Review Article

The Role of Leptin-Melanocortin System and Human Weight Regulation: Lessons from Experiments of Nature

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Abstract

Introduction: Common obesity is a multi-factorial trait, contributed by the "obesogenic" environment of caloric abundance and increasing automation, sedentary lifestyle and an underlying genetic susceptibility. There have been major advances in the past decade in our understanding of the human weight regulation mechanism and pathogenesis of obesity, abetted by discoveries of genetic defects which lead to human obesity. Materials and Methods: Reports of genetic mutations causing obesity in humans and murine models were reviewed. Results: Humans with genetic defects resulting in leptin deficiency, leptin receptor deficiency, pro-opiomelanocortin deficiency (POMC), and melanocortin 4 receptor (MC4R) deficiency developed severe obesity as the dominant phenotypic feature, though these are rare autosomal recessive conditions, except MC4R deficiency which is inherited in an autosomal co-dominant fashion. Common and rare variants of the *POMC* and melanocortin 3 receptor genes may be predisposing factors in the development of common obesity. Recent reports of human obesity associated with thyrosine kinase B (TrkB) defect and brain derived neurotrophic factor (BDNF) disruption, coupled with other murine studies, supported the role of BDNF/TrkB as effectors downstream of the melanocortin receptors. Conclusion: Despite exciting discoveries of single gene mutations resulting in human obesity, most cases of obesity are likely the result of subtle interactions of several related genetic variants with environmental factors which favour the net deposition of calories as fat, culminating in the obese phenotype. The mechanisms of action of these genes in the development of obesity are now being examined, with the aim of eventually discovering a therapeutic intervention for obesity.

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Introduction

Obesity as a Multi-factorial Trait

Obesity predisposes to significant morbidities and premature mortality, and the increasing obesity prevalence all over the world has been attributed to industrialisation and modernisation which encourages a sedentary lifestyle and increased calorie intake.[1,2] This results in energy intake and expenditure imbalance and the net deposition of calories as fat. Although this trend of increasing body girth is very much driven by the "obesogenic" environment, it is facilitated by the individual's genetic susceptibility to excessive weight gain.[3]

Common obesity is widely regarded as a complex, multi-factorial disorder with high heritability, which evolved from interaction between the modern "obesogenic" environment and the individual's genetic susceptibility to excessive weight gain.[3] Studies of twins, adoptees and families indicate that as much as 80 per cent of the variance in the body mass index (BMI) is attributable to genetic factors.[4-11] These studies, as well as numerous linkage and association studies, supported the role of genes in the pathogenesis of human obesity. The relative contribution of the environment and genetic susceptibility towards the pathogenesis of obesity varies between different obese individuals, and genetic predisposition to weight gain may account for severity of phenotype, such as the age of onset of obesity (early or childhood onset versus late or adult onset),[8] and even the individual's response to weight losing measures.[12,13]

The role of these genetic variants associated with obesity (labelled "obesity genes" henceforth in this article) in the current obesity epidemic is addressed in another review article of this issue. The stable gene pool of humans in the

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short period of time in which the current obesity epidemic ravages the world could only mean that these obesity genes play a passive role.[14,15] However, individuals with these genetic variants may be predisposed to severe or even morbid obesity when exposed to the "obesogenic" environment conducive for weight gain.[14,16] These individuals may possess the "thrifty genes" (obesity predisposing genetic variants) which would otherwise be protective against starvation (and therefore confer selection advantage historically), but in the present day "obesogenic" environment might develop severe obesity, as observed in ethnic groups at high risk of obesity such as the Pima Indians, Pacific Islanders, Afro-Americans and Hispanic-Americans.[17]

Research into the genetic basis of obesity has advanced rapidly over the past 2 decades, and provided revelations with regards to the molecular mechanism of energy homeostasis in the process. Traditional methods employed to uncover these genetic variants associated with obesity include genome-wide scans which studied unrelated obese individuals, linkage studies which examined related pairs or families with obesity, and association studies which investigated the association between obesity and polymorphic variants of candidate genes predicted to affect weight regulation. The search for genetic factors predisposing to common obesity is challenging and progress has been slow, as it is likely that each individual genetic variant exerts subtle effect on body weight and thus proving its association with increased adiposity can be difficult. The obese phenotype is very heterogeneous, even within the same family, and there is variable contribution from genetic, environmental and behavioural influences which differ for every obese individual, confounding efforts to analyse this condition.

Monogenic Obesity Illuminates the Molecular Circuitary of Energy Homeostasis

While the search for genetic variants associated with obesity poses a major challenge, we have witnessed significant milestones in obesity gene research in the last decade – the discovery of novel single gene defects which result in human obesity, namely leptin deficiency, leptin receptor deficiency, pro-opiomelanocortin (POMC) deficiency, prohormone convertase 1 deficiency (PC1), melanocortin 4 receptor deficiency and tyrosine kinase B (TrkB) deficiency. These monogenic forms of human obesity resulted in the deficiency of critical molecules and disruption of the leptin-melanocortin system which lead to the obese phenotype, and thus provide validation of the role of the leptin-melanocortin system in energy homeostasis, and unravel the molecular circuitary of human weight regulation.

The Leptin-Melanocortin System

Various human and murine genetic studies have shed light on the biological weight regulation mechanism, akin to pieces of jigsaw puzzle being put together which progressively unravel this integrated system. Excess nutrients are stored in adipose tissue. Adipose tissue secretes leptin in response to increased fat storage, which circulates as an afferent satiety signal and activates hypothalamic neurons expressing POMC located in the arcuate nucleus, which innervates other hypothalamic regions known to regulate feeding behaviour.[18-20] POMC is a polypeptide that undergoes tissue-specific post-translational processing, the products of which include the melanocortin peptides α, β, and γ-melanocyte-stimulating hormones (MSH),[21] and 1 or more of the 3 melanocortin peptides are involved in the anorectic response (i.e. reduced feeding) by stimulating melanocortin-4 receptors (MC4R) on neurons downstream in the paraventricular nucleus (PVN),[22-26] and melanocortin-3 receptors (MC3R) to reduce feed efficiency, which is the ability to convert food to fat stores.[27-31] The melanocortin system thus mediates the anorexigenic (inhibition of feeding) effects of leptin, reducing food intake and increasing reducing energy expenditure. MC3R is also located on POMC expressing neurons in the arcuate nucleus, and may form part of a feedback loop which negatively regulates the anorexic tone of the POMC expressing neurons,[32] where melanocortin peptides from activated POMC neurons negatively autoregulate further POMC expression through their inhibitory actions at the MC3R. Recent evidence suggests that tyrosine kinase B receptor, brain derived neurotrophic factor,[33,34] and nesfatin[35] are critical mediators downstream of MC4R. Leptin also inhibits neurons co-expressing the orexigenic (feed promoting) neuropeptide Y and agouti-related peptide in the arcuate nucleus, which will otherwise promote feeding activity.[36]

While the leptin and melanocortin pathways are thought to be one functional unit, there is evidence that there is some degree of independence between the 2 systems. Leptin deficient *ob/ob* mice are more obese than the mouse models knock out for *POMC* or *MC4R*,[37] and removal of leptin receptors specifically from the ARC POMC neurons resulted in only modest obesity.[38] The melanocortin pathway, though an important distal mediator of leptin signalling in the hypothalamus, may not be the only one. Much is yet to be discovered.

Leptin

The discovery of leptin[39] and the leptin receptor[40] heralded a new era in obesity research. The protein, "leptin", is derived from the Greek word "leptos", meaning thin. The etymology of the word "leptin" implies that its physiological role is primarily to suppress body fat, by decreasing food

intake and increasing energy expenditure. Leptin is a 167 amino acid peptide made exclusively in adipose tissue in a wide range of animal species, including humans. The *ob* gene encoding leptin is located on the mouse chromosome 6, and the human homologue of the *ob* gene has been mapped to chromosome 7q31.3.[41,42] Northern blot or RT-PCR analysis of the messenger ribonucleic acid (mRNA) for the *ob* gene showed that it was expressed only in adipose tissue.[39]

Leptin is secreted by adipocytes as an afferent satiety signal, produced in proportion to the mass of adipose tissue, which acts as an endocrine organ. Both human and animal studies have demonstrated the close association between body fat, leptin mRNA and the plasma leptin levels.[43-45] Increase in fat storage will lead to increased leptin, which inhibits the satiety centre in the hypothalamus, and also influences other neuroendocrine systems, including those related to puberty and fertility. There appears to be a lipostatic set point for weight regulation, in which the body will maintain a certain weight and body composition. Several observations, including the variable and modest response of human obesity to supraphysiological doses of recombinant leptin,[46] have suggested that the primary energy homeostatic function of leptin is to maintain adequate fat stores for times of starvation, rather than preventing obesity in times of excess and food aplenty, in keeping with the theory that evolutionary pressures have geared our system to favour fat storage over consumption which confers survival advantage.

Leptin Deficiency

Leptin deficiency from disruption of both leptin genes results in severe obesity in mice and humans. The *ob/ob* (leptin deficient) mouse is characterised by obesity, hyperphagia, hyperglycaemia,[47] hyperinsulinemia due to insulin resistance,[48] hypothermia,[49] impaired hypothalamic-pituitary-thyroid axis,[50] defective T cell mediated immunity, and hypogonadotropic hypogonadism causing infertility,[51] and leptin replacement reverses these endocrine and metabolic defects.[43,52,53]

Human congenital leptin deficiency is rare. Five children from 3 consanguineous Pakistani families were reported to be homozygous for a frameshift *Lep* mutation comprising of a single G deletion affecting codon 133 (Δ133G), which led to 14 aberrant amino acids, followed by a premature stop codon.[54-57] The mutant leptin was not secreted, but accumulated intracellularly as a consequence of misfolding and aggregation, and was subsequently degraded by the proteasome.[58] There were also 3 related Turkish subjects homozygous for a missense mutation Arg105Trp due to a C to G transition, which resulted in impaired processing and secretion of leptin.[59]

These individuals had undetectable or very low leptin levels, and exhibit extreme early onset obesity. Their birth weights were within normal limits, but rapidly become obese by 3 to 4 months of age, with marked hyperphagia and were constantly hungry. They have high percentage body fat of 54% to 57%, and linear growth was not stunted with IGF-I levels within normal range. Bone age was advanced by about 2 years, but bone mineral density (BMD) was appropriate for age and gender. There was possible abnormal function of the hypothalamic-pituitary-thyroid axis, and there was also an overall reduction in T cell number and function. There was no evidence of impairment in basal or total energy expenditure, and body temperature was normal, unlike the ob/ob mice, whose oxygen consumption, energy expenditure and body temperature were low.[54] Thus, leptin may be less central to the regulation of energy expenditure in humans than in mice. Another difference in humans with either leptin or leptin receptor mutations was the consistently normal glucocorticoid concentrations, in contrast to the marked excess in ob/ob mice. The human cases with leptin deficiency had hypogonadotropic hypogonadism, with delayed puberty or primary amenorrhoea, yet had normal responses to short human chorionic gonadotropin stimulation test (test of gonadal function) and gonadotropin releasing hormone stimulation test (test of pituitary function), which were suggestive of a hypothalamic defect. There were interesting reports of gradual amelioration of the phenotype in the leptin deficient adults over the years, including late sexual development with an improvement in thyroid and immune function. It was postulated that other processes could have compensated for the regulatory function of leptin.[60]

Three leptin deficiency children homozygous for the Δ133G mutation received up to 50 months of recombinant human methionyl leptin (R-metHuLeptin) replacement, administered as subcutaneous injections once daily, in escalating doses if weight gain was documented over 2 successive 2-month periods, to achieve 10%, 20%, 50%, 100% and 150% of predicted leptin concentration based on height and weight.[55] There was dramatic weight loss that started within 2 weeks of initiation, and sustained through the trial period. Refractory periods of weight gain did occur but were overcome with increases in leptin doses. Fat mass represented 98% of the weight loss, with reciprocal increase in lean mass, and there was reduced food intake up to 84% during ad libitum meal tests. It was so successful that a morbidly obese boy weighing 42 kg at 3 years of age achieved normal weight after 2 years of therapy, weighing 32 kg (75th percentile on local weight chart) after 48 months of treatment. There was no discernible change in basal and total energy expenditure. An 11-year-old pre-pubertal girl with congenital leptin deficiency who received

leptin replacement began to manifest pubertal progress subsequently, with gradual increase in pulsatility of gonadotropins, and achieved regular menstrual cycles by 12.1 years. The other 2 younger children (<6 years) remained appropriately pre-pubertal during leptin replacement. Congenital leptin deficency caused hypogonadism and pubertal failure, but leptin replacement permitted puberty to progress appropriately in the 11-year-old girl, yet did not cause precocious puberty in the other 2 younger children. The authors proposed that leptin may act as a permissive metabolic gate which allowed progression of appropriately timed pubertal development. These reports of human leptin deficiency and the effects of leptin replacement provided the first evidence of the importance of leptin in energy regulation as well as endocrine functions in humans.

Family studies showed that leptin deficiency was inherited in an autosomal recessive fashion. However, though heterozygotes for the mutant *Lep* gene were not morbidly obese, 76% of them were obese with BMI of more than 30 kg/m^2. The blood leptin levels of these heterozygotes were lower than matched controls, with poor correlation between body fat mass and leptin levels, and their body fat percentage exceeded the predicted body fat percentage.[61] This observation demonstrated that haploinsufficiency of 1 *Lep* gene may result in increased adiposity, lower leptin and higher likelihood of obesity, but not to the extent of an intermediate phenotype typical of an autosomal co-dominant condition. The study of this group of individuals who are partially deficient in leptin also showed that differences in circulating leptin levels, within the range found in normal humans, can directly influence adiposity.

Leptin Receptor Deficiency

The leptin receptor is a member of the cytokine family of receptors with several splice variants. The long leptin receptor isoform is considered the principal signalling isoform. The intracellular signalling system for leptin appears to involve activation of the JAK-STAT system, with Stat-3 being the phosphorylated intermediate. The human *LEPR* gene has been mapped to chromosome 1p31. Leptin receptors are distributed widely, including brain and many peripheral tissues (lungs, kidneys, muscle and adipose tissue), suggesting that this peptide may provide a wide range of tissues with information about fat stores. If obesity in humans were due to leptin receptor mutations, then one would expect a much higher leptin concentration than predicted, based on fat mass, but this is not the case.[62] Considine et al[63] examined expression of the *LEPR* gene in the hypothalamic tissue from 7 lean and 8 obese humans obtained shortly after autopsy. Using RT-PCR, there was no difference in the amount of *LEPR* mRNA between the lean and obese subjects. A Gln23Arg polymorphism due to A-to-G substitution at nucleotide 668 of the *LEPR* cDNA was detected, where 11 subjects were heterozygous and 3 were homozygous. The occurrence of the polymorphic allele(s) did not correlate with the BMI in the patients studied. The results suggested that leptin resistance observed in obese humans is unlikely to be due to a defect in the leptin receptor. Gotoda et al[64] determined the entire coding sequence of the human leptin receptor cDNA from peripheral blood lymphocytes of 22 morbidly obese patients. Five common DNA sequence variants were found to be distributed throughout the coding sequence at codons 109, 223, 343, 656, and 1019, with one rare silent mutation at codon 986, as well as a novel alternatively spliced form of transcript. None of the 5 common variants, including 3 that predict amino acid changes, were null mutations causing morbid obesity, because homozygotes for the variant sequences were also found in lean subjects. Furthermore, the frequency of each variant allele and the distribution of genotypes and haplotypes were similar in 190 obese and 132 lean white British males selected from a population-based epidemiologic survey. The results suggested that these are polymorphisms, and that mutations in the leptin receptor gene are not a common cause of human obesity.

The diabetes (*db/db*) mouse had abnormal splicing of the long (hypothalamic) leptin receptors,[65] and had features similar to that of the *ob/ob* (leptin deficient) mouse. There was early onset morbid obesity with hyperphagia and reduced energy expenditure, infertility secondary to hypogonadotropic hypogonadism, diabetes with dyslipidaemia, hypercortisolism and decreased growth hormone production with stunted linear growth.[66] The first human *LEPR* mutation was discovered in a consanguineous family of Kabylian origin (northern Algeria) where 3 siblings had morbid obesity since early childhood.[67] This *LEPR* gene mutation resulted from G to A substitution at the +1 position of intron 16 (one base after exon 16), which led to exon skipping and loss of transmembrane and cytoplasmic domains. The truncated protein might be secreted as leptin bind protein (like the short isoform) which trapped serum leptin in bound form and prolonged its half-life, contributing to the very high leptin levels observed. The homozygous mutation caused severe obesity and pituitary dysfunction. The affected sisters had normal birth weight but rapidly gained weight in the first few months of life. Manifestations included bizarre eating behaviour, fighting for food, impulsivity and stubbornness, labile emotions and social dysfunction, reminiscent of the Prader-Willi Syndrome. However, they were not mentally retarded, and had resting metabolic rates similar to that predicted, with normal core temperature. Growth hormone and thyrotropin levels were low, with the presence of hypogonadotropic hypogonadism. These findings supported leptin and its receptor as important physiological regulators of several endocrine functions.

Farooqi et al[68] screened the *LEPR* genes of 300 subjects with severe early onset obesity and hyperphagia, inclusive of 90 probands from cansanguineous families, and reported 7 homozygotes and 1 compound heterozygote for nonsense or missense *LEPR* mutations which resulted in impaired receptor signalling. Affected subjects also had delayed puberty due to hypogonadotropic hypogonadism. Unexpectedly, serum leptin levels were within the range predicted by the elevated fat mass in these subjects. Thus, serum leptin levels cannot be used as a marker for leptin receptor deficiency. The clinical features of leptin receptor deficient subjects were less severe than those with congenital leptin deficiency.

The Role of Leptin and Leptin Receptor Deficiencies in Common Obesity

Majority (>90%) of people with common obesity were hyperleptinemic, and do not have low leptin levels or mutations of the leptin gene.[69,70] On the contrary, there is strong positive correlation between serum leptin concentrations and the percentage body fat, BMI, and basal insulin concentrations,[71,72] and leptin levels are reduced in obese individuals who lose weight.[43] There is thus a hypothesis that these individuals developed resistance to the action of leptin, so that the increase in adipose tissue mass is maintained. As the vast majority of obese individuals also do not have defective leptin receptors and therefore not contributory to the hyperleptinemia observed,[63,64] the relative insensitivity of the hypothalamic satiety centre to leptin action may be due to abnormal carriage of leptin across the blood brain barrier,[73,74] or more intriguing, defective mediators in the pathway distal to the leptin receptor. This hypothesis is obviously shared by many, given the myriad of research in quest of genetic defects downstream to the leptin receptors.

Pro-opiomelanocortin Deficiency

The pro-opiomelanocortin (*POMC*) gene is expressed in neurons originating in the arcuate nucleus of the hypothalamus and in the nucleus tractus solitarius (NTS) of the caudal medulla, as well as in the corticotrophs of the anterior pituitary and in the skin and lymphoid system.[75] The human *POMC* gene is located on chromosome 2p23.3 and spans 7665 kb. It comprises of 3 exons, and though all 3 exons are transcribed, only part of the mRNA is translated. Exon 1 only contains untranslated sequences, part of exon 2 codes for signalling peptide and the first few amino acid residues of the N-terminal peptide (NT), and exon 3 codes for most of the translated mRNA, namely the C-terminal of NT, joining peptide (JP), adrenocorticotropic hormone (ACTH) and β-lipotropic hormone (β-LPH). The structure of POMC and its derivatives is illustrated in figure 1. The signalling peptide is necessary for the translocation of the nascent protein through the membrane of the rough endoplasmic reticulcum (RER), after which it is rapidly leaved. The POMC protein (accession no. NP_000930) is then engaged in the secretory pathway and ready for processing by the prohormone convertases 1 and 2 (PC1 and PC2). POMC undergoes extensive and tissue-specific posttranslational processing by proprotein convertases (PCs) to yield a range of biologically active peptides.[21,76,77]

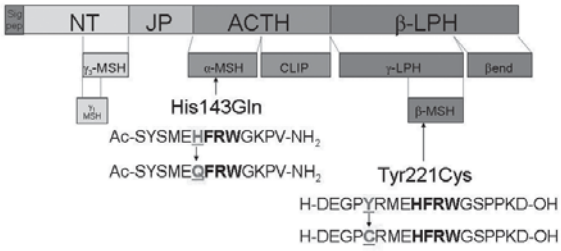

Fig. 1. Structure of POMC and location of 2 missense mutations – His143Gln and Tyr221Cys.

Pituitary corticotrophs express prohormone convertase 1 (PC1), but not PC2, which cleaves 4 specific dibasic Lys-Arg cleavage sites and results in the production of NT, JP, ACTH and β-LPH. In contrast, the expression of PC2 (in addition to PC1) within the hypothalamus results in additional processing and cleavage of all dibasic Lys-Arg and Arg-Arg sites of the precursor polypeptide, leading to the production of α-, β- and γ-MSH (the melanocortins) but not ACTH. The melanocortins mediate their effects through a family of 5 related G protein-coupled receptors, 2 of which, MC3R and MC4R, are highly expressed within the central nervous system.[78] Genetic defects impairing the synthesis and processing of POMC and in the receptors for its constituent melanocortin peptides have clearly established that the melanocortin system plays a critical role in energy homeostasis in rodents and humans.[79]

POMC mutations have helped to shed light on the role of the melanocortin system in energy homeostasis.[80] Mice[81,82] and humans[83-85] genetically lacking POMC-derived peptides are severely obese from early age, and were associated with severe glucocorticoid deficiency due to concomitant absence of ACTH, and may present with neonatal hypoglycaemia, cholestasis and liver failure. These children had repeatedly elevated TSH and borderline low free T4, and normal TRH stimulation test response, suggestive of subclinical hypothyroidism of hypothalamic origin, where elevated biologically inactive TSH has been observed. This is in keeping with recent findings that the melanocortin system exerts influence on TRH expressing neurons of the PVN, and also the possible role of POMC-derived peptides on the maturation of the hypothalamic-pituitary-thyroid axis. The first report of human cases of POMC deficiency also

described fair skin and red hair due to lack of MSH stimulation of melanocortin 1 receptors of melanocytes, resulting in reduced eumelanin and increased red pheomelanin. However, a Turkish child homozygous for POMC null mutation was described to have severe obesity and ACTH deficiency, but had dark hair.[85] This suggested that skin pigmentation is dependent on the genetic background of the individual, and the phenotype was modified by other genetic factors. This is reinforced by the observation where *Pomc*$^{-/-}$ mice generated on a 129 (*A*^{w}/*A*^{w}) genetic background back-crossed and bred onto the C57BL/6 (*a*/*a*) genetic background had a black coat color indistinguishable from that of the wild-type C57BL/6 mice.[86]

Importantly, while heterozygous null POMC mutations in mice[82] and humans[84] do not necessarily result in a severe or intermediate phenotype, and do not exhibit cortisol deficiency, these heterozygous mutations predispose to obesity. The heterozygous *Pomc*$^{+/-}$ mice had similar phenotype to wildtype mice on standard diet,[81,82] but developed obesity when put on a high-fat diet,[82] exhibiting an intermediate obese phenotype between wild-type and *Pomc*$^{-/-}$ mice. Heterozygous parents of obese children homozygous for *POMC* null mutations had high normal to high BMI,[84] and other family members heterozygous for *POMC* null allele were more overweight than those with wildtype alleles.[85] Heterozygous partially inactivating *POMC* mutations Arg236Gly were also found more frequently in obese children.[87] Thus it can be inferred that a single functional copy of the *POMC* gene may not be sufficient for maintaining normal energy homeostasis under certain conditions such as in an "obesogenic" environment, and haploinsufficiency can interact with dietary factors to increase body weight. Given that the region of human chromosome 2 containing the POMC gene has been identified by several studies as a susceptibility locus for common human obesity,[88-90] it is very possible that genetic variants within the melanocortin peptides or the promoter regions might predispose to common obesity.

Until recently, there is still uncertainty regarding the relative importance of particular POMC-derived melanocortin ligands in the control of energy balance. Attention has been principally focused on α-MSH as the probable endogenous ligand in rodents,[91] and presumed to be the de facto ligand in the human melanocortin system as well. This is largely because rodents (the most common experimental species) lack the proximal dibasic site that is necessary for the proteolytic cleavage event that produces β-MSH in humans (mouse accession number P01193, rat accession number AAA41903). Thus the role for β-MSH in the control of energy balance has been largely overlooked.

Recently, we[92] and others[93] independently reported a heterozygous missense mutation Tyr221Cys in the region of *POMC* encoding β-MSH (Fig. 1), which highlighted the importance of this molecule in human energy homeostasis. The tyrosine residue at position 221 is highly conserved, and substitution with cysteine impaired the variant peptide's ability to bind to and activate signalling from the MC4R. Out of 538 patients with severe early-onset obesity screened, 5 unrelated probands (0.9%) were heterozygous for this missense mutation Tyr221Cys, and this frequency was significantly increased (P <0.001) compared to that of the general UK Caucasian population (0.08%). Moreover, the variant cosegregated with obesity/overweight in affected family members. Obese children carrying the Tyr221Cys variant were hyperphagic and showed increased linear growth, both of which are features of MC4R deficiency. These studies support β-MSH as an important ligand of MC4R *in vivo*, and its role in the control of human energy homeostasis.

Interestingly, we also found a missense mutation His143Gln in the core binding domain of α-MSH in the same study (Fig. 1),[92] also resulting in reduced binding and signalling through MC4R, but this mutation did not co-segregate with obesity in the family, providing more compelling evidence for a specific role of β-MSH than α-MSH in human weight regulation. Thus analogues of β-MSH may be a more realistic therapeutic option for the future. This study is an example of how human genetic studies can provide insights into the human physiology which cannot be obtained through the study of rodent models.[15]

Melanocortin 4 Receptor Deficiency

The human melanocortin 4 receptor (MC4R) is a 332 amino acid protein encoded by a single exon localised on chromosome 18q22.[94,95] The MC4R is a 7 transmembrane G-protein coupled receptor highly expressed in hypothalamic nuclei which regulate energy homeostasis.[96,97] MC4R is modulated by the endogenous agonist α-MSH and antagonist agouti-related protein, and signals through activation of adenylate cyclase.[22] Mice with inactivation of both copies of the *MC4R* genes produced an obesity syndrome with hyperphagia associated with a pathological lack of satiety, hyperinsulinaemia with hyperglycaemia and increased linear growth, but unlike leptin deficient mice, had normal reproductive function.[23] Heterozygotes had an intermediate weight between the homozygotes and wild-type mice, and females were more affected than males. *MC4R* knockout mice continue to increase feeding on a high fat diet, but do not increase thermogenesis. Interestingly, the MC4R knockout mouse exhibits normal feeding and returns to previous weight in response to food restriction. Thus, MC4R does not appear to be required for normal feeding or metabolic response to fasting. However,

MC4R is required for normal response to a high fat diet by maintaining satiety, and increasing thermogenesis and metabolic rate.

MC4R deficiency resulting from the disruption of 1 or both *MC4R* alleles represents the commonest monogenic form of human obesity.[24,26] Human MC4R deficiency was reported to affect 4% and 5.8% of severely obese French and British populations respectively.[24,26] However, studies elsewhere reported low incidence of *MC4R* mutations in their respective obese populations.[98-106] Obese individuals with MC4R deficiency displayed a common, non-syndromic form of obesity and were not characterised by any peculiar phenotypic abnormalities. The subjects with *MC4R* mutations were obese from an early age, but with the increase in both fat and lean masses, were excessively hungry from 6 to 8 months of age with persistent food-seeking behaviour, and become distressed if food was not provided. They had higher food intake when compared to obese controls when assessed with ad-libitum meals. There was increased growth velocity in childhood, where those with *MC4R* mutations were taller than matched obese controls, and the bone age exceeded the chronological age by 1 to 4.9 years. Pubertal onset and secondary sexual characteristics were normal. They also had significantly higher insulin levels compared to matched controls, but the majority were not diabetic. The proportions of type 2 diabetic or glucose intolerant subjects, triglyceride levels and leptin levels were not statistically different between both mutated and non-mutated obese groups. The affected subjects did not have any developmental, intellectual or behavioural problems, and there were no dysmorphic features.

In vitro function of mutant MC4-receptors correlated with the severity of the clinical phenotype, indicating that weight regulation is sensitive to the amount of functional MC4 receptors. Subjects with inactivating (null) MC4R mutations were heavier, taller and more hyperphagic than those with partially active *MC4R* mutations.[24]

Most family studies revealed autosomal co-dominant pattern of inheritance. Homozygotes for *MC4R* mutations exhibited a more severe phenotype than heterozygotes, where they were heavier and taller. Transmission of the mutations in the affected families indicated variable penetrance and expressivity that is not related to the functional severity of the mutations *in vitro*. Family studies of heterozygous probands demonstrated co-segregation of mutation with early onset obesity with 100% penetrance, while that of homozygous probands demonstrated early onset obesity only in 68% of heterozygous family members. There is variable age of onset of obesity as well as its severity, even for the same mutation within the same family. The reason for this variability in penetrance and expressivity is yet to be fully elucidated.

A recent study of 227 obese local children and adolescents screened for *MC4R* gene mutations by direct sequencing identified 3 mutations in 3 subjects: 4 bp deletion from nucleotides 631-634 (c.631-634delCTCT), Tyr157Ser (c.470A>C), and 1 bp deletion at nucleotide 976 (c.976delT) (1.3% of study subjects).[106] *In vitro* transient transfection studies supported the pathogenic role of both novel mutations Tyr157Ser and c.976delT, where the signalling activities of the mutant receptors were impaired. These *MC4R* mutations were associated with early onset severe obesity in heterozygosity; the proband with the mutation Tyr157Ser was homozygous for this mutation and had morbid obesity, while the other members of the consanguineous family were heterozygotes and their obese phenotype was less severe. Thus the *MC4R* mutations resulted in an autosomal co-dominant form of obesity, with variable expressivity evident in the family studies. Interestingly, our family studies revealed that adults heterozygous for the mutations were less obese compared to the heterozygote children, similar to observations made in other bigger studies. We hypothesise that this may be due to amelioration of phenotype severity with age, genetic anticipation, or difference in exposure to modifying factors at critical stages of childhood such as the environment.

Melanocortin 3 Receptor deficiency

MC3R is a 7 transmembrane G-protein coupled receptor[107] expressed in hypothalamic nuclei known to regulate energy homeostasis. It exhibits a more restricted distribution than MC4R in the central nervous system,[108] and has a dominant role in the inhibition of energy storage.[27,28] *Mc3r*$^{-/-}$ mice homozygous for knockout mutations of *MC3R* gene had increased body fat[27,28] not caused by increased food intake but by increased feed efficiency. The *Mc3r*$^{-/-}$ mice were hypophagic with hyperleptinemia compared to wildtype littermates.[27] These mice were unusually susceptible to high fat diet-induced obesity, and were relatively inactive, which partly explained the obesity. The mice showed no perturbations in metabolic rate, thyroid hormone levels, respiratory exchange ratio or body temperature. Male mice developed mild hyperinsulinaemia. Mice lacking both MC3R and MC4R have exacerbated obesity, which supports the notion that both are important and non-redundant.[27]

MC4R mutations causing human obesity are well described,[24,26] but the search for human *MC3R* mutations has not been very fruitful.[29,109-112] Two common single nucleotide polymorphisms in almost complete linkage dysequilibirum, Thr6Lys and Val81Ile within the coding region of *MC3R*, were associated with higher body fat and leptin levels in obese children,[29,30] and the causative role of the 6Lys/81Ile variants is supported by the presence of an

additive effect, where heterozygotes had an intermediate phenotype compared to homozygotes,[30] as well as impaired signalling activity of the variant MC3 receptor when stimulated by α-MSH *in vitro*. We recently identified 3 novel heterozygous missense mutations (Ile183Asn, Ala70Thr, and Met134Ile) in 3 unrelated subjects, which were not found in 188 controls.[30,31] *In vitro* functional studies of the resultant mutant receptors revealed impaired signalling activity but normal ligand binding and cell surface expression. The heterozygotes demonstrated higher leptin levels and adiposity and less hunger, compared to obese controls, reminiscent of the *MC3R* knockout mice. Family studies did not show good co-segregation of obesity with genotype in the adults, but of great interest is the co-segregation of these mutations with childhood or early onset obesity. A recent Italian study also found 3 novel *MC3R* mutations (Ala293Thr, Ile335Ser and X361Ser) in 290 obese subjects.[113] The mutations segregated with obesity in the members of the families studied. However, only the Ile335Ser mutant receptor demonstrated a loss of function in *in vitro* expression studies.

Common obesity is a polygenic trait resulting from the interaction of multiple genetic loci with the environment. Sequence variants in a large set of genes implicated in energy regulation could predispose an individual to excessive weight gain in a given environment. While *MC3R* mutations are unlikely to result in an autosomal dominant form of monogenic obesity given the lack of strong co-segregation in family studies, the studies so far provided evidence that *MC3R* can be 1 of the predisposing genes which contributes to increased adiposity, and the wide variation in the adiposity of the individuals with common and rare variants may be due to other modifying genetic and environmental factors. Just like the mice and humans heterozygous for *POMC* inactivating mutations,[82,84,85,87,92] partially inactivating genetic variants of *MC3R* may likewise exert a significant effect on the phenotype even in the heterozygous state, in the "obesogenic" environment. This notion was supported by the linkage of a locus encoding *MC3R* on human chromosome 20q13.2 to the regulation of BMI, subcutaneous fat mass and fasting insulin.[114] *MC3R* mutations may not result in autosomal dominant forms of obesity, but may contribute as a predisposing factor to childhood obesity, and exert an effect on the human phenotype.

Downstream of the Melanocortin Receptors

Recent studies continued to shed light on the mediators and pathways distal to the melanocortin receptors. The brain-derived neurotrophic factor (BDNF) is a neurotrophin (neural growth factor) which exerts its effect via the tyrosine kinase B receptor (TrkB), regulates neuronal development and modulates synaptic plasticity, contributing to memory and learning. However, BDNF deficient mice (heterozygotes as well as conditional deletion in post-natal brain) demonstrated hyperactivity, hyperhagia and obesity,[33,115] while TrkB hypomorph rats (25% of normal activity) were obese and exhibited a complex neurobehavioural phenotype,[33] implicating BDNF and TrkB in the regulation of mammalian eating behaviour and energy homeostasis. BDNF expression in the hypothalamic VMH, an integral part of the feeding centre, was regulated by nutritional status. Central infusion of BDNF suppressed hyperphagia and weight gain in MC4R deficient mice,[33] which strongly support BDNF and TrkB as downstream mediators of the melanocortin receptors.

The relevance of BDNF/TrkB in human weight regulation was supported by a case report of an 8-year-old Caucasian boy with a *de novo* heterozygous TrkB mutation Tyr772Cys affecting the highly conserved tyrosine residue located in the activation loop of catalytic domain, which disrupted receptor signalling *in vitro*, abolishing the basal phosphorylation activity.[34] The boy had severe obesity (BMI z-score +4) and hyperphagia, and had developmental delay with impaired learning and memory. There were also absence seizures, impaired immediate/short-term memory, repetitive speech, labile mood and impaired nociception. Another supporting evidence is the report of an 8-year-old girl with obesity, hyperphagia, impaired cognitive function and hyperactivity, who had a *de novo* inversion of a region in chromosome 11p, 46,XX,inv(11)(p13p15.3), encompassing the BDNF locus.[116]

Another recent relevant discovery is the neuropeptide nesfatin-1, which is derived from the previously described protein nucleobindin-2 (NUCB2).[35] This protein is expressed in the hypothalamic nuclei known to regulate appetite, and fasting suppressed NUCB2 expression in the hypothalamic PVN, which receives inputs from the POMC neurons in the arcuate nucleus, where MSH stimulates MC3R/MC4R in the PVN to reduce feeding. Injection of NUCB2 directly into the brains of rats promotes anorexia, while injection of neutralising antibodies to endogenous NUCB2 in rat brain stimulates feeding. Central injection of α-MSH elevates NUCB2 gene expression in the PVN, the satiety effect of nesfatin-1 is blocked by MC3R/MC4R antagonist, and nesfatin-1 does not directly activate melanocortin receptor dependent signalling in cultured cells. A hypothesis to reconcile these findings is that nesfatin could be expressed in melanocortin responsive PVN neurons, and nesfatin-1 could act locally to potentiate the action of melanocortins on MC3R/MC4R signalling.[117] An alternative is that nesfatin-1 requires coordinated output by melanocortin dependent signals to exert its effect.

The Quest to Complete the Unfinished Jigsaw Puzzle

The discovery of leptin and its receptor heralded a new era in obesity research, as it inspired an unprecedented surge of research activities leading to an explosion of new knowledge about the intricate molecular mechanism of weight regulation. The subsequent human and murine genetics studies performed in the quest to elucidate the genes governing obesity have improved our understanding of weight regulation. Whether this knowledge can be effectively applied to clinical practice remains to be seen. What is clear from these genetic studies is that they have challenged our traditional views of the pathogenesis of obesity, and changed our view of obesity as just a psychological disorder arising from sheer gluttony and poor discipline. The multitude of evidence now supports a physiological basis for weight dysregulation, where individuals may be predisposed to varying degree of obesity due to their genetic make-up, especially when exposed to the "right" environmental conditions. Some individuals may be simply predisposed to obesity at multiple genetic levels, and this may also make it very difficult for them to lose weight. Current research reinforces the view that obesity is caused by both genetic and environment determinants.

Although much attention has been focused on leptin-melanocortin system, there are a large number of other unidentified hormones, receptors and enzymes that are involved in the complex regulating mechanism governing energy homeostasis, waiting to be discovered. While the weight regulation mechanism is similar between rodents and man, differences may exist, and thus mere experimentation with knockout obese mouse models may not be adequate. The search for human genetic mutations in candidate genes predicted to affect weight regulation serves a very important purpose. By showing that loss-of-function mutations in these candidate genes, which occur naturally in humans, result in or predispose to human obesity, these "experiments of nature" validate the products of these genes as critical mediators of the weight regulation mechanism in humans. The discovery of these novel gene products will also collectively help to complete this unfinished jigsaw puzzle. These critical mediators in turn will become the focuses of intense, million dollar research efforts by pharmaceutical companies to produce new drugs to combat the worldwide epidemic of obesity. Indeed, elucidation of the complete human weight regulation mechanism and the pathogenesis of obesity will facilitate the design of novel therapeutic agents as adjuncts in the treatment of obesity. By identifying these genes and their products, and understanding how they confer susceptibility and interact with other factors to lead to obesity, we can then devise more effective prevention and treatment strategies to combat this epidemic.

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