# *ob* gene mutations and human obesity

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## Evidence for a genetic basis to human obesity

Whilst recent changes in the prevalence of human obesity point to the importance of environmental determinants, genetic susceptibility has been identified as a major contributing factor (Bouchard, 1996). A number of monogenic and polygenic effects are likely to be involved, with their expression likely to vary with diet and the level of physical activity. These genetic influences are not confined to obesity, but exert their effect across the whole range of body weight, and are consistent with a polygenic inheritance of fat mass. Results from both twin and adoption studies suggest a heritability of fat mass of approximately 30–40 %. Data from large twin studies have consistently noted a concordance of 0·7–0·9 between monozygotic twins and of 0·35–0·45 between dizygotic twins (Borjeson, 1976; Stunkard *et al.* 1986*a*). Furthermore, a large Danish study demonstrated a clear relationship between the weight of biological parents (not adoptive parents) and the weight of adoptees (Stunkard *et al.* 1986*b*); they found no difference between the intra-pair correlation coefficients of identical twins reared apart compared with those reared together, suggesting that sharing the same childhood environment did not contribute to the similarity of BMI of twins later on in life.

### *Single gene defects*

Although human obesity is clearly a complex multifactorial disease, this does not preclude a contribution by a major single gene or genes. There is evidence to suggest that some genes account for most of the risk of obesity in families in which they are segregating, and more than seventy genes or chromosomal regions have been implicated as having some role in obesity in animals and man (Lee *et al.* 1997). Recent studies of genetic syndromes of obesity in rodents have provided several novel insights into molecules which may be involved in the control of energy intake and energy expenditure. These include *agouti*, a secreted melanocyte-stimulating hormone-receptor antagonist whose ectopic expression leads to severe obesity in mice (Huszar *et al.* 1997), *tubby/tubby* mice harbour homozygous mutations for a phosphodiesterase(*EC* 3.1.4.1)-like molecule which is expressed in the hypothalamus (Nobentrauth *et al.* 1996), and the *fat/fat* mouse has a genetically-defective form of carboxypeptidase E which results in the impaired processing of prohormones (Fricker *et al.* 1996).

The most exciting insights emanating from the study of murine obese models come from the findings in the *ob/ob* and *db/db* mice. The *ob/ob* mice are normal weight at birth, then become hyperphagic, markedly obese, hyperinsulinaemic and remain infertile with evidence of hypogonadotropic hypogonadism (Ahima *et al.* 1996). In 1994, Friedman's group used positional cloning methods to clone the mouse *ob* gene and its human homologue (Zhang *et al.* 1994). Two strains of *ob/ob* mice were found to be deficient in the 16 kDa protein product of the *ob* gene (leptin) as a result of mutations in the *ob* gene. Leptin has subsequently been shown to be an adipocyte-specific hormone which acts largely at the level of the hypothalamus, to control appetite and energy expenditure (Maffei *et al.* 1995; Stephens *et al.* 1995; Vaisse *et al.* 1996). The importance of the hypothalamic form of the leptin receptor in the control of body weight has been confirmed by the finding that the very obese *db/db* mice harbour a homozygous mutation in the hypothalamic form of the cell surface receptor for leptin (Chen *et al.* 1996). Confirmation of the importance of leptin in the regulation of energy balance in these rodent models was provided by the simultaneous publication by three groups showing that the administration of parenteral leptin reduces food intake, increases energy expenditure, and corrects the obesity and associated metabolic disturbances found in *ob/ob* mice (Campfield *et al.* 1995; Halaas *et al.* 1995; Pelleymounter *et al.* 1995). As expected, leptin administration had no effect on the *db/db* mouse.

## The *ob* gene in human subjects

The discovery of leptin as a potential controller of the 'adipostat' in mice has generated enormous interest, and has been rapidly succeeded by a large number of studies attempting to define the role of leptin in the control of human fat mass. Studies of the leptin gene in hundreds of obese human subjects had until recently failed to identify any subjects with a mutation within the coding region of the leptin gene (Considine *et al.* 1995*a*; Maffei *et al.* 1996). In human subjects plasma leptin levels have been found, in general, to correlate positively with several indices of obesity, such as BMI and percentage body fat (Considine *et al.* 1995*b*). This had led to the suggestion that 'leptin resistance' rather than leptin deficiency, is more likely to represent the underlying mechanism in most cases of human obesity.

**Abbreviations:** Ob1, Ob2, first cousins (girl and boy respectively) with severe obesity.

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### *Clinical cases*

We were recently referred an 8-year-old girl (Ob1) for investigation of her severe obesity; at the time she weighed 86 kg (> 99·6th centile). Her height (1·37 m) was on the 75th centile for her age and her percentage body fat was calculated to be 57, based on reference data (reference range for children 15–25; Fomon *et al.* 1982). She had a normal birth weight of 3·46 kg and had an unremarkable postnatal period, until 4 months of age, when her weight deviated steeply from predicted centiles (Fig. 1). Further questioning revealed a history of marked hyperphagia as the child was noted to be constantly hungry, demanding food continuously and eating considerably more than her siblings. She was never distracted from her food, which became the focus of her daily routine. There was also a history of impaired satiety, as even after a large meal she was never full and was demanding more immediately after having eaten. Her weight continued to increase exponentially throughout childhood, despite dietary advice; indeed, a period of hospital admission for marked energy restriction (2100 kJ/d) only resulted in a slight reduction in the rate of weight gain. As a result of her weight she developed marked valgus deformities of the legs for which she required bilateral proximal tibial osteotomies. She suffered substantial morbidity as a result of her extreme obesity and underwent liposuction of lower limb fat in 1994 in an attempt to improve her mobility.

Ob1 comes from a highly consanguineous family of Pakistani origin. Her parents are first cousins, have normal dietary practices and are not severely obese. She has two normal-weight siblings who are well. Ob1 has a first cousin, Ob2, who is now aged 2 years. He had a normal weight at birth (3·53 kg) but rapidly became obese, deviating from predicted centiles for weight by 3 months of age. He currently weighs 29 kg (> 99·6th centile), his height is 0·89 m (75th centile); percentage body fat was calculated as 54 using Fomon *et al.* (1982) reference data. Ob2 also has two normal-weight siblings and his parents are first cousins; they have normal eating habits and are not morbidly obese.

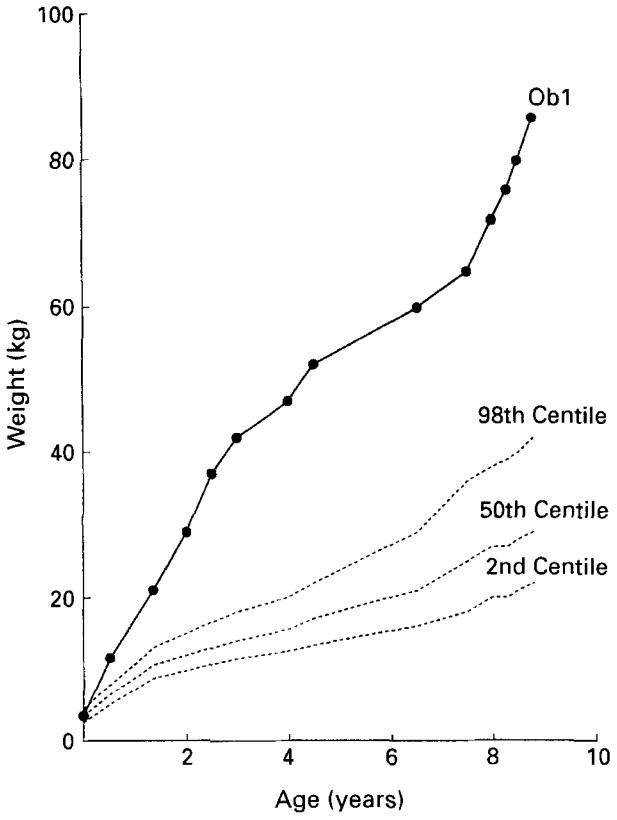


**Fig. 1.** Weight of Ob1 (8-year-old girl with severe obesity; for details, see p. 472) compared with normal centiles for girls.

Clinical examination of both children revealed no evidence of acanthosis nigricans, no evidence of any endocrine abnormality, nor features to suggest any of the recognized syndromes associated with severe childhood obesity such as Alstroms, Carpenters or Bardet-Biedl. Ob1 and Ob2 were both clinically pre-pubertal.

Previous investigations had included a normal karyotype and a normal computerized tomography scan of the brain on Ob1. Serum concentrations of luteinizing hormone, follicle-stimulating hormone, oestradiol and testosterone were at pre-pubertal levels. The 09.00 hours plasma cortisol levels in both children were within the reference range, with Ob1 having a slight elevation of plasma adrenocorticotrophic hormone. However, the administration of 1 mg dexamethasone at 24.00 hours to Ob1 completely suppressed urinary free cortisol to < 17 nmol/l. While fasting plasma glucose was normal in both children, fasting insulin levels were elevated in Ob1 (plasma insulin 158 pmol/l; normal range 0–60 pmol/l). A slight elevation of thyroid-stimulating hormone was noted for both children, but a subsequent thyrotrophin-releasing hormone test in Ob1 showed no evidence of thyroid hormone deficiency.

Not only was the pattern of weight gain similar in these two children, but the history of hyperphagia and impaired satiety was identical. In view of the highly consanguineous nature of the family, we wondered about a genetic cause for their severe, early-onset obesity, for which no other cause had been identified.

### *Leptin levels*

In view of the similarity with the phenotype of *ob/ob* mice, serum leptin levels for Ob1 and Ob2 were measured by radioimmunoassay (Linco Research, St Charles, MO, USA). They were found to be close to the limits of detection of this assay at 1·0 and 0·7 ng/ml respectively. Since serum leptin levels show a strong positive correlation with indices of obesity (Fig. 2), the finding of barely detectable levels of serum leptin in these two extremely obese children was notable.

Samples from two control groups were assayed to establish an 'in-house' set of data which is consistent with the almost linear relationship that has previously been observed between serum leptin concentration and percentage body fat. Adult control subjects were healthy UK Caucasians aged 30–39 years (*n* 30). Pre-pubertal control subjects were UK Caucasian children aged 1–11 years undergoing elective surgery (*n* 16). No control subject had undergone any recent marked change in body weight. Height (m) and weight (kg) were measured; percentage body fat was calculated for adults using the formula of Garrow & Webster (1985) and estimated for children using reference data of Fomon *et al.*


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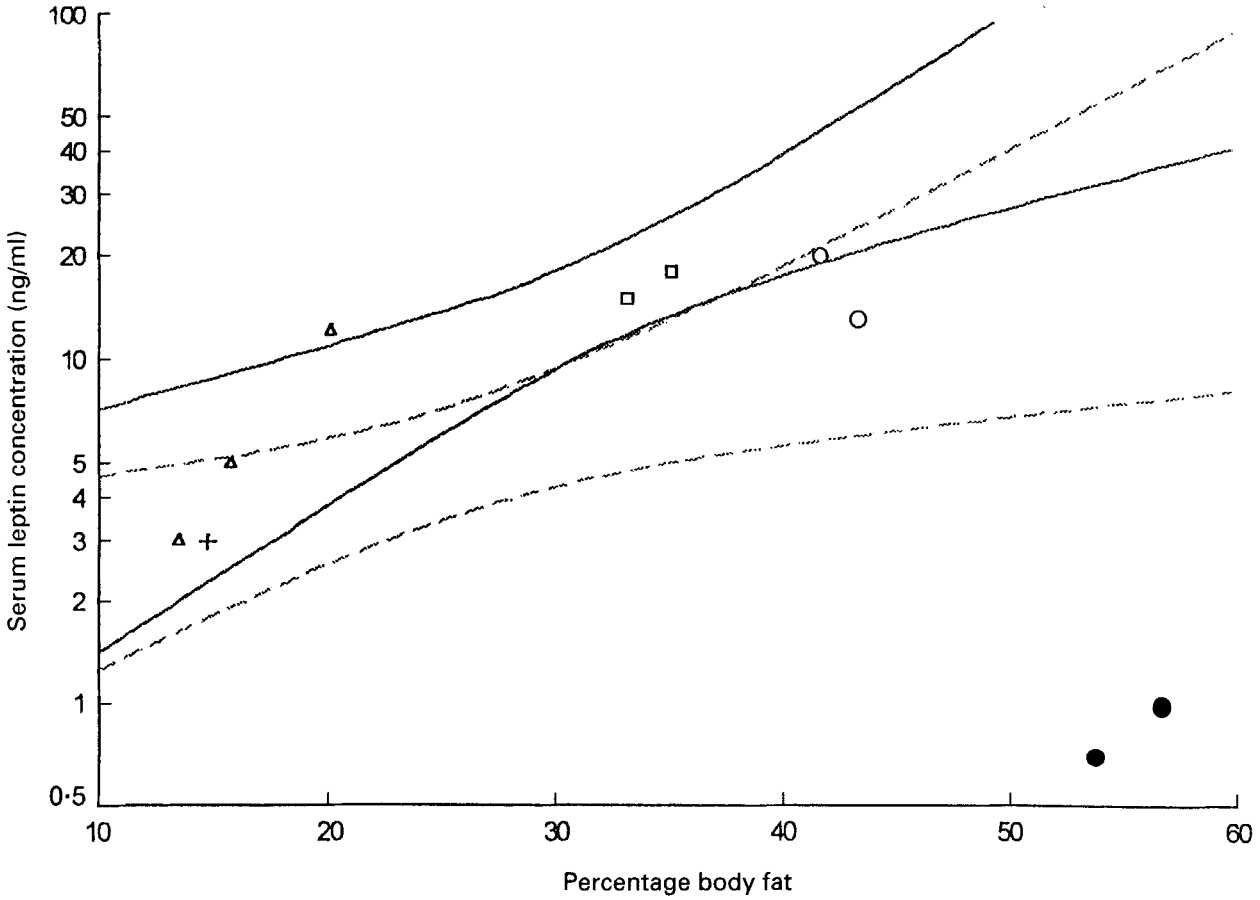


**Fig. 2.** Relationship between serum leptin concentrations and percentage body fat for two extremely-obese closely-related children (Ob1 and Ob2 (girl and boy respectively; first cousins); ●), a heterozygote sibling (+), normal siblings (△), heterozygote mothers (○), heterozygote fathers (□). (—), 95 % CI of the mean for normal adults; (~ ~), 95 % CI of the mean for normal children. For details of subjects and normal controls, see pp. 472–473.

(1982) with calculations for obese children based on the assumption that excess weight was 80 % fat. The average percentage body fat for a 70 kg UK Caucasian male is 17 and for a 60 kg UK Caucasian female is 22–25. Percentage body fat in pre-pubertal children ranges from 15 to 25. The mean serum leptin level found in sixteen normal pre-pubertal children was 8 (SD 4·5) ng/ml and in thirty adults was 23 (SD 10) ng/ml.

Serum leptin levels for two of the parents and all four siblings were within the 95 % CI of the mean for adults and children respectively. Two parents had slightly lower leptin levels than would be expected for their percentage body fat; this may be a consequence of the differences in body fat distribution in Asians compared with Caucasians (Asians have a predominantly central body fat distribution). Differences in leptin gene expression between subcutaneous and omental fat have been identified in studies based on human adipose tissue depots (Montague *et al.* 1997*b*).

As the leptin levels measured by radioimmunoassay were very low, plasma leptin levels in Ob1 and Ob2 were examined further using a highly-sensitive solid-phase sandwich enzyme immunoassay. Plasma leptin levels in Ob1 and Ob2 were 0·11 and 0·38 ng/ml respectively, again close to the detection limit of this assay (0·07 ng/ml). Finally, no immunoreactive leptin was detected in the serum of Ob2 by Western blotting (M Nicolson (Amgen, Thousands Oaks, CA, USA), unpublished results).

## Leptin gene mutation

The human leptin gene on chromosome 7q comprises three exons and encodes a 167 amino acid polypeptide. In view of the undetectable leptin levels in these two subjects, the coding region of the leptin gene was analysed by direct sequencing of polymerase chain reaction products amplified from genomic DNA from both Ob1 and Ob2. Both children were found to be homozygous for a mutation in the leptin gene; the mutation is a deletion of a single guanine nucleotide in codon 133 of the leptin gene, which disrupts the reading frame, leading to the introduction of fourteen aberrant amino acids after glycine 132, followed by a premature stop codon. It is unlikely that the mutant leptin would have any biological activity as it lacks the C-terminal cysteine involved in intra-chain disulfide bonding. Mutation of this cysteine has been reported to render the protein biologically inactive (Zhang *et al.* 1997).

All four parents were heterozygous for this mutation. Of the four siblings of the probands, one was a heterozygote and three were homozygous for the wild-type sequence. The genotype of all family members was confirmed by nucleotide

sequencing (Montague *et al.* 1997*a*). The mutation was not found by single-strand conformational polymorphism analysis in 108 alleles of UK control subjects of Pakistani origin.

*Functional studies of the mutant leptin*

Chinese hamster ovary cells were transiently transfected with expression vectors encoding either wild-type or the mutant leptin complementary DNA. Whilst 16 kDa wild-type leptin was readily detectable in the medium by immunoprecipitation, SDS–PAGE and Western blotting, no secreted mutant leptin was detected. When cells expressing the mutant leptin were lysed, a 14 kDa truncated species was readily detected, suggesting that the absence of mutant leptin in the medium was not due to a failure of the antibodies to recognize the mutant form. Thus, it is likely that the frame-shift mutation results in a form of leptin which is not targeted normally for secretion.

## Discussion

The co-existence in Ob1 and Ob2 of low immunoreactive serum leptin levels, severe obesity and a homozygous frame-shift mutation of the leptin gene are likely, therefore, to be causally linked. Thus, congenital deficiency of leptin in human subjects results in a phenotype with striking similarities to that seen in *ob/ob* mice. The *ob/ob* mouse has a normal birth weight, but rapidly exhibits hyperphagia, and is severely obese and hyperinsulinaemic (Ahima *et al.* 1996); a similar picture was observed in these two cousins who were notably born of normal weight, had a comparable pattern of eating behaviour and subsequently gained weight resulting in extreme obesity.

In contrast to *ob/ob* mice which show stunted linear growth, the heights of both Ob1 and Ob2 are at the 75th centile. However, given the age of the subjects it is not possible to comment on the future effects of leptin deficiency on the pubertal growth spurt and final adult height of these children.

The *ob/ob* mice are also characterized by hypogonadotropic hypogonadism resulting in infertility (Ahima *et al.* 1996). As Ob1 and Ob2 are both clinically pre-pubertal, with serum concentrations of luteinizing hormone, follicle-stimulating hormone, oestradiol and testosterone at pre-pubertal levels, the effects of congenital leptin deficiency on the human reproductive axis cannot, at present, be established in these children.

While fasting plasma glucose was normal in both children, fasting insulin levels were elevated in Ob1, consistent with the hyperinsulinaemia and insulin resistance seen in *ob/ob* mice. Furthermore, the markedly higher plasma insulin concentration in Ob1 (158 pmol/l) compared with Ob2 (46 pmol/l) suggests that insulin resistance may worsen with age.

The fact that all four parents and one of the four siblings of the probands were heterozygous for the frame-shift mutation in the leptin gene provided an opportunity to examine whether this genotype was associated with any phenotypic abnormalities in human subjects. None of the four heterozygous parents, nor the one heterozygous sibling, were morbidly obese, a finding which is consistent with the absence of severe obesity in the murine *ob* heterozygotes (Coleman, 1979). However, leptin levels for two of the heterozygote parents were slightly less than would be predicted by their percentage body fat and this observation will provide the basis of further studies.

This finding represents the first description in human subjects of leptin deficiency arising from a mutation in the leptin gene and in association with severe early-onset obesity. Although such mutations are likely to represent a rare cause of obesity in human subjects their true prevalence remains undetermined, and we are currently conducting a comprehensive evaluation of candidate genes in a cohort of severely-obese children to address some of these questions. These subjects illustrate how such single gene defects are able to provide unique insights into the role of leptin and other peptides in the regulation of energy balance, and allow us to explore the interaction between genes and the environment in the aetiology of human obesity.

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