Genetic, molecular and physiological mechanisms involved in human obesity: Society for Endocrinology Medal Lecture 2012

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Summary
The health consequences of obesity represent one of the major public health challenges of our time. Whilst the role of environmental drivers such as reduced physical activity and increased food intake is widely acknowledged, the importance of biological factors which influence individual variation in weight is less readily recognised. Considerable evidence suggests that genetic factors influence a person’s weight in a given environment and that these genetic influences are more potent at the extremes of the body mass index (BMI) distribution. The discovery that genetic disruption of certain pathways can lead to severe obesity has informed our current understanding of how body weight is regulated by brain circuits that regulate appetite and energy expenditure. These studies provide a framework for investigating patients and ultimately may guide the development of more rational-targeted therapies for genetically susceptible individuals with severe obesity.

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Introduction
Obesity is defined as an increase in fat mass that is sufficient to adversely affect health. Whilst measurement of body fat mass is not routinely possible outside of the research setting, body mass index (BMI; weight in kg/height in metres²) is a useful surrogate marker. Current prevalence data from the Health Survey of England using the WHO definition of a BMI more than 30 kg/m² to define obesity, indicate that 24% of men and 25% of women in the UK are obese (www.nso.org.uk), figures that are consistent with other European countries. As body mass index increases, so does the relative risk of type 2 diabetes, hypertension, and cardiovascular disease. As such, the rising prevalence of obesity represents a major threat to public health. At an individual level, severe obesity is often associated with a multitude of problems including sleep disturbance and respiratory difficulties, and joint and mobility issues as well as mood disturbance. Severe obesity is also associated with considerable social stigma which can affect quality of life, job opportunities and interactions with health care professionals.

Genes and environment
The recent rise in obesity prevalence is largely driven by increases in energy intake associated with the availability of inexpensive, highly palatable foods and a reduction in energy expenditure associated with reduced voluntary physical activity. In fact, only small changes in overall energy balance (7–10 kcal/day surplus to requirements) are sufficient to account for the increase in the mean BMI of the UK population over the last 30 years (Fig. 1). In addition to changes in the mean BMI of the population, there has also been an increase in the proportion of people at the tail end of the BMI distribution, those with severe obesity. The susceptibility of some individuals to gain weight in a particular environment is likely to represent a complex interaction of multiple biological, social and environmental factors, but there is evidence that severe obesity is also strongly influenced by genetic factors. Indeed, family, twin and adoption studies all indicate that body weight is highly heritable and that this heritability is more notable at the extremes of the BMI distribution.

These observations have formed the basis of our approach to use genetic strategies to investigate the mechanisms involved in the regulation of body weight and the disruption of these mechanisms in patients with severe obesity. Working with colleagues in Cambridge and many collaborators around the World, we have discovered several genes whose disruption leads to severe obesity which begins in childhood (Fig. 2). The study of these patients and of the molecular and physiological mechanisms that link genetic changes to obesity and associated clinical phenotypes have informed our understanding of the systems that are involved in the regulation of body weight and the coupling of changes in energy balance to the changes in neuroendocrine axes, immunity and other parameters.
Homeostatic pathways involved in the regulation of weight

In the early 1900s, clinical studies of patients with brain tumours suggested that the hypothalamus may be involved in the regulation of appetite and body weight. However, the precise nature of these neural pathways has only been elucidated in the last 20 years. Experimental studies in highly inbred strains of severely obese mice led to the identification of the hormone leptin in 1994. Leptin is a hormone that is secreted by adipocytes and in general, leptin mRNA concentrations in adipose tissue and serum leptin concentrations correlate positively and closely with fat mass in humans. Leptin’s physiological role is primarily to signal in response to nutritional depletion. In fasting, leptin levels fall acutely, which triggers a series of changes in energy intake, energy expenditure and neuroendocrine function in order to restore energy homeostasis.

Many of the physiological effects of leptin are mediated through hypothalamic neurons which express the long signalling form of the leptin receptor. Leptin stimulates the expression of pro-opiomelanocortin (POMC) in neurons located in the arcuate nucleus of the hypothalamus. POMC is extensively post-translationally modified to generate the melanocortin peptides which activate the melanocortin receptors to modulate diverse functions in the central nervous system, the adrenal gland and skin. In addition, leptin inhibits orexigenic pathways mediated by neurons expressing the melanocortin antagonist Agouti-related peptide (AgRP) and Neuropeptide Y (NPY); NPY can suppress the expression of POMC. These two sets of primary leptin-responsive neurons project to other hypothalamic sites.

Fig. 1 Schematic depicting changes in BMI distribution in the UK.

Fig. 2 Schematic of the hypothalamic leptin-melanocortin pathway. POMC (pro-opiomelanocortin); PC1/3 (prohormoneconvertase 1/3); MC4R (melanocortin 4 receptor); SIM1 (single-minded one).
expressing the melanocortin 4 receptor (MC4R). One important site is the paraventricular nucleus (PVN), which is the primary central regulator of the sympathetic nervous system, and regulates neuroendocrine signalling via the hypothalamo-pituitary-thyroid axis and the hypothalamo-pituitary-adrenal axis.

The downstream targets of melanocortin signalling still remain relatively obscure, although brain-derived neurotrophic factor (BDNF)-mediated signalling through the TrkB receptor may play a role. Whilst it is clear from rodent studies that leptin-responsive hypothalamic circuits interact with other brain centres to co-ordinate food intake and modulate efferent signals to the periphery to regulate energy expenditure and intermediary metabolism, the relevance of these pathways and others to the regulation of human energy homeostasis remains to be fully explored.

**Mutations in the leptin pathway cause severe human obesity**

We showed that homozygous mutations in the genes encoding the hormone leptin result in severe obesity from a young age.\(^9,10\) The key clinical features seen in these patients are an intense drive to eat (hyperphagia), with aggressive behaviour when food is denied. Patients also exhibit impaired satiety, demanding food soon after the end of a meal. Mutations in the gene encoding the leptin receptor result in a very similar phenotype characterised by hyperphagia and severe early-onset obesity.\(^11,12\) Leptin and leptin receptor deficiency are associated with hypogonadotropic hypogonadism and a failure of normal pubertal development. However, there is some evidence for the delayed but spontaneous onset of menses in some leptin receptor-deficient adults.\(^13\) In these patients, the excess adipose tissue mass may lead to the production of sufficient oestrogen to result in uterine development and irregular menses in the absence of fully developed secondary sexual characteristics. Leptin may exert these effects on the reproductive system through a number of molecules including kisspeptin,\(^14\) which signals through GPR54, to modify the release of gonadotrophin-releasing hormone.

Although congenital leptin deficiency is rare, we are able to demonstrate that it is entirely treatable with daily subcutaneous injections of recombinant human leptin (Fig. 3) with beneficial effects on the degree of hyperphagia, reversal of the immune defects and infection risk seen in leptin-deficient patients and permissive effects on the appropriate development of puberty (Fig. 2).\(^10,15\) Such treatment is currently available to patients on a named patient basis. We showed that the major effect of leptin administration is on food intake, with normalisation of hyperphagia and enhanced satiety. In the leptin-deficient state, basal metabolic rate (BMR) and free-living energy expenditure were appropriate for age and body composition. However, as weight loss by other means is associated with a decrease in BMR,\(^16\) the fact that energy expenditure did not fall in our leptin-deficient subjects is notable. The administration of leptin permits progression of appropriately timed pubertal development, suggesting that leptin is a permissive factor for the development of puberty.

These studies provided the first proof of principle that leptin is an essential regulator of body weight in humans.\(^10,15,17\) Recombinant human leptin has led to sustained, beneficial effects on appetite and body weight for up to fifteen years.

These observations demonstrated that the study of the extreme phenotype of severe early-onset obesity could reveal major, highly penetrant genetic effects and that the functional and phenotypic characterisation of genetic obesity syndromes could provide valuable insights into the mechanisms involved in energy homeostasis and the pathophysiology of obesity. We went on establishing a comprehensive international study of patients with severe, early-onset obesity (BMI standard deviation score >3, onset<10 years), which has involved the collection of clinical details, serum and DNA samples on over 5000 patients, making this Genetics of Obesity Study (GOOS), one of the largest cohorts of its kind in the World. In further, studies in patients with severe early-onset obesity, we have identified mutations in a number of genes involved in pathways that regulate appetite downstream of leptin (Fig. 2).

**MC4R deficiency represents the commonest genetic form of severe obesity**

Patients with homozygous/compound heterozygous mutations that disrupt POMC present with cortisol deficiency due to ACTH deficiency, red/light coloured hair and a lack of skin pigmentation, as well as severe obesity in early life. We and others have reported that heterozygous mutations in MC4R are found in 2–3% of children in obesity clinics and up to 5% of patients with severe early-onset obesity, making MC4R deficiency the commonest genetic form of severe obesity.\(^18\)

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significant mutations in MC4R are found at a frequency of approximately 1/1,000 in the general UK population. MC4R mutations are inherited in a co-dominant manner, with variable penetrance and expression in heterozygous carriers. As a result of this growing body of information, assessment of the sequence of the MC4R is increasingly recognised as a necessary part of the clinical evaluation of the severely obese child.

MC4R mutation carriers are objectively hyperphagic, but the degree of hyperphagia is not as severe as that seen in leptin deficiency. We found that the severity of receptor dysfunction seen in in vitro assays predicted the food intake at a test meal, suggesting that signalling through this pathway is a major mechanism for the regulation of appetite. We found that MC4R-deficient patients have a lower prevalence of hypertension and lower systolic and diastolic blood pressures when compared to equally obese volunteers, which may be explained by altered sympathetic nervous system activation. These studies indicated that the melanocortin system is a key link between changes in weight and changes in blood pressure.

Ultimately, the hope is that the finding of specific genetic causes of obesity can inform the development of rational mechanism-based therapies for these patients. At present, Roux-en-Y bypass surgery has been shown to be effective in adults with MC4R mutations. A number of drugs that target MC4R are being developed for the potential treatment of this group of patients including pharmacological chaperones and a selective melanocortin receptor agonist that is likely to enter Phase 2 clinical trials in the near future.

Modulation of leptin and melanocortin signalling

Additional genetic studies in the GOOS cohort have led to the identification of mutations in a number of molecules that modulate leptin-melanocortin signalling. Leptin mediates its effects on energy balance by binding to the long form of the LEPR and activating associated Janus kinase 2 (JAK2). JAK2 phosphorylates multiple tyrosines in LEPR enabling recruitment of downstream effectors. JAK2 also autophosphorylates allowing the binding of the adapter protein Src homology 2 (SH2) B adapter protein 1 (SH2B1), which enhances JAK2 activation and helps recruit insulin receptor substrate (IRS)-1 and IRS-2 to the LEPRb/JAK2 complex, thereby acting as a key endogenous positive regulator of leptin sensitivity. Targeted deletion of Sh2b1 in mice results in impaired leptin signalling and severe obesity. SH2B1 null mice are also insulin resistant as a result of impaired insulin signalling.

The first evidence for the role of SH2B1 in human energy balance came with our identification of copy number variants (CNVs), specifically deletions of a 220-kb segment of chromosome 16p11.2, which were associated with highly penetrant severe early-onset obesity and severe insulin resistance. This deletion included a small number of genes, one of which was SH2B1. We subsequently identified several coding heterozygous-mutations in the SH2B1 gene which resulted in a loss of function in a number of invitro assays. Mutation carriers were disproportionately hyperinsulinemic for the degree of obesity, with some developing type 2 diabetes at an early age. Unexpect-edly, we found that mutation carriers had behavioural abnormalities including delayed speech and language development, aggressive behaviour, social isolation and, in some instances, criminal behaviour as adults. These phenotypes are not seen in association with the other genetic obesity syndromes we have characterised to date (11, 12), although the socio-economic status of mutation carriers is comparable. As we observed impaired NGF-induced neuronal differentiation in vitro, a similar response to other ligands, such as centrally expressed neurotrophins such as BDNF, could contribute to these features.

MRAP2

There is increasing recognition that accessory proteins can modulate GPCR trafficking, as well as ligand binding and signalling. An accessory protein for MC2R, MC2R accessory protein (MRAP), is required for the trafficking of MC2R to the surface of adrenal cells and for signalling in response to ACTH. All mammals have a paralogous gene, MRAP2, which, like MC3R and MC4R, is predominately expressed in the brain including in regions involved in energy homeostasis such as the hypothalamus. We showed that MRAP2 interacts with MC4R and in collaboration with mice lacking Mrap2 are obese. Rare variants in MRAP2 have been associated with severe human obesity, but further work will be needed to characterise the mechanisms underlying this association.

Brain-derived neurotrophic factor (BDNF) and tyrosine kinase receptor tropomycin-related kinase B (TrkB)

BDNF is one of the several nerve growth factors which activate signalling by the tyrosine kinase receptor tropomycin-related kinase B (TrkB) which may lie distal to MC4R signalling. We reported a child with severe obesity, impaired short-term memory, and developmental delay who had a de novo missense mutation impairing the function of TrkB. We also identified a patient with a de novo chromosomal inversion, which encompasses the BDNF locus and disrupts BDNF expression. Yanovski and colleagues showed that in patients with WAGR syndrome, a subset of chromosome 11p.12 deletions encompassing the BDNF locus were associated with early-onset obesity. Defects in this pathway have severe consequences on development, and as such, are likely to be very rare and may often occur de novo.

SIM1 deficiency

We have recently reported multiple heterozygous obesity-associated mutations in single-minded 1 (SIM1), a basic helix-loop-helix transcription factor involved in the development and function of the paraventricular nucleus (PVN) of the hypothalamus. Several lines of evidence suggest that SIM1 may influence energy homeostasis by interacting with pathways involved in central melanocortin signalling. Similarly, we found that heterozygous carriers of loss of function SIM1 mutations share many clinical features with patients with MC4R deficiency including hyperphagia and
autonomic dysfunction. In addition, we observed a variable phenotype of developmental delay with some autistic like features in some, but not all, SIM1 mutation patients. Patients with chromosomal deletions involving 6q14–q21, a region which encompasses several genes including SIM1, have been reported to develop early-onset obesity and developmental delay with some reports suggesting that there is a degree of phenotypic overlap with Prader-Willi syndrome. Impaired oxytocinergic signalling is one mechanism implicated in the hyperphagia and obesity seen in Prader-Willi syndrome (PWS), a clinical syndrome caused by lack of expression of a cluster of maternally imprinted snoRNAs on chromosome 15 which are thought to be involved in alternative mRNA splicing. Some of these features are reminiscent of the cognitive and behavioural deficits seen in SIM1 variant carriers. Interestingly, the hyperphagia of sim1 haplo-insufficient mice is partly ameliorated by the central administration of oxytocin and exacerbated by the administration of an oxytocin receptor antagonist. Further, characterisation of these features will have implications for the identification of patients in whom variants in SIM1 should be considered and for genetic counselling of SIM1 variant carriers.

Genetic influences on metabolic rate
To date, all the genetic forms of severe obesity seem to act predominantly by impacting on the regulation of appetite. However, given the homology between the energy balance pathways in different species, it is plausible that genetic variants may impact on energy expenditure. Basal metabolic rate accounts for approximately 70% of daily energy expenditure, with the remainder being attributable to voluntary physical activity (20%) and non-exercise-related activity thermogenesis (10%). Whilst differences in muscle mass account for 60-70% of the inter-individual variability in basal metabolic rate, genetic differences may explain some of the variability.

We recently identified multiple mutations in the gene encoding KSR2 (Kinase Suppressor of Ras2), a cellular scaffolding protein that is involved in the Ras-Raf-MEK signalling pathway, implicated in cell division, differentiation and growth. Basal metabolic rate (BMR) correlates very closely with energy expenditure, on the basis of age, gender and body composition; the cellular fuel sensor and AMP-activated protein kinase (AMPK). Under conditions of nutrient deprivation, intracellular ATP levels fall and levels of AMP rise, promoting AMPK activation which in turn promotes catabolic processes and inhibits anabolic pathways. Whilst some of the variants reduced the interaction between KSR2 and AMPK, when compared to wild-type KSR2, almost all the KSR2 variants impaired glucose oxidation and palmitate-stimulated fatty acid oxidation in cells. These observations indicate that multiple molecular and cellular mechanisms underlie the phenotype associated with disruption of KSR2 in humans.

Clinical reports suggested that some KSR2 mutation carriers experienced marked weight loss in childhood when prescribed the anti-diabetic drug metformin (for severe insulin resistance). We found that the reduced basal level of fatty acid oxidation seen with the KSR2 mutations was completely rescued in all cases by the addition of metformin in cells. Further work will be needed to see if these observations can be replicated in formal experimental clinical studies and to investigate the cellular mechanisms underlying these effects.

Conclusions
It is important not to lose sight of the fact that some individuals are particularly susceptible to the development of severe obesity. Some genetic susceptibility may be explained by an accumulation of common genetic variants in these patients. Methods have been developed to predict the impact of common variants using susceptibility scores, however, these common variants, even in combination, are not sufficient to explain the genetic susceptibility to severe obesity, which is more likely to be driven by rare variants that are more highly penetrant. Our work has emphasised the need to recognise and characterise the heterogeneity of obesity and to define subgroups of patients at risk of different metabolic and cardiovascular complications who may benefit from targeted preventative and therapeutic strategies. Future strategies to treat and support this group of patients, whose numbers are increasing, will need to take into account the major biological influences on the drive to eat and how heritable differences between individuals influence their risk of obesity.

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