Chapter 2

Genetic variation in the hypothalamic pathways and its role on obesity, review

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Abstract

Over recent decades the prevalence of obesity has increased dramatically worldwide. Although this epidemic is mainly attributable to modern (Western) life style, multiple twin and adoption studies indicate the significant role of genes in the individual’s predisposition to becoming obese. As the hypothalamus plays a central role in controlling body weight, its regulatory circuits may represent a crucial system in the pathogenesis of the disorder. Genetic variations in genes in the hypothalamic pathways may therefore contribute to the susceptibility for obesity in humans and animals.

We summarize current knowledge on the physiological role of the hypothalamus in body weight regulation, and review genetic studies on the hypothalamic candidate genes in relation to obesity. Together, data from functional and genetic studies, as well as the new, common, obesity loci identified in genome-wide association scans, support an important role for the hypothalamic genes in predisposing to obesity. However, findings are still inconclusive for many candidate genes. To improve our understanding of the genetic architecture of common obesity, we suggest specific obesity phenotypes should be considered and different analytical approaches used. Such studies should consider multiple genes from the same physiological pathways, together with environmental risk factors.
Introduction

Over the past two decades, the prevalence of overweight and obesity has increased dramatically worldwide (1, 2). According to the World Health Organization there are currently more than 1 billion overweight adults (body mass index (BMI) ≥25 kg/m²), of which at least 300 million are clinically obese (BMI ≥30 kg/m²) (1). This epidemic is mainly attributable to changes in our life style – eating habits have shifted to greater consumption of foods high in fats and sugars while, at the same time, physical activity has decreased (1, 3-5). Since obesity is strongly associated with a higher risk for various chronic diseases, including cardiovascular disease, hypertension and type 2 diabetes (6, 7), it has been declared one of the major public health problems of this century (1).

Overweight and obesity result from a long-lasting imbalance between food intake (or consumed calories) and energy expenditure, leading to storage of excess calories as body fat (1). Both food intake and energy expenditure are controlled by the brain, which regulates the homeostasis of energy metabolism in the body (8, 9). The hypothalamus plays a key role in this complex physiological process in which multiple signals from peripheral systems are received, interpreted and transmitted to induce changes in behavior and metabolism. As a result, body weight remains remarkably stable most of the time in most people (8), but any defects in this system can lead to a deregulation of body weight. Indeed, single mutations disrupting the normal functioning of genes in these regulatory pathways are known to cause severe monogenic forms of obesity in humans (10).

Contrary to the rare monogenic forms of obesity, the nature of common obesity is more intricate. Although a chronic disruption of energy balance is required for disease development, there is a wide individual variability in the predisposition for increased body weight due to overfeeding. Many twin-, adoption- and family studies have indicated a significant contribution of genetic factors to obesity pathogenesis. Therefore, differences in genetic susceptibility may explain variation in the predisposition for obesity (11, 12). Altogether, the current obesity epidemic is most likely the result of a complex interplay between multiple genetic, behavioral and environmental factors that, in part, affect energy balance and thus regulation of body weight (11, 12).

Since the hypothalamus represents the crucial link in the regulation of body weight, altered efficiency in its regulatory pathways due to genetic variation may contribute significantly to the obesity epidemic.

Here we examine the impact of genetic variation in the hypothalamic signaling pathways regulating body weight in the etiology of obesity. We have chosen to focus
on the genes encoding the key players in these pathways: (1) the major neuropeptides from the hypothalamic signaling network involved in regulating food intake, and (2) the receptors for the peripheral hormones involved in monitoring energy homeostasis. First we will explore the role of the peptides and receptors in regulating body weight, and then review the genetic association studies performed on these genes. We will discuss several important issues that should be considered in genetic studies on obesity, such as definition of the obesity phenotype and statistical strategies to analyze the relationship between genetic variants and complex diseases.

The role of the hypothalamus in regulating energy balance

Animal studies have demonstrated the key role of the hypothalamus in the regulation of feeding and metabolism. During this process the hypothalamus is constantly receiving signals from the peripheral system and it responds to these by adjusting behavior (e.g. searching for food or satiety feeling) and the metabolic rate.

Input signals from the periphery

Control of energy balance involves the integration of satiety signals from the gastrointestinal tract (GI), adiposity and nutrient related signals. In response to food intake the gut releases cholecystokinin (CCK) and peptide YY (PYY) that inhibit food intake via by mediating the satiety feeling and, hence, prevent over-consumption. In contrast to these peptides, ghrelin is secreted by the stomach when hungry and stimulates appetite (13). Adiposity signals provide feedback information from body energy stores to the brain and are mediated via circulating hormones – leptin, produced by adipocytes, and pancreatic insulin (14). Leptin circulates at levels proportional to body fat, and its high concentration in plasma inhibits feeding. In contrast, when food is restricted, the level of leptin falls, which activates appetite-stimulating pathways in the hypothalamus via the leptin receptors. Insulin acts in a similar way via the same pathways (15). It is known that hypothalamic defects in either insulin or leptin signaling are associated with increased food intake or heavier body weight (14-16). In addition, the availability of blood glucose or fatty acids is monitored via specific sensing mechanisms described in different hypothalamic regions (17).
The integration of peripheral signals in the hypothalamus (see Figure 1)

Two different neuronal populations in the arcuate nucleus (ARC) of the hypothalamus, sensing multiple signals from the periphery, are “first-order” neurons (18). The orexigenic or appetite-stimulating neurons, producing the neuropeptide Y (NPY) and agouti-related protein (AGRP), are drivers of food intake as their neuronal activity is observed in conditions of weight loss and increased hunger (17). Other anorexigenic or appetite-suppressing neurons act via pro-opiomelanocortin (POMC), that suppress food intake via the secreted alpha-melanocyte stimulating hormone (α-MSH), and cocaine- and amphetamine-regulated transcript (CART), and are stimulated under conditions of positive energy balance (18). The NPY/AGPR neurons have an inhibitory effect on the activity of POMC/CART cells mediated by gamma-aminobutyric acid (GABA) (19). Both sets of ARC neurons express many receptors for input signals from the peripheral system. These signals transmitted via CCK, PYY, leptin and insulin suppress the activation of NPY/AGRP neurons and switch the anorexigenic pathway mediated by POMC/CART cells, leading to reduction of food intake.

Downstream signaling of the arcuate nucleus

Signals from the ARC neurons are sent to “second-order” neurons expressing melanocortin receptors MC3R and MC4R and to other parts of the hypothalamus, such as the paraventricular nucleus (PVN) and the lateral hypothalamus (LHA). Especially MC4R is important in controlling the energy balance, with its major effect being on decreasing food intake and increasing metabolic rate. Functional studies in rodents indicate the contribution of melanocortin signaling in different brain regions to different characteristics of the obesity syndrome, e.g. its disruption in the PVN induces hyperphagia and gain in body weight, while in the LHA it accelerates the development of obesity on a high-fat diet (20).

In addition, many other peripheral hormones and neuropeptides from the hypo/thalamic pathways are involved in regulating food intake; these circuits have been reviewed in (17, 18, 21). Among important players in appetite regulation are orexin, which is expressed widely in LHA (22) and stimulates food-seeking behavior under conditions of fasting (21); the brain-derived neurotrophic factor (BDNF), which is secreted in the VMN nucleus and decreases food intake in response to nutritional status via MC4R signaling (23), and 5-hydroxytryptamine (5-HT) receptors, which are located in different hypothalamic regions and through which serotonin from the reward circuit regulates satiety (18).
Chapter 2: Genetic variation in the hypothalamic pathways and its role on obesity

The hypothalamus

New obesity genes: GNPD2, KCTD15, NEGR1, NPC1L1, PTER, TMEM18, ??

The hypothalamic pathways

Food intake

Energy expenditure

Stomach

GI tract

Adipose tissue

Pancreas
Figure 1: The interactions between the neuropeptides in the hypothalamus regulate energy homeostasis and eating behavior.

Signals related to diet and circulating nutrients (shown at the bottom) that stimulate (+) or inhibit (–) the production of peptides are received by the various hormonal receptors in the ARC nuclei, which contain NPY/AGRP- and POMC/CART-producing groups of cells. The activation of NPY/AGRP neurons promotes food intake, whereas POMC/CART neurons have the opposite effect. Both signals project on to different hypothalamic areas, such as the lateral hypothalamic area (LHA), known to be a “hunger center” and containing neurons that stimulate food intake and promoting weight gain; the paraventricular nucleus (PVN), where neurons reducing food intake are located; or the ventromedial nucleus (VMN), expressing neuropeptides regulated by energy status. In addition, the hypothalamus receives signals that modulate reward-inducing feeding behavior (e.g. via serotonin). These multiple signals are transmitted and transformed to changes in food intake and energy expenditure providing energy balance. New genes from the hypothalamus are reported to be associated with obesity, however their role in the body weight regulating pathways is still unknown (highlighted in red).

Abbreviations: AGRP, agouti-related protein; ARC, arcuate nucleus; BDNF, brain-derived neurotrophic factor; CART, cocaine-amphetamine-regulated transcript; CCK, cholecystokinin; FTO, fat mass and obesity-associated; GABA, gamma-aminobutyric acid; GAD2, glutamate decarboxylase 2; GI, gastrointestinal tract; GHSR, growth hormone secretagogue receptor; HTR2A, 5-hydroxytryptamine (serotonin) receptor; KCTD15, potassium channel tetramerisation domain containing 15; INSR, insulin receptor; LEPR, leptin receptor; LHA, lateral hypothalamic area; MCHR2, melanin-concentrating hormone receptor; MC1R, melanocortin-1 receptor; MC2R, melanocortin-2 receptor; MC3R, melanocortin-3 receptor; MC4R, melanocortin-4 receptor; MTMR9, myotubularin related protein 9; α-MSH, alpha-melanocyte stimulating hormone; NEGR1, neuronal growth regulator 1; NMB, neuromedin B; NMU, neuromedin U; NPC1, niemann-Pick disease, type C1; NPY, neuropeptide Y; NPY1R, neuropeptide Y1 receptor; NPY2R, neuropeptide Y receptor Y2; NPY5R, neuropeptide Y receptor Y5; POMC, proopiomelanocortin; PTER, phosphotriesterase-related gene; PVN, paraventricular nucleus of the hypothalamus; PYY, peptide YY; SH2B1, SH2-B homolog; TMEM18, transmembrane protein 18; SCG3, secretogranin III, TUB, tubby homolog; VMN, ventromedial nucleus of the hypothalamus; UBL5, ubiquitin-like 5.
Severe obesity due to mutations in the hypothalamic genes

The patients with severe obesity caused by a single gene mutation represent only a very small percentage of the obese population. However, the study of extreme human phenotypes has provided novel insights into the pathogenesis of obesity and highlighted the fundamental nature of hypothalamic pathways in regulating energy balance. For example, single mutations disrupting the normal functioning in genes, including \( \text{MC4R} \) (the most common monogenic form of obesity), \( \text{POMC} \), or leptin receptor, lead to extremely severe obesity (for a comprehensive review see the paper by Farooqi and O’Rahilly (10)).

In summary, studies in animals and severely obese patients have demonstrated that the hypothalamic pathways play a crucial role in regulating energy balance and any defects in this system lead to a deregulation of body weight and, thus, to obesity.

Association studies in genes in the hypothalamic pathways

We performed a large-scale literature review to examine the contribution of genetic variants in the hypothalamic regulatory pathways to the obesity pathogenesis. Since 1996, the Human Obesity Gene Map (HOGM) project has extensively evaluated all published results in the research areas relevant to the genetics of obesity. We therefore only explored genetic association studies published after the last comprehensive literature review carried out by the HOGM project in 2005 (24), and combined our results with the information from that HOGM update. We performed a PubMed search using a combination of keywords for genetic studies, different obesity phenotypes, and individual gene names for the hypothalamic neuropeptides (specified in Figure 2). For the review 107 publications published between November 2005 and January 2009 were selected.

Candidate gene association studies for common obesity

As of 1 January 2009, a total of 27 candidate genes for obesity from the hypothalamic pathways have been examined (Supplementary Table 1, available online: http://www3.interscience.wiley.com/journal/122440250/suppinfo), including 21 genes reported to be associated with obesity-related phenotypes in the last HOGM update (24). Since November 2005 new studies on 11 of these 21 genes have been published, showing that variants in the following genes are associated with the risk of obesity: \( \text{AGRP} \) (25, 26), \( \text{BDNF} \) (26-29), \( \text{GAD2} \) (30), \( \text{HTR2A} \) (31, 32), \( \text{LEPR} \) (33-41), \( \text{MC4R} \) (42-53), \( \text{NPY} \)
Figure 2: Criteria and strategy used in the PubMed search for relevant publications.

A PubMed search was performed using a combination of keywords for genetic studies, different obesity phenotypes, and individual gene names for the hypothalamic neuropeptides. In order to identify the names of the genes coding these proteins, we used the Entrez Gene database at the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/sites/entrez). Multiple gene names were found for several proteins, for which we then selected the human gene names using the HuGE Navigator database (http://www.hugenavigator.net/HuGENavigator/startPagePedia.do), and the HOGM database (http://obesitygene.pbrc.edu/). Altogether, 40 gene names were included in the search based on their function, including two newly identified candidate genes for obesity, FTO and TUB.

Since the last update of HOGM (in November 2005), 576 studies were retrieved in PubMed using the keywords combination. Since only the articles that were indexed for MEDLINE were found in that search, we also screened relevant publications by checking all the studies published during the last year that were not yet indexed. After carefully reading the abstracts, 106 publications were selected for further reviewing, these were published between November 2005 and 1 January 2009, plus one just published genome-wide association on early onset and morbid obesity (135).
(54-56), NPY2R (57-60), POMC (61, 62), and UBL5 (63) (Suppl Table 1). However, in addition to these positive studies, a few other studies were unable to reproduce the associations between genetic markers in AGRP (54), BDNF (26), GAD2 (64, 65), LEPR (41, 66-68), MC3R (49), MC4R (51, 69-71), NPY (57, 72), and NPY2R (73) with obesity-related phenotypes. Negative findings were also reported for BDNF, LEPR, NPY in the last HOGM update (24).

After the last HOGM update, six new genes from the hypothalamic pathways were investigated in candidate gene association studies: FTO (52, 66, 74-105), GHSR (106-109), MCHR2 (110), NMU (111), PCSK1 (112) and TUB (113-115). Of the 107 papers selected for this review, one-third (36 papers) were studies replicating the original genome-wide association studies (GWAS) finding for the FTO gene and the majority confirmed the original association. The association between the variants in GHSR and obesity was investigated in four recent studies. While one large, population-based study, comprising more than 3600 individuals from 10 Western European countries, and two moderate sample size studies in Finnish and Chinese populations reported a positive association (106-108), no relationship between common GHSR variation and body weight or BMI was found in another study of more than 6600 adults and children from a UK population cohort (109). This lack of consistency in the results requires further investigation of the role of GHSR in obesity susceptibility. Next, candidate gene PCSK1, known to cause monogenic obesity, was investigated in a total of 13,659 individuals of European ancestry (112). The associated nonsynonymous SNP rs6232 encodes for N221D amino change, which leads to the reduced enzymatic activity of P1 protein involved in the processing of the neuropeptides from the melanocortin pathway (116).

Other new candidate genes – MCHR2, NMU, and TUB – are highly expressed in different areas of the hypothalamus and were previously studied in mice models for obesity (117-121). Two independent studies supported the original findings of the effect of TUB on BMI (113) and also reported the association with waist circumference and percentage central fat (114, 115). No replication studies have been published for MCHR2 and NMU yet, hence their role in obesity genetics remains to be clarified.

Several meta-analyses were published between November 2005 and August 2008 that provided support for the protective role of the V103I and the I251L polymorphisms in the MC4R gene against human obesity at a population level (43, 122).

To prioritize the importance of the 27 candidate genes regarding their contribution to obesity, we have summarized all the evidence for the association from the reviewed literature and from the human obesity gene map (Table 1). Based on the literature reviewed, we consider five (FTO, HTR2C, LEPR, MC4R, PCSK1) of the 32 genes examined from the hypothalamic pathways to be of particular interest. The role of FTO and MC4R
in obesity genetics has been strongly established in multiple candidate gene- and genome-wide association studies. For \textit{HTR2C} and \textit{LEPR}, the association with weight gain and different obesity phenotypes, respectively, was positively replicated in 10 and more studies, supporting their contribution to disease. Finally, the association of the functional variant in \textit{PCSK1}, an attractive positional and functional gene, was reported in a large-scale study from eight independent case-control and family-based cohorts. The conclusions for the other 22 genes remain open, either because of lack of information or because the associations were not always confirmed by later studies. Thus, replication studies in large populations that take specific obesity phenotypes into account are needed to clarify the genetic contribution to the pathogenesis of obesity.

**Inconsistency between association studies**

Exploring the selected literature on candidate gene studies clearly showed that the lack of reproducibility of associations remains a major problem in genetic studies of common obesity. For example, from the reviewed literature, there is no compelling evidence that variation in \textit{GHSR} (as discussed above) and \textit{GAD2} contribute to obesity. For the latter, two studies reported the association of rs2236418 (243A→G) with obesity in the opposite direction (64, 65) compared to the original finding and the positive replication published recently (24, 30). Several explanations for this reversal of association direction were suggested, such as differences in genetic background, patterns of interacting environmental exposures, clinical features between the two samples (64) or interpopulation differences in linkage disequilibrium (LD) structure within this genomic region (65). Indeed, opposite directions of association for the same allele with the disease in different populations, or flip-flop phenomena, can be due to differences in the correlation of the examined genetic variant with another causal variant (123).

In summary, inconsistency between candidate gene association studies does not necessarily imply false-positive findings, while potential reasons for non-replication could be: (1) ethnicity ascertainment criteria leading to population stratification, (2) the underlying LD structure between populations, (3) the different obesity phenotypes analyzed in studies, (4) the inadequate control of the type I error rate, or (5) the lack of power of the replication studies (124). While reviewing the selected studies, we also observed that, in almost one-third of the publications, ethnical homogeneity of the population studied, genotyping call rate, or the control for Hardy-Weinberg equilibrium (\textit{HWE}) were not mentioned by the researchers. This straightforward but important information is necessary to judge the quality of the genetic association study performed (125).
**Table 1:** Evidence of association between genes from the hypothalamic pathways and obesity-related phenotypes based on genetic association studies.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Candidate gene studies (positive/ negative results)</th>
<th>Associated obesity phenotype</th>
<th>Additional information**</th>
<th>Ranking</th>
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<td>OV, BC</td>
<td>A candidate gene from the identified locus</td>
<td>Promising genes with a strong evidence for their contribution to common obesity, reported in genome-wide association studies</td>
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<td><em>KCTD15</em></td>
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<td>Monogenic obesity in humans, animal model</td>
<td>Promising genes for common obesity, showing replications in ten and more candidate gene studies</td>
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<td>Additional information**</td>
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* Genetic association studies published up to January 2009 (all relevant studies published until October 2005 were reviewed based on the last update of the Human Obesity Gene Map project (24)). ** The information on animal models of obesity and severe forms of obesity is based on the last update of the Human Obesity Gene Map project and the review by Farooqi and O'Rahilly (10, 24). Abbreviations: OO overall obesity, CO central obesity, BC body composition.
Genome-wide association studies for common obesity

Genome-wide association studies (GWAS) combine the latest advances in genotyping technology with a comprehensive catalogue of common genetic variants – the International HapMap project – (126), representing a powerful, gene-discovery approach. To date, several new loci for common obesity were identified in GWA studies carried out in Caucasian populations (127-134). Notably, the majority of associated loci highlight genes that are highly expressed in the brain (and particularly in the hypothalamus), again suggesting a key role for genes involved in regulating energy balance over those involved in metabolism.

So far, published large-scale GWAS have reported eight loci for common obesity in or near genes that may play a role in the hypothalamus – BDNF, FTO, GNPDA2, KCTD15, MC4R, NEGR1, SH2B1, TMEM18 (127, 128, 130, 133-135), and two new loci for early-onset and morbid obesity – NPC1 and PTER (135) (Supplementary Table 1, available online: http://www3.interscience.wiley.com/journal/122440250/suppinfo). In addition, two small-scale GWAS conducted in Japanese populations with more than 60,000 SNPs identified MTMR9 and SCG3 as obesity susceptibility genes, although replications from independent studies are needed to confirm these findings (136, 137).

The role of the BDNF, MC4R and SH2B1 genes in obesity pathogenesis is well known from animal models studies (24). The functions of the other nine genes are not yet clear: FTO was shown to be regulated by feeding and fasting (138), NEGR1 and TMEM18 are known to be involved in neural development (133, 134), NPC1 is involved in lipid transport (135), whereas the functions of GNPDA2, KCTD15, MTMR9, PTER and SCG3 are unknown. However, all these novel genes are highly expressed in the hypothalamus (133, 134, 136-140).

Some of these novel obesity genes were identified through a meta-analysis of 15 GWA studies of over 32,000 individuals, with further replication in large-scale follow-up studies (n >59,000). Each of the associated variants has a very modest effect ranging from 0.06 to 0.33 units of BMI per allele, and their combined effect explains only a small proportion of the variation in adult BMI. These results indicate that a large sample size is required to provide reliable evidence on new common variants that play a role in complex obesity. Furthermore, the majority of the identified variants are located in noncoding regions suggesting (1) that these intronic SNPs reflect the importance of regulating protein products rather than structural protein differences in disease susceptibility, and (2) the presence of causal variants within a haplotype cluster. Additional functional analyses will be needed to understand the physiological mechanisms underlying the observed associations.
It is also important to remember that current-generation GWA genotyping platforms are based on HapMap data, which is based on the “common disease, common variant” hypothesis and, thus, target SNPs with minor allele frequency ≥5% (141). In contrast, the hypotheses on slightly deleterious SNPs that underwent weak purifying selection (142), or the “Predation Release” hypothesis (e.g. the absence of predation led to a change in genes predisposing to fat gain due to random mutations and genetic drift) (143) highlight the importance of rare variants in susceptibility to common diseases. An extensive search for genetic variants associated with the extremes of human body mass, the only large-scale study to date, revealed an excess of rare, nonsynonymous SNPs among obese individuals compared with lean individuals (144). Therefore, the inclusion of rare and structural variants, such as insertions, deletions and copy number variants, in large-scale association studies and deep sequencing will provide more insight into the relative contribution of common and rare variation to the genetic structure of obesity.

Consistency between genome-wide association studies and candidate studies

So far, GWAS have confirmed the association of the hypothalamic genes BDNF, MC4R and SH2B1 with obesity that were previously examined in candidate gene studies (26-29, 42-48, 145), although not all the candidate gene studies reported a positive association with BDNF or MC4R. Another interesting observation is that SNPs near MC4R also contribute to the risk for common obesity. Coding mutations in this gene are the most frequent cause for severe obesity. This provides evidence of possible overlap between monogenic and complex forms of the disease. Rare variants in MC4R (V103I and I251L) are also shown to be protective for obesity (43, 122) suggesting the genes have a “balanced effect” on a metabolic trait: whereas loss-of function mutations lead to severe obesity, gain-of-function variants may be protective against the disorder (43).

Several factors may explain why genes consistently reported to be associated with obesity-related phenotypes were not found to be associated in GWAS. First, it is expected that common variants contributing to obesity will have only a moderate effect (the estimated odds ratios are probably 1.2-1.3 or lower), and large-scale studies are therefore required to provide sufficient statistical power for their identification (146). Secondly, hundreds of thousands of SNPs are genotyped in GWAS and only markers that pass a strict threshold for multiple-test correction in the statistical analysis are considered to be associated with the phenotype studied. This correction of the type 1 error rate automatically leads to reduced power. Thus, GWAS are often underpowered
to detect genes with small effect size (146). This means that several candidate genes with small effect sizes that alter the risk are not picked up in GWAS but they might well be associated with the disease. A good example of this is \textit{PPARG}, a gene with a well-established role in the development of type 2 diabetes that was also known to be associated with the disease from candidate gene studies, but was not found to be significantly associated by the many type 2 diabetes GWAS. Collaborations in which several large GWAS are meta-analyzed will increase the power to detect variants and might confirm several of the genes found by candidate gene studies. Thirdly, even high-density SNP chips do not capture all the common variations in the human genome (estimated coverage is \textasciitilde80\%) and thus absence of good coverage for the true, disease-associated variant in the genotyping platforms could lead to false-negative results (146, 147). Finally, all causes of inconsistency between candidate gene studies (such as differences in the frequency of the risk allele, in ascertainment of cases and controls, in populations studied, and undetected population stratification (146-148), also apply to the inconsistency between candidate gene- and GWAS. For instance, the association between \textit{FTO} and obesity has never been found in non-Caucasian populations, such as Chinese Han, Asian Indians or Oceanic populations (132, 149-151), indicating that variants associated with a disease in one population do not necessarily have the same effect or association in another population. Thus, it is important to keep in mind that false-negative findings in a GWAS cannot be excluded due to such study design limitations (141, 146). To help avoid all these factors leading to inconsistency in genetic association studies, a group of experts from diverse disciplines, including biostatistics, clinical medicine, epidemiology, genetics and scientific publishing, proposed a list of criteria of best practices for the design, conduct and publication of replication studies (152).

\textbf{Pitfalls in genetic association studies on obesity}

As we saw in the reviewed literature, non-replication remains the major problem in genetic association studies related to common obesity. And, as discussed earlier, such inconsistency can be explained by population stratification, study heterogeneity, lack of type 1 error control, lack of power to detect small effects, and publication bias. However, there are still two other important problems which are often not recognized: the definition of the obesity phenotype and methodological issues.
Phenotype definition
According to the World Health Organization guidelines, the most common definition of overweight and obesity is a body mass index (BMI) $\geq 25 \text{ kg/m}^2$ and $\geq 30 \text{ kg/m}^2$, respectively (1). BMI is the individual’s body weight divided by the square of their height (1, 153). As a clinical tool, BMI is simple to calculate, easy to measure, and a well-defined criterion for diagnosing the severity of obesity. However, since BMI does not directly incorporate body fat or fat distribution levels, it does not differentiate between lean and fat tissue (e.g. a misclassification is possible between overweight and muscular individuals), nor does it delineate the pattern of fat distribution (154). It has also been shown that relative fatness in adults increases with age in a sex-specific way, even when BMI remains constant (i.e. there is a greater relative increase in men than in women) and that there are national- or ethnic differences in the relationship between BMI and percentage body fat (155). Thus BMI, which measures overall obesity only, might not be the best marker for obesity phenotype. In addition, unmeasured or poorly defined environmental factors contribute strongly to the problems of correctly defining obesity phenotypes: two individuals may have the same weight or BMI through different combinations of multiple factors, such as physical activity, dietary patterns, stress or smoking (156).

Other common obesity-related parameters are waist circumference and waist-to-hip ratio used to measure abdominal obesity, and skinfold thickness to quantify subcutaneous fat. More accurate methods for quantifying central fat mass and visceral fat are CT (computed tomography), MRI (magnetic resonance imaging) (11) and ultrasonography (157), but these methods are expensive and time-consuming and therefore unsuitable for large-scale studies.

Many of the association studies presented here investigate the relation between genes and BMI as a marker of obesity. As BMI might not be the best marker, misclassification of obese subjects based on their BMI could be one of the reasons for lack of replication. The same explanation was suggested in a recent meta-analysis of genome-wide linkage studies in BMI and obesity (158). This analysis combined the results from 37 linkage studies, encompassing data from more than 10,000 families and over 31,000 individuals, but researchers failed to find any strongly positive loci for BMI. Moreover, it was shown later that there are high genetic correlations between different measurements of obesity, indicating that genetic contribution may be specific for each trait (159). Hasselbalch et al. highlighted the importance of the specific obesity phenotypes to be taken into account in genetic studies on obesity (159).
To investigate whether different genetic variants from the hypothalamic pathway contribute to different measures of obesity, we explored the associations of each gene with specific obesity phenotypes (Figure 3). The analysis revealed no obvious pattern of relationship with a specific obesity phenotype and the genes, possibly due to the differences between the study designs: although some studies did include different obesity-related traits, not every study chose the same phenotype, making it difficult to compare results. However, the GWAS results indicate that the association of many novel obesity genes with BMI is mainly driven by effect on weight, suggesting their effect on overall obesity.

In conclusion, to improve our understanding of obesity pathogenesis, we need a better definition of obesity phenotypes, combined with detailed epidemiological data on life style and dietary patterns for different ethnic groups in different subpopulations.

Figure 3: The relationship between genes from the hypothalamic pathways and obesity-related phenotypes. Overall (or general) obesity phenotypes are indicated by BMI and body weight; central obesity phenotypes – by waist and hip circumferences and waist-hip ratio; body composition phenotypes – by different measurements such as fat mass, fat free mass, body fat percentage, lean mass, sagittal abdominal diameter, subcutaneous abdominal, abdominal total fat, total fat mass, body fat.
Methodological issues
As discussed above, the hypothalamus is a highly complex physiological system that regulates energy balance. To protect the proper regulation of its neuronal circuits, a strong compensatory mechanism is required. Thus, loss of function in one gene in this system will probably be compensated by altered expression of another gene (9), and it is therefore essential to analyze epistasis or interactions between genes to understand the role of the hypothalamic pathways as a whole system in obesity etiology (160).

Current statistical strategies to analyze the association of gene variants in relation to complex traits such as obesity still focus on single locus methods, such as $\chi^2$ statistics (161). Given the complexity of the system and the fact that both genetic variation and an imbalance in energy intake and expenditure need to be present, single locus methods may not be based on the correct underlying model of association. In complex traits, gene-gene and gene-environment interactions are likely to contribute to development of the trait (162, 163). This insight has resulted in a growing recognition that multi-locus analysis tools and pathway-based approaches, in which gene-gene, gene-environment, and even higher order interactions can be incorporated, are needed to unravel the complex etiology of these diseases (164, 165). Recently, network analysis that takes the complexity of the neuronal feeding pathways into account as well as a combination drug approach was suggested to improve anti-obesity pharmacotherapy (166, 167). This may be a more efficient strategy since it involves compensatory or counter-regulatory neuropeptide signaling mechanisms involved in the processes of appetite control. We suggest using a similar approach in genetic studies of obesity.

Conclusions
This review shows that genetic variation in the different hypothalamic pathways that sense and integrate the peripheral signals reflecting metabolic status are important in the development of obesity phenotypes. So far, discoveries of novel obesity genes by genome-wide association studies suggest a key role for the hypothalamic pathways in regulating food intake and energy homeostasis in the genetic architecture of common obesity. The importance of some of these genes is further highlighted by candidate gene and functional studies. However, the verdict is still open on many other promising candidate genes. Apart from all the methodological issues in genetic association studies, we propose that future studies should take the complexity of the hypothalamic system into account. This can be done by network analysis, multi-locus tools, and
pathway-based approaches, which not only incorporate the interactions between the genes from this system, but can also take environmental risk factors into account. More attention should also be paid to defining the specific obesity phenotypes studied. We have clearly shown that genes from the hypothalamic pathways play an important role in the etiology of obesity and this knowledge may help develop prevention strategies and pharmaceutical agents that specifically target these pathways.
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