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Neural Activity in Response to Food Images is Mediated by the ASB9 and DNM3 Gene and is Associated with Methylation and Ghrelin.

Further Support for Important Genetic Effects in Obesity

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Master's thesis

Human-Machine Communication

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Neural Activity in Response to Food Images is Mediated by the ASB9 and DNM3 Gene and is Associated with Methylation and Ghrelin; further Support for Important Genetic Effects in Obesity

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Abstract

Gaining insights in how genetics influence obesity, brain activity, and eating behavior will add important knowledge for developing strategies for weight-loss treatment, as obesity may stem from different causes and as individual feeding behavior may depend on genetic differences. To this end, we have examined if the ASB9, DNM3 and RSPO04 gene affects neural activity via fMRI when individuals are presented with images of food. Recently five DNA methylation sites related to BMI have been discovered. Directly expanding upon these results, we are the first to test whether methylation levels of these five sites are predictive of brain responses to food images, and if they correlate with the metabolic hormone ghrelin. We found that individuals with the high risk allele for the DNM3 or ASB9 showed increased neural activity in widespread regions that have been associated with emotion, memory, self-image and executive functioning. Next, we found that as methylation at the cg07814318 site decreased, brain activity increased in brain areas important for salience as well as in areas with previous associations to food signaling and obesity. Moreover, methylation levels at cg07814318 also strongly correlate with ghrelin. Our results suggest that the genotypes of the ASB9 and DNM3 gene are associated with differential neural processing of food images. We might conclude that that food images are less salient for people with the ASB9 and DNM3 risk alleles, coinciding with diminished emotional response and impulse control. Our findings are novel for being the first to detect brain areas affected by methylation in overweight individuals, and add support to previous reports which attempt to link ghrelin, brain activity, and genetic differences between people of different weight categories.

Keywords: Obesity - Neural Activity - ASB9 - DNM3 - Methylation - cg07814318 - Genetic Risk Score.

Introduction

Background

Environmental, genetic, and behavioral factors are all related to the prevailing epidemia of obesity. According to the World Health Organization 39% of adults aged 18 years and over were overweight in 2014, and 13% were obese. Obese is defined as a BMI above 30, BMI above 25 is defined as overweight, normal weight is defined as a BMI between 18,5 and 25. Obesity facilitating environmental factors such as fast food restaurants, where 1500-calorie meals are not exceptional (e.g., BigMac+fries+drink), are commonly available. However, while we can effortlessly get high-calories meals, not every individual becomes obese. The reason that not everybody becomes obese could be a result of behavioral or genetic differences. The risk of becoming obese could be mediated by epigenetics, which is the the study of physiological phenotypic trait variations that are caused by the environment or external factors that can switch genes off or on, and how the environment affects cell functionality instead of that cell variations are caused by changes in DNA sequences. The risk of obesity could also be mediated by genetic variation or their expression in the brain, which in their turn affect behavior and metabolism or brain activity itself. Obesity it is said to be a highly heritable trait although it is not sure whether obesogenetics influence the food expenditure or intake in a direct manner (Wardle et al., 2008). (Karra et al., 2013) have discovered and identified multiple neuropeptides (i.e., Ghrelin and Leptin) as orexigenic signaling hormones that are important for the central regulation of food intake. Findings like such provide us with information that not only genetic profiles but also hormones influence feeding behavior and that consequences of obesity are highly complex relating to the nature of driving forces. Eating because of hunger, reward or of other stimuli that involve memory and higher cortical functions (i.e. top down regulation

to inhibit intake) are examples of those forces. Even though associations between body mass index and several genetic variants have been identified, there is not much known about the epigenetic changes that are related to BMI.

Genetic risk scores for the overweight

The link between brain activity and genes or single nucleotide polymorphisms (SNPs) can be summarized via a calculated genetic risk score (GRS). Genetic risk scores are generally able to summarize risk associated variation across a human genome (Horne et al., 2005) by aggregating information from multiple-risk SNPs. In general, genetic risk scores tend to group information from multiple single nucleotide polymorphisms, resulting in a lesser importance from each individual SNP to the summary measurement, this makes them more robust to imperfect linkage for any one SNP. When more SNPs are included in a GRS, the distribution of values approaches a normal distribution, even when individual risk SNPs are (relatively) uncommon (Belsky et al., 2013). In the lab at the department of Functional Pharmacology and Neuroscience at the University of Uppsala, Sweden, a GRS for BMI has been calculated based on initial BMI versus weighted summed risk alleles. The SNPs used for this GRS were selected from Speliotes et al. (2010) with a minor allele frequency of $>10\%$. The correlation of this GRS for obesity was calculated on a subset of 121 SNPs available from Speliotes et al. (2010) and shown to be near zero (Bandstein, 2014 not published). After a close inspection of the SNPs that were used for this GRS it was found that only 66 are associated with weight, this could have influenced the correlation. One of the minor aims of this study is to recalculate the previous discussed GRS in order to improve the correlation between this GRS and BMI via selection and usage of only weight associated SNPs in the GRS calculation. This could generate yet another bit of proof that BMI is -at least partly- regulated by a genetic profile.

Current knowledge on neural activity for the overweight

Current literature on genetic influences on metabolism, satiety and neural responses elicited via food image presentation have suggested that genotype variation could mediate those influences through different brain activity patterns (Wiemerslage et al., 2015 in press), though so far it has been unknown which exchange of information is influenced by genetics or epigenetics. Wiemerslage and colleagues (2015 in press) have found that genotypes for the *fat mass and obesity associated gene* (FTO) also known as the single nucleotide polymorphism (SNP); rs9939609 show different neuronal activation patterns when overweight- or obese individuals are presented with images of different caloric content as measured via fMRI. Thus, previous studies have provided the insight into genetic profiles or genotypes of certain genes that are associated with different patterns of brain activity, and that the differences in neural activity could mediate the intake of food, but not how the flow of information is manipulated by different genes. Since causal relations are not yet known we are not able to pinpoint which exact genes mediate body mass index (BMI) and in which manner.

Brain regions such as the dlPFC, MPFC OFC and the ACC, are regions in which brain activity have been found to be higher when non obese participants are presented with high-calorie (HC) pictures in comparison to when they are presented with low-calorie (LC) images (Wiemerslage et al., 2015 in press). Brooks and colleagues have found that neural activity is higher for individuals with the AA genotype of the FTO gene when they are presented with HC food images(Brooks, Rask-Andersen, Benedict, & Schiöth, 2012). Others have argued that when individuals with both A alleles of the FTO gene where presented with images of food lessened activation in top down regulatory brain areas was observed, which in their turn could mediate or inhibit consumption (Carnell, Gibson, Benson, Ochner, & Geliebter, 2012). That the activity is higher for the

FTO-AA's than for the TT's implies is that restriction and control of appetite could be higher for TT individuals and as such, that AA individuals could lack control and might therefore have excessive appetite impulses. This lack of control can be the result of the lessened activity in the more frontal brain regions which in general regulate executive behavior. Another modulation of behavior to consume food could arise from the heightened activity in the Dorsal Striatum, when perceiving high caloric foods, since this brain structure has been linked to the process of rewarding action-outcomes and with habit learning and craving in addiction (Stoeckel et al., 2008). In overweight individuals (BMI>25) we can observe different levels of response activity within those regions when participants are presented with images of different caloric content. These responses could in their turn promote or demote the willingness of the intake of food. Overweight people (BMI>25) in comparison to normal weight people reflect less activity in brain areas responsible for the maintenance of attention (i.e., dlPFC) and in brain areas involved in object processing (i.e., parietal lobe) (Stingl et al., 2012). This could potentially reflect a relative absence of objective evaluation of presented stimuli, thereby giving way to the much stronger hedonic responses (Ochner et al., 2011). An overly active bottom-up reward system, in combination with a less active top-down control system, could lead to impulsive behaviors such as excessive food intake.

Previous fMRI studies on obesity investigated neuronal activity of satiation and hunger (Burger & Berner, 2014; Stingl et al., 2012; Karra et al., 2013; Rothemund et al., 2007). In some of these studies, participants were food-deprived and either received a liquid meal or observed food cues alternating with neutral pictures following the fast (cf. (Karhunen, Lappalainen, Vanninen, Kuikka, & Uusitupa, 1997)). They found that the brain regions that were activated when overweight individuals where presented with images of food where Parietal, Temporal and Prefrontal cortices, Cingulate Cortex, Hypothalamus, Nucleus Accumbens (NA) and Amygdala, Midbrain, Insula, and Orbitofrontal Cortex (OFC) (Rothemund et al., 2007; Karhunen et al., 1997). Recapitulating previous studies and result, "hunger", the psychological state, recruits a network of regions that have been associated with emotion and reward processing, with internal state perception, classical conditioning and goal-directed behavior. It is important to note that in these previous studies the stimuli were kept constant whereas physiological states such as hunger and satiation were manipulated. It is not known if obese and normal-weight individuals differ in processing cues of food or appetite concerning solely the motivation to consume -or not to- but not physiological states. Therefore, using functional magnetic resonance imaging (fMRI), we assessed whether neural activity following visual presentation of food images differs for participants with different genotypes and BMI categories when intrinsic states were kept constant but the type of stimulus was manipulated.

To elaborate on previous findings that certain genes influence neural responses in food image paradigms we will study the effects of the ASB9, DNM3, RSP04 and MC4R genes on neural responses. These genes where chosen based on their availability within the lab at department of Neuroscience at the University of Uppsala, Sweden, and on their previous discovered link to obesity. In our analysis will consider four SNPs of which two have been previously linked to several obesity measures in Genome Wide Association Study studies (GWAS); the ASB9rs5980075 and the DNM3rs1011371 (Heid et al., 2010).

Link between the ASB9, DNM3 and BMI The rs5980075 has been found to be linked to waist-to-hip circumference ratio (WHR) and dietary energy intake in a study on post-menopausal African-American and Hispanic women (Edwards et al., 2013). This SNP is located on chromosome X position 14932409, proximate to this SNP is the ASB9 gene (ankyrin repeat

and SOCS box containing 9), which encodes a signaling protein involved in the process of protein modification (ubiquitination). Although the role and pathological mechanism implications of these specific variations in the genome are still to be elucidated, this specific variation is most likely to affect the proximate ASB9 gene. The DNM3-rs1011371 has been previously correlated with BMI and WHR by means of a genome wide association study, this correlation was confirmed in a clinical study in Hispanic US women (Graff et al., 2013). This SNP is located on the dynamin 3 gene, also known as the DNM3 gene. In close proximity to the DNM3 there is the phosphatidylinositol N-acetylglucosaminyltransferase sub-unit C (PIGC) gene, which encodes the expression of a protein involved in anchoring proteins to the cell membrane and which has been suggested to be responsible for the link between the DNM3-rs1011371 SNP and WHR (Heid et al., 2010).

Current knowledge of methylation effects for the overweight

Next to the influence of genotypes on brain activity, this neural activity can also be mediated by alterations in DNA as a result of methylation. Methylation of DNA is the heritable and reversible attachment of a methyl group to a nucleotide. Both environmental and genetic factors could affect the process and extend of DNA methylation (Dick et al., 2014). The impact of both genetic and environmental factors could be integrated on a phenotype due to the effect of DNA methylation, this is one of the potential downstream functional outcomes of this epigenetic change. Alternatively, phenotypes can cause epigenetic changes which could in their turn mediate downstream effects by ways of changing a certain gene expression (Dick et al., 2014). As methylation sites could implicate different genes than a GWAS using SNPs can, it is an important development to use methylation data in association studies. Studies on methylation may also offer more information on the regulation of the genes they link to. However, the use of methylation data as an experimental variable is still relatively new, especially for brain imaging studies - in which there is ”guarded optimism” for its use in predicting brain function (Nikolova & Hariri, 2015). Dick et al. (2014) have associated five methylation sites with body-mass index. These same authors argue in the *The Lancet* that they found two cohorts of altered DNA by methylation; a discovery cohort in which they found five islands that correlate with body mass index those where; cg07814318, cg09664445, cg16672562, cg22891070 and cg27146050. In the second cohort; the replication cohort, they found only the latter three to correlate.

Hormones as BMI moderators

Since not only genetics, epigenetics and/or behavior seems to influence weight status, orexigenic signaling molecules or hormones could also have affect BMI. Ghrelin has been suggested as a predictor of BMI (Tschöp et al., 2001), in this study Ghrelin will also be used as a regressor in brain activity analysis to infer about the effect of this orexigenic signaling hormone on neural responses. The choice to use ghrelin was based on knowledge gained from Tschöp et al. (2001), since they argue that ghrelin is an important signaling hormone for eating behavior, previous fMRI studies also have found ghrelin to be of influence on brain activity (Burger & Berner, 2014), and it may be a regulatory link between genetic background and brain responses to food (Karra et al., 2013). Another reason that Ghrelin has been used is the availability of blood-ghrelin levels from participants that participated in the FTO study performed by this lab; the functional Pharmacology and Neuroscience lab at the University of Uppsala, Sweden.

Aims of this study

The current research project tends to correlate genotypes, hormones and BMI with patterns of neural responses.

Based on previous findings by (Brooks et al., 2012;

Karra et al., 2013) and (Wiemerslage et al., 2015 in press), we hypothesized that striatal activation following image presentation should be greater in obese than in not obese individuals when feelings of hunger and satiation are kept constant. Stimulus value manipulation was expected to result in different activation of reward-processing regions, particularly the Striatum, with HC stimuli yielding greater regional brain activation than LC stimuli and eating-related utensils. Furthermore, activation was expected in connected dopaminergic regions such as Insula and Amygdala.

We hypothesize that specific patterns of brain activity reflect mechanisms such as the amount of craving that a person experiences, self reflection or the power to inhibit food consumption, which could have an effect on BMI. Brain areas that are relevant are those that have been associated with eating and food consumption, for example Carnell et al. (2012) have found that images of food can trigger responses in many brain areas including emotion/memory areas (i.e. Amygdala, Hippocampus), in reward/motivation areas (i.e. Nucleus Accumbens, Dorsal Striatum, Caudate/Putamen), impulse control areas (dlPFC, MFC, OFC) and a region for contextual understanding (Retrospleneal cortex). What is expected is that participants carrying two copies of the risk alleles for the ASB9, DNM3, RPSO3 and MC4R show higher activity in the dorsal striatum, the amygdala and the thalamus when they are presented with pictures of high calorie foods in comparison to when they are presented with low calorie food pictures. Since those brain regions have been associated with reward and feelings of craving, a higher activity here can be related to more impulsive food consuming.

To the best of our knowledge, this study is the first to use methylation data combined with brain imaging data in examining neural responses to food and is a direct expansion of previous results which associated five methylation sites with body-mass index (BMI) (Dick et al., 2014). Since -to the best of our knowledge- Ghrelin nor methylation data has been used before in brain activity analysis no hypothesis where thought of in advance. Against this background, we sought to determine if the BMI-associated methylation sites were also associated with brain activity important for processing food images or other biological measures such as ghrelin levels in blood.

Methods

Participants The data that was used in the present study (fMRI as well as the genetic (SNP) and blood data) had already been acquired by the department of Functional Pharmacology and Neuroscience at the University of Uppsala, Sweden, before the start of this project. The data consists of brain activity- and genetic data of 30 participants. All of the participants are males with a mean age of 25,8 (SD = 2,7; (age range: 20 to 33)) with an average BMI of 25,3 (SD = 0,46; (BMI range: 20,4 to 35,7)). Individuals with a BMI above 25 are overweight and above 30 are defined as obese. The selection criteria for participating in the fMRI study was that participants were that they were either homozygous for the non-risk or the risk allele of the *fat mass and obesity associated*(FTO) SNP rs9939609. Next, individuals were genotyped for the ASB9−rs5980075, DNM3−rs1011371, MC4R−rs2331841 and RSPO3−rs9491696 single nucleotide polymorphisms, and blood ghrelin levels where measured. From the participants blood we also measured methylation levels of five sites; the cg07814318, cg09664445, cg16672562, cg22891070 and cg27146050. Participants were recruited by advertisements within the area surrounding Uppsala, Sweden. All of the participants were right-handed and had normal vision. Participants were scanned in the morning, in a fasted state. Both methylation and ghrelin data were also collected from participants while they were in a fasted state. Prior to any experimental procedure, all participants signed an informed consent to the study that conformed to the Declaration of Helsinki and approved by the local ethics committee.

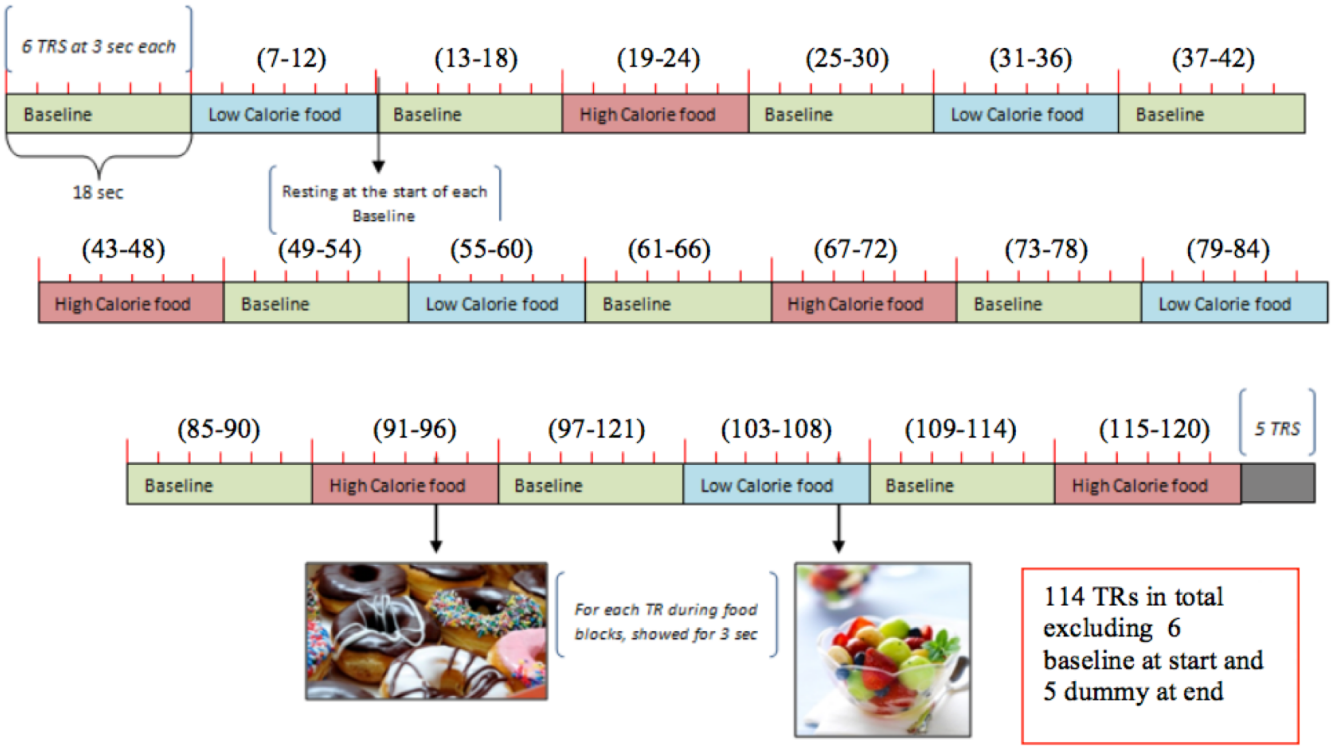

Figure 1: Paradigm design, TR = Red lines = Repetition time = 3 seconds

Task and Stimuli

Blood−oxygen−level−dependent (BOLD) signals were be measured while participants were presented with images of low−calorie (LC) food, high−calorie (HC) food or baseline images, images were presented in a block format. Figure 1 illustrates the design of the experiment. The images were presented in five cycles of the following block design pattern: C, LC, C, HC and each block contained six images, as is depicted in 1. In total, the number of measurements collected for each group were 54 for control, 30 for LC, and 30 for HC. Low and high calorie food images were determined by caloric content, and were controlled for visual features (color, size, etc.). Caloric content of the HC and LC images were confirmed by their perceived caloric content in focus groups representative of the population to be studied. High calorie images were cakes, pies, ice cream, candy, fried foods, and hamburgers for example. Low calorie images were, for example vegetables, fruits, and salads without high-calorie dressing. Control images/baseline images were a gray screen with a cross-hair in the center. The stimuli were presented in the scanner using MRI-compatible goggles (NordicNeuroLab, Bergen, Norway) attached to the headcoil. All participants were in a fasted state and instructed to imagine the feeling of eating the food presented.

Brain image acquisition

Structural and functional brain images were acquired with a Philips 3-Tesla (Achieva, Philips Healthcare, Best, Netherlands) using a standard head coil. 125 volumes were registered during the T2-weighted echo-planar imaging (EPI) sequence with whole brain coverage of 30 slices (slice thickness = 3mm; 1 mm gap, interleaved scan order, in-plane resolution: (3mm x 3mm), repetition time (TR) = 3000 msec; echo time (TE) = 35 msec, flip angle = 90). Tissue probability

maps were introduced in the segmentation step (see *preprocessing*) to differentiate between gray matter, white matter and cerebrospinal fluid in each individual. The volumes (in liters) will be calculated based on the extracted tissue maps of each subject. The gray matter and white matter volumes are added together to find the total brain volume (TBV).

Genetic risk score

A Genetic Risk Score (GRS) will be calculated to use this vector as a covariate in the neural analysis. The risk score will be based on a sub selection of 66 SNPs from a total of 136 that have been provided by the department of Functional Pharmacology and Neuroscience at the University of Uppsala, Sweden. The selected 66 SNPs where chosen based on literature in which these SNPs have been associated with BMI (Speliotes et al., 2010; Thorleifsson et al., 2009; Okada et al., 2012; Johansson et al., 2010; Willer et al., 2009). For each participant the GRS was calculated based on the selection of SNPs; each copy of the risk allele for each individual SNP added a score of 1 to the final risk score. Via this manner the lowest GRS possible was 0 (when a participant did not carry any of the risk alleles), the highest score possible was 132 (if a participant carried all the risk alleles), so, the higher the risk score the more copies of risk alleles one carries. The average genetic risk score for this set of participants was 59,5 (GRS range: 47 to 70). See the appendix for a list of the SNPs that have been used to calculate this GRS.

Data analysis

Preprocessing All preprocessing steps will be performed using software package Statistical Parametric Mapping (SPM, version 8, http://www.fil.ion.ucl.ac.uk/spm/), implemented in MATLAB (version R2014a, 11 FEB 2014, 8.3.0.532, 64-bit). The images were realigned and estimated to remove movement artefacts in the data. EPI images are further matched with the structural image by using co-registration, which makes the cost function as smooth as possible. The anatomical image was segmented to strip away unnecessary tissue in the images. Tissue probability maps were introduced in the segmentation step to differentiate between gray matter, white matter and cerebrospinal fluid in each individual. Volume was calculated based on the extracted tissue maps of each subject. The gray matter and white matter volumes were added together to find the total brain volume (TBV; see 'Statistical analysis'). Then, functional images were normalized to fit the segmented anatomical image. Finally, images were smoothed using a Gaussian function (8 mm) to minimize noise and bias.

Statistical analysis fMRI statistical analysis were done using the same versions of SPM and MATLAB as previous described. For all whole-brain results, a family wise error (FWE) corrected significance level can be set at .005 to correct for multiple testing. For all results, a p-value $<.05$ will be considered significant. From our 3 variables (HC, LC, baseline), we will be able to test 4 contrasts in our brain activity analysis: LC versus baseline, HC versus baseline, both LC and HC versus baseline, and HC versus LC. Those four contrasts will be tested with multiple regression analysis. For the regressions, groups will be determined by genotype, three of the four contrasts will be tested: Low-Calorie versus baseline, High-Calorie versus baseline, and the comparison between Low Calorie and High Calorie for neural responses. The contrasts will be be analyzed with full factorial designs if there are more than two groups to be compared, if not then a simple regression for t-values will be used. The ASB9 was divided in two groups, AA (N=12) or GG (N=18). The DNM3 and RSPO3 were divided in three groups; AA (N=11 for DNM3 and N=10 for RSPO3),AG (N=11 for DNM3 and N=10 for RSPO4) or GG (N=8 for DNM3 and N=10 for RSPO3). The ASB9 and DNM3 risk allele is the G allele, so the GG genotype is the high risk group

for both these genes. For the MC4R and RSPO3 there is no risk allele known. For all the analysis total brain volume (TBV) and body-mass index (BMI) were used as covariates. TBV is taken in the analysis to control for the effect of brain volume but volume itself is not of interest. The calculated GRS will be used for a simple correlation analysis with BMI.

Results

Genetic risk score

To analyze the effect of genes on body mass index and on neural responses, multiple regression- and correlation analysis have been performed. The first step to answer the influence of genes on BMI was to correlate a newly calculated genetic risk score to BMI. We found that this genetic risk score correlates highly significant with BMI ($r(28)=.47$; $p<.001$, SD=5,9). This correlation shows that there is a clear relation between SNPs and body mass. For a complete list of the SNPs that have been used for the calculation of the genetic risk score, including their corresponding risk allele see table S7.

Influence of genes on neural responses

In previous studies, performed by researchers at the department of Functional Pharmacology and Neuroscience at the University of Uppsala, Sweden, a link between weight and brain activity has been found. Based on these non published results and that of others who have found that genes correlate with BMI, for example Horne et al. (2005); Belsky et al. (2013); Thorleifsson et al. (2009), we have performed brain activity analyses with a subset of the genes used in the GRS as a regressor.

Blood sampling from the participants who participated in the FTO study (a study previously performed within the lab of Functional Pharmacology and Neuroscience at the University of Uppsala, Sweden (Wiemerslage et al., 2015 in press)) did not only provide genotypes for the FTO gene, also for four others; the ASB9−rs5980075, DNM3−rs1011371, MC4R−rs2331841 and RSPO3−rs9491696. Song et al. (2008) have shown that each copy of the FTO risk-allele A was significantly associated with a raise of .45 kg/m(2) in BMI. Since the FTO gene has already been processed in this lab before (the same neuroscience lab of the University of Uppsala) and have shown to correlate with differentiated brain activity patterns for different genotypes this gave rise to the idea that the other available genes might do the same, therefore we analyzed whether the subgroups within the genes (as divided by genotype) showed differentiated patterns of activity too. We have compared neural activation of of participants grouped by genotype for those four genes that where chosen based upon their previous associations with BMI and the availability of the genetic data of the participants who have undergone fMRI scanning in the food image paradigm. Since the MC4R did not show any effects in brain activity between genotype groups this gene will be omitted from further brain activity analysis.

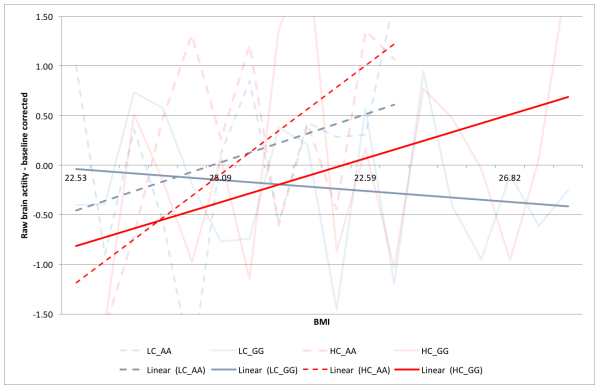

Figure 2: Interaction effect of ASB9 genotype for raw, baseline corrected brain activity as a function of BMI for the Cingulate Cortex depicted as estimated linear effect lines. (LC stands for Low-Calorie, AA or GG stands for genotypte, high transparent lines are the original, the non-transparent the estimated effect lines)

We were able to test four contrasts within the neural response analysis as the paradigm differentiated two types of caloric images and a baseline image. As described before, we were able to distinguish brain activity differences for both types of food images (i.e. high-

Table 1: This table lists the brain regions in which the neural activity is higher for participants that have both copies of the non risk allele (AA, N=12) and have a relatively high BMI in comparison to participants that have both copies of the risk allele (GG, N=18) and have a high BMI. The activity represented in this table is the outcome of the regression for the contrast in which high-calorie activity is compared to baseline activity for the ASB9 gene (BA= Brodman area).

Region	BA	Laterality	Cluster Size (voxels)	MNI coordinates			p-value (FWE corr.)		t
				x	y	z	Cluster level	Peak Voxel	
Medial Frontal Gyrus	6	R	1416	15	-19	58	0,000	0,04	6,0
Cingulate Gyrus	31	L		-3	-28	37		0,42	4,7
Anterior Cingulate	24	R		3	26	13		0,47	4,7
Thalamus	NA	R	158	18	-19	16	0,034	0,62	4,4
Caudate	NA	R		15	2	10		0,90	4,0
Thalamus	NA	L		-12	-1	10		0,95	3,9

calorie and low-calorie images) versus baseline activity and against each other.

Neural main effects of genotype for the high calorie versus baseline activity contrast

Neural activity in high caloric discrimination contrast showed to be heightened in multiple regions for both the ASB9 and DNM3 risk genotype. For the ASB9 risk group (the GG genotype) we found that there were two clusters of heightened activity in comparison to the non risk group, the first in the Medial Frontal Gyrus ($p<.0001$) and the second in the Anterior Cingulate ($p<.0001$). These clusters both represent activity as a function of the genotype, therefore this activity is a main effect of genotype for the ASB9. Based on this finding we can argue that activation in those clusters of brain activity is less of the risk allele (GG) group in comparison to the non-risk allele (AA). See supplementary Figure S1 for an overview of the active clusters, coordinates an p-values. The DNM3 risk group shows a cluster of heightened activity (in comparison to the non risk group) that includes the Insula ($p<.0005$) for this same HC versus baseline contrast.

Neural main effects of genotype for the average of food image presentation versus baseline activity

The ASB9 nor the RSPO4 gene show any main effects of genotype for this contrast, the DNM3 however does seems to influence brain activity patterns for the contrast in which the average of food images is contrasted to the baseline. For this HCLC versus baseline contrast, participants with the DNM3 risk allele (GG) showed a higher brain activity in four clusters which include the Superior Frontal Gyrus ($p<.0001$), Middle- and Superior Temporal Gyrus($p=.01$ and $p=.025$ respectively), the Insula ($p=.004$) and a trend towards significance in the Posterior Cingulate ($p=.08$). These are main effects for the DNM3 since they represent activity modulation as a function of genotype. See supplementary Table S2 for an overview of the active clusters, coordinates an p-values corresponding to this main effect.

BMI modulation on neural responses

To test the hypothesis whether BMI has a modulatory effect on neural responses, BMI has been added as a variable in the regression. We found that there was an interaction effect between neural activity and BMI for the ASB9 to be observed in two clusters that include the Medial frontal Gyrus ($p=.0002$), which also includes the (anterior) Cingulate Gyrus, and a cluster that includes

the Thalamus (p=.03) and Caudate Nucleus, see Table 1. This interaction effect shows an increased brain activity when individuals have both copies of the non-risk alleles as a function of BMI in comparison to participants with the risk genotype but a lower BMI. Participants in the high risk genotype group with a relatively high BMI show lessened activity in the Cingulate Cortex in comparison to the low risk genotype participants with a relatively high BMI see for a graphical representation of this interaction Figure 2. We found that the ASB9 gene also regulated the relation between brain activity, genotype and BMI in such a way that when a participant carries the high risk genotype but has a low BMI brain activity drops in comparison to the low risk genotype and a low BMI. This effect was observed in two clusters; in the Medial Frontal Cortex (p<.0001) and B) in the Anterior Cingulate Cortex (p<.0001) see Table S3 in the supplementary material. Both those relations show that BMI has a strong modulatory effect on neural activity for the risk group of the ASB9 gene. For the DNM3 gene the interaction between genotype, BMI and brain activity showed three clusters of heightened activity in the same brain regions as the main effect; a cluster in the Superior Frontal Gyrus (p<.0001) and two in the Middle Temporal Gyrus bilaterally (left- p<.0001 and Right Temporal Gyrus p=.005) see supplementary Table S4 for a complete effect overview. That the interaction effect for the DNM3 includes the same anatomical regions as the main effect for the DNM3, could imply that the observed activity is stronger mediated by genotype then by BMI, this because the regions and effect sizes show to be similar when BMI is not taken into account.

The only effect of RSPO3 was a numerically significant higher cluster of activity in the Cuneus and Precuneus(p=.06) for a main effect of genotype in the high calorie versus baseline contrast. This cluster contains 141 voxels and is previously described as one of the regions that mediates self-reflections and aspects of consciousness.

Effect of methylation on neural activity

Methylation is thought to reflect involvement in regulation or gene expression, protein function and RNA processing. According to Dick et al. (2014) DNA methylation plays a part in transcriptional regulation of genes and miRNAs, control of alternative promoter usage and alternative splicing. They also state that both environmental as well as genetic factors can affect the extend in which DNA methylation occurs. An effect on DNA methylation could integrate the impact of the environment and genetic factors on a phenotype when seen in view of the range of potential functional outcomes of this epigenetic change. On the other hand, epigenetic changes that are caused by a phenotype could mediate their effects by changing the gene expression.

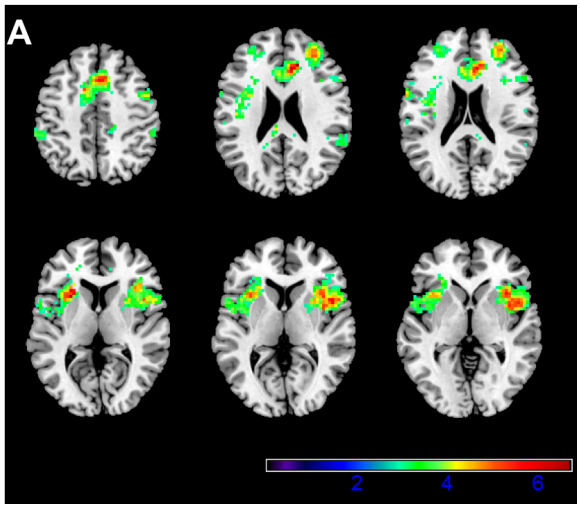

Figure 3: This image represent axial slices of heightened neural activity for significant clusters as a result of the regression of the methylated cg07814318 island over BMI and neural responses to high calorie food cues in comparison to activity to low caloric images. (BA= Brodmann area, slice numbers represent MNI z-coordinate heights and t-statistics indicated by color-coded scale bar)

As described earlier, we sought to determine if the methylation sites, which were previously associated with BMI by Dick et al. (2014), were also associated with brain activity important for processing food images or other biological measures such as blood ghrelin levels. Five BMI associated methylation sites were

tested for effects on brain activity in response to food images measured by fMRI, these five DNA methylation sites are the cg07814318, cg09664445, cg16672562, cg22891070 and cg27146050. We have used the same paradigm and data of the same participants for the analysis of the effects of methylation on neural activation. We have used a multiple regression analysis using the five previously mentioned methylation island values and BMI as regressors. We found that when the cg07814318 was regressed against brain activity for the contrast of brain activity between HC and LC that there is a main effect of methylation in multiple highly significant clusters of brain activity (all below $p<.0001$). This main effect of methylation of neural activity include brain areas such as the Cingulate Gyrus, the Precentral Gyrus, the Inferior Parietal Lobe and the Claustrum. For an overview of these regions, coordinates and p-values see supplementary Table S6. Further independent regressions found an inverse relationship between methylation and brain activity in such a way that as the value of the methylated cg07814318 site rises, brain activity in previously mentioned regions drops as a function of BMI, the neural activity belonging to this effect can be seen in Figure 3. In Figure 4 a representative fitted plot showing a near linear, negative relationship between activity at the global maximum coordinate (located in the Claustrum) and methylation is shown. Because methylation is associated with gene expression, protein function and RNA processing, this could mediate the effects as observed.

Relationship between methylation and ghrelin After finding results for the cg07814318 site via fMRI, we tested if methylation levels for this site correlated with ghrelin levels or BMI. We chose ghrelin as it is involved in eating behavior (Tschöp et al., 2001), has previously been correlated with results from previous MRI studies (Burger & Berner, 2014), and may be a regulatory link between genetic background and brain responses to food (Karra et al., 2013). Of the 30 participants that have performed in the fMRI study 22 have undergone

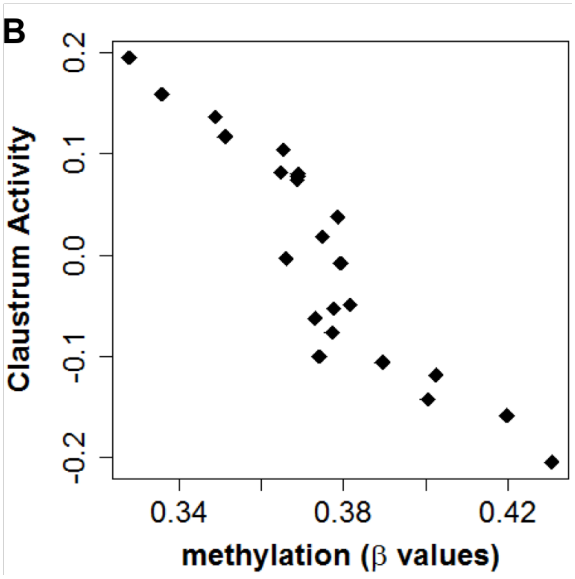

Figure 4: Brain activity in the Claustrum regressed over methylation of the KLF13 cg07814318 site. A representative fitted plot showing a near linear, negative relationship between activity at the global maximum coordinate (n = 23; y-axis is response at MNI coordinates: x = -24, y = 23, z = 7)

blood-sampling of these 22 data sets two were omitted due to measurement errors. A multiple regression analysis of all scanned participants found a that both methylation at the cg07814318 site and ghrelin blood levels decreased as BMI increased ($p<.05$, Multiple R squared $=.20$, $DF=20$) see Figure 5. The cp07814318 does not only seem to be a predictor of brain activity it also correlates with fasted Ghrelin levels ($r=.56$, $p<.05$, $DF=20$). A correlation analysis between the methylated cg07814318 island and BMI gave a non-significant correlation ($r=-.33$, $p>.05$. $DF=20$).

Discussion

In this study multiple ways of examining the effects of genes on neural correlates of appetitive processing in over weight individuals are analyzed. In the first part of the study we sought to test whether genotypes that are associated with obesity affects the neural processing of food images with different caloric content and to what extent BMI is an important factor. Of the four genes that have been tested, two genes showed to mediate neural

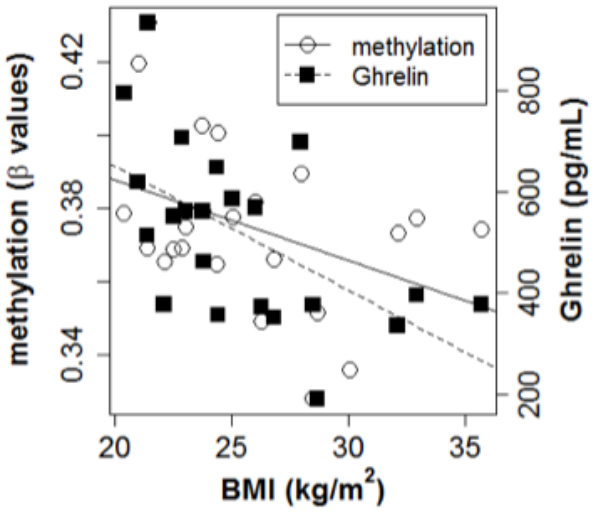

Figure 5: *Positive relationship between methylation at a site near the KLF13 gene (cp07814318) and ghrelin levels in relation to body mass index.* Both methylation at the cg07814318 site and ghrelin blood levels decreased as BMI increased. Both methylation and ghrelin data was collected from participants while they were in a fasted state. A multiple regression analysis of all scanned participants found a positive relationship between methylation and ghrelin levels against BMI ($p<.05$, Multiple R squared $=.20$, $DF=21$)

response patterns to food cues in regions that have been associated with emotion, self reflection, craving and top-down control. Those two genes were the ASB9 and DNM3, putatively linked to obesity. In the second part of this study we have used methylation data combined with brain imaging data in examining neural responses to food images and is a direct expansion of previous results that associated five methylation sites with BMI (Dick et al., 2014). Dick et al. (2014) argue in the *The Lancet* that they found two cohorts of altered DNA by methylation; a discovery cohort in which they found five islands that correlate to BMI; cg07814318, cg09664445, cg16672562, cg22891070 and cg27146050. In the second cohort; the replication cohort, they found only the latter three to correlate. We have found that of the five methylation sites tested, only one had a relationship to brain activity, which was the cg07814318 site, putatively linked to the KLF13 gene (Dick et al., 2014).

Before an in depth description of what the methylation effects might imply, first the effects of genotyping in relation to brain activity will be discussed. In the last part of this discussion the link between Ghrelin and methylation will be described.

Neural activity as mediated by genotypes

To examine the effect of genotypes on neural response patterns and to what extend BMI influences this relation, an fMRI analysis has been performed. The fMRI testing sessions included 30 individuals, they where presented with images of high-calorie food, low-calorie food or with a baseline image while blood-oxygen-level dependent signals were measured. We performed analysis on four SNPs to check whether they showed different neural activity patterns; the ASB9rs5980075, DNM3rs1011371, RSPO3rs9491696 and MC4Rrs2331841, of which only the first two showed different neural patterns when presented with different caloric images.

For the ASB9-rs5980075 we have found that the AA-genotype had a different response pattern of brain activity in comparison to the GG-genotype when the participants when viewing low calorie food images in comparison to baseline pictures, specifically in areas areas such as the bilateral Medial- and Superior Frontal Gyrus, Thalamus, Caudate Nucleus, Precuneus, Insula and the Anterior Cingulate Cortex (ACC) and dlPFC. These regions have been associated with emotion, memory, self-image and executive functioning (Goldberg, Harel, & Malach, 2006). Previous fMRI studies involving food images and/or obesity also implicate these brain areas in the neural processing of food images (Karra et al., 2013), although these studies did not examine the influence of the genotypes that have been used in this paradigm. Moreover, while both genotypes had a similar distribution of BMI, ranging between normal-weight to over-weight in each genotype, we found that BMI was associated with differential activity within each genotype with comparatively more brain regions associated with BMI in the AA genotype than in the GG allele. These results are in line with results found for the FTO polymorphism which suggest that polymorphisms of the

ASB9 affect the processing of visual food stimuli. Our results show that the at-risk ASB9 genotype (AA) is associated with increased BMI and that, for both the AA and GG genotypes, elevated BMI differentially affects the perception of food based on brain functions related to impulse control and reward processing.

Cingulate Cortex activity The Cingulate Cortex shows a clear discrimination in brain activity for the different genotypes of the ASB9 and DNM3 risk groups. This part of the cortex is a multifunctional brain region that has many connections to areas associated with emotional processing. The Cingulate Cortex functions as a central node in the default mode network (DMN) that is involved in arousal and awareness, balancing external and internal thought, and emotion (Leech & Sharp, 2014). Fiset et al. (1999) have found that low levels of arousal are associated with decreased activity in the Cingulate, this implies that individuals with the risk alleles may require higher caloric salience to get their Cingulate Cortex activated as high as the non risk-group individuals. Previous MRI studies have shed light on the link of the Cingulate Cortex to feeding behavior or obesity, they have shown that the Cingulate Cortex may affect obesity through reward modulation and sensory processing of body state, as is described in a meta-analysis done by Brooks, Cedernaes, and Schioth (2013).

Precuneus activity Other brain regions, such as the Precuneus has previously been correlated to obesity, Gearhardt, Yokum, Stice, Harris, and Brownell (2014) showed that obese individuals showed less activity in the Precuneus and Cuneus when viewing nonfood versus food commercials. This is in line with results we found. Our results imply that simple visual stimulation with food stimuli in obese individuals activates regions that are related to reward anticipation and procedural memory or habit learning. Additionally, images of high-caloric foods yielded activations which are dependent on BMI in brain regions responsible or of influence on processing of taste information (anterior) Insula, motivation, memory functions as well as emotion (Cingulate Cortex).

Insular activity The Insula 'plays a crucial part in conscious urges to take drugs' (Naqvi & Bechara, 2009), Gearhardt, Grilo, DiLeone, Brownell, and Potenza (2011) have shown that food can, for some individual, elicit the same strong responses as drugs including the craving, which in his turn could motivate those individuals to over consume. We have found that the risk group of the DNM3 (GG genotype) show heightened activity in the Insula in response to high caloric food image discrimination. This could imply that these individuals have stronger feelings of addiction or craving when they see high caloric food, in combination with diminished activity in top down control areas (dlPFC) this could lead to overconsumption.

Methylation effects on neural responses

This study was a direct expansion of a previous study in which five methylation sites have been associated with body-mass index (Dick et al., 2014) by ways of a genome wide association study (GWAS), they found five methylation islands that correlate to BMI; cg07814318, cg09664445, cg16672562, cg22891070 and cg27146050. Our expansion on these findings lies in the use brain imaging data combined with methylation data in order to examen neural responses to food cues. We found that only one of the five tested methylation sites had a relationship to brain activity; the cg07814318 site, putatively linked to the KLF13 gene (Dick et al., 2014). It has been suggested that overexpression and/or underexpression of KLF13 in porcine fat cells either promotes or inhibits adipocyte differentiation respectively through effects on PPARy (Jiang et al., 2015). And KLF13 is involved in the immune pathway of activated T cells in Systemic Lupus Erythematosus (SLE). However, direct effects of KLF13 in feeding behavior or effects in the brain remain to be tested.

We have tested the effects of methylation as a regres-

sor in the analysis of brain activity in the same paradigm that has been used for the rest of our analysis. The results showed a negative relationship with brain activity and the cg07814318 in four brain areas: the Cingulate Cortex, the Precentral Gyrus, Inferior Parietal Lobe (IPL) and the Claustrum. The modulatory effect of the Cingulate Cortex in relation to obesity has already been described in the subsection above.

IPL activity The Inferior Parietal Lobe has been studied in relation to obesity too, effects related to food consumption and/or obesity have been reported over almost two decades (Gautier et al., 1999). They have argued about the IPL showing heightened activity when fasted participants taste high glucose or high fat foods during a liquid-meal taste perception task.

Claustrum activity The Claustrum was famously singled-out by Francis Crick to be a critically important structure for consciousness (Crick & Koch, 2005), and has the highest connectivity in the brain per regional volume (Torgerson, Irimia, Goh, & Van Horn, 2015), therefore brain activity in the Claustrum can easily be suspected. However, little evidence is currently available that links the Claustrum to obesity or feeding behavior. Recently, this highly connected brain region has been described as a gain control for sensory information input processing such as location, and emotional- and physical states processing, or to promote a preferred modality in its output somewhat analogous to the thalamus. Based on the areas involved in this study, brain activity relevant to the cg07814318 methylation site appears to center around salience of hedonic food images, as analysis was performed in a paradigm that contrasts brain activity when presented with high- versus low-calorie food images. As can be seen in Figure 4 we have found that neural activity in the Claustrum drops as a function of methylation at the cg07814318 island when participants are presented with a high calorie discrimination task. This relation can give rise to the idea that less methylated participants need a stronger saliency in the image to 'activate' conscious perception.

Ghrelin and its' effect

To help explain how brain activity might be influenced by methylation, we have also tested relations between methylation levels and the orexigenic signaling hormone ghrelin. We found a positive correlation between ghrelin and methylation levels of the cg07814318 site against BMI, as well as between only methylation values and ghrelin. Our results also support the finding that the obese have lower levels of fasting ghrelin (Tschöp et al., 2001). Ghrelin, or perhaps other metabolic hormones, may offer some insight into the relationship between methylation levels and brain activity as it may a regulating-link between the two phenomena. Karra et al. (2013) have suggested this relationship with the fat mass and obesity related gene. Although our results do not provide any direct evidence of ghrelin influencing brain activity, we further support its association, and suggest that augmented methylation may be a possible effect of ghrelin signaling important in the brains' responses to food. Our results suggest that, even though the sample size is small, the methylated gene cp07814318 influences the way visual stimuli are processed such that when methylation rises participants have a higher activity in regions that inhibits the control food intake which in turn can result in impulsive eating behavior. Whether methylation of cg07814318 in the cells of these brain areas has a direct effect on brain signaling is unknown.

It is likely that the of Ghrelin and methylation on neural activity are acting either in concert or through several intermediary signaling pathways. The strong correlation of cg07814318 methylation and ghrelin perhaps argues for a metabolic/hormonal regulation of methylation which in turn could mediate neural responses to food consumption. Thus, we suggest that metabolic signaling molecules may alter methylation levels, and hence regulation, of genes involved in the brains responses to images of food particularly for brain areas related to judging the salience of such food images. In

our particular experiment, decreased ghrelin levels in the obese may result in decreased methylation of the cg07814318 site for the KLF13 gene, resulting in increased signaling in brain areas important for controlling the salience of intrinsic motivation to food signaling. Without knowing the function of methylation at the cg07814318 site or of the KLF13 gene, it is difficult to predict what this signaling may be important for. Future studies should expand upon KLF13s relationship with obesity and perhaps also test for hormonal effects on the methylation of the cg07814318 site.

Conclusions

In line with findings of Tuulari et al. (2015), who have concluded that Superior Frontal Cortices, Premotor areas and Precuneus support cognitive control of appetite when individuals encounter visual food cues or stimuli, we found the same regions to be activated. Even though the brain regions seem to be of similar function in both obese and normal-weight participants, responses in the Dorsal Striatal regions during food image presentation seem to be reduced in obese individuals. This could imply that altered homeostatic and reward signaling in combination with increased functional connectivity within circuits of cognitive control are likely to play an important role in the drive of excessive eating and play an important role in the physiopathology of obesity, and may contribute to the development and maintenance of obesity, even though frontal inhibitory function seems to be largely preserved in obesity.

Limitations

Some limitations of this study are the use of only males in the fMRI experiments, as well as the exclusion of the AG genotype for the ASB9. Because our study involved only males, it is possible that the results may not be generalizable to females. Prior studies related to differences in cortical processes associated with appetite control have indeed shown gender effects(Wang et al., 2009).

Despite statistical significance, it is unknown if the relationship between methylation levels of the cg07814318 site and brain activity is physiologically significant. The range of methylation values in relation with brain activity was small. However, the small range but a clear linear pattern in the relationship with brain activity may signify an on/off type of effect with tight top down regulation. Unfortunately, not enough is known about the values of methylation levels of individual genes to either support or refute this hypothesis.

Summary

We were able to provide evidence that normal weight and obese individuals differ in their brain activation related to cue-induced food motivation. A conclusion can been drawn that both the ASB9 and DNM3 risk alleles for obesity are associated with reduced neural activity in regions that are associated with impulsivity, emotion and reward during a caloric image discrimination task in comparison to the neural responses of lowrisk genotypes, next to this, it seems that BMI is a mediating factor for each genotype on brain activity. This implies that overweight or obese individuals with the risk alleles for the DNM3 and ASB9 gene might be more prone to unhealthy eating behavior due to food images being less salient at evoking normal appetitive responses compared to overweight/obese people with the AA/GG for the DMN3 and ASB9 genotype respectively. We maintain that BMI is an important factor in fMRI research as well as in the relationship between brain activity and genotype. Our findings offer insight into the relationship between DNM3, ASB9, obesity, brain activity, methylation and the food signaling hormone Ghrelin; and suggest that overweight/obese populations have different attitudes and functional processing for food images depending on genetic background, or gene modification by ways of methylation or hormonal effects.

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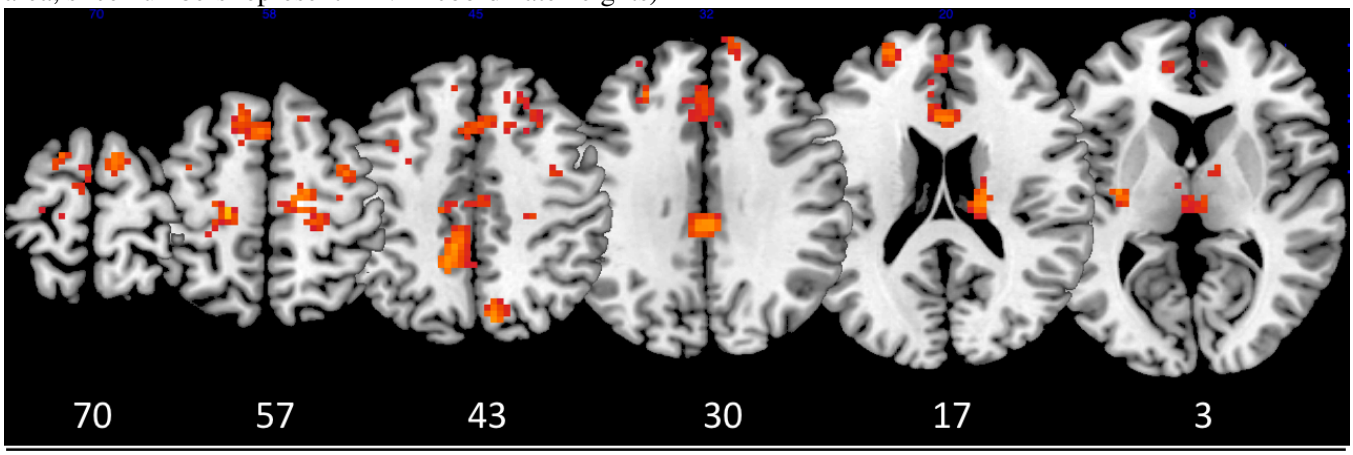
Supplementary

Table S1: ASB9-AA group (non-risk genotype, N=12) effect of for high-calorie food in comparison to Baseline activity, listed regions show higher activity for the non-risk (AA) then the risk genotype (GG) group (BA= Brodmann area, slice numbers represent MNI z-coordinate heights)



Region	BA	Laterality	Cluster Size (voxels)	MNI coordinates			p-value (FWE corr.)		t
				x	y	z	Cluster level	Peak Voxel	
Medial Frontal Gyrus	6	R	416	15	-19	58	0,000	0,02	6,3
Cingulate Gyrus	31	L		-3	-28	37		0,52	4,6
Cingulate Gyrus	31	L		-9	-43	43		0,76	4,3
Anterior Cingulate	24	R	706	3	26	13	0,000	0,47	4,7
Superior Frontal Gyrus	6	L		-12	20	64		0,75	4,3
Superior Frontal Gyrus	6	R		9	14	67		0,83	4,1

Table S2: Table of the clusters that show a higher activity for the DNM3 risk-allele (GG, N=8) in comparison to the non-risk genotype (AA) for the contrast that contrasts high-calorie versus Baseline activity

Region	BA	Laterality	Cluster Size (voxels)	MNI coordinates			p-value (FWE corr.)		t
				x	y	z	Cluster level	Peak Voxel	
Superior Frontal Gyrus	8	R	492	21	56	34	0,000	0,30	4,9
Superior Frontal Gyrus	8	R		24	41	46		0,42	4,7
Superior Frontal Gyrus	8	L		-15	44	49		0,72	4,3
Superior Temporal Gyrus	22	L	171	-33	-55	22	0,025	0,56	4,5
Insula	13	L	253	-45	2	-5	0,004	0,62	4,4
Thalamus	NA	L		-18	-16	-5		0,64	4,4
Insula	13	L		-45	-7	-5		0,90	4,0
Middle Temporal Gyrus	39	R	205	45	-61	22	0,011	0,94	3,9
Insula	13	R		48	-43	28		0,98	3,7
Superior Temporal Gyrus	39	R		45	-52	28		0,99	3,6

Table S3: Table of brain regions in which brain regions are shown in which the ASB9 gene regulated the relation between brain activity, genotype and BMI in terms of when a participant carries the low risk genotype but has a low BMI brain activity rises in comparison to the high risk genotype and a low BMI (BA= Brodman area)

Region	BA	Laterality	Cluster Size (voxels)	MNI coordinates			p-value (FWE corr.)		t
				x	y	z	Cluster level	Peak Voxel	
Medial Frontal Gyrus	6	R	387	15	-19	58	0,000	0,01	6,5
Cingulate Gyrus	31	L		-3	-28	37		0,67	4,4
Precuneus	7	L		-12	-46	43		0,80	4,2
Anterior Cingulate	24	R	455	3	26	13	0,000	0,52	4,6
Superior Frontal Gyrus	6	L		-12	20	64		0,91	4,0
Superior Frontal Gyrus	6	R		9	14	67		0,93	3,9

Table S4: Table of clusters that show higher activity for the DNM3 non-risk genotype (AA) for the interaction between the non-risk-Genotype and a relative high BMI for the high-calorie versus Baseline activity opposed to the high risk genotype with relative low BMI (BA= Brodman area)

Region	BA	Laterality	Cluster Size (voxels)	MNI coordinates			p-value (FWE corr.)		t
				x	y	z	Cluster level	Peak Voxel	
Superior Frontal Gyrus	8	R	649	24	41	46	0,000	0,27	5,0
Superior Frontal Gyrus	8	L		-30	35	49		0,31	4,9
Superior Frontal Gyrus	8	R		21	56	34		0,34	4,9
Middle Temporal Gyrus	39	L	416	-36	-58	25	0,000	0,80	4,3
Insula	13	L		-45	2	-5		0,92	4,3
Insula	13	L		-45	-7	-5		0,98	4,3
Middle Temporal Gyrus	39	R	230	45	-61	22	0,006	0,80	4,2
Supramarginal Gyrus	40	R		48	-46	34		0,92	4,0
Superior Temporal Gyrus	39	R		51	-52	28		0,98	3,7

Table S5: Table of clusters that show higher activity for the DNM3 risk group (GG) for the relation between the risk-Genotype and a relative high BMI for the high-calorie versus Baseline activity opposed to low risk genotype with relative low BMI (BA= Brodman area)

Region	BA	Laterality	Cluster Size (voxels)	MNI coordinates			p-value (FWE corr.)		t
				x	y	z	Cluster level	Peak Voxel	
Thalamus	NA	L	246	-18	-16	-5	0,004	0,33	4,9
Insula	13	L		-45	2	-5		0,62	4,4
Superior Temporal Gyrus	13	L		-42	-25	7		0,92	4,0
Superior Frontal Gyrus	8	R	317	21	56	34	0,001	0,35	4,8
Superior Frontal Gyrus	8	R		24	41	46		0,72	4,3
Superior Frontal Gyrus	6	R		15	41	52		0,92	3,9
Middle Temporal Gyrus	39	R	155	45	-61	31	0,037	0,99	3,6
Middle Temporal Gyrus	39	R		45	-61	22		1,00	3,5
Insula	13	R		51	-40	25		1,00	3,4

Table S6: Table of the clusters of brain activity that show a higher activity when the methylation at the cg07814318 site is relatively higher when brain activity between High-Calorie and Low-Calorie images are presented (BA= Brodman area)

Region	BA	Laterality	Cluster Size (voxels)	MNI coordinates			p-value (FWE corr.)		t
				x	y	z	Cluster level	Peak Voxel	
Claustrum	NA	L	642	-24	23	7	0,000	0,04	6,7
Claustrum	NA	L		-27	14	7		0,10	6,3
Precentral Gyrus	6	L		-60	5	19		0,28	5,6
Cingulate Gyrus	32	R	1360	15	32	25	0,000	0,05	6,7
Cingulate Gyrus	24	L		-12	8	43		0,06	6,5
Cingulate Gyrus	32	R		9	17	40		0,08	6,4
Precentral Gyrus	44	R	395	48	8	4	0,000	0,20	5,8
Claustrum	NA	R		36	17	1		0,27	5,6
Claustrum	NA	R		39	5	1		0,35	5,4
Inferior Parietal Lobu	40	R	146	54	-40	31	0,033	0,97	4,2
NA	NA	NA		45	-40	31		0,98	4,1
Inferior Parietal Lobu	40	R		54	-34	43		1,00	3,6

Table S7: Table of the single nucleotide polymorphisms that have been used for the calculation of the Genetic Risk Score with their corresponding risk alle. The calculation for the GRS was calcualted by adding each SNP score, for example, if a partcipant had a CC allele combination for the first SNP in the list the ”rs10150332” then he scores ”2” for this snip, if that individual had a GG then a score of ”0” was added to the final score for that individual, if a CG was present a score of ”1” was added. This was done for each SNP which means that a maximum score of 132 and a minimum of 0 was computable. The higher the score the more risk alleles that individual carries, which in turn correlates highly significant with BMI.

rs10150332 C	rs4929949 C	rs6794092 G	rs925946 T	rs6545814 G	rs2444217 A	rs7498665 G	rs2331841 A	rs7138803 A
rs10767664 A	rs713586 C	rs10458787 G	rs1024889 G	rs9356744 T	rs2867125 C	rs7647305 C	rs4377469 T	rs7481311 T
rs10938397 G	rs7359397 T	rs2275215 T	rs1152846 G	rs11142387 C	rs2922763 T	rs12597579 C	rs6265 G	
rs13078807 G	rs867559 G	rs10783050 C	rs1458095 G	rs10769908 C	rs3810291 A	rs261967 C	rs6499640 A	
rs13107325 T	rs887912 T	rs12970134 A	rs1878047 G	rs10838738 G	rs516636 A	rs4776970 A	rs3934834 G	
rs1514175 A	rs9816226 T	rs2568958 A	rs1927702 G	rs12324805 C	rs4549702 G	rs652722 C	rs824931 G	
rs206936 G	rs10993160 A	rs2844479 T	rs2383393 G	rs17782313 C	rs7864204 A	rs2241423 G	rs6548238 C	
rs2112347 T	rs11671664 G	rs29941 C	rs3803915 C	rs2815752 A	rs2033195 C	rs2287019 C	rs9939609 A	