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ARTICLE

Association of TMEM18 variants with BMI and waist circumference in children and correlation of mRNA expression in the PFC with body weight in rats

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Genome-wide association studies have shown a strong association of single-nucleotide polymorphisms (SNPs) in the near vicinity of the *TMEM18* gene. The effects of the TMEM18-associated variants are more readily observed in children. TMEM18 encodes a 3TM protein, which locates to the nuclear membrane. The functional context of TMEM18 and the effects of its associated variants are as of yet undetermined. To further explore the effects of near-TMEM18 variants, we have genotyped two TMEM18-associated SNPs, rs6548238 and rs4854344, in a cohort of 2352 Greek children (Healthy Growth Study). Included in this study are data on anthropomorphic traits body weight, BMI *z*-score and waist circumference. Also included are dietary energy and macronutrient intake as measured via 24-h recall interviews. Major alleles of rs6548238 and rs4854344 were significantly associated with an increased risk of obesity (odds ratio=1.489 (1.161–1.910) and 1.494 (1.165–1.917), respectively), and positively correlated to body weight (*P*=0.017, *P*=0.010) and waist circumference (*P*=0.003, *P*=0.003). An association to energy and macronutrient intake was not observed in this cohort. We also correlated food intake and body weight in a food choice model in rats to Tmem18 expression in central regions involved in feeding behavior. We observed a strong positive correlation between TMEM18 expression and body weight in the prefrontal cortex (PFC) (*r*=0.5694, *P*=0.0003) indicating a potential role for TMEM18 in higher functions related to feeding involving the PFC.
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INTRODUCTION

Genome-wide association (GWA) studies have led to the identification of several loci in the human genome containing genetic variants conferring an increased risk of developing overweight and obesity.[1–2] One such gene to be associated with a higher BMI, the fat mass and obesity-associated gene (*FTO*) was identified by Frayling *et al* in 2007.[3] FTO has since been shown to be involved in regulation of feeding behavior via homeostatic hypothalamic pathways,[4–6] and its obesity-associated variants to confer gain-of-function by increasing gene transcription.[7] The most recent meta analyses of GWA studies on obesity, which included genetic information from 249 796 individuals, identified variants at a total of 32 genetic loci affecting the development of BMI.[2] Single-nucleotide polymorphisms (SNPs) in the *FTO* gene, followed by SNPs in the proximity of TMEM18 and MC4R, have consistently given the strongest associations to obesity in large-scale GWA studies. TMEM18-associated SNPs were first found to be associated with a higher BMI in 2009 by the GIANT consortium,[1] and this was confirmed in the same year by an independent group.[8]

Results from child cohorts have shown stronger effects of near-TMEM18 SNPs to obesity, compared with adults. Zhao *et al*[9] found near-TMEM18 SNPs to confer the strongest effect on pediatric BMI out of 25 studied obesity-associated variants in a cohort of 6078 children of European descent. A similar effect was observed in a cohort of 2042 children and adolescents from four European countries when the effects of 17 variants were studied. The strongest effect size for BMI and sum of skin folds observed in this study was for the near-TMEM18 SNP rs6548238.[10] The rs6548238 variant also had a moderate effect on waist circumference and height. However, no correction was made for multiple testing in this study. Results from this group also showed an association of rs6548238 and rs7561317 with obesity in a cohort of 1027 children from the Stockholm area, but no association to anthropomorphic traits was detected in this study.[11] Results from adult cohorts have been more inconsistent. A study on 4923 adults from northern Sweden was unable to detect an association of rs6548238 to obesity, type 2 diabetes or measures of body composition and adipose distribution.[12] Another study on a cohort of 6013 Chinese type 2 diabetes patients, 1087 healthy adolescents and 605 healthy adults was also unable to detect an association of the rs7561317 genotype to type 2 diabetes, waist circumference and waist-to-hip ratio. There was a trend toward an association to BMI but this did not reach statistical significance.[13] In a Japanese adult cohort of 1129 obese and 1736 normal weight controls, the TMEM18-associated SNPs, rs2867125, rs6548238, rs4854344 and rs7561317, were all observed to be associated to obesity.[14] The near-TMEM18 SNP rs7561317 was

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also tested for association to phenotypes of metabolic disorder in this study, owing to its previous association to type II diabetes. BMI, blood pressure, fasting plasma glucose, triglycerides, total cholesterol and HDL cholesterol were compared in obese and controls, but no association to rs7561317 genotype was observed.

TMEM18 is a highly conserved transmembrane protein containing three membrane-spanning regions containing a nuclear localization signal, as well as a coiled-coil domain, at the C-terminal.[11] It most likely localizes to the nuclear membrane after translation, and was discovered to be involved in the migration of neural stem cells and neural progenitor cells toward glioma cells as part of the downstream signaling pathway of chemokine receptors.[15] Expression profiling in rat and mouse showed TMEM18 to be expressed in all tissues tested. It is also abundantly expressed in the brain and in central regions including those involved in food intake. However, in contrast to, for example: FTO, NPY, POMC, AgRP or MC4R; TMEM18 has not been found to be regulated in brain regions regulating food intake. Our earlier studies found no regulation of TMEM18 to take place in the hypothalamus or brain stem during food deprivation or consumption of a palatable high-fat diet,[11] this was in contrast to canonical feeding-regulatory peptides such as POMC, NPY, MC4R and AgRP,[16] as well as FTO.[4,5] The anatomical, molecular and functional basis of how TMEM18 may be involved in regulating BMI is thus obscure.

In the present study, we genotyped two TMEM18 SNPs previously associated with BMI: rs6548238 and rs4854344, in a cohort of 2352 Greek schoolchildren containing anthropomorphic data on body weight, BMI and waist circumferences as well as dietary intake data, such as energy and macronutrient intake as determined by 24-h recall interviews. Food preference and body weight in a rodent food choice model[17] were also correlated to expression levels of Tmem18 in several tissues involved in different aspects of feeding, such as components of the mesolimbic and mesocortical pathways: the amygdala, nucleus accumbens and hippocampus, as well as the prefrontal cortex (PFC), which is involved in behavioral inhibition and executive function.

MATERIALS AND METHODS

Subjects and phenotype characterization

The cohort of Greek children comprised 2658 schoolchildren, attending the 5th and 6th grades of primary schools (Table 1). This cohort was part of the 'Healthy Growth Study', a large-scale cross-sectional epidemiological study initiated in May 2007 as described previously.[17,18] An extended letter explaining the aims of the current study and a consent form were provided to each parent who had a child in one of the primary schools participating in the study. Those parents who agreed to participate in the study gave their informed consent by signing the consent form, and provided their contact details. Body weight and height were measured in all study participants using standard procedures and equipment. Body weight was measured to the nearest 10 g and height was measured to the nearest 0.1 cm in standing position. BMI *z*-score was calculated relative to the International Obesity Task Force (IOTF) definitions.[19] Subjects were categorized as normal weight and obese using IOTF BMI cut-off values equivalent to adult BMI of 24 and 30, respectively. Waist circumference was measured to the nearest 0.1 cm with the use of a non-elastic tape (Hoechstmass, Sulzback, Germany) around the trunk, at the level of umbilicus midway between the lower rib margin and the iliac crest, and with the subject at a standing position. Dietary intake data were obtained by trained dieticians and nutritionists by morning interviews with the children at school site for two consecutive weekdays and one weekend day, using the 24-h recall technique. All study participants were asked to describe the type and amount of foods, as well as all beverages consumed during the previous day, provided that it was a usual day according to the participant's perception. To improve the accuracy of food descriptions, standard household measures (cups, tablespoons, etc) and food models were used to define amounts when appropriate. At the end of each interview, the interviewers, who were dieticians rigorously trained to minimize interviewer effect, reviewed the collected food intake data with the respondent in order to clarify entries, servings and possible forgotten foods. The ratio of reported energy intake and predicted basal metabolic rate was used to asses underreporting of calorie intake. Basal metabolic rate was estimated according to Schofield equations,[20] taking into account age, sex, and body weight and with cut-off limits developed by Goldberg *et al.*[21]

Food intake data were analyzed using the Nutritionist V diet analysis software (version 2.1, 1999, First Databank, San Bruno, CA, USA), which was extensively amended to include traditional Greek recipes, as described in Food Composition Tables of Greek Cooked Foods and Dishes.[22] Furthermore, the database was updated with nutritional information of processed foods provided by independent research institutes, food companies and fast-food chains. DNA for genotyping was available for 2352 subjects (1311 girls and 1064 boys). Approval of the consent procedure, and to conduct the study, was granted by the Greek Ministry of National Education and the Ethical Committee of Harokopio, University of Athens.

Genotyping and linkage disequilibrium analysis

The genotyping of *TMEM18* SNPs was carried out with pre-designed Taqman single-nucleotide polymorphism genotyping assays (Applied Biosystems, Foster City, CA, USA) and an ABI7900 genetic analyzer with SDS 2.2. software at the Uppsala Genome Center (http://www.genpat.uu.se/node462). The genotype call rate was 99.6%. Test for deviation from Hardy–Weinberg equilibrium

Table 1 Descriptive characteristics of the cohort of 2352 Greek children, stratified according to weight status

Characteristics	*All*	*Normal weight*	*Obese*
N	2352	2080	272
Age (years)	11.2 ± 0.7	11.2 ± 0.7	11.1 ± 0.6
Body weight (kg)	45.3 ± 11.1	38.7 ± 6.3	64.5 ± 9.5
Height (cm)	148.7 ± 7.8	147.3 ± 7.7	152.3 ± 7.4
BMI *z*-score	0.9 ± 1.2	−0.01 ± 0.8	2.8 ± 0.3
BMI	20.3 ± 3.8	20.3 ± 3.8	27.6 ± 2.5
Average dietary energy intake (kcal/day)	1791.6 ± 553.7	1801.5 ± 553.8	1715.9 ± 547.6
Fiber (g)	13.7 ± 7.5	13.8 ± 7.6	12.8 ± 6.5
Cholesterol (mg)	230.9 ± 127.7	284.1 ± 138.2	226.1 ± 120.8
% Total energy from			
Fat	40.9 ± 7.3	40.9 ± 7.2	40.8 ± 7.9
Carbohydrates	45.6 ± 8.5	45.7 ± 8.4	45.2 ± 9.2
Protein	15.6 ± 3.5	15.6 ± 3.4	16.1 ± 3.7

Values are geometric means ± SD.

was performed using the Pearson's χ^2-test (1 d.f), and none of the SNPs did deviate from Hardy–Weinberg equilibrium ($P>0.4$). Haploview[23] was used for linkage disequilibrium (LD) measurements according to confidence intervals (CIs)by Gabriel *et al*,[24] as well as graphical representation of the LD structure indicated as r^2. The LD pattern was generated using HapMap data version 2, release 21 and CEU as analysis panel.

Statistical analyses

Association with obesity was analyzed with logistic regression and odds ratio with a 95% CI. Associations between genotypes and phenotypes were analyzed with linear regression, assuming an additive model. Quantitative skewed variables were normalized by transformation before analysis. The models were adjusted for age, gender, BMI and length when needed. Statistical analyses were performed with PLINK (http://pngu.mgh.harvard.edu/purcell/plink/).[25] Multiple testing was adjusted with the false discovery rate according to the procedure of Benjamini and Hochberg,[26] and a *P*-value <0.005 was considered statistically significant.

Power calculations

For the case/control analyses, power calculations were carried out with the CaTS power calculator (http://www.sph.umich.edu/csg/abecasis/CaTS/index.html).[27] We had 80% power to detect association with obesity with a relative risk of 1.3.

Food choice model

Thirty-six outbred male Wistar rats (Scanbur BK AB, Sollentuna, Sweden) were housed in standard macrolon cages at constant temperature ($22\pm1\,^{\circ}C$) and humidity ($50\pm5\%$). Rats were 10 weeks old at the start of the study, and housed individually for 8 days during the food preference paradigm. Water and standard food chow (R36, Lactamin, Lidköping, Sweden) was supplied *ad libitum* during the entire experiment except for the 5-day food preference test, during which the animals had free access to three diets: a palatable high-fat diet and two reference diets with high amounts of either casein (high-protein diet) or maize starch (high-carbohydrate diet). Compositions of the different diets are described in Alsiö *et al*.[28] The food was provided in bowls and weighed each day to measure the ingested amount. This model allowed the determination of the following outcomes: body weight at endpoint, total food intake during palatable diet presentation, high-fat diet preference (high-fat diet consumption divided by total intake) and consumption of standard chow. Subsequent to the 5-day food choice, the animals were kept on standard chow for 12 days; this 'washout' period was inserted to allow effects of the food choice diets on gene expression to subside. Animals were then killed by decapitation. All samples were collected immediately following decapitation. Dissection of the brain is described by Alsiö *et al*.[28] Samples were then immersed in RNAlater (Applied Biosystems/Ambion, Austin, TX, USA) and stored at room temperature for 2 h before being stored at $-20\,^{\circ}C$ until preparation. All animal procedures were approved by the Uppsala Animal Ethical Committee (ID: C 285/5) and followed the guidelines of Swedish legislation on animal experimentation (Animal Welfare Act SFS1998:56) and European Union legislation (Convention ETS123 and Directive 86/609/EEC).

Generation of complementary DNA (cDNA)

Tissues were homogenized by sonification (Branson sonifier B 15), and RNA was purified from the samples using the TRIzol method (Sigma-Aldrich, Stockholm, Sweden).[29] Samples were then treated with DNase I (Roche Diagnostics, Bromma, Scandinavia) to remove residual DNA contamination. Presence of residual DNA contamination after DNase I treatment was checked by using PCR, and products were run on agarose gel. cDNA was then generated using reverse MLV reverse transcriptase (Invitrogen, Lidingö, Sweden) according to the manufacturer's specifications.

Quantitative real-time PCR

PCR reactions were run in a total volume of 20µl using Taq polymerase kits (Biotools, Madrid, Spain). Each reaction was performed in duplicate according to the manufacturer's specifications, and contained 75 mM Tris/HCL, 50 mM KCl and 20 mM $(NH_4)_2SO_4$, 4 mM $MgCl_2$, 0.25 mM dNTPs, 1:20 DMSO, 20 mU/µl Taq polymerase, 50 mM forward and reverse primer and 1:4 SYBR-green (Invitrogen). Reactions were run on iCycler temperature cyclers, and fluorescence was measured using MyiQ single color real-time PCR detection system. Data were analyzed using iQ5 software (BioRad, Sundbyberg, Sweden). Primer temperatures were optimized for specificity using temperature gradients and analyzing melting curves of the PCR products.

Statistical analysis

Gene expression was normalized using the geometric mean of the most stable housekeeping genes ($M<1.5$), in accordance with the geNorm-method as previously published by Vandesompele *et al*.[30] Statistical analysis was performed in Prism v 5.02 for Windows (GraphPad Software, San Diego, CA, USA; http://www.graphpad.com). The non-parametric test for correlation (Spearman's test, Prism v. 5.02) was used to test for correlation between mRNA expression and model parameters: body weight at the end of the food preference paradigm, total amount of food consumed, preference for the high-fat diet and the amount of chow consumed during the food preference paradigm.

RESULTS

TMEM18-associated SNPs rs4854344 and rs6548238 are associated to obesity, BMI and waist circumference but not dietary intake

Cohort characteristics are displayed in Table 1. rs6548238 and rs4854344 are located about 30 kb downstream of TMEM18 and in almost absolute LD with each other, $r^2=0.98$ (Supplementary Figure 1). Association to obesity was determined by comparing normal weight subjects (BMI<25) with obese (BMI>30). Analysis showed major alleles of rs6548238 and rs4854344 to be significantly associated with an increased risk of obesity (rs6548238: 1.489 (1.161–1.910), $P=0.002$; rs4854344: 1.494 (1.165–1.917), $P=0.001$) (Table 2), which is in line with previous results on a cohort of 1027 children and adolescents from the Stockholm area.[11] By using linear regression analysis, we also observed higher BMI (rs6548238: $P=0.006$, rs4854344: $P=0.005$) and waist circumference (rs6548238: $P=0.003$,

Table 2 Genotype distribution and odds ratio of two SNPs downstream *TMEM18* between obese and normal-weight Greek children

			Genotype, n *(%)*						
		N	*G1*	*G2*	*G3*	*MAF, %*	*OR (95% CI)*	P	*HWE*
rs6548238	Normal weight	2073	82 (4.0)	689 (33.2)	1302 (62.8)	20.6			
A>G	Obese	270	9 (3.3)	62 (23.0)	199 (73.7)	14.7	1.489 (1.161–1.910)	0.002	0.461
rs4854344	Normal weight	2072	83 (4.0)	686 (33.1)	1303 (62.9)	20.5			
G>T	Obese	271	9 (3.3)	62 (22.9)	200 (73.8)	14.7	1.494 (1.165–1.917)	0.001	0.591

Abbreviations: CI, confidence interval; MAF, minor allele frequency; OR, odds ratio.
Data are number of subjects in each group and number of subjects for each genotype (G) (% in each group). MAF for each group is given in percentage. OR with a 95% CI was calculated assuming an additive model. Association with obesity was determined comparing subjects with normal weight (BMI $<25\,kg/m^2$) and obesity (BMI $30\,kg/m^2$). HWE indicate *P*-values for deviation from Hardy–Weinberg Equilibrium, excluded if $P\leq0.001$.

Table 3 Association between two SNPs downstream TMEM18 and body weight, BMI *z*-score, waist circumference and dietary energy intake in 2352 Greek children

	Beta	*95% CI*	P *Add*
rs6548238			
Body weight (kg)	0.063	0.011–0.115	0.017
BMI *z*-score	0.097	0.026–0.169	0.005
Waist circumference (cm)	0.111	0.039–0.182	0.003
Average dietary energy intake (kcal/day)	−0.034	−0.036–0.106	0.343
Fiber (g)	−0.007	−0.064–0.078	0.845
Cholesterol (mg)	0.063	−0.008–0.135	0.081
% Total energy from			
Fat	−0.239	−0.047–0.096	0.513
Carbohydrates	0.034	−0.106–0.036	0.342
Protein	−0.039	−0.033–0.112	0.289
rs4854344			
Body weight (kg)	0.068	0.016–0.120	0.01
BMI *z*-score	0.102	0.031–0.173	0.005
Waist circumference (cm)	0.110	0.039–0.182	0.003
Average dietary energy intake (kcal/day)	−0.038	−0.03–0.109	0.291
Fiber (g)	−0.003	−0.068–0.075	0.926
Cholesterol (mg)	0.065	−0.006–0.136	0.071
% Total energy from			
Fat	−0.029	−0.042–0.101	0.422
Carbohydrates	0.041	−0.112–0.030	0.261
Protein	−0.042	−0.030–0.114	0.260

Beta indicates transformed beta values. *P* indicates *P*-values adjusted for significant covariates.

rs4854344: $P=0.003$) for carriers of the major allele of rs6548238 and rs4854344, and trends for higher body weight (rs6548238: $P=0.017$, rs4854344: $P=0.010$) (Table 3). The two SNPs were not associated with the average dietary energy intake (kcal/day). Similarly, daily intake of fiber and cholesterol, as well as percentages of energy intake derived from total fat and protein, were independent on TMEM18 genotypes in this cohort (Table 3). Underreporting of calorie intake was assessed for 22.6% of the entire cohort and 12.1% of the cohort for which genotype data were available. However, no effect on the outcome was observed when we removed subjects assessed to be underreporting from the analysis. This indicates that the lack of an association between food intake and TMEM18-related SNPs observed in our cohort was likely not caused by the underreporting of calorie intake by the participants.

Expression of PFC TMEM18 correlates to body weight in rats

The rodent food choice paradigm was previously employed to identify associations between hypothalamic gene expression levels and food intake, as well as body weight of rats.[28] We did observe expression of TMEM18 in all the tissues studied. However, we observed no correlation for TMEM18 expression with high-fat diet preference, amount of chow, amount of total food ingested or body weight in the hypothalamus, nucleus accumbens, amygdala or hippocampus. We did, however, observe a strong correlation between TMEM18 expression and body weight in the rat PFC ($r=0.5694$, $P=0.0003$) (Figure 1). We also investigated the expression of several proteins known to be involved in cortical signaling (Supplementary Table 1). Body weight was significantly correlated to expression of GABA-receptor subunit α-3 (Gabra3) ($P=0.0052$), as well as 5-HT_{2A} receptor ($P=0.049$), α-1B adrenergic receptor ($P=0.0168$) and GABA B receptor 2 ($P=0.0426$) (Supplementary Figure 1).

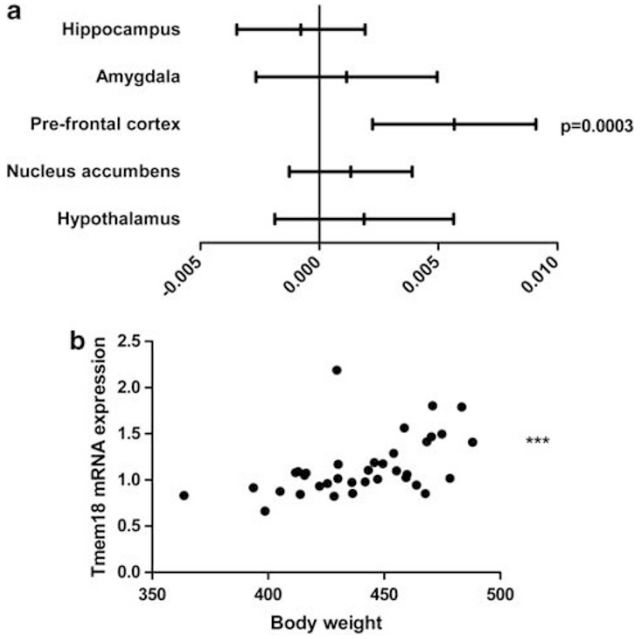

Figure 1 : **a** Slopes for best-fit curves ± 95% CI, generated from linear regression analysis of mRNA expression of TMEM18 in regions of the brain in rats ($n=36$) as measured by quantitative reverse transcriptase PCR, against body weight in grams. Linear regression was performed in Prism v5.02 (GraphPad Software). **b** Relative expression of TMEM18 mRNA in the rat PFC, as measured by quantitative reverse transcriptase PCR, plotted against body weight in grams. Results from linear regression analysis of TMEM18 mRNA expression against body weight at the end of the food preference paradigm shows a significant correlation between body weight and expression of TMEM18. ***$P<0.001$.

DISCUSSION

Here, we show that the major alleles of the near-TMEM18 SNPs: rs6548238 and rs4854344, were significantly associated with an increased risk to develop obesity in the studied cohort of 2352 Greek children. These results are in line with the previous results on a cohort of children from the Stockholm area,[11] as well as with the results from Zhao *et al*[9] who observed strong associations of three TMEM18-associated SNPs to obesity in a cohort of 6078 children of European descent. We also observed associations of rs6548238 and rs4854344 to anthropomorphic traits: waist circumference, body weight and BMI *z*-score, which were not detected in the Stockholm cohort. We did however not observe any association to dietary energy and macronutrient intake (Table 3).

Studies on adult cohorts have yielded more inconsistent results indicating that the effects of TMEM18-associated variants may be more pronounced in early childhood and adolescence. This is supported by a study by Elks *et al*[31] who observed obesity-risk alleles identified through GWA studies, including TMEM18-associated SNPs, to confer an effect on weight gain also in early childhood and adolescence. This effect was observed as early as in the first weeks after birth. An association study for BMI-increasing alleles and birth weight reported a slight effect of rs6548238 on birth weight, but not when multiple testing was accounted for.[32]

rs6548238 and rs4854344 are located about 30 kb downstream of TMEM18. Haplotype analysis showed rs6548238 and rs4854344 to be in almost complete LD. Both SNPs are located in a haplotype block, which does not encompass the *TMEM18* gene (see Supplementary Figure 2). It is thus unlikely that rs6548238 and rs4854344 are linked

to causative SNPs inside the *TMEM18* gene. They may however have an effect on the transcription of TMEM18 by affecting the binding of transcription factors or co-regulators of transcription. At this stage, the mechanisms behind the effect of these variants are, as of yet, undetermined.

We present for the first time an association of body weight in rats with gene expression of TMEM18. This association was neuroanatomically localized to the PFC. The PFC has been proposed to have an important role in integrating behavioral cues from multiple sources in the brain (reviewed by Miller and Cohen[33]). It receives information from various sources, among these are the hippocampus, hypothalamus, mesolimbic circuit, motor cortex and sensory cortices. In the context of feeding behavior, a model for the PFC in prioritization of long-term goals by inhibiting behavioral cues from the hypothalamus, brainstem and mesolimbic pathway, that is, hunger and craving, has been suggested. This has been termed 'top-down' control of, or inhibition of, behavior, which denotes higher cognitive function inhibiting behavioral cues from the basal ganglia, brain stem and hypothalamus. Other aspects of PFC function includes what has been described as impulse control and working memory. A functional role for TMEM18 in the PFC is, as of yet, unclear. Earlier reports of involvement of TMEM18s in neural migration may be a valuable lead and point to a role in the development of the PFC, or in dynamic plasticity related to memory formation and learning. Learning-dependent cortical remodeling has been reported in the primate PFC in response to afferent dopamine signaling,[34] and synaptic plasticity in the PFC has been shown to be modulated by monoaminergic signaling from PFC afferents.[35]

In this context, it is of interest that we observe concomitant correlation in the rat PFC of GABA-receptor transcripts Gabra3, Gabbr2, and monoamine receptor transcripts 5Ht2a and Adrb1, with body weight at the end of the feeding paradigm. The α_{1A} adrenergic receptor and the 5-HT_{2A}-receptor both act as excitatory G protein-coupled receptors coupling to the $G\alpha_q$ signaling cascade, and elicits phospholipase C-mediated Ca^+ release.[36] Postsynaptically, they act to stimulate GABA release from cortical interneurons and also glutamate release from pyramidal neurons.[37] Stimulation of α_1-adrenergic receptors in the rat PFC has also been shown to increase local release of 5-HT.[36] The GABA receptor is a ligand-gated ion channel commonly composed of five subunits: two α, two β and one γ subunit. Each subunit also has several subtypes, which creates a large number of combinations making up the GABA-receptor isoform diversity. The subunit composition of the receptor in turn affects ligand affinity and can also affect its electrophysiological properties.[38] The α3 GABA-receptor subunit is frequently a component of the $\alpha_3\beta_2\gamma_2$ GABA-receptor subtype, which is expressed in the cerebral cortex and in monoaminergic neurons of the basal forebrain. Knockout studies have shown this subunit to be involved in sensorimotor signaling and a hyperdopaminergic phenotype, which has been suggested to be related to schizophrenia.[39] The correlation of the expression of these genes in the PFC of rats with high body weight indicates PFC serotonergic and adrenergic signaling to be affected in these animals with concurrent effects on monoaminergic receptor expression and subsequent effects on GABA-receptor gene transcription.

In summary, we show that SNPs in TMEM18 are associated with obesity and body weight, as well as anthropomorphic measures of adiposity: waist circumference, in a cohort of 2352 Greek children. No association was found to behavioral phenotypes: dietary energy and macronutrient intake as measured by 24-h recall interviews. It should be mentioned that every evaluation of dietary intake is limited, and the limitations of 24-h recalls in capturing habitual intake at the individual level are well known, because of the large day-to-day variation in intake that exists and possible difficulties in remembering what has actually been ingested. However, for the purpose of this study, three 24-h recalls on a reasonably large group, with no obvious bias, was deemed appropriate to study a plausible association with energy intake and TMEM18. However, replication of this with other estimates of energy intake would be valuable. Surprisingly, we found that the expression of TMEM18 in the rat PFC is correlated to body weight in our animal model. This is the first functional data showing a link between expression of TMEM18 and phenotype. Concomitant regulation of monoaminergic- and GABA-ergic receptor transcripts could prompt further studies for a role on TMEM18 in synaptic plasticity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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