Endocrine Reviews. First published ahead of print November 22, 2006 as doi:10.1210/er.2006-0040

# Genetics of Obesity in Humans

I.Sadaf Farooqi & Stephen O'Rahilly

University Departments of Medicine and Clinical Biochemistry

Box 232, Addenbrooke's Hospital

Cambridge

CB2 2QQ

United Kingdom

Tel – 44-1223-762634

Fax – 44-1223-762657

Email: so104@medschl.cam.ac.uk

DISCLOSURE STATEMENT: S.F. has nothing to disclose. S.R. consults for Merck and Cambridge Antibody Technology, and has equity interest in Prosidion.

**ABSTRACT**

Considerable attention has focused on deciphering the hypothalamic pathways that mediate the behavioural and metabolic effects of leptin. We and others have identified several single gene defects that disrupt the molecules in the leptin-melanocortin pathway causing severe obesity in humans. In this review, we consider these human monogenic obesity syndromes and discuss how far the characterization of these patients has informed our understanding of the physiological role of leptin and the melanocortins in the regulation of human body weight and neuroendocrine function.

## Introduction

Changes in diet and physical activity have driven the rise in the prevalence of adult and childhood obesity over a relatively short time interval (1, 2). However, it is important to recognise the significant contribution of hereditary influences on weight especially at a time when we are beginning to develop an understanding of the molecules involved in the control of energy homeostasis and how genetic variation within them can influence human obesity (3, 4). The genetic contribution to body weight has been established through family studies, investigating parent-offspring relationships, the study of twins and adopted children (5, 6). These studies consistently report heritability estimates of 40–70% (3). Thus, weight is a highly heritable trait, with a heritability that is only slightly less than for that for height (7, 8). As is the case for height, where nutritional changes in the last fifty years have contributed to substantial increases in mean final height in many populations, environmentally-driven changes in body weight in the population occur against a background of susceptibility to weight gain that is determined by genetic factors. Thus, genetic approaches can be applied to understand both the molecular and physiological mechanisms involved in human obesity.

Progress to date has mostly been in more severe familial forms of obesity presenting in childhood. Although these only represent a small fraction of those with obesity (albeit a group with disproportionate physical and psychosocial morbidity and health costs) the implications of these discoveries have been profound (9). We have explored the genetic basis of severe childhood obesity

where we considered that major and more highly penetrant genetic effects were likely to be found. In 1997, we established the Genetics of Obesity Study (GOOS) to recruit patients with severe obesity (BMI sds>3) of early-onset (< 10 years). We were particularly interested in children with a strong family history of obesity and those from consanguinous families. Our intention was to use a candidate gene approach to look for mutations in genes thought to play a role in the regulation of body weight based on evidence primarily from rodent models at the time. With the help of colleagues throughout the world, we have to date recruited over 2200 patients to the GOOS cohort. In the past nine years, several human disorders of energy balance that arise from genetic defects have been described by ourselves and others (9). All of these are in molecules identical or similar to those known to cause obesity in genetic and experimental syndromes of obesity in rodents and all have been identified using a candidate gene approach. These mutations all result in severe obesity in childhood without developmental pleiotropic features.

**MUTATIONS IN GENES ENCODING LEPTIN AND THE LEPTIN RECEPTOR**

In 1997, we reported two severely obese cousins from a highly consanguineous family of Pakistani origin (10). Both children had undetectable levels of serum leptin and were found to be homozygous for a frameshift mutation in the *LEP* gene (ΔG133), which resulted in a truncated protein that was not secreted. We have since identified five further affected individuals from four other families (11,

12) (and unpublished observations) who are also homozygous for the same mutation in the leptin gene. All the families are of Pakistani origin but not known to be related over five generations. A large Turkish family in which three adults carry a homozygous missense mutation (C-->T substitution at codon 105 resulting in Arg-->Trp) in the LEP gene have also been described (13).

To date, only one mutation in the leptin receptor gene has been published (14). The mutation was found in homozygous form in three severely obese adult siblings from a consanguineous family of Algerian origin. This mutation results in abnormal splicing of leptin receptor transcripts and generates a mutant leptin receptor that lacks both transmembrane and intracellular domains. The mutant receptor circulates at high concentrations bound to leptin, resulting in very elevated serum leptin concentrations (15).

## CLINICAL PHENOTYPES ASSOCIATED WITH LEPTIN AND LEPTIN RECEPTOR DEFICIENCY

The clinical phenotypes associated with congenital leptin and leptin receptor deficiencies are similar. Leptin and Leptin receptor deficient subjects are of normal birthweight but exhibit rapid weight gain in the first few months of life resulting in severe obesity (11). Body composition measurements show that leptin deficiency is characterised by the preferential deposition of fat mass giving a distinct clinical appearance with excessive amounts of subcutaneous fat over

the trunk and limbs (Figure 1) (11). All patients were hyperinsulinemic consistent with the severity of obesity and some adults have developed type 2 diabetes in the 3rd - 4th decade (11).

All subjects in these families are characterised by intense hyperphagia with food seeking behaviour and aggressive behaviour when food is denied (11) and energy intake at an adlibitum meal is markedly elevated.

In leptin deficient humans we found no detectable changes in resting metabolic rate using indirect calorimetry or total energy expenditure using chamber calorimetry (16). Free-living energy expenditure measured using the doubly labelled water method was not significantly different after adjustment for body composition. However, Ozata et al reported abnormalities of sympathetic nerve function in leptin deficient adults consistent with defects in the efferent sympathetic limb of thermogenesis (17).

Leptin and leptin receptor deficiency are associated with hypothalamic hypothyroidism and hypogonadotropic hypogonadism. Evidence from rodents suggests that leptin is necessary for the normal biosynthesis and secretion of TRH (18). Complete leptin deficiency is associated with a moderate degree of hypothalamic hypothyroidism characterised by low free thyroxine and high serum thyroid stimulating hormone (TSH) which is bioinactive. In leptin deficient children, plasma free thyroxine concentrations are within the normal range, but

four children had significantly elevated TSH levels (11) and the pulsatility of TSH secretion, studied in a single adult with congenital leptin deficiency, was characterized by a markedly disorganized secretory pattern (19). Two subjects homozygous for a nonsense mutation in the leptin receptor were diagnosed with hypothyroidism in childhood and thyroid hormone replacement therapy commenced (14).

Normal pubertal development does not occur in adults with leptin or leptin receptor deficiency, with biochemical evidence of hypogonadotropic hypogonadism (13). However, there is some evidence for the delayed but spontaneous onset of menses in leptin and leptin receptor deficient adults (17).

Leptin deficient children have normal linear growth in childhood and normal IGF-1 levels. However, because of the absence of a pubertal growth spurt, the final height of adult subjects is reduced. In the one previously reported leptin receptor deficient family, short stature and abnormal serum levels of growth hormone (GH) and insulin-like growth factor (IGF) binding protein 3 were noted in childhood (14). However, assessment of the GH/IGF axis is difficult in obese children and adults as obesity itself is associated with abnormalities in basal and dynamic tests of the GH/IGF axis.

We demonstrated that children with leptin deficiency had profound abnormalities of T cell number and function (11), consistent with high rates of childhood

infection and a high reported rate of childhood mortality from infection in obese Turkish subjects (17).

**RESPONSE TO LEPTIN ADMINISTRATION IN LEPTIN DEFICIENCY**

We have reported the dramatic and beneficial effects of daily subcutaneous injections of recombinant human leptin leading to a reduction in body weight and fat mass in three congenitally leptin deficient children (11, 16). We have recently commenced therapy in two other children and seen comparably beneficial results (personal observations) (Figure 1). All children showed a response to initial leptin doses that were designed to produce plasma leptin levels at only 10% of those predicted by height and weight (i.e. approximately 0.01mg/kg of lean body mass). Leptin therapy has also been successfully used in the three Turkish leptin deficient adults (20).

The major effect of leptin was on appetite with normalisation of hyperphagia. Leptin therapy reduced energy intake during an 18MJ *adlibitum* test meal by up to 84% (5MJ ingested pre-treatment vs 0.8MJ post-treatment in the child with the greatest response)(11). Leptin treatment was associated with reduced hunger scores with no change in satiety in adults with leptin deficiency (20). We were unable to demonstrate a major effect of leptin on basal metabolic rate or free-living energy expenditure, but, as weight loss by other means is associated with a decrease in (BMR) basal metabolic rate, the fact that energy expenditure did not fall in our leptin deficient subjects is notable.

The administration of leptin permitted progression of appropriately timed pubertal development in the single child of appropriate age and did not cause the early onset of puberty in the younger children (11). In adults with leptin deficiency, leptin induced the development of secondary sexual charateristics and pulsatile gonadotrophin secretion.

In the three previously reported children there were small, but sustained, increases in free $T_4$, free $T_3$, and TSH that occurred within 1 month of leptin therapy. These observations are fully consistent with an effect of leptin at the hypothalamic level. A fourth patient had substantial elevation of TSH before treatment, such that thyroxine therapy was commenced (12). However, replacement therapy was stopped when thyroid function tests normalised after leptin treatment.

**PARTIAL LEPTIN DEFICIENY IN HETEROZYGOTE CARRIERS**

The major question with respect to the potential therapeutic use of leptin in more common forms of obesity relates to the shape of the leptin dose response curve. We have clearly shown that at the lower end of plasma leptin levels, raising leptin levels from undetectable to detectable has profound effects on appetite and weight. Heymsfield et al administered supraphysiological doses (0.1–0.3 mg/kg body weight) of leptin to obese subjects for 28 weeks (21). On average, some

subjects lost weight, but the extent of weight loss and the variability between subjects has led many to conclude that the leptin resistance of common obesity cannot be usefully overcome by leptin supplementation, at least when administered peripherally. However, it is of interest that there was a significant effect on weight in some subjects with low serum leptin levels, suggesting that leptin can continue to have a dose/response effect on energy homeostasis across a wide serum concentration range. To test this hypothesis, we studied the heterozygous relatives of our leptin deficient subjects (22). Serum leptin levels in the heterozygous subjects were found to be significantly lower than expected for percent body fat and they had a higher prevalence of obesity than seen in a control population of similar age, sex and ethnicity (22). Additionally, % body fat was higher than predicted from their height and weight in the heterozygous subjects compared to control subjects of the same ethnicity (22). These findings closely parallel those in heterozygous ob/- and db/- mice (23). These data provide further support for the possibility that leptin can produce a graded response in terms of body composition across a broad range of plasma concentrations.

**COMPLETE POMC DEFICIENCY**

In 1998, Krude *et al.* provided the first description of humans congenitally lacking *POMC* gene products (24). One proband was a compound heterozygote for two nonsense mutations and a second patient was homozygous for a mutation in the 5'-untranslated region that introduced an additional out-of-frame start site, thus

interfering with *POMC* translational initiation (Figure 2). Subsequently, Krude et al have reported three additional unrelated European children with congenital POMC deficiency who were either homozygous or compound heterozygous for *POMC* mutations (Figure 2). We have recently identified a sixth patient with complete POMC deficiency, being homozygous for a complete loss of function mutation which results in the loss of all POMC-derived peptides (25).

These patients all presented in early life with features of hypocortisolaemia secondary to ACTH deficiency, leading to hypoglycemia, prolonged jaundice, susceptibility to the effects of infection and in one case, neonatal death. The children responded well to physiological replacement with glucocorticoids but all subsequently developed marked obesity in association with hyperphagia. In this disorder, in both humans and murine models, obesity occurs despite profound glucocorticoid deficiency, a condition normally associated with severe weight loss. In our patient, weight gain was documented prior to the commencement of hydrocortisone replacement therapy. Notably, in *pomc* null mice, restoration of relatively normal glucocorticoid levels results in a marked worsening of the obesity and insulin resistance (26), suggesting that the glucocorticoid deficiency modulates the severity of the metabolic phenotype.

Notably, all children thus far reported have pale skin and red hair, features consistent with the known role of POMC-derived peptides in the determination of the phaeomelanin to eumelanin ratio in melanocytes. Our Turkish proband is the

first reported patient with POMC deficiency who does not have red hair (25). It is likely this can be explained by his differing genetic background as the other reported patients were all white Caucasian subjects of European ancestry. The retention of dark hair in this child and his similarly affected deceased sibling indicates that the synthesis of eumelanin in humans is not absolutely dependent on the presence of melanocortin peptides. It can be assumed, that in ethnic groups that are predominantly characterized by dark hair, other genetic variants act to maintain eumelanin synthesis in the absence of POMC derived ligand, while in Northern European races, such eumelanin synthesis is more critically dependent on the presence of such ligands (27).

Thus, the cardinal features of congenital POMC deficiency are isolated ACTH deficiency, hyperphagia and severe early onset obesity. Although red hair may be an important diagnostic clue in patients of Caucasian origin, its absence in patients originating from other ethnic groups should not result in this diagnostic consideration being excluded.

**POMC HAPLOINSUFFICIENCY**

Krude *et al* have previously attempted to assess the impact of loss of one *POMC* allele in the parents and heterozygous relatives of their probands. They estimated the maximum lifetime BMI standard deviation score (BMI SDS) in adult *POMC* heterozygotes and suggested that most had a maximum lifetime BMI SDS of 1, which is at the upper end of the normal range (28). We had the

opportunity to study a large Turkish consanguineous pedigree with 12 heterozygote carriers and seven wild type subjects (25). The significantly higher prevalence of obesity/overweight in the carriers provides compelling support for the idea that loss of one copy of *POMC* is sufficient to markedly predispose to obesity.

This is particularly relevant as we and others have described a variety of heterozygous point mutations in *POMC*, including mutations in alpha- and beta-MSH, which significantly increase obesity risk but are not invariably associated with obesity.

## POMC MUTATIONS AFFECTING SPECIFIC MELANOCORTIN PEPTIDES

In order to determine whether missense/nonsense mutations within the melanocortin peptides might predispose to obesity, we screened the coding regions of the POMC gene for mutations in over 600 UK Caucasian subjects with severe early-onset obesity. We identified a number of sequence variants in *POMC* in severely obese children. Three of these missense mutations directly affect regions of the POMC gene that encode melanocortin peptides (Figure 3). R236G was identified in three patients but also two controls. We have previously shown that this mutation disrupts a di-basic cleavage site between β-MSH and β-endorphin, resulting in a β-MSH/ β-endorphin fusion protein that binds to MC4R but has reduced ability to activate the receptor (29). Its presence in both obese probands and controls reflects previous studies that show that this is not a highly

penetrant cause of inherited obesity but may increase the risk of obesity in carriers.

We identified five unrelated probands who were heterozygous for a rare missense variant in the region encoding β-MSH, Tyr221Cys (30). This frequency was significantly increased ($p < 0.001$) compared to the general UK Caucasian population and the variant co-segregated with obesity/overweight in affected family members. The overrepresentation of this mutation in obese subjects is supported by independent studies in a German population (31). Compared to wild-type β-MSH, the variant peptide was impaired in its ability to bind to and activate signaling from the MC4R (30). Obese children carrying the Tyr221Cys variant were hyperphagic and showed increased linear growth, both of which are features of MC4R deficiency. These studies support a role for β-MSH in the control of human energy homeostasis.

Interestingly, we found a missense mutation in α-MSH in a single proband, which had a major deleterious effect on its function (30). However, this variant was found in one lean family member and one lean unrelated control. While it is likely that this variant is contributing to the obesity of the proband, it is notable that our studies provide more compelling evidence for a specific role for β-MSH than α-MSH in the control of human energy balance.

It is possible that an important role for β-MSH in the control of energy balance has been overlooked as attention has been principally focused on α-MSH as the probable endogenous ligand in rodents (32). This is largely because rodents lack

the proximal di-basic site that is necessary for the proteolytic cleavage event that produces β-MSH in humans. Analogues of β-MSH have been shown to have beneficial effects in vitro and invivo in mice (33) and may offer a realistic therapeutic option in these patients.

## GENETIC VARIATION AT THE POMC LOCUS AND COMMON OBESITY

It is notable that a number of genetic linkage studies have identified chromosome 2p22 (a region encompassing the *POMC* gene) as the site of a gene or genes influencing common obesity and obesity-related traits. The strongest evidence for a quantitative trait locus (QTL) influencing obesity-related phenotypes comes from the San Antonio Family Heart Study undertaken in Mexican American extended families, with a Log Odds Ratio (LOD) score of 7.5 for serum leptin levels on chromosome 2p22 (34). Strong evidence for linkage of plasma leptin levels, one of the most robust markers of fat mass, to this region of chromosome 2 was also seen in a genome wide scan performed in French obese sibling pairs (35). Association studies of the *POMC* gene and indices of adiposity have been inconsistent (36, 37) but most have been underpowered. The extent to which these effects are the consequence of variation in or around the *POMC* locus itself has yet to be determined but the knowledge that the control of human energy balance is sensitive to *POMC* gene dose strengthens the candidacy of *POMC* as a site where variants affecting expression could influence body weight.

It is plausible that genetic variation around the *POMC* locus might confer a risk of obesity through a gene-environment interaction. We recently reported that 129 mice heterozygous for a null mutation in the *pomc* gene become significantly hyperphagic and obese on a high fat diet but not on normal chow (38). Interestingly, in a recent genome wide scan analysis in Mexican American families, suggestive evidence of linkage with saturated fat intake was found on chromosome 2p22 (39).

**MUTATIONS IN PRO-HORMONE CONVERTASE 1**

Many biologically inactive prohormones and neuropeptides are cleaved by serine endoproteases to release biologically active peptides. The prohormone convertases (PC1 and 2) are expressed in neuroendocrine tissues and act upon a range of substrates including proinsulin, proglucagon and pro-opiomelanocortin (POMC) (40). Prohormone convertase 1 (PC1) is itself synthesised as an inactive precursor, then undergoes two autocatalytic events, firstly within the endoplasmic reticulum (ER) and then within the secretory vesicles of the regulated secretory pathway (RSP) to generate a fully active 66kDa isoform that is stored in mature secretory granules.

We have previously reported an adult female with severe early-onset obesity, hypogonadotropic hypogonadism, postprandial hypoglycemia, hypocortisolemia, and evidence of impaired processing of POMC and proinsulin (41). She was found to be a compound heterozygote for *PC1* mutations: $Gly^{593}Arg$, which causes failure of maturation of the inactive propeptide form of PC1 (pro-PC1) and

its retention in the endoplasmic reticulum, and $A \rightarrow C^{+4}$ in the donor splice site of intron 5, resulting in exon skipping, a frameshift, and a premature stop codon in the catalytic domain (42). We have described the second case of congenital PC1 deficiency, in a patient who was a compound heterozygote for two loss of function mutations: $Glu^{250}$stop, which is predicted to truncate the PC1 protein within the catalytic domain, and $Ala^{213}$del, which deletes a highly conserved alanine residue near the catalytically essential $His^{208}$ residue (43). Intriguingly, this patient suffered from severe small intestinal absorptive dysfunction as well as the characteristic severe early-onset obesity, impaired prohormone processing, and hypocortisolemia. We hypothesized that the small intestinal dysfunction seen in this patient and, to a lesser extent, in the first patient we described may be the result of a failure of maturation of propeptides within the enteroendocrine cells and nerves that express PC1 throughout the gut. The finding of elevated levels of progastrin and proglucagon provided *in vivo* evidence that prohormone processing in enteroendocrine cells was abnormal (43).

PC1 deficiency has been described in two independent mouse models with notable differences in phenotype. In one mouse model, about 40% of *PC1* null embryos die before birth, and another 40% within 6 days. The remaining pups appear normal at birth but are only 60% of the size of heterozygous or wild-type littermates with reduced growth associated with decreased levels of growth hormone (GH) mRNA and decreased circulating GH (44). They suffer from chronic mild diarrhea associated with bulky moist stools. Blood glucose levels are normal despite a severe impairment in proinsulin processing, which results in

accumulation of immature secretory granules in the pancreatic beta cells. However, these pc1 null mice were not reported to be obese, leading to the suggestion that PC1 may serve different functions in rodents compared to humans. Recently, a second mouse model of pc1 deficiency has been generated spontaneously by random mutagenesis resulting in a homozygous missense mutation (N222D) in the catalytic domain (45). These pc1 knockouts are hyperproinsulinaemic, are 30% heavier than wild type littermates with an increase in food intake.

An important caveat regarding prohormone processing disorders is that it is very difficult to state with certainty which altered phenotypes are due to which altered processing events. This uncertainty derives from the fact that these enzymes act on multiple precursors, some of which may not as yet be fully characterized.

**HUMAN MC4R DEFICIENCY**

Of the five known melanocortin receptors, the melanocortin 4 receptor (MC4R) has been most closely linked to control energy balance in rodents (46). Mice homozygous for a deleted MC4 receptor become severely obese; heterozygotes have body weights intermediate between wild type and homozygote null animals (47). In 1998, two groups reported heterozygous mutations in the MC4 receptor in humans which were associated with dominantly inherited obesity (48, 49). Since then, heterozygous mutations in MC4R have been reported in obese humans from various ethnic groups (50-52).

We have studied over 2000 severely obese probands and found that approximately 5-6% have pathogenic MC4R mutations that are non-conservative in nature, not found in control subjects from the background population and co-segregate with obesity in families (53). The prevalence of MC4R mutations has varied from 0.5% of obese adults to 6% in patients with severe childhood obesity (53, 54). Recent studies provide an important indication of the true population prevalence of this disorder: 1-2.5% of people with a BMI>30 kg/$m^2$ being found to harbour pathogenic mutations in MC4R in UK and European populations (54), confirming that MC4R deficiency is the most common obesity syndrome described to date and is one of the most common genetic diseases with a higher prevalence than more familiar diseases such as cystic fibrosis.

While we found a 100% penetrance of early-onset obesity in heterozygous probands, others have described obligate carriers who were not obese (51). Given the large number of potential influences on body weight, it is perhaps not surprising that both genetic and environmental modifiers will have important effects in some pedigrees. Indeed we have now studied 6 families in whom the probands were homozygotes and in all of these, the homozygotes were more obese than heterozygotes (53). Interestingly, in these families, some heterozygous carriers were not obese. Taking account of all of these observations, co-dominance, with modulation of expressivity and penetrance of the phenotype, is the most appropriate descriptor for the mode of inheritance.

This finding is supported by the pattern of inheritance of obesity seen in heterozygous and homozygous MC4R knockout mice (47).

We have now studied over 150 MC4R deficient subjects in our Clinical Research Facility. The clinical features of MC4R deficiency include hyperphagia, which invariably starts in the first year of life (53). Alongside the increase in fat mass, MC4R-deficient subjects also have an increase in lean mass and a marked increase in bone mineral density, thus they often appear 'big-boned'. They exhibit accelerated linear growth in early childhood, which does not appear to be due to dysfunction of the GH axis and may be a consequence of the disproportionate early hyperinsulinemia seen in these patients (53). The accelerated linear growth and the disproportionate early hyperinsulinemia are consistent with observations in the MC4R KO mouse (55).

Although affected subjects are objectively hyperphagic, adlibitum energy intake at a test meal is not as severe great as that seen with leptin deficiency (53). Of particular note is the finding that the severity of receptor dysfunction seen in *in vitro* assays can predict the amount of food ingested at a test meal by the subject harbouring that particular mutation (Figure 4). One notable feature of this syndrome is that the severity of many of the phenotypic features appears to partially ameliorate with time. Thus, obese adult mutation carriers report less intense feelings of hunger and are less hyperinsulinemic than children with the same mutation (personal observations).

We have studied in detail the signalling properties of many of these mutant receptors and this information should help to advance the understanding of structure/function relationships within the receptor (56). Importantly, we have been unable to demonstrate evidence for dominant negativity associated with these mutants, which suggests that MC4R mutations are more likely to result in a phenotype through haplo-insufficiency (56). About 70% of missense mutations in MC4R are retained intracellularly (57).

While, at present, there is no specific therapy for MC4R deficiency, it is highly likely that these subjects would respond well to pharmacotherapy that overcame the reduction in the hypothalamic melanocortinergic tone that exists in these patients. As most patients are heterozygotes with one functional allele intact, it is possible that small molecule MC4R agonists might, in future, be excellent treatments for this disorder (58).

**MUTATIONS IN THE NEUROTROPHIN RECEPTOR TRKB**

Recently, the concept that hypothalamic neuronal networks involved in energy homeostasis are “hardwired” has been challenged. In mice, hypothalamic neurones projecting from the arcuate nucleus to the paraventricular nucleus develop after birth and their development is regulated by leptin (59). In addition, synaptic plasticity in the mature rodent brain has been identified as a component of the neuronal regulation of energy homeostasis as leptin has been shown to

acutely modulate excitatory and inhibitory synaptic inputs at the level of first order arcuate neurones (60). However, it is difficult to establish whether synaptic plasticity plays a role in the physiological regulation of energy homeostasis in humans and whether under pathological conditions, hypothalamic neuronal networks and plasticity may be impaired and contribute to human obesity.

Brain-derived neurotrophic factor (BDNF) regulates the development, survival and differentiation of neurons through its high-affinity receptor, TrkB (tropomyosin related kinase B). Unlike other neurotrophins, BDNF is secreted in an activity-dependent manner that allows for highly controlled release. Recently, BDNF has been implicated in the regulation of body weight, as its expression is reduced by fasting (61) and BDNF administration causes weight loss in wild type mice through a reduction in food intake. BDNF has also been implicated in memory and a range of behaviours using a number of conditional knockout models (62)

We previously 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, the tyrosine kinase receptor that mediates the effects of both BDNF and the neurotrophin, NT4/5 (63). We have also identified a patient with severe hyperphagia and obesity and a complex neurobehavioural phenotype including impaired cognitive function and memory as well as distinctive hyperactive behaviour. Interestingly, this patient has a *de novo* paracentric inversion, 46,XX,inv(11)(p13p15.3), which encompasses the BDNF locus and

disrupts BDNF expression (64). Although to date only two such patients have been identified, understanding the mechanisms whereby BDNF regulates hypothalamic neuronal circuits may have potential therapeutic benefits for the treatment of more common forms of human obesity.

## CONCLUSIONS

In practical terms the discovery of these genetic disorders has helped de-stigmatise human obesity and allow it to be seen as a medical condition rather than simply a moral failing. The clinical evaluation of the severely obese child is becoming increasingly sophisticated and will require the development of expertise in the recognition of these emerging syndromes together with the incorporation of novel biochemical and molecular genetic diagnostics. These approaches will need to be combined with the more traditional nutritional and behavioural approaches to optimize treatment for individual children. Although there is no accepted definition for severe or morbid obesity in childhood, a BMI s.d. >2.5 (weight off the chart) is often used in specialist centres and the crossing of major growth percentile lines upward is an early indication of risk of severe obesity. The assessment of severely obese children and indeed adults should be directed at screening for potentially treatable endocrine and neurological conditions and identifying genetic conditions so that appropriate genetic counselling and in some cases treatment can be instituted (65). The presence of hyperphagia and severe obesity in a young child (<5 y) and a positive family history of early-onset obesity support a genetic diagnosis.

The genetic defects found to date all affect the drive to eat resulting in hyperphagia in affected subjects. Thus human food intake should not be considered as an entirely voluntarily controllable phenomenon but one driven by powerful biological signals. It is likely that further discovery of causative genetic

defects in humans and experimental animals will continue to highlight other molecular elements of the pathways involved in the regulation of body weight. To date candidate gene studies in the GOOS cohort have led to the identification of seven monogenic disorders, including the most recent disorders involving the neurotrophin receptor TrkB and its ligand BDNF. However, these disorders only account for 7% of patients in the GOOS cohort (mean BMI sds 4.5, mean age of onset 5 years). Many of the remaining patients have a history of early onset obesity inherited in a Mendelian manner or have subphenotypes that overlap with those seen in the known monogenic obesity syndromes, suggesting that there are many other genes and gene products to identify and characterise and we are undertaking a number of approaches to find novel obesity genes. There are many patients from consanguinous families in whom severe obesity segregates in an autosomal recessive manner and in whom mutations in the known obesity genes have been excluded. The development of high density single nucleotide polymorphism microarrays and their application in autozygosity mapping provides an efficient way to identify obesity genes in such pedigrees. Comparative genomic hybridisation (CGH) arrays can be used to identify subtle chromosomal rearrangements (less than 5 Mb) that cannot be identified by conventional karyotyping. We are using these arrays in patients with severe obesity, developmental delay and structural/dysmorphic features. These patients are often uniquely affected in their families which can be indicative of a denovo mutation or rearrangement.

Progress in the discovery of the genetic factors underlying more common forms of obesity, largely of onset in adolescence or adulthood, has not been so swift. It is likely that the effects of any individual genetic variant will be more subtle and therefore proving its association with an alteration in body weight will be more challenging. In fact, studies of this nature, involving populations of sufficient size to address these questions, have only begun to be undertaken. It is not yet clear whether the genetic architecture of common obesity will conform more to the "common variant-common disease" model in which some relatively common polymorphisms have modest but widespread effects on risk or the "multiple rare variants-common disease" model where multiple different rare alleles underlie genetic susceptibility. It is likely that common genetic variants will selectively influence an individual's response to environmental stimuli and that those of a particular genotype, for example, will be less likely to respond to particular subtypes of dietary intervention than others. Knowledge of gene-environment interaction will increasingly play a role in the improved targeting of behavioural interventions for the prevention of obesity.

Another approach to identifying genes implicated in the regulation of body weight may be to look at the opposite end of the spectrum. Marked thinness appears to be a trait that is as stable and heritable as obesity (66) and evidence from animal models suggests that searching for genes influencing adiposity may be profitability pursued by studying very thin healthy individuals (67). The

identification of such genes may provide important insights into the pathophysiological of obesity and could help explain why some individuals do not become obese despite environmental provocation. It will be interesting to see whether insights into the biology of thinness and obesity resistance may ultimately lead to preventative and management strategies for obesity.

Legend for Figure 1.

Effects of recombinant human leptin treatment in leptin deficiency

Legend for Figure 2.

Mutations in POMC resulting in a complete loss of function

Legend for Figure 3.

Mutations in POMC derived melanocortin peptides

Legend for Figure 4.

Genotype-phenotype correlations in Human MC4R deficiency.

Adlibitum food intake at an 18MJ test meal for patients with leptin deficiency and complete and partial loss of function MC4R mutations.

## REFERENCES


1. **Kopelman PG** 2000 Obesity as a medical problem. Nature 404:635-43.
2. **Ogden CL, Flegal KM, Carroll MD, Johnson CL** 2002 Prevalence and trends in overweight among US children and adolescents, 1999-2000. Jama 288:1728-32
3. **Barsh GS, Farooqi IS, O'Rahilly S** 2000 Genetics of body-weight regulation. Nature 404:644-51.
4. **Comuzzie AG** 2002 The emerging pattern of the genetic contribution to human obesity. Best Pract Res Clin Endocrinol Metab 16:611-21
5. **Maes HH, Neale MC, Eaves LJ** 1997 Genetic and environmental factors in relative body weight and human adiposity. Behav Genet 27:325-51
6. **Stunkard AJ, Harris JR, Pedersen NL, McClearn GE** 1990 The body-mass index of twins who have been reared apart. N Engl J Med 322:1483-7
7. **Silventoinen K, Kaprio J, Lahelma E** 2000 Genetic and environmental contributions to the association between body height and educational attainment: a study of adult Finnish twins. Behav Genet 30:477-85
8. **Carmichael CM, McGue M** 1995 A cross-sectional examination of height, weight, and body mass index in adult twins. J Gerontol A Biol Sci Med Sci 50:B237-44
9. **Farooqi IS, O'Rahilly S** 2005 Monogenic obesity in humans. Annu Rev Med 56:443-58
10. **Montague CT, Farooqi IS, Whitehead JP, et al.** 1997 Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature 387:903-8
11. **Farooqi IS, Matarese G, Lord GM, et al.** 2002 Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. J Clin Invest 110:1093-103
12. **Gibson WT, Farooqi IS, Moreau M, et al.** 2004 Congenital leptin deficiency due to homozygosity for the Delta133G mutation: report of another case and evaluation of response to four years of leptin therapy. J Clin Endocrinol Metab 89:4821-6
13. **Strobel A, Issad T, Camoin L, Ozata M, Strosberg AD** 1998 A leptin missense mutation associated with hypogonadism and morbid obesity. Nat Genet 18:213-5.
14. **Clement K, Vaisse C, Lahlou N, et al.** 1998 A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. Nature 392:398-401
15. **Lahlou N, Landais P, De Boissieu D, Bougneres PF** 1997 Circulating leptin in normal children and during the dynamic phase of juvenile obesity: relation to body fatness, energy metabolism, caloric intake, and sexual dimorphism. Diabetes 46:989-93
16. **Farooqi IS, Jebb SA, Langmack G, et al.** 1999 Effects of recombinant leptin therapy in a child with congenital leptin deficiency. N Engl J Med 341:879-84.
17. **Ozata M, Ozdemir IC, Licinio J** 1999 Human leptin deficiency caused by a missense mutation: multiple endocrine defects, decreased sympathetic tone, and immune system dysfunction indicate new targets for leptin action, greater central

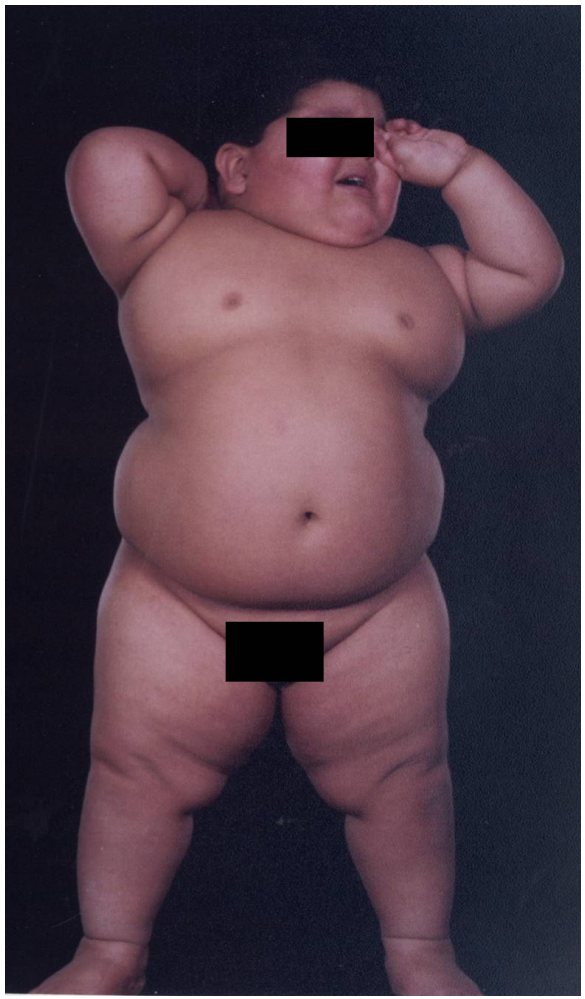
than peripheral resistance to the effects of leptin, and spontaneous correction of leptin-mediated defects. J Clin Endocrinol Metab 84:3686-95.
18. **Flier JS, Harris M, Hollenberg AN** 2000 Leptin, nutrition, and the thyroid: the why, the wherefore, and the wiring. J Clin Invest 105:859-61
19. **Mantzoros CS, Ozata M, Negrao AB, et al.** 2001 Synchronicity of frequently sampled thyrotropin (TSH) and leptin concentrations in healthy adults and leptin-deficient subjects: evidence for possible partial TSH regulation by leptin in humans. J Clin Endocrinol Metab 86:3284-91.
20. **Licinio J, Caglayan S, Ozata M, et al.** 2004 Phenotypic effects of leptin replacement on morbid obesity, diabetes mellitus, hypogonadism, and behavior in leptin-deficient adults. Proc Natl Acad Sci U S A 101:4531-6
21. **Heymsfield SB, Greenberg AS, Fujioka K, et al.** 1999 Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. JAMA 282:1568-75.
22. **Farooqi IS, Keogh JM, Kamath S, et al.** 2001 Partial leptin deficiency and human adiposity. Nature 414:34-5
23. **Chung WK, Belfi K, Chua M, et al.** 1998 Heterozygosity for Lep(ob) or Lep(rdb) affects body composition and leptin homeostasis in adult mice. Am J Physiol 274:R985-90.
24. **Krude H, Biebermann H, Luck W, Horn R, Brabant G, Gruters A** 1998 Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans. Nat Genet 19:155-7
25. **Farooqi IS, Drop S, Clements A, et al.** 2006 Heterozygosity for a POMC-null mutation and increased obesity risk in humans. Diabetes 55:2549-53
26. **Coll AP, Challis BG, Lopez M, Piper S, Yeo GS, O'Rahilly S** 2005 Proopiomelanocortin-deficient mice are hypersensitive to the adverse metabolic effects of glucocorticoids. Diabetes 54:2269-76
27. **Rees JL** 2003 Genetics of hair and skin color. Annu Rev Genet 37:67-90
28. **Krude H, Biebermann H, Schnabel D, et al.** 2003 Obesity due to proopiomelanocortin deficiency: three new cases and treatment trials with thyroid hormone and ACTH4-10. J Clin Endocrinol Metab 88:4633-40
29. **Challis BG, Pritchard LE, Creemers JW, et al.** 2002 A missense mutation disrupting a dibasic prohormone processing site in pro-opiomelanocortin (POMC) increases susceptibility to early-onset obesity through a novel molecular mechanism. Hum Mol Genet 11:1997-2004
30. **Lee YS, Challis BG, Thompson DA, et al.** 2006 A POMC variant implicates beta-melanocyte-stimulating hormone in the control of human energy balance. Cell Metab 3:135-40
31. **Biebermann H, Castaneda TR, van Landeghem F, et al.** 2006 A role for beta-melanocyte-stimulating hormone in human body-weight regulation. Cell Metab 3:141-6
32. **Mountjoy KG, Wong J** 1997 Obesity, diabetes and functions for proopiomelanocortin-derived peptides. Mol Cell Endocrinol 128:171-7
33. **Hsiung HM, Hertel J, Zhang XY, et al.** 2005 A novel and selective beta-melanocyte-stimulating hormone-derived peptide agonist for melanocortin 4

receptor potently decreased food intake and body weight gain in diet-induced obese rats. Endocrinology 146:5257-66
34. **Comuzzie AG, Hixson JE, Almasy L, et al.** 1997 A major quantitative trait locus determining serum leptin levels and fat mass is located on human chromosome 2. Nat Genet 15:273-6
35. **Delplanque J, Barat-Houari M, Dina C, et al.** 2000 Linkage and association studies between the proopiomelanocortin (POMC) gene and obesity in caucasian families. Diabetologia 43:1554-7
36. **Hixson JE, Almasy L, Cole S, et al.** 1999 Normal variation in leptin levels in associated with polymorphisms in the proopiomelanocortin gene, POMC. J Clin Endocrinol Metab 84:3187-91
37. **Baker M, Gaukrodger N, Mayosi BM, et al.** 2005 Association between common polymorphisms of the proopiomelanocortin gene and body fat distribution: a family study. Diabetes 54:2492-6
38. **Challis BG, Coll AP, Yeo GS, et al.** 2004 Mice lacking pro-opiomelanocortin are sensitive to high-fat feeding but respond normally to the acute anorectic effects of peptide-YY(3-36). Proc Natl Acad Sci U S A 101:4695-700
39. **Cai G, Cole SA, Bastarrachea-Sosa RA, Maccluer JW, Blangero J, Comuzzie AG** 2004 Quantitative trait locus determining dietary macronutrient intakes is located on human chromosome 2p22. Am J Clin Nutr 80:1410-4
40. **Seidah NG, Chretien M** 1999 Proprotein and prohormone convertases: a family of subtilases generating diverse bioactive polypeptides. Brain Res 848:45-62
41. **O'Rahilly S, Gray H, Humphreys PJ, et al.** 1995 Brief report: impaired processing of prohormones associated with abnormalities of glucose homeostasis and adrenal function. N Engl J Med 333:1386-90
42. **Jackson RS, Creemers JW, Ohagi S, et al.** 1997 Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. Nat Genet 16:303-6
43. **Jackson RS, Creemers JW, Farooqi IS, et al.** 2003 Small-intestinal dysfunction accompanies the complex endocrinopathy of human proprotein convertase 1 deficiency. J Clin Invest 112:1550-60
44. **Zhu X, Zhou A, Dey A, et al.** 2002 Disruption of PC1/3 expression in mice causes dwarfism and multiple neuroendocrine peptide processing defects. Proc Natl Acad Sci U S A 99:10293-8
45. **Lloyd DJ, Bohan S, Gekakis N** 2006 Obesity, hyperphagia and increased metabolic efficiency in Pc1 mutant mice. Hum Mol Genet 15:1884-93
46. **Yeo GS, Farooqi IS, Challis BG, Jackson RS, O'Rahilly S** 2000 The role of melanocortin signalling in the control of body weight: evidence from human and murine genetic models. Qjm 93:7-14.
47. **Huszar D, Lynch CA, Fairchild-Huntress V, et al.** 1997 Targeted disruption of the melanocortin-4 receptor results in obesity in mice. Cell 88:131-41
48. **Yeo GS, Farooqi IS, Aminian S, Halsall DJ, Stanhope RG, O'Rahilly S** 1998 A frameshift mutation in MC4R associated with dominantly inherited human obesity. Nat Genet 20:111-2
49. **Vaisse C, Clement K, Guy-Grand B, Froguel P** 1998 A frameshift mutation in human MC4R is associated with a dominant form of obesity. Nat Genet 20:113-4

50. **Farooqi IS, Yeo GS, Keogh JM, et al.** 2000 Dominant and recessive inheritance of morbid obesity associated with melanocortin 4 receptor deficiency. J Clin Invest 106:271-9.
51. **Vaisse C, Clement K, Durand E, Hercberg S, Guy-Grand B, Froguel P** 2000 Melanocortin-4 receptor mutations are a frequent and heterogeneous cause of morbid obesity. J Clin Invest 106:253-62.
52. **Hinney A, Schmidt A, Nottebom K, et al.** 1999 Several mutations in the melanocortin-4 receptor gene including a nonsense and a frameshift mutation associated with dominantly inherited obesity in humans. J Clin Endocrinol Metab 84:1483-6
53. **Farooqi IS, Keogh JM, Yeo GS, Lank EJ, Cheetham T, O'Rahilly S** 2003 Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. N Engl J Med 348:1085-95
54. **Larsen LH, Echwald SM, Sorensen TI, Andersen T, Wulff BS, Pedersen O** 2005 Prevalence of mutations and functional analyses of melanocortin 4 receptor variants identified among 750 men with juvenile-onset obesity. J Clin Endocrinol Metab 90:219-24
55. **Fan W, Dinulescu DM, Butler AA, Zhou J, Marks DL, Cone RD** 2000 The central melanocortin system can directly regulate serum insulin levels. Endocrinology 141:3072-9.
56. **Yeo GS, Lank EJ, Farooqi IS, Keogh J, Challis BG, O'Rahilly S** 2003 Mutations in the human melanocortin-4 receptor gene associated with severe familial obesity disrupts receptor function through multiple molecular mechanisms. Hum Mol Genet 12:561-74
57. **Lubrano-Berthelier C, Durand E, Dubern B, et al.** 2003 Intracellular retention is a common characteristic of childhood obesity-associated MC4R mutations. Hum Mol Genet 12:145-53
58. **Nargund RP, Strack AM, Fong TM** 2006 Melanocortin-4 receptor (MC4R) agonists for the treatment of obesity. J Med Chem 49:4035-43
59. **Bouret SG, Draper SJ, Simerly RB** 2004 Trophic action of leptin on hypothalamic neurons that regulate feeding. Science 304:108-10
60. **Pinto S, Roseberry AG, Liu H, et al.** 2004 Rapid rewiring of arcuate nucleus feeding circuits by leptin. Science 304:110-5
61. **Xu B, Goulding EH, Zang K, et al.** 2003 Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. Nat Neurosci 6:736-42
62. **Snider WD** 1994 Functions of the neurotrophins during nervous system development: what the knockouts are teaching us. Cell 77:627-38
63. **Yeo GS, Connie Hung CC, Rochford J, et al.** 2004 A de novo mutation affecting human TrkB associated with severe obesity and developmental delay. Nat Neurosci 7:1187-9
64. **Gray J, Yeo GSH, Cox JJ, Keogh JM, Morton J, Adlam ALR, Yanovski JA, El Gharbawy A, Han C, Tung YCL, Hodges JR, Raymond FL, O' Rahilly S, Farooqi IS.** 2006 Impaired cognitive function, hyperactivity and severe obesity in a child with functional loss of one copy of *BDNF*. Diabetes (in press).

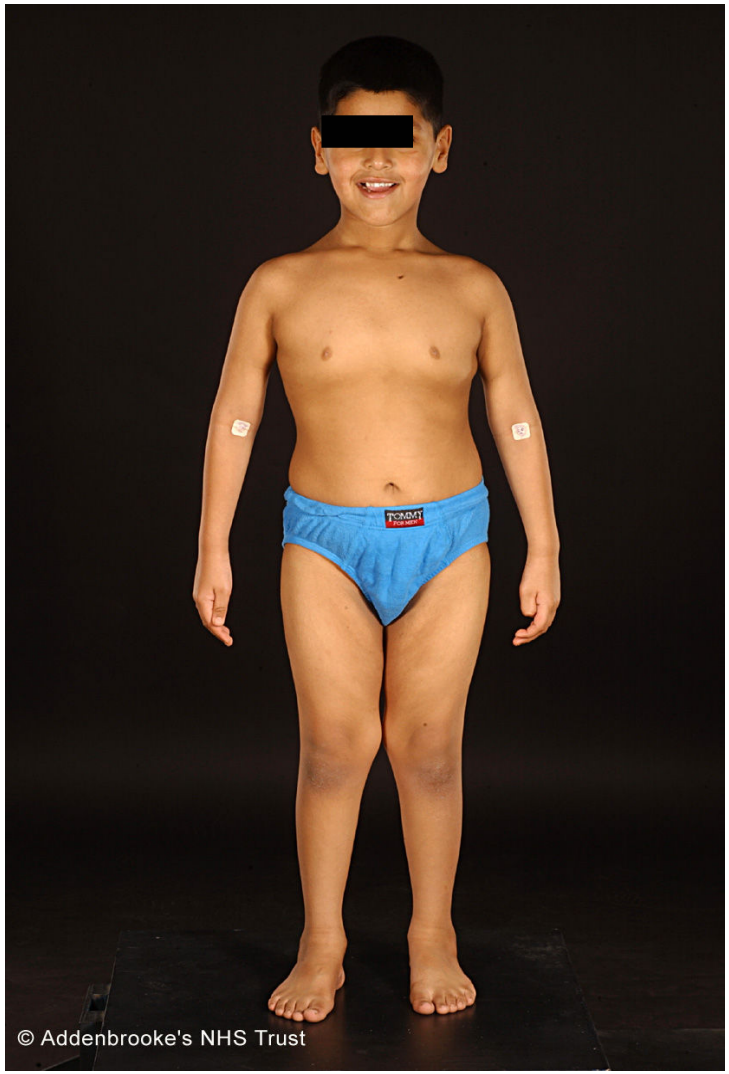
65. **Farooqi IS** 2006 The severely obese patient--a genetic work-up. Nat Clin Pract Endocrinol Metab 2:172-7.
66. **Forbes GB, Sauer EP, Weitkamp LR** 1995 Lean body mass in twins. Metabolism 44:1442-6
67. **Laskarzewski PM, Khoury P, Morrison JA, Kelly K, Mellies MJ, Glueck CJ** 1983 Familial obesity and leanness. Int J Obes 7:505-27

Figure 1. Response to leptin administration in leptin deficiency



weight = 40kg, age 3yrs

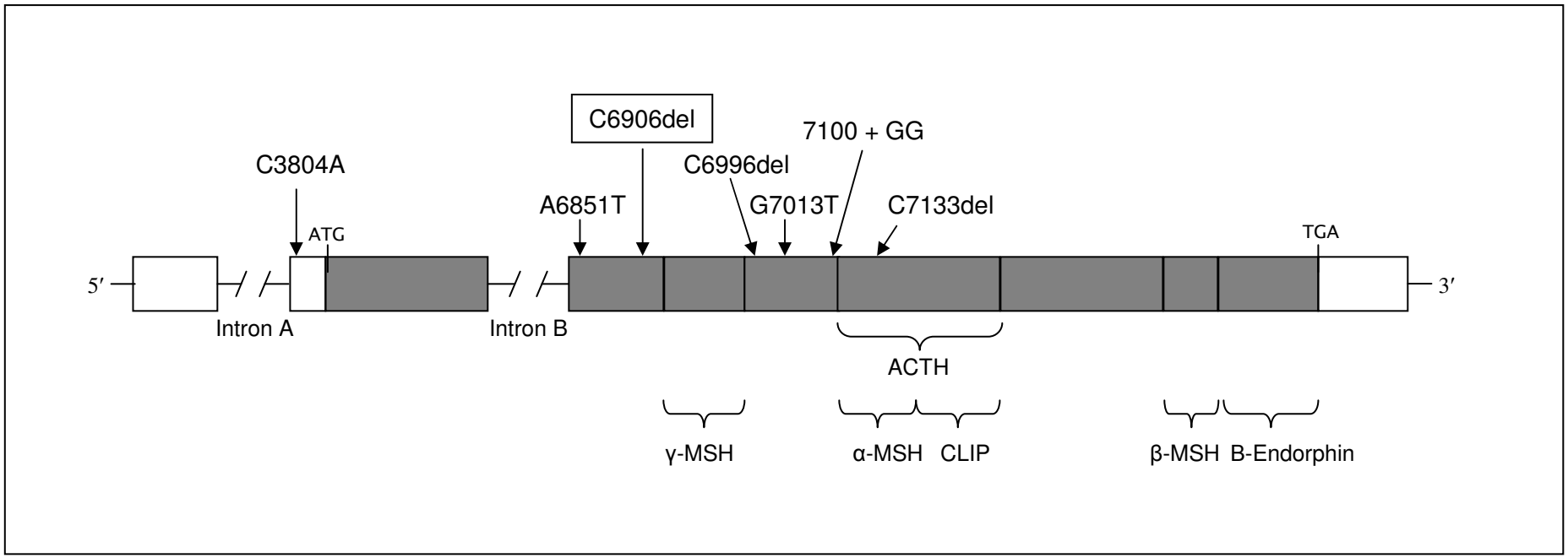
BEFORE LEPTIN




weight = 29kg, age 6yrs

AFTER LEPTIN

Figure 2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7100 + GG
C3804A
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A6851T
G7013T
C7133del
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3′
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Intron B
ACTH
γ-MSH
α-MSH
CLIP
β-MSH
B-Endorphin

Figure 3

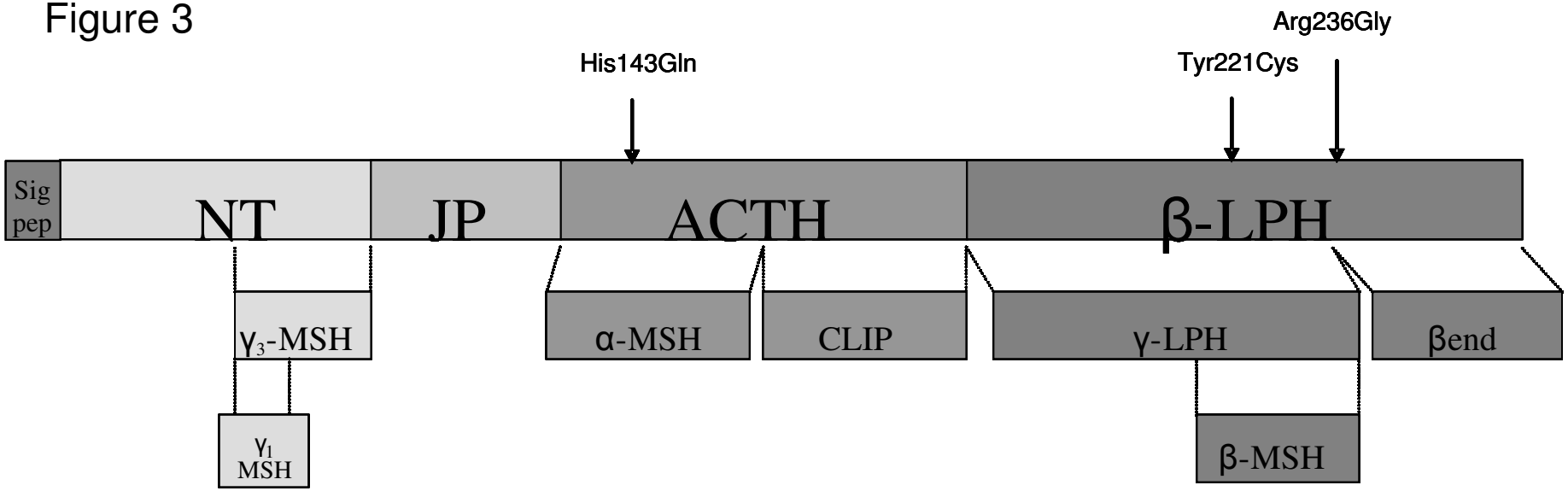
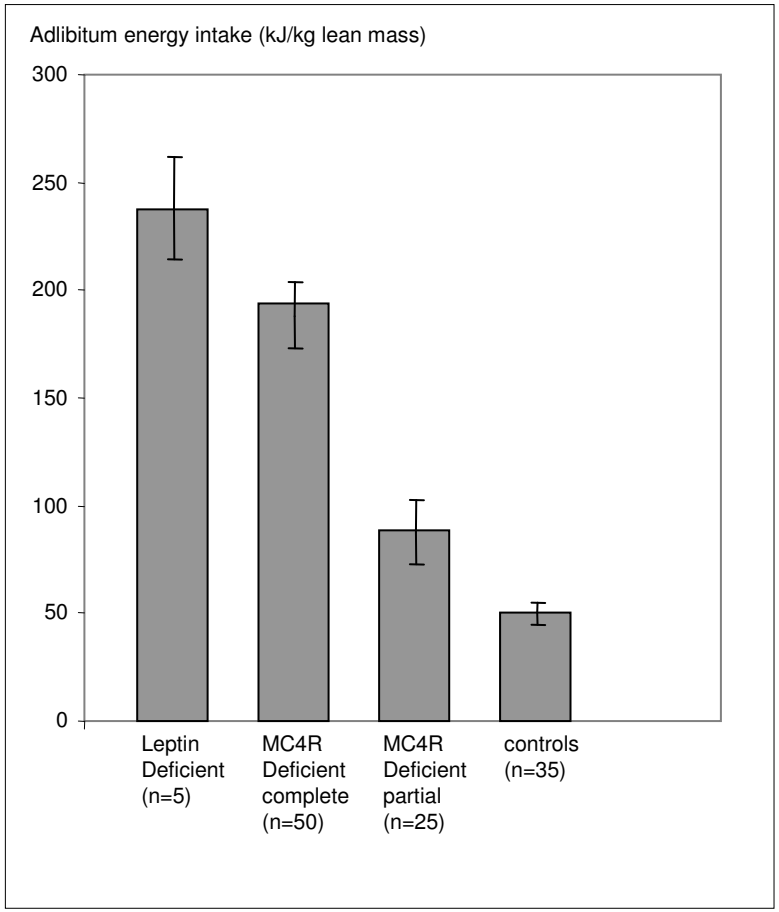

Arg236Gly
His143Gln
Tyr221Cys
Sig pep
NT
JP
ACTH
β-LPH
γ3-MSH
α-MSH
CLIP
γ-LPH
βend
γ1 MSH
β-MSH

Figure 4


Adlibitum energy intake (kJ/kg lean mass)
300
250
200
150
100
50
0
Leptin Deficient (n=5)
MC4R Deficient complete (n=50)
MC4R Deficient partial (n=25)
controls (n=35)