

Minireview: Human Obesity—Lessons from Monogenic Disorders

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Genetic influences on the determination of human fat mass are profound and powerful, a statement that does not conflict with the obvious influence of environmental factors that drive recent changes in the prevalence of obesity. The assertion of the importance of genetic factors has, until recently, largely been based on twin and adoption studies. However, in the last 6 yr, a number of human genes have been identified in which major missense or nonsense mutations are sufficient in themselves to result in severe early-onset obesity, usually associated with disruption of normal appetite control mechanisms.

Progress in the identification of more common, subtler genetic variants that influence fat mass in larger numbers of people has been slower, but discernible. Human genetics will continue to make an invaluable contribution to the study of human obesity by identifying critical molecular components of the human energy balance regulatory systems, pointing the way toward more targeted and effective therapies and assisting the prediction of individual responses to environmental manipulations. (*Endocrinology* 144: 3757–3764, 2003)

THERE IS NOW widespread recognition that the continuing increase in the prevalence of obesity seen in many countries is likely to have major adverse effects on public health (1). In the search for the environmental drivers of this epidemiological phenomenon, there is some danger that we may overlook the critical importance of inherited factors in the determination of interindividual differences in fat mass. The identification of such factors is of great clinical, as well as theoretical, importance for a number of reasons. Firstly, genetic influences are likely to be particularly powerful in people with severe and early-onset obesity, the group most likely to suffer adverse clinical consequences. Secondly, the use of genetics to identify critical molecular components of the human control system for energy homeostasis may help to target safe and specific drug development. Finally, it is known that diet and exercise programs, while frequently effective in inducing weight loss, rarely maintain this. It is very likely that the genetic makeup of an individual may influence their response to particular measures. Ultimately, it should be possible to identify genetic subgroups of subjects who might be particularly responsive or resistant to specific environmental modulations.

Evidence for the Heritability of Human Fat Mass

Traditionally, the most favored model for separation of the genetic component of phenotypic variance between individuals (heritability) is based on studies of twins, as monozygotic cotwins share 100% of their genes and dizygotes 50% on average. Twin studies suggest a heritability of fat mass of between 40 and 70% with a concordance of 0.7–0.9 between monozygotic twins compared with 0.35–0.45 between dizygotic twins (2, 3). Correlation of monozygotic twins reared apart is virtually a direct estimate of the heritability (al-

though monozygotic twins do share the intrauterine environment, which may contribute to lasting differences in body mass in later life). Estimates vary from 40–70%, depending on age of separation of twins and the length of follow-up (reviewed in Ref. 4).

Complete adoption studies are useful in separating the common environmental effects because adoptive parents and their adoptive offspring share only environmental sources of variance, whereas the adoptees and their biological parents share only genetic sources of variance. One of the largest series, based on over 5000 subjects from the Danish adoption register that contains complete and detailed information on the biological parents, showed a strong relationship between the body mass index (BMI) of adoptees and biological parents across the whole range of body fatness but none when compared with the adoptive parents (5). The Danish group have also shown a close correlation between BMI of adoptees and their biological full siblings who were reared separately by the biological parents of the adoptees, and a similar, but weaker relationship with half-siblings (6).

This is not to say that environmental forces are unimportant. The progressive increase in mean BMI in the United States since records began is very likely to be driven by a combination of increased food availability and palatability and decreased physical activity. However, this needs to be put in perspective. Between 1991 and 2000, the mean weight of an American adult increased by about 4 kg. However, American adults vary in weight between 50 and 300 kg, suggesting hugely differing susceptibilities to weight gain between individuals within the same environment (7).

We already have an excellent precedent for a phenotype that is well accepted as being largely genetically determined, yet shows secular changes with time, presumably based on changing environmental influences. In humans, height is highly heritable (75–90%) (8–10); indeed, clinically we use mid-parental height to predict the ultimate stature of a child.

Abbreviations: BMI, Body mass index; MC4R, melanocortin 4 receptor; PC, prohormone convertase; POMC, proopiomelanocortin; SDS, sd score.

However, young adults in most Westernized countries have shown a marked and progressive increase in height over the past century, presumably as a result of improved nutrition in prenatal and early postnatal life. It is interesting that we have no difficulty in accepting that height is largely genetically determined, yet in the face of a highly analogous data set for weight, there is sometimes considerable reluctance to accept the profound influence of heredity.

Identification of Human Obesity Genes

This review will largely focus on a group of recently described human monogenic obesities resulting from mutations in critical molecular elements of the homeostatic control mechanism regulating energy balance. Before dealing with these, we will briefly touch on recent advances in discovery of the genes responsible for complex pleiotropic syndromic obesity, and we will finish with some short comments on polygenic obesity.

Pleiotropic syndromes

Pleiotropic syndromes refer to complex and often long-described clinical syndromes in which obesity is only one of

a constellation of physical and developmental anomalies (*e.g.* Prader Willi, Bardet-Biedl syndromes). There are about 30 Mendelian disorders with obesity reported as an omnipresent or variable clinical feature. Positional genetic strategies have led to the recent identification of several different causative genetic defects underlying such syndromes (Table 1). In most cases, the defective gene product is an intracellular protein that is expressed throughout the body and is of unknown function. As yet, the mechanistic link between such defective gene products and dysregulation of energy balance is obscure.

Monogenic Human Obesity Syndromes

The field of human obesity has benefited enormously from the recent advances in rodent genetics (11). The 1990s brought the positional identification of a series of murine obesity genes including leptin (12), the leptin receptor (13), carboxypeptidase E (14) and agouti (15), and targeted genetic manipulation, established the critical regulatory role of molecules such as the melanocortin 4 receptor (MC4R) (16) and agouti-related protein (17). Many of these discoveries were rapidly followed by the finding of mutations in homologous

TABLE 1. Pleiotropic obesity syndromes

Syndrome	Additional clinical features	Locus	Gene	Refs.
Autosomal dominant				
Prader-Willi syndrome (PWS) ^a	Hypotonia, mental retardation, short stature, and hypogonadism	15q11.2-q12	Unknown	60–64
Albright hereditary osteodystrophy (AHO) ^b	Short stature, skeletal defects, and impaired olfaction	20q13.2	GNAS1	65,66
Fragile X syndrome	Mental retardation, macro-orchidism, and high-pitched jocular speech	Xq27.3	FMR1	67
Ulnar-mammary syndrome	Ulnar defects, delayed puberty, and hypoplastic nipples	12q24.1	TBX3	68
Autosomal recessive				
Bardet-Biedl syndrome				
	Mental retardation, dysphormic extremities, retinal dystrophy or pigmentary retinopathy, hypogonadism, and structural abnormalities of the kidney or functional renal impairment	11q13 (BBS1)	BBS1	69
		16q21 (BBS2)	BBS2	70
		3p13 (BBS3)	Unknown	
		15q22 (BBS4)	BBS4	71
		2q31 (BBS5)	Unknown	
		20p12 (BBS6)	BBS6 (MKKS)	72–74
Alstrom syndrome	Retinal dystrophy, neurosensory deafness, and diabetes	2p13	ALMS1	75,76
Cohen syndrome	Prominent central incisors, ophthalmopathy, and microcephaly	8q22	Unknown	77
X-linked				
Borjeson-Forssman-Lehmann syndrome	Mental retardation, hypogonadism, large ears	Xq26	PHF6	78
Mehmo syndrome	Mental retardation, epilepsy, hypogonadism, and microcephaly	Xp22.13	Unknown no.	79,80
Simpson-Golabi-Behmel, type 2	Craniofacial defects, and skeletal and visceral abnormalities	Xp22	Unknown	81
Wilson-Turner syndrome	Mental retardation, tapering fingers, and gynaecomastia	Xp21.2	Unknown	82

In each column, references for the description of the clinical syndrome, identification of putative loci, and specific genes are indicated. Where the responsible gene is unknown, studies examining the role of candidate genes contained within the relevant locus are referenced.

^a Deletion of the paternal segment or loss of the entire paternal chromosome 15 with presence of two maternal homologs (uniparental maternal disomy) leads to the phenotype of PWS due to the effects of imprinted genes.

^b Maternal transmission of GNAS1 (Gs α subunit) mutations leads to AHO plus resistance to several hormones (*e.g.* PTH) that activate Gs in their target tissues (pseudohypoparathyroidism type IA), whereas paternal transmission leads only to the AHO phenotype (pseudopseudohypoparathyroidism).

^c An X-linked mitochondrial disorder.

genes or pathways causing human disease. Such mutations tend to result in morbid obesity presenting in childhood without the developmental pleiotropic features characteristic of the recognized syndromes of childhood obesity, although there are distinct and characteristic clinical, neuroendocrine, and biochemical features. Notably, they mostly appear to have their most striking effects on energy intake.

Congenital leptin deficiency

Congenital leptin deficiency was the first of these monogenic syndromes to be described (18), and the clinical features of this condition have now been reported in two UK families of Pakistani origin (three affected individuals) and one Turkish family (two living affected subjects) (19). We have recently identified two further cases, one of whom is unrelated to either of our original probands (our unpublished data). Although the Pakistani families are not known to be related over five generations, they all carry the same frameshift mutation (Δ G133). This suggests either a founder effect in that population or, more likely in our view, that this G deletion, occurring as it does in a run of six guanines, represents a hotspot (*i.e.* region in the genome where there is a greater chance of DNA replication error, predisposing to insertion or deletion mutations). High levels of consanguinity, even after migration to developed countries, may be sufficient to explain the apparently disproportionate mutational prevalence among this ethnic group. The Δ G133 mutation results in a truncated form of leptin that is misfolded and not secreted (20). Obese subjects in the single consanguineous Turkish family with leptin deficiency are homozygous for a missense mutation that appears to be associated with low leptin levels, although the precise molecular mechanisms have not been studied (19).

The clinical phenotype of human congenital leptin deficiency is very similar to that seen in the *ob/ob* mouse. Thus, in common with the mouse, leptin-deficient humans have early-onset obesity, increased food intake, hypogonadotropic hypogonadism, hyperinsulinaemia, defective function of the hypothalamo-pituitary thyroidal axis, and defects in T cell number and function (21–23). The T cell hyporesponsiveness, which we recently demonstrated in two leptin-deficient children, was profound and provokes questions regarding susceptibility of such children to infectious disease. Parents from the UK families report more frequent and severe respiratory tract infections in their affected than their unaffected children. In the Turkish family, who live in a remote area where medical care is not readily accessible, there is a history of high early mortality in obese children from that large kindred.

There are some phenotypes where the parallels between human and mice are not as clear-cut. Thus, *ob/ob* mice are stunted and have elevated corticosterone levels (24), neither of which appears to be the case in humans. The contribution of reduced energy expenditure to the obesity of the *ob/ob* mouse is reasonably well established (25, 26); however, we were unable to detect any major reductions in resting or free-living energy expenditure (22), although we were unable to examine how such systems adapted to stressors such as cold.

Studies of the heterozygote members of the Δ G133 families

indicate that their leptin levels are lower than ethnically matched control subjects, and this partial leptin deficiency is associated with a mean fat mass 23% greater than predicted by their height and weight (27). This is very similar to the subtle phenotype seen in *ob* heterozygote mice (28).

Of the human monogenic obesity syndromes identified thus far, leptin deficiency is unique in being amenable to mechanism-based therapy. We have recently reported the dramatically beneficial effects of daily sc injections of leptin in three children (22) and are obtaining similar results in another two (our unpublished data). These effects were seen at a dose equivalent to 10% predicted serum leptin concentration (0.01 mg/kg lean body weight) in contrast to the supraphysiological doses (0.1–0.3 mg/kg body weight) required to induce weight loss in leptin-sufficient obesity (29). In the most dramatic example of its effects, a 3-yr-old boy who was severely disabled by gross obesity (42 kg) now weighs 32 kg (75th centile for weight) after 48 months of leptin therapy (Fig. 1).

Of particular note, the major effect of leptin was on appetite with normalization of hyperphagia. Leptin therapy reduced energy intake during an 18 MJ *ad libitum* test meal by up to 84% (5 MJ ingested before treatment *vs.* 0.8 MJ post treatment in the child with the greatest response). In contrast to the dramatic effects of leptin on human energy intake, we were unable to demonstrate a major effect of leptin on basal metabolic rate or free-living energy expenditure. Because weight loss by other means is reported to be associated with a fall in basal metabolic rate, the failure of decline in energy expenditure in these subjects is likely, in itself, be significant.

Leptin administration permitted the full progression of appropriately timed pubertal development but did not appear to cause precocious activation of the pubertal process in younger children (22). Free T₄ levels, although being in the normal range before treatment, consistently showed an increase at the earliest posttreatment time point and subsequently stabilized at that new state (22). These findings are consistent with evidence from animal models that leptin profoundly influences TRH release from the hypothalamus (30–32).

Weight loss continued in all subjects throughout the trial, albeit with some refractory periods, which were overcome by increases in leptin dose. The UK families all carry the Δ G133 frameshift mutation, and thus wild-type leptin is a novel antigen to them. Unsurprisingly, all subjects developed anti-leptin antibodies after 6 wk of leptin therapy, which hampered the interpretation of serum leptin levels and in some cases were capable of neutralizing leptin in a bioassay (22). The fluctuating nature of the antibodies may reflect the complicating factor that leptin deficiency is itself an immunodeficient state and that leptin administration leads to a switch from the secretion of predominantly Th2 to Th1 cytokines (33), which may directly influence antibody production. This may explain refractory periods during therapy in some subjects when weight was gained despite continuing treatment. Thus far, in all cases we have been able to regain control of weight by increasing the delivered dose of leptin.

Although congenital leptin deficiency is rare, the response to leptin administration in these patients has provided an important proof of principle and highlighted some of the biological functions of leptin in humans. Our studies suggest

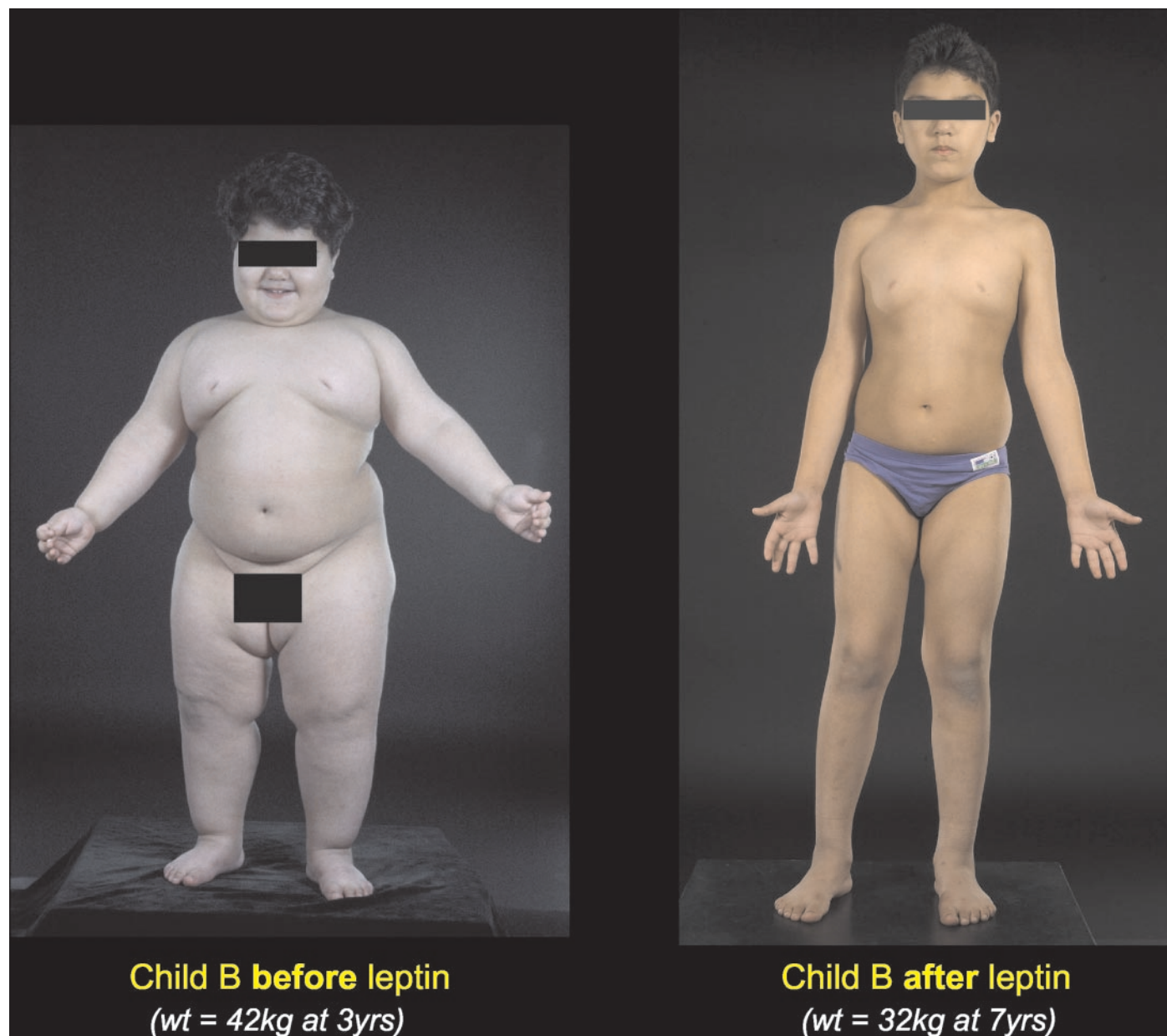


FIG. 1. Clinical response to leptin administration in congenital leptin deficiency

that leptin may be involved in dynamic changes in energy expenditure and thyroid function. Furthermore, in a recent study of leptin-sufficient subjects, administration of twice-daily recombinant human leptin prevented the fall in energy expenditure and thyroid hormones seen after 10% weight loss (34). Thus, the weight-reduced state may be considered a state of relative leptin deficiency associated with subtle changes in energy expenditure and thyroid function.

Leptin receptor deficiency

In a consanguineous family of Kabilian origin, three severely obese subjects were found to be homozygous for a mutation that truncates the leptin receptor before the transmembrane domain (35). Leptin receptor-deficient subjects were born of normal birth weight, exhibited rapid weight gain in the first few

months of life, with severe hyperphagia and aggressive behavior when denied food. Basal temperature and resting metabolic rate were normal, cortisol levels were in the normal range and all subjects were normoglycaemic with elevated plasma insulins as seen in leptin-deficient subjects. In contrast, the presence of mild growth retardation in early childhood with impaired basal and stimulated GH secretion and decreased IGF-1 and IGF binding protein 3 levels, and evidence of central hypothyroidism in these subjects, suggest that loss of the leptin receptor may result in a more diverse phenotype than loss of its ligand leptin.

Proopiomelanocortin (POMC)

The first-order neuronal targets of leptin action in the brain are catabolic POMC and anabolic neuropeptide Y/agouti-

related protein neurons (36–38). These reside in the hypothalamic arcuate nucleus, where the signaling isoform of the leptin receptor is highly expressed. POMC is sequentially cleaved by prohormone convertases to yield peptides including α -MSH that have been shown to play a role in feeding behavior. Forty percent of POMC neurons in the arcuate nucleus express the mRNA for the long form of the leptin receptor and POMC expression is regulated positively by leptin (39). There is clear evidence in rodents that α -MSH, a melanocortin peptide produced from POMC, acts as a suppressor of feeding behavior, probably through the MC4R (reviewed in Ref. 40). Targeted disruption of MC4R in rodents leads to obesity, severe hyperinsulinaemia, and increased linear growth; heterozygotes have an intermediate phenotype compared with homozygotes and wild-type mice (16).

The first evidence for the involvement of POMC-derived peptides in human energy homeostasis came from the description of two children harboring complete loss of function mutations in the POMC gene (41). The affected children presented with hypocortisolemia early in life and had undetectable levels of plasma cortisol and ACTH, consistent with isolated ACTH deficiency. Interestingly, both probands had very pale skin and red hair, developed hyperphagia, and became severely obese. In these two probands, the failure of adrenal steroidogenesis was a consequence of the lack of ACTH signaling through the adrenal MC2R, whereas the red hair and pale skin were due to the lack of α -MSH induced activation of the MC1R in skin melanocytes. Hyperphagia and weight gain is likely to be the result of reduced hypothalamic melanocortinergic signaling, presumably through the MC4R. Recently, we identified a heterozygous missense mutation (Arg236Gly) in POMC that disrupts the dibasic amino acid processing site motif between β -MSH and β -endorphin (42). In a pooled analysis of published data (42–44), mutations disrupting this site were found in 0.9% of children with severe early-onset obesity and 0.2% of normal weight subjects. Functional studies demonstrated that the mutation completely prevents the processing between β -MSH and β -endorphin, resulting in an aberrant β -MSH/ β -endorphin fusion peptide. In *in vitro* studies, this fusion protein bound to the MC4R with an affinity identical to that of α - and β -MSH but had a markedly reduced ability to activate the receptor. These results suggested that mutations at ARG236 may confer an inherited susceptibility to obesity through a novel mechanism whereby the production of an aberrant fusion protein has the capacity to interfere with central melanocortinergic signaling, thus predisposing to obesity in certain individuals.

Prohormone Convertase (PC)1

Further evidence for the role of the melanocortin system in the regulation of body weight in humans comes from the description of a 47-yr-old woman with severe childhood obesity, abnormal glucose homeostasis, and very low plasma insulin but elevated levels of proinsulin, hypogonadotropic hypogonadism, and hypocortisolemia associated with elevated levels of POMC. This subject was found to be a compound heterozygote (*i.e.* heterozygous for two mutations,

each occurring on a different chromosome), for mutations in prohormone convertase 1 (45), which cleaves prohormones at pairs of basic amino acids, leaving C-terminal basic residues that are then excised by carboxypeptidase E. We have recently identified a child with severe, early-onset obesity who was a compound heterozygote for complete loss of function mutations in PC1 (personal observations). Although failure to cleave POMC is a likely mechanism for the obesity in these patients, PC1 cleaves a number of other neuropeptides in the hypothalamus, such as glucagon-like peptide 1, which may influence feeding behavior. The phenotype of these subjects is somewhat similar to that seen in the carboxypeptidase E-deficient *fat/fat* mouse (14). However, interestingly, mice that have been rendered totally deficient in PC1 by gene targeting while showing biochemical abnormalities of prohormone processing very similar to our patients have some markedly different phenotypic features (46). For example, PC1-deficient mice, in contrast to humans, are short but not obese (46). Thus, it appears that normal PC1 function is more essential for the maintenance of normal energy homeostasis in humans than in mice.

MC4R

Several groups have identified mutations in *MC4R* in obese subjects from different ethnic groups (47–52). In a recent study, we screened 500 subjects with severe, early-onset obesity for mutations in *MC4R* and found that approximately 6% of such subjects had mutations that were likely to be causative of the condition (53, 54). The criteria for this are important to state explicitly as there are several relatively common but functionally irrelevant amino acid variants in human *MC4R*. The mutations we identified are not conservative in nature, are not found in control subjects from the background population, and cosegregate with obesity in families. *MC4R* deficiency represents the most commonly known monogenic disorder presenting as morbid obesity (53). The lower prevalence observed in some studies may be explained by the differing prevalence in certain ethnic groups but is most likely to reflect the later onset and reduced severity of obesity of subjects in these studies. Whereas we found a 100% penetrance of early-onset obesity in heterozygous probands, others have described obligate carriers who were not obese (48). Given the large number of potential influences on body weight, it is not surprising that genetic and environmental modifiers will have important effects in some pedigrees. Notably, we have now seen six families in whom the proband was a homozygote and in all of these, the homozygotes were more obese than heterozygotes (53). Interestingly, in these families, some heterozygous carriers were not obese. This may reflect ethnic-specific effects as all these families were of Indo origin. Taking all of these observations together, codominance, with modulation of expressivity and penetrance of the phenotype, is the most appropriate descriptor for the mode of inheritance, a finding supported by the pattern of inheritance of obesity seen in heterozygous and homozygous *MC4R* knockout mice (16).

At this time, we have examined over 70 *MC4R* mutant carriers in our Clinical Research Facility. In addition to the increase in fat mass, *MC4R* mutant subjects have a parallel

increase in lean mass that is not seen in leptin deficiency (53). Linear growth of these subjects is also striking with affected children having a height SD score (SDS) of +2 compared with population standards (mean height SDS of other obese children in our cohort = +0.5). In addition, MC4R deficient subjects have higher levels of fasting insulin than age-, sex-, and BMI SDS-matched children (53). The acceleration in linear growth and the disproportionate early hyperinsulinaemia are consistent with the findings in the MC4R knockout mouse.

Affected subjects are objectively hyperphagic, but not as severely as those with leptin deficiency. Of particular note is the finding that severity of receptor dysfunction demonstrated in *in vitro* assays predicts the amount of food ingested at a test meal by the subject harboring that mutation (53). One notable feature of this syndrome is the finding that the severity of many of the phenotypic features appears to partially ameliorate with time. Thus, obese adult mutation carriers report less intense hunger and are less hyperinsulinaemic than are children carrying the same mutation (personal observations). We have studied the signaling property of many of these mutant receptors in detail, and the information gleaned from those studies should help advance knowledge of structure/function relationship within the receptor. Importantly, we have been unable to demonstrate any evidence for dominant negativity associated with these mutants, suggesting that MC4R mutations are more likely to result in a phenotype through haploinsufficiency (54).

Polygenic Obesity

The genetic determinants of interindividual variation in body fat mass are likely to be multiple and interacting, with each single variant producing only a moderate effect. Because of this complexity, the search for genes predisposing to common obesity has been a challenging undertaking.

Results from reported genome-wide linkage studies that have examined obesity and/or related intermediate traits have identified several loci that show positive evidence for linkage with a LOD score of at least 2.6 (55). Only a single locus has been highlighted in more than one of the genome-wide scans reported to date. In two studies, one of extended, Mexican-American pedigrees (56) and the other of French sibling-pairs (57), significant linkage of serum leptin levels to chromosome 2p21 was found. In the Mexican-American study, suggestive evidence of linkage of fat mass to 2p21 was reported, whereas in the French study no hint of linkage to BMI was identified. The potential importance of this locus is supported by a study of African-Americans, which confirmed linkage with serum leptin levels in this population (58). This region of human chromosome 2 includes the POMC gene, in which loss-of-function mutations have been demonstrated as rare Mendelian causes of obesity in humans. Of note, POMC mutations affecting the β -MSH/ β -endorphin processing site have been reported in around 0.8% of obese children and 0.2% controls, considering four different published studies (42). While these relatively low prevalence variants could not explain linkage results, they do provide yet more biological support that subtle alterations in POMC could influence body fat mass.

To some, the apparently slow progress in polygenics suggests that this approach will not ultimately bear fruit. The recent striking success in inflammatory bowel disease should be a powerful rebuttal to the skeptics (59). In our view, it is only a matter of time (and unfortunately money!) before the combination of linkage and large-scale association studies in multiple ethnic groups begins to reliably uncover the genetic substrate for common forms of obesity.

Conclusions

As a result of the studies in human genetic obesity that we have described, considerable progress has been made. Firstly, we now know, beyond any doubt, that humans can become severely obese directly as a result of genetic disruption of a single element of a homeostatic system regulating energy balance. Secondly, we know that those critical regulatory molecules are as important for human energy balance as they are in lower species. Thirdly, it is notable that all of the known genetic defects resulting in severe human obesity do so largely through disruption of the normal controls of ingestive behavior. A further illustration of the potency and potential importance of these central mechanisms is the clear correlation between a particular MC4R mutation on *in vitro* signaling and the amount of food ingested at a test meal (53). Finally, and most importantly, the paradigm of leptin deficiency illustrates how the identification of genetically defined subtypes among the obese can lead to the institution of highly effective and life-saving therapy (22).

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