, provide further evidence that variations in *FTO* influence dopamine signalling in humans in the same way as predicted from rodents [14]. Specifically, on the basis of the associations between *ANKK1* variations and D2 receptor availability [34, 35], as well as D2 receptor binding potential in humans [36]. Our results indicate that the *FTO* polymorphism investigated here may be less critical when sufficient D2 receptors are available but it may have adverse effects when dopaminergic transmission is limited. Given the high frequency of both polymorphisms in the general population, a substantial number of people will carry the risk alleles for both polymorphisms, i.e. in our study population >20% of the participants carried both risk alleles. Our results demonstrate that these individuals will be at increased risk for obesity, insulin resistance and type 2 diabetes. Our data also highlight the possibility that in addition to other known environmental perturbations, the identified interaction contributes to the known association of the *FTO* polymorphism with both obesity and diabetes [2, 4].

Table 3 Associations between *FTO* rs8050136 and metabolic traits after stratification for *ANKK1* rs1800497 in the MDC cohort

<i>FTO</i> rs8050136	CC			XT			Interaction (<i>ANKK1</i> × <i>FTO</i>)				
	<i>ANKK1/TaqIA</i> rs1800497		XA <i>n</i> = 1030	<i>ANKK1/TaqIA</i> rs1800497		XA <i>n</i> = 514	$\beta \pm SE$	$P_{\text{add}}/P_{\text{dom}}$	$P_{\text{add}}/P_{\text{dom}}$	P_{add} for interaction	P_{dom} for interaction
	CC <i>n</i> = 537	XA <i>n</i> = 1030		CC <i>n</i> = 302	XA <i>n</i> = 514						
Height (cm)	167.9 ± 9.0	167.3 ± 9.0	167.3 ± 9.0	168.0 ± 9.1	168.1 ± 9.2	0.356 ± 0.47	0.4/0.6	0.4/0.5	0.3	0.3	
Weight (kg)	75.1 ± 13.6	74.4 ± 13.6	74.4 ± 13.6	74.9 ± 13.9	76.0 ± 13.6	0.019 ± 0.011	0.5/0.7	0.08/0.1	0.1	0.1	
BMI (kg/m ²)	26.6 ± 4.1	26.5 ± 4.3	26.5 ± 4.3	26.5 ± 4.1	26.9 ± 3.9	0.015 ± 0.01	0.8/0.9	0.13/0.2	0.2	0.2	
Percentage body fat (%)	25.1 ± 6.7	25.5 ± 7.0	25.5 ± 7.0	25.2 ± 6.8	24.6 ± 6.8	-0.354 ± 0.42	0.5/0.6	0.8/0.4	0.9	0.7	
Lean body mass (kg)	55.7 ± 11.5	53.8 ± 10.6	53.8 ± 10.6	55.0 ± 11.0	56.1 ± 10.7	0.7 ± 0.6	0.1/0.06	0.2/0.3	0.02	0.04	
Waist circumference (cm)	91.9 ± 11.5	91.3 ± 12.1	91.3 ± 12.1	91.3 ± 11.5	92.8 ± 12.0	0.019 ± 0.008	0.2/0.4	0.025/0.023	0.021	0.026	
Waist-to-hip ratio	0.89 ± 0.08	0.89 ± 0.09	0.89 ± 0.09	0.88 ± 0.08	0.90 ± 0.09	0.012 ± 0.005	0.5/0.7	0.05/0.009	0.086	0.024	
Fasting glucose (mmol/l)	5.9 ± 0.7	5.9 ± 0.8	5.9 ± 0.8	5.8 ± 0.7	5.8 ± 0.7	0.017 ± 0.05	0.3/0.8	0.09/0.7	0.1	0.7	
Glucose 120 min OGTT (mmol/l)	7.1 ± 2.4	7.2 ± 3.3	7.2 ± 3.3	7.1 ± 2.3	7.1 ± 2.4	0.069 ± 0.17	0.8/0.5	0.4/0.7	0.6	0.9	
2-point OGTT-derived insulin sensitivity index (AU)	6.2 ± 4.3	6.3 ± 4.3	6.3 ± 4.3	6.3 ± 4.2	6.0 ± 3.9	-0.084 ± 0.05	0.7/0.5	0.02/0.08	0.077	0.073	
Fasting insulin (pmol/l)	59.9 ± 33.5	59.8 ± 33.8	59.8 ± 33.8	59.2 ± 35.1	63.6 ± 36.2	0.078 ± 0.04	0.9/1.0	0.04/0.03	0.2	0.093	
Insulin 30 min OGTT (pmol/l)	354.6 ± 244.4	339.0 ± 225.1	339.0 ± 225.1	336.9 ± 202.7	368.7 ± 221.5	0.091 ± 0.04	0.4/0.3	0.034/0.022	0.052	0.015	
Insulin 120 min OGTT (pmol/l)	357.4 ± 278.2	352.5 ± 336.4	352.5 ± 336.4	344.7 ± 276.0	374.7 ± 327.1	0.08 ± 0.05	0.5/0.16	0.04/0.12	0.09	0.04	

Data are presented as unadjusted means ± SD

Genotypes were compared by multiple linear regression analysis with adjustments for sex and age

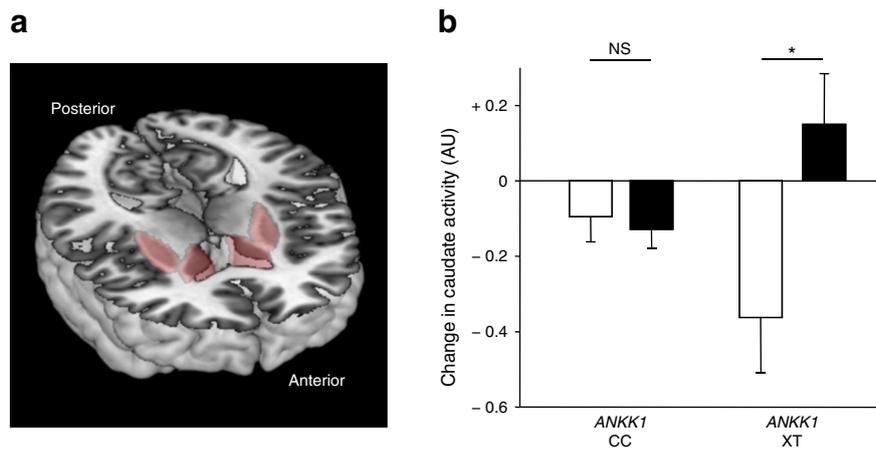


Fig. 2 Insulin sensitivity in the caudate nucleus of the striatum (**a**) is determined by the obesity-risk polymorphism in *FTO* and the D2 receptor polymorphism in *ANKK1*. Insulin effects on the caudate nucleus were assessed by fMRI combined with intranasal insulin administration in 45 healthy volunteers. (**b**) Insulin-induced change in caudate activity was determined and participants were stratified according to their *ANKK1* rs1800497 genotype in CC and XT carriers. *FTO* rs8050136 genotype is indicated by bar colour: white bars, *FTO* CC allele carriers; black bars, *FTO* XA

obesity-risk allele carriers. There was no association with the *FTO* SNP in *ANKK1* CC carriers, whereas *FTO* differed in the striatal response to insulin under the *ANKK1* XT background. Here, *FTO* non-risk allele carriers had a reduction in activity while *FTO* obesity-risk allele carriers showed an increase. This figure shows unadjusted means \pm SEM. *p* values are taken from an unadjusted additive inheritance model. AU, arbitrary units; NS, non-significant ($p > 0.05$), * $p < 0.05$

Complementing our findings of peripheral markers of metabolism and obesity, our neuroimaging results show that the effect of the detected interaction between *FTO* and *ANKK1* on insulin sensitivity is not limited to peripheral tissues, but that it also exists in the D2-receptor rich caudate nucleus. The *ANKK1* genotype influenced D2 receptor binding and BOLD responses to the receipt of palatable and energy-dense food in this region [41, 46]. This is of particular interest since both BOLD response [47] and dopamine release [48] in the caudate nucleus following palatable food intake was related to ratings of meal pleasantness. This demonstrates the importance of D2 receptor signalling in the caudate nucleus in eating behaviour and food reward. Interactions between insulin and dopaminergic networks contribute to both these brain functions accordingly [20]. Even though brain insulin sensitivity was not specifically tested in animals lacking *Fto* in dopaminergic neurons [14], it is tempting to speculate that altered insulin responses contributed to the observed phenotype.

There are at least two plausible explanations as to how the detected interactions in the brain could lead to the observed peripheral associations with regard to body fat distribution and systemic metabolism. Dopamine signalling in the brain is a determinant of peripheral insulin sensitivity [18]. Accordingly, circulating prolactin, a proxy for dopaminergic tone, has an age-dependent association with peripheral insulin sensitivity and glycaemia [49]. Based on our current results, dopaminergic control over peripheral glucose metabolism could be affected by *FTO* genotype.

However, we observed that the known association of the *FTO* SNP rs8050136 with brain insulin resistance depends on functional dopamine signalling, as determined by the *ANKK1* polymorphism. The *FTO* SNP was not associated with insulin responsiveness in the caudate nucleus in persons carrying the *ANKK1* non-risk allele, while it was associated with regional brain insulin sensitivity in carriers of the risk allele. In addition to various other functions, such as memory and reward, insulin delivery to the brain induced weight loss [29, 50]. Brain insulin sensitivity even predicts loss of metabolically unhealthy visceral fat during a lifestyle intervention [51]. Furthermore, insulin in the brain was recently shown to modulate peripheral insulin sensitivity in humans [28, 29]. Impaired brain insulin signalling could therefore contribute to increased body fat content and increased waist circumference, as well as to reduced peripheral insulin sensitivity in carriers of both risk alleles. In addition, our results in combination with the finding of an altered midbrain response in participants carrying both polymorphisms during a reward learning task [42] indicate that the interaction of *FTO* and *ANKK1* affects metabolic and behavioural brain networks.

The alterations that we observed in the brain in carriers of both risk alleles might trigger the alterations in peripheral metabolism, but we cannot rule out the possibility that the detected associations in regional brain activity are secondary to peripheral changes, e.g. altered body fat content. How *FTO* variations influence *IRX3/5* or *RPGRIP1L* expression in dopaminergic neurons and how this relates to their functional properties has not yet been addressed. Further studies are required to gain a better understanding of the mechanisms

underlying our results. Both cohorts that were analysed in this work only included individuals of European descent, and SNP rs8050136 may not be a good proxy for the association of the *FTO* locus with obesity in cohorts of different ethnicity. Therefore, our results cannot necessarily be transferred to another population. Thus, replication studies are needed, especially with participants of different genetic origin. Since we used a candidate gene approach, additional contributions of other genetic variants will be missed.

In summary, we report an interaction between the obesity-risk polymorphism in *FTO* and the *ANKK1/TaqIA* SNP indicating that the ~20% of the population carrying both risk alleles have a markedly higher risk for obesity and diabetes. Our data also suggest that the *ANKK1* risk allele may be a major contributor to the previously reported influence of *FTO* on obesity and metabolism.

Acknowledgements We thank all study participants for their cooperation in this project. We gratefully acknowledge the excellent technical assistance of Maike Borutta, Anna Bury, Anja Dessecker, Dr Louise Fritsche, Alke Guirguis, Ellen Kollmar, Corinna Sailer, Andreas Vosseler and Roman-Georg Werner (all University of Tübingen, Germany).

Parts of this study were presented in abstract form at the 75th Scientific Sessions of the American Diabetes Association in June 2015.

Funding This study was supported in part by a grant (01GI0925) from the Federal Ministry of Education and Research (BMBF) to the German Center for Diabetes Research (DZD e.V.) and the Helmholtz Alliance ICEMED-Imaging and Curing Environmental Metabolic Diseases and through an EFSD Rising Star award supported by Novo Nordisk to MH. The MDCS was supported by the Swedish Research Council's grant, a Linné grant and an ERC Advanced Research Grant (269045) to LG. EA was supported by the Albert Pahlsson Foundation and the Bo & Kerstin Hjelt Diabetes Foundation.

Author contributions MH performed the study, analysed the data and wrote the manuscript. SK performed the study, analysed the fMRI data and contributed to discussions. EA and PA analysed the MDC data and contributed to discussions. RW, FM, HS and AF acquired data and contributed to discussions. DMS contributed to the interpretation of the data and contributed to discussions. HUH, LCG, AF and HP designed the studies and contributed to discussions. All authors revised the manuscript and approved the final version before submission. HP is the guarantor for the data from Tübingen and LCG is the guarantor for the data from Malmö.

Duality of interest All authors declare that there are no conflicts of interest that are directly relevant to the content of this study.

References

- Walley AJ, Asher JE, Froguel P (2009) The genetic contribution to non-syndromic human obesity. *Nat Rev Genet* 10:431–442. doi:10.1038/nrg2594
- 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. doi:10.1126/science.1141634
- 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. doi:10.1038/ng2048
- Loos RJF, Yeo GSH (2014) The bigger picture of *FTO*: the first GWAS-identified obesity gene. *Nat Rev Endocrinol* 10:51–61. doi:10.1038/nrendo.2013.227
- Haupt A, Thamer C, Staiger H et al (2009) Variation in the *FTO* gene influences food intake but not energy expenditure. *Exp Clin Endocrinol Diabetes Off J Ger Soc Endocrinol Ger Diabetes Assoc* 117:194–197. doi:10.1055/s-0028-1087176
- Cecil JE, Tavendale R, Watt P et al (2008) An obesity-associated *FTO* gene variant and increased energy intake in children. *N Engl J Med* 359:2558–2566. doi:10.1056/NEJMoa0803839
- Wardle J, Carnell S, Haworth CMA et al (2008) Obesity associated genetic variation in *FTO* is associated with diminished satiety. *J Clin Endocrinol Metab* 93:3640–3643. doi:10.1210/jc.2008-0472
- Heni M, Kullmann S, Veit R et al (2013) Variation in the obesity risk gene *FTO* determines the postprandial cerebral processing of food stimuli in the prefrontal cortex. *Mol Metab* 3:109–113. doi:10.1016/j.molmet.2013.11.009
- Karra E, O'Daly OG, Choudhury AI et al (2013) A link between *FTO*, ghrelin, and impaired brain food-cue responsivity. *J Clin Invest* 123:3539–3551. doi:10.1172/JCI44403
- Staiger H, Machicao F, Fritsche A, Häring H-U (2009) Pathomechanisms of type 2 diabetes genes. *Endocr Rev* 30:557–585. doi:10.1210/er.2009-0017
- Smemo S, Tena JJ, Kim K-H et al (2014) Obesity-associated variants within *FTO* form long-range functional connections with *IRX3*. *Nature* 507:371–375. doi:10.1038/nature13138
- Claussnitzer M, Dankel SN, Kim K-H et al (2015) *FTO* Obesity Variant Circuitry and Adipocyte Browning in Humans. *N Engl J Med* 373:895–907. doi:10.1056/NEJMoa1502214
- Stratigopoulos G, Burnett LC, Rausch R et al (2016) Hypomorphism of *Fto* and *Rpgrip11* causes obesity in mice. *J Clin Invest* 126:1897–1910. doi:10.1172/JCI85526
- Hess ME, Hess S, Meyer KD et al (2013) The fat mass and obesity associated gene (*Fto*) regulates activity of the dopaminergic mid-brain circuitry. *Nat Neurosci* 16:1042–1048. doi:10.1038/nn.3449
- Luo S, Luo J, Meier AH, Cincotta AH (1997) Dopaminergic neurotoxin administration to the area of the suprachiasmatic nuclei induces insulin resistance. *Neuroreport* 8:3495–3499
- Diepenbroek C, van der Plassé G, Eggels L et al (2013) Alterations in blood glucose and plasma glucagon concentrations during deep brain stimulation in the shell region of the nucleus accumbens in rats. *Front Neurosci* 7:226. doi:10.3389/fnins.2013.00226
- Halpern CH, Tekriwal A, Santollo J et al (2013) Amelioration of binge eating by nucleus accumbens shell deep brain stimulation in mice involves D2 receptor modulation. *J Neurosci Off J Soc Neurosci* 33:7122–7129. doi:10.1523/JNEUROSCI.3237-12.2013
- DeFronzo RA (2011) Bromocriptine: a sympatholytic, d2-dopamine agonist for the treatment of type 2 diabetes. *Diabetes Care* 34:789–794. doi:10.2337/dc11-0064
- Figlewicz DP, Szot P, Chavez M et al (1994) Intraventricular insulin increases dopamine transporter mRNA in rat VTA/substantia nigra. *Brain Res* 644:331–334
- Figlewicz DP, Sipols AJ (2010) ENERGY REGULATORY SIGNALS AND FOOD REWARD. *Pharmacol Biochem Behav* 97:15–24. doi:10.1016/j.pbb.2010.03.002
- Speed NK, Matthies HJG, Kennedy JP et al (2010) Akt-dependent and isoform-specific regulation of dopamine transporter cell surface

- expression. *ACS Chem Neurosci* 1:476–481. doi:10.1021/cn100031t
22. Palmiter RD (2007) Is dopamine a physiologically relevant mediator of feeding behavior? *Trends Neurosci* 30:375–381. doi:10.1016/j.tins.2007.06.004
 23. Könnner AC, Hess S, Tovar S et al (2011) Role for insulin signaling in catecholaminergic neurons in control of energy homeostasis. *Cell Metab* 13:720–728. doi:10.1016/j.cmet.2011.03.021
 24. Tschritter O, Preissl H, Yokoyama Y et al (2007) Variation in the FTO gene locus is associated with cerebrocortical insulin resistance in humans. *Diabetologia* 50:2602–2603. doi:10.1007/s00125-007-0839-1
 25. Obici S, Feng Z, Karkhanian G et al (2002) Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. *Nat Neurosci* 5:566–572. doi:10.1038/mm861
 26. Pocai A, Lam TKT, Gutierrez-Juarez R et al (2005) Hypothalamic K(ATP) channels control hepatic glucose production. *Nature* 434:1026–1031. doi:10.1038/nature03439
 27. Pocai A, Obici S, Schwartz GJ, Rossetti L (2005) A brain–liver circuit regulates glucose homeostasis. *Cell Metab* 1:53–61. doi:10.1016/j.cmet.2004.11.001
 28. Heni M, Wagner R, Kullmann S et al (2014) Central insulin administration improves whole-body insulin sensitivity via hypothalamus and parasympathetic outputs in men. *Diabetes* 63:4083–4088. doi:10.2337/db14-0477
 29. Heni M, Kullmann S, Preissl H et al (2015) Impaired insulin action in the human brain: causes and metabolic consequences. *Nat Rev Endocrinol* 11:701–711. doi:10.1038/nrendo.2015.173
 30. Do R, Bailey SD, Desbiens K et al (2008) Genetic variants of FTO influence adiposity, insulin sensitivity, leptin levels, and resting metabolic rate in the Quebec Family Study. *Diabetes* 57:1147–1150. doi:10.2337/db07-1267
 31. Haupt A, Thamer C, Machann J et al (2008) Impact of variation in the FTO gene on whole body fat distribution, ectopic fat, and weight loss. *Obes Silver Spring Md* 16:1969–1972. doi:10.1038/oby.2008.283
 32. Scott LJ, Mohlke KL, Bonnycastle LL et al (2007) A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* 316:1341–1345. doi:10.1126/science.1142382
 33. Neville MJ, Johnstone EC, Walton RT (2004) Identification and characterization of ANKK1: a novel kinase gene closely linked to DRD2 on chromosome band 11q23.1. *Hum Mutat* 23:540–545. doi:10.1002/humu.20039
 34. Jönsson EG, Nöthen MM, Grünhage F et al (1999) Polymorphisms in the dopamine D2 receptor gene and their relationships to striatal dopamine receptor density of healthy volunteers. *Mol Psychiatry* 4:290–296
 35. Zhang Y, Bertolino A, Fazio L et al (2007) Polymorphisms in human dopamine D2 receptor gene affect gene expression, splicing, and neuronal activity during working memory. *Proc Natl Acad Sci U S A* 104:20552–20557. doi:10.1073/pnas.0707106104
 36. Gluskin BS, Mickey BJ (2016) Genetic variation and dopamine D2 receptor availability: a systematic review and meta-analysis of human in vivo molecular imaging studies. *Transl Psychiatry* 6:e747. doi:10.1038/tp.2016.22
 37. Comings DE, Muhleman D, Ahn C et al (1994) The dopamine D2 receptor gene: a genetic risk factor in substance abuse. *Drug Alcohol Depend* 34:175–180
 38. Felsted JA, Ren X, Chouinard-Decorte F, Small DM (2010) Genetically determined differences in brain response to a primary food reward. *J Neurosci Off J Soc Neurosci* 30:2428–2432. doi:10.1523/JNEUROSCI.5483-09.2010
 39. Benton D, Young HA (2016) A meta-analysis of the relationship between brain dopamine receptors and obesity: a matter of changes in behavior rather than food addiction? *Int J Obes* 40:S12–S21. doi:10.1038/ijo.2016.9
 40. Winkler JK, Woehning A, Schultz J-H et al (2012) TaqIA polymorphism in dopamine D2 receptor gene complicates weight maintenance in younger obese patients. *Nutr Burbank Los Angel Cty Calif* 28:996–1001. doi:10.1016/j.nut.2011.12.018
 41. Stice E, Spoor S, Bohon C, Small DM (2008) Relation between obesity and blunted striatal response to food is moderated by TaqIA A1 allele. *Science* 322:449–452. doi:10.1126/science.1161550
 42. Sevgi M, Rigoux L, Kühn AB et al (2015) An Obesity-Predisposing Variant of the FTO Gene Regulates D2R-Dependent Reward Learning. *J Neurosci Off J Soc Neurosci* 35:12584–12592. doi:10.1523/JNEUROSCI.1589-15.2015
 43. Goldstein JI, Crenshaw A, Carey J et al (2012) zCall: a rare variant caller for array-based genotyping: genetics and population analysis. *Bioinforma Oxf Engl* 28:2543–2545
 44. Stefan N, Machicao F, Staiger H et al (2005) Polymorphisms in the gene encoding adiponectin receptor 1 are associated with insulin resistance and high liver fat. *Diabetologia* 48:2282–2291. doi:10.1007/s00125-005-1948-3
 45. Kullmann S, Frank S, Heni M et al (2013) Intranasal insulin modulates intrinsic reward and prefrontal circuitry of the human brain in lean women. *Neuroendocrinology* 97:176–182. doi:10.1159/000341406
 46. Roth CL, Hinney A, Schur EA et al (2013) Association analyses for dopamine receptor gene polymorphisms and weight status in a longitudinal analysis in obese children before and after lifestyle intervention. *BMC Pediatr* 13:197. doi:10.1186/1471-2431-13-197
 47. Small DM, Zatorre RJ, Dagher A et al (2001) Changes in brain activity related to eating chocolate: from pleasure to aversion. *Brain J Neurol* 124:1720–1733
 48. Small DM, Jones-Gotman M, Dagher A (2003) Feeding-induced dopamine release in dorsal striatum correlates with meal pleasantness ratings in healthy human volunteers. *Neuroimage* 19:1709–1715
 49. Wagner R, Heni M, Linder K et al (2014) Age-dependent association of serum prolactin with glycaemia and insulin sensitivity in humans. *Acta Diabetol* 51:71–78. doi:10.1007/s00592-013-0493-7
 50. Hallschmid M, Benedict C, Schultes B et al (2004) Intranasal insulin reduces body fat in men but not in women. *Diabetes* 53:3024–3029
 51. Tschritter O, Preissl H, Hennige AM et al (2012) High cerebral insulin sensitivity is associated with loss of body fat during lifestyle intervention. *Diabetologia* 55:175–182. doi:10.1007/s00125-011-2309-z