

Obesity – is it a genetic disorder?

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Obesity is one of the most pressing problems in the industrialized world. Twin, adoption and family studies have shown that genetic factors play a significant role in the pathogenesis of obesity. Rare mutations in humans and model organisms have provided insights into the pathways involved in body weight regulation. Studies of candidate genes indicate that some of the genes involved in pathways regulating energy expenditure and food intake may play a role in the predisposition to obesity. Amongst these genes, sequence variations in the adrenergic

receptors, uncoupling proteins, peroxisome proliferator-activated receptor, and the leptin receptor genes are of particular relevance. Results that have been replicated in at least three genome-wide scans suggest that key genes are located on chromosomes 2p, 3q, 5p, 6p, 7q, 10p, 11q, 17p and 20q. We conclude that the currently available evidence suggests four levels of genetic determination of obesity: genetic obesity, strong genetic predisposition, slight genetic predisposition, and genetically resistant. This growing body of research may help in the development of anti-obesity agents and perhaps genetic tests to predict the risk for obesity.

Keywords: candidate genes, familial risk, genetic testing, genetics, obesity, positional cloning.

Introduction

According to the National Center of Health Statistics (2002) almost 65% of adults in the United States are overweight [body mass index (BMI) ≥ 25] and 31% of them are obese (BMI ≥ 30) [1]. In European countries, the prevalence of obesity increased also dramatically in the past 10 years. Current data from individual national studies suggest that the prevalence of obesity in western European countries ranges between 10 and 20% for men and between 10 and 25% for women. In central and eastern European women, the prevalence is even higher, ranging between 20 and 30% [2, 3]. Of particular concern for the future is the alarming rise of obesity in children and adolescents. The prevalence of

overweight children [BMI ≥ 95 th percentile BMI cut-off Centres for Disease Control and Prevention (CDC) growth charts] in the United States has more than doubled since 1976, currently exceeding 15% [1]. In 1999, the prevalence of obesity in 15–24 year olds in Europe was reported to be as high as 8% in Ireland and 11% in Greece [4]. In France, the number of obese children has increased fivefold during the past decade [5]. No longer solely confined to Western societies, obesity has increased worldwide by more than 75% since 1980 [6]. Urbanization, rapid shifts in technology and abundant availability of low-cost, highly palatable foods have altered the way people live. These changes are fuelling the obesity epidemic [7–9]. Worldwide, more than one billion adults are overweight or

obese, and there is no sign that the rapid increase in obesity seen over the past two decades is abating. The World Health Organization has declared overweight as one of the top 10 risk conditions for the overall burden of disease in the world and one of the top five in developed nations [10].

The major culprit of the recent obesity epidemic appears to be a changing environment that promotes excessive calorie intake and discourages physical activity, conditions that are poorly compensated for under the prevailing characteristics of our genome [11, 12]. Indeed, our genome has evolved under times of privation, when food was only periodically available and the risk of famine was ever present. In addition, large amounts of physical effort were required to obtain food and to fight or flight. As a consequence, modern human species are populated with individuals who are likely to be endowed with the ability to sustain biological functions with efficiency, and to store excess energy in adipose tissue and triglycerides in nonadipose tissue as well [13]. Genes that predispose to obesity may have provided survival advantage in times of famine. Adults of present-day industrialized countries have a hunter-gatherer genome but live in a sedentary, food-abundant society [14]. This mismatch between our ancient biology and present-day living circumstances can lead to energy imbalance and eventually to obesity.

Although the so called 'thrifty genotype hypothesis', put forward by James Neel in 1962 [15], postulated that such an imbalance could be traced to a genotype, the hypothesis needs to be revised to accommodate more complex multifactorial systems and the polygenic nature of the genetic predisposition to obesity. One evolutionary acquisition of *Homo sapiens* has been the ability to consume and store large amounts of dietary fats in adipose tissue. In contrast, carbohydrate and protein storage is limited and more tightly regulated [16]. Although rapid globalization of the westernized way of life is responsible for its increasing prevalence, obesity is a typical common multifactorial disease that arises through the joint actions of multiple genetic and environmental factors.

In this paper, we review the role of genetic factors in the development of obesity based on genetic epidemiological studies, monogenic forms of obesity and association and linkage studies for common types of obesity. Furthermore, we attempt to assess

how such information can be used to predict the risk of becoming obese, which may eventually be helpful in making decisions about prevention and therapy.

Genetic predisposition and interactions with the environment

Obesity results from a chronic disruption of the energy balance. The long-term relations amongst energy intake, energy expenditure, nutrient partitioning and adipogenesis determine the amount of energy stored in the body. When energy intake chronically exceeds energy expenditure and when fuel partitioning favours lipid storage and carbohydrate oxidation, the resulting imbalance causes expansion of fat cells and increased number of fat cells. Figure 1 describes a model integrating the various classes of affectors and the paths leading to obesity.

It is accepted that lifestyle and environmental factors have an influence on the determinants identified in Fig. 1. However, human obesity has also important genetic correlates that interact with relevant environmental factors. In other words, the susceptibility to obesity is partly determined by genetic factors, but an 'obesogenic' environment is typically necessary for its phenotypic expression. There is a synergistic relationship between genes and environment: in the presence of a genetic predisposition to obesity, the severity of the disease is largely determined by lifestyle and environmental conditions. When individuals living in a 'restrictive' environment evolve towards an 'obesogenic' environment, such as that found in industrialized countries, most are likely to gain weight. However, those with a high genetic predisposition for obesity

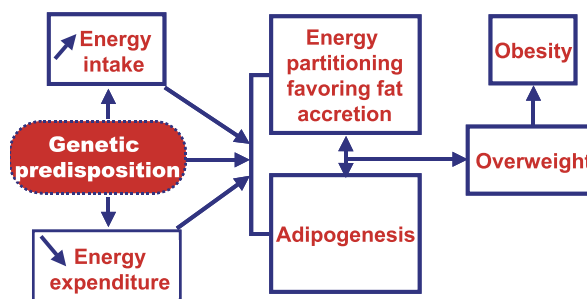


Fig. 1 Diagram of the determinants of positive energy balance and fat deposition with indication about the sites of action of a genetic predisposition (from Ref. 233).

will gain the most weight, whereas those resistant to obesity will gain little if any weight.

An example of this gene–environment interaction effect can be derived from a population apparently predisposed to obesity and type 2 diabetes, such as the Pima Indians. Pima Indians living in the ‘restrictive’ environment of the remote Mexican Sierra Madre mountains have a much lower prevalence of obesity and type 2 diabetes mellitus than those living in the ‘obesogenic’ environment of Arizona, in the south western United States [17]. Another illustration of the gene–lifestyle interaction phenomenon relevant to obesity can be found in experimental studies conducted in pairs of identical twins. The response to a positive [18] or a negative [19] energy balance treatment was found to be heterogeneous amongst twin pairs, but more homogeneous within members of the same pair. In a 100-day overfeeding study [18], 12 male monozygotic pairs ate a 1000 kcal per day surplus (6 days per week) over the energy cost for weight maintenance. There was at least three times more variance in response between pairs than within pairs for the gains in body weight, sum of skinfolds, fat mass and fat-free mass (Fig. 2). These data demonstrate that some individuals are more likely to gain fat in an obesogenic environment than others. Along the same line, seven pairs of young adult male monozygotic twins completed a negative energy balance protocol during which they exercised on cycle ergometers twice a day, nine of 10 days, over a period of 93 days, whilst being kept on a constant

daily energy and nutrient intake [19]. The exercise caused a mean total energy deficit above the energy cost for body weight maintenance of 58 000 kcal. Again, the data showed large interindividual differences in weight loss, but only small differences within pairs (Fig. 2).

These experiments confirm that the magnitude of a subject’s response to changes in lifestyle or environmental conditions (from rather restrictive to more obesogenic and vice versa) depends on a genetic predisposition thought to be largely inherited.

Evidence from genetic epidemiology

Genetic epidemiology has been helpful in defining the magnitude of the genetic contribution to obesity in a population perspective. The level of heritability has been considered in a large number of twin, adoption and family studies. Heritability is simply the fraction of the population variation in a trait (e.g. BMI) that can be explained by genetic transmission. Several studies [20–22] have indicated that genetic factors account for a substantial portion of variation in human adiposity [18, 23]. However, the reported heritability estimates range from as low as 5% to as high as 90%. Plausible explanations for this wide range of heritability estimates include the way the study was conducted and the kinds of relatives upon which they are based. For instance, studies conducted with monozygotic and dizygotic twins [24], or monozygotic twins reared apart [20, 25]

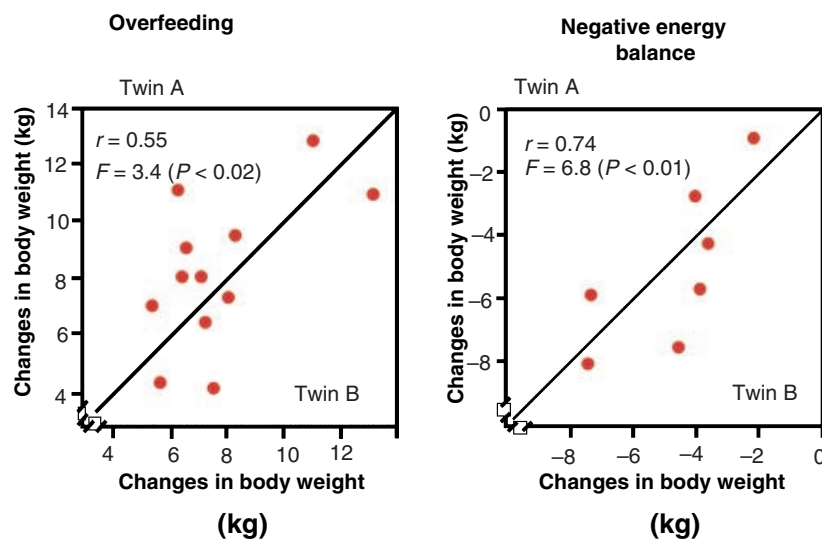


Fig. 2 Intrapair resemblance in the response of identical twins to long-term changes in energy balance. Left, 12 pairs of identical twins were submitted to an 84 000 kcal energy intake surplus over 100 days. Right, seven pairs were subjected to a negative energy balance protocol caused by exercise. The energy deficit was 58 000 kcal over 93 days. Reproduced from Bouchard [18, 19].

have yielded the highest heritability levels, with values clustering around 70%. In contrast, adoption studies have generated the lowest heritability estimates, of the order of 30% or less [26–28]. Family studies have generally found levels of heritability intermediate between twin and adoption study reports [29]. It is fair to say that, based on the recent dramatic increases in the prevalence of obesity, serious doubts have been raised in many quarters concerning high heritability values for weight-for-height phenotypes such as BMI.

Another way to estimate the importance of genes in the development of obesity is to calculate the familial risk. The risk of becoming obese when a first-degree relative is overweight or obese can be quantified using a statistic called the lambda coefficient (λ_R) or the standardized relative risk ratio. Lambda is defined by the ratio of the risk of being obese when a biological relative proband is obese compared with the risk in the population at large, i.e. the overall prevalence of obesity [30]. Estimates of λ_R based on BMI data from twin and family studies suggested that the risk of obesity, as defined by the 90th BMI percentile, is about two to three times higher for an individual with a family history of obesity. This risk tended to increase with the severity of the obesity, with estimates of λ_R of about 3–6 for the 95th percentile cut-off point [31]. Similar results have been reported in the National Health and Nutrition Examination Survey III (NHANES III). Data obtained from 2349 first-degree relatives of 840 obese probands and 5851 participants of NHANES III showed that the prevalence of obesity (BMI \geq 30) is twice as high in families of obese individuals than in the population at large [32]. Moreover, the risk of extreme obesity (BMI \geq 45) is about seven to eight times higher in families of extremely obese subjects. Data from the 1981 Canada Fitness Survey, which included 15 245 participants aged from 7 to 69 years, showed that the familial risk of obesity was five times higher for relatives in the upper 1% distribution of BMI than in the general Canadian population [33]. However, the latter study suggested that the familial risk was not entirely due to genetic factors as the spouses of probands were also characterized by an elevated risk.

These studies clearly support the contribution of genetic factors in the development of obesity, although there is an uncertainty concerning the magnitude of the genetic risk. The ultimate goal,

however, is to identify the specific genes involved. The approach to the identification of genes causing obesity is multifaceted. As no single strategy can provide all the answers, we have to rely on a variety of complementary approaches to zero in on the genes and, subsequently, on the mutations with functional implications. These main results are reviewed in the following sections. More detailed information on genes, mutations and quantitative trait loci (QTLs) for various obesity-related phenotypes are described in 'The human obesity gene map' [34] that can also be accessed on-line (<http://obesitygene.pbrc.edu>).

Monogenic forms of obesity

With few exceptions, obesity is a complex multifactorial disease. As for other complex human diseases, the identification of genes and mutations causing obesity in a small number of cases does not directly address genetic causes in the population as a whole, but can illuminate candidate pathways involved in the pathophysiology of obesity.

Mendelian disorders

In this section, we describe some of the Mendelian disorders for which obesity is a clinical manifestation but not a dominant feature, and for which causal genes have been identified. Figure 3 shows the chromosomal location of the genes and loci that have been identified for these Mendelian disorders on the ideogram of the human karyotype.

Amongst them, the Prader–Willi syndrome (PWS) is the most common (estimated prevalence of 1 : 25 000) and best characterized of the human obesity syndromes. PWS is an autosomal dominant disorder characterized by obesity, reduced foetal activity, muscular hypotonia at birth, short stature, hypogonadism, mental retardation, small hands and feet, and hyperphagia that usually develops between 12 and 18 months. Most patients (70%) have a deletion or disruption of several genes on the proximal long arm of the paternal chromosome 15 (15q11–q13) and most of the remainder have maternal disomy, i.e. two maternally derived chromosomal regions at 15q11. This 4.5-Mb PWS region at 15q11–q13 contains at least seven imprinted genes, of which five are expressed exclusively from the paternal chromosome. In some

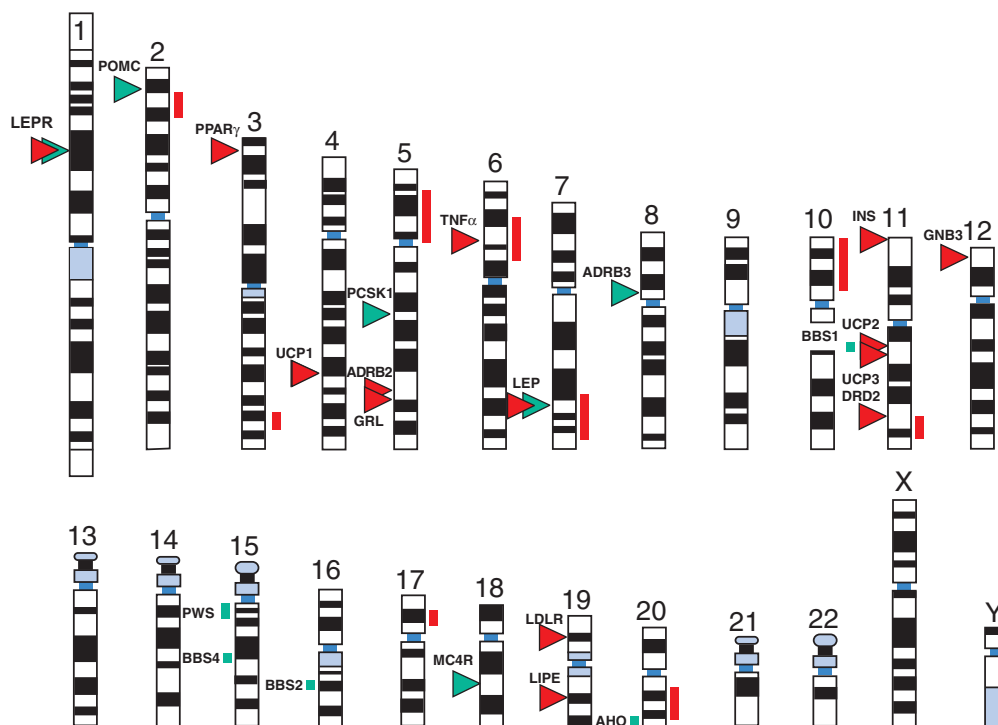


Fig. 3 Chromosomal location of obesity genes. Ideogram of human karyotype with loci for Prader–Willi Syndrome (PWS), Albright hereditary osteodystrophy (AHO), Bardet–Biedl Syndrome (BBS) (green line), monogenic mutations (green triangle), selected obesity candidate genes (red triangle) and QTLs identified by genome-wide linkage scans (red line).

patients, microdeletions of only 7.5 kb were found. This has allowed narrowing the critical PWS region to less than 4.3 kb, spanning the promoter and exon 1 of the small nucleoriboprotein N (*SNRPN*) gene [35, 36]. Recent studies suggest that deletions, mutations and/or translocations in the C/D box small nucleolar RNAs within this *SNRPN* gene cause critical loss of function for the development of the syndrome [37, 38].

The Albright hereditary osteodystrophy (AHO) is an autosomal dominant disorder. AHO is characterized by obesity, round facies, short stature, brachydactyly, subcutaneous calcifications, mental retardation in some cases, hypocalcaemia, elevated serum parathyroid hormone (PTH) level and parathyroid hyperplasia. In Japan, the prevalence of AHO is estimated at 1 in 139 000. No prevalence data in other populations have been reported yet. The AHO is due to parental imprinting of mutations in the *GNAS1* gene (guanine nucleotide-binding protein, α -stimulating activity polypeptide 1). *GNAS1* is widely expressed and encodes the α -subunit of the heterotrimeric G protein, Gs, which transduces signals between certain cell-surface receptors

(including those for PTH, thyroid-stimulating hormone and thyrotropin-releasing hormone) and intracellular adenylyl cyclase. Mutations in the gene result in reduced expression or altered function of the α -subunit of the Gs-protein. The *GNAS1* gene is located at chromosome 20q13.3, and a whole series of variants in the gene have been associated with AHO, but only one, a recurring 4 bp deletion in exon 7, is common amongst AHO patients [39]. Two phenotypical variants of AHO exist, depending on the parental origin of the mutated allele in the *GNAS1* gene. The most severe form is pseudohypoparathyroidism (PHP), in which hypocalcaemia and peripheral resistance to parathyroid hormone (PTH) are present. This form of AHO is caused by mutations in the maternal *GNAS1* [40–42]. The less-severe form is pseudopseudohypoparathyroidism (PPHP), in which no hormonal resistance is observed. It is caused by a mutated paternal allele.

The Bardet–Biedl syndrome (BBS), characterized by obesity, mental retardation, pigmentary retinopathy, polydactyly and hypogenitalism, has been the subject of intense research. British studies found

a prevalence of 1 in 160 000, whereas in the Middle East the occurrence is 1 in 13 500 due to co-sanguinity. BBS is a genetically heterogeneous disorder linked to at least seven loci [34]. Although BBS was originally thought to be a recessive disorder, the clinical manifestation of some BBS forms (BBS2, BBS4 and BBS6) requires recessive mutations at one of the loci plus an additional mutation at a second locus [43]. The *BBS1* locus was narrowed down to a 1 cM region on 11q13 [44, 45]. By positional cloning, Mykytyn *et al.* [46] identified the mutated gene (designated *BBS1* gene), on chromosome 11q34. Several mutations have been reported, amongst which the methionine-to-arginine conversion at codon 390 (Met390Arg) accounts for approximately 80% of all *BBS1* mutations [46, 47]. The *BBS2* gene maps on 16q21. Numerous mutations have been found that segregate with the syndrome. However, the function of the gene has not been determined yet [43, 48]. Interestingly, Katsanis *et al.* reported that 40% of the *BBS2* patients had also a mutation in another *BBS* gene [43]. Mykytyn *et al.* identified the gene related to *BBS4* (designated *BBS4* gene) on chromosome 15q22.3–q23 [49]. The predicted protein shows strong homology to O-linked *N*-acetylglucosamine transferase (OGT) from several species. In humans, OGT has been implicated in insulin resistance and may play a role in diabetes [50]. However, four mutations in the *BBS4* gene contributed less than 3% of affected families. Mutational data from other *BBS* genes raised the possibility that *BBS4* may participate in triallelic inheritance with *BBS2* and *BBS1* [51]. *BBS6* is caused by mutations in the *MKKS* (McKusick–Kaufman Syndrome) gene [52, 53] which encodes for a chaperonin protein that plays a role in protein integrity [54].

Although causative mutations underlying the above obesity syndromes have been identified, no clear mechanistic link between the product of mutant gene and disordered energy balance has been defined [55]. Furthermore, attempts to link loci involved in these obesity syndromes to obesity in otherwise clinically normal subjects have led to negative results. Reed *et al.* investigated the linkage relations between 17 genetic markers spanning chromosomal regions implicated in five different obesity syndromes (PWS, BBS, Cohen, Borjeson and Wilson–Turner) and the BMI in 44 families segregating for morbid obesity (average BMI of the

proband was 49.8) [56]. Sib-pair linkage analyses revealed no evidence of linkage between any of the markers and obesity in these families. These results suggest that the genetic loci contributing to obesity in these families are not the same as those involved in the most common form of obesity. However, before excluding these chromosomal regions as potential carriers of loci predisposing to common obesity, other studies with genetic markers within the syndrome causative genes must be performed.

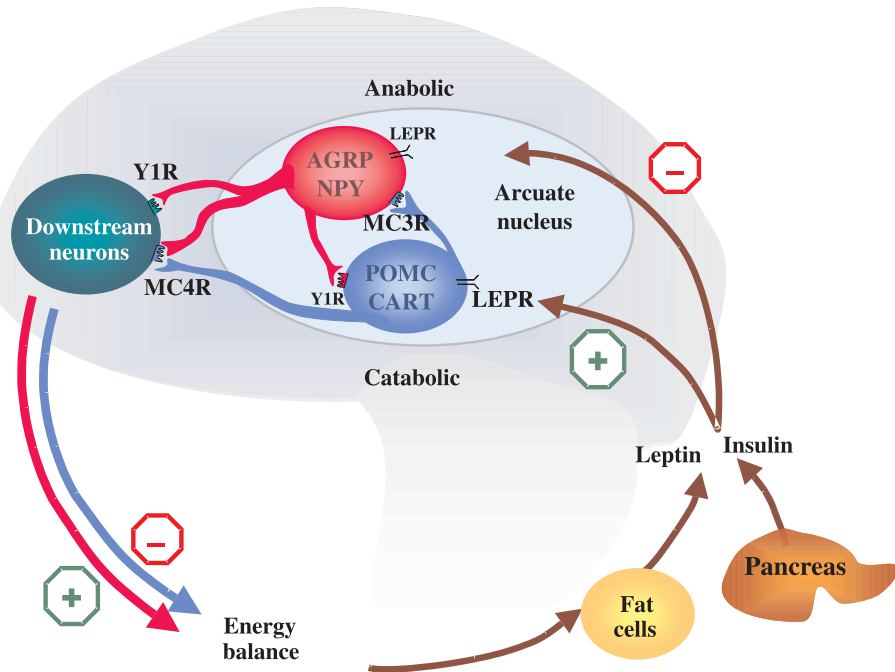
Single-gene disorders

Several mutations in human genes with homology to obesity-causing genes in mice or in genes involved in the same metabolic pathways have been identified. In such cases, obesity is the dominant feature and is largely independent of environmental factors. Although these cases are rare, they have led to a better understanding of physiological regulatory pathways of appetite and energy homeostasis. Finding that mutations in a gene cause similar phenotypes in rodents and humans underscores the fundamental and highly conserved nature of the pathway regulating energy balance. Figure 3 shows the chromosomal location of these obesity genes on the ideogram of the human karyotype.

Leptin and leptin receptor. Several mutations that arose spontaneously in mice have been shown to cause obesity. The first breakthrough resulted from innovative parabiosis experiments performed with obese *ob/ob* and *db/db* mice [57]. By surgical cross-anastomosis between the circulatory systems of obese and wild-type animals, an adiposity-sensing pathway, regulated by a circulating hormone and its cognate receptor, was predicted. This prediction was confirmed by the cloning of the *ob* [58] and *db* [59] genes and the characterization of their products, known as leptin and leptin receptor, respectively.

Leptin is an endocrine hormone primarily secreted by adipocytes. Leptin participates in many biological pathways [60]. One of its key roles is that of communicating to the brain information on long-term energy stores. Leptin circulates at levels proportional to body fat content and enters the central nervous system (CNS) in proportion to its plasma concentration. The primary site of leptin action is in the hypothalamus, where its absence triggers a

Fig. 4 Central pathways contributing towards the regulation of energy intake and energy expenditure. Insulin and leptin are hormones that circulate in proportion to body fat stores; they inhibit AGRP/NPY neurones and stimulate adjacent POMC/CART neurones. Lower insulin and leptin levels are predicted to activate AGRP/NPY neurones, whilst inhibiting POMC/CART neurones. AGRP and NPY are neuropeptides that stimulate food intake and decrease energy expenditure, whereas α -melanocyte stimulating hormone and CART are neuropeptides that inhibit food intake and increase energy expenditure. AGRP, agouti-related protein; CART, cocaine- and amphetamine-regulated transcript; Lepr, leptin receptor; MC3R/MC4R, melanocortin 3/4 receptor; NPY, neuropeptide Y; POMC, Pro-opiomelanocortin; Y1r, neuropeptide Y1 receptor.



series of neuroendocrine responses that conserve energy when food availability is limited. As illustrated in Fig. 4, the anabolic NPY- and AGRP-containing neurones and the catabolic POMC-containing neurones are direct targets of leptin action [60, 61].

Mice carrying the *ob* or *db* mutations exhibit identical phenotypes, characterized by early-onset obesity, hyperphagia, low core temperature, insulin resistance and susceptibility to diabetes mellitus [58, 59]. The *ob* mutation results in a lack of leptin production.

In humans, only a few patients carrying a mutation in the leptin (*LEP*) gene have been described. Three children, two cousins and one unrelated boy from Pakistani origin, were homozygous for a frameshift mutation (G398) that resulted in a truncated leptin [62, 63] with an aberrant C-terminus [64]. The children were extremely obese from a young age and had severe hyperphagia. Heterozygous relatives of these children had subnormal leptin levels, a higher than expected prevalence of obesity and a higher percentage of body fat compared with ethnicity-matched controls [65]. In addition, three adults and one child of a highly consanguineous kindred homozygous for a mutation (C105T) in exon 3 of the *LEP* gene have been reported [66, 67]. All four had extremely low serum leptin levels and exhibited early-onset morbid

obesity with hyperphagia. These patients were also characterized by hypogonadotropic hypogonadism, indicating a possible role of leptin in the initiation of puberty and reproduction [66]. Interestingly, subcutaneous administration of recombinant human leptin for up to 4 years had major and sustained beneficial effects on the multiple phenotypic abnormalities associated with the congenital human leptin deficiency [63, 68].

The *db* mutation in mice is a single base substitution in the leptin receptor gene, which deranges the normal splicing of the gene. The subsequent abnormal splicing creates a transcript with a premature stop codon encoding for a receptor form lacking the normal intracellular C-terminus motif, which is critical for tyrosine kinase activation [69, 70]. Different mutations in the leptin receptor gene have also been identified in two rat models of obesity, the Zucker and Koletsky rats. Both the *fa/fa* mutation in the Zucker rat and the *f/f* mutation in the Koletsky rat are mutations in the extracellular domain of the leptin receptor, resulting in decreased expression of the receptor on the cell surface and hence a diminished leptin signal [71]. Interestingly, the phenotype of these two obese rat strains is additionally characterized by dyslipidaemia and, in case of the Koletsky rat, by hypertension.

In humans, only one family to date has been identified with a leptin receptor (LEPR) mutation [72]. In homozygotes for the mutation, a truncation of the receptor before the transmembrane domain abolishes leptin signalling leading to hyperphagia and massive obesity soon after birth. The homozygous patients did not spontaneously undergo puberty. These symptoms are similar to those of individuals with a LEP deficiency. A striking difference with LEP patients is that homozygous LEPR patients show significant growth retardation and hypothalamic hypothyroidism. This suggests that in humans, even in the absence of circulating leptin, an intact leptin receptor maintains its capacity to stimulate certain hypothalamic-releasing factors. This would explain why the loss of function of the leptin receptor results in a more severe phenotype compared with that caused solely by the lack of leptin. The heterozygous LEPR family members were overweight or moderately obese. Their leptin levels were about half those of the homozygous LEPR patients, indicating that the LEPR mutation may have a pleiotropic effect.

Following the cloning of the *ob* and *db* genes, considerable attention is now focused on deciphering the neural pathways that mediate the neuroendocrine and metabolic responses to the action of leptin (Fig. 4).

Pro-opiomelanocortin. Pro-opiomelanocortin (POMC) is post-transcriptionally processed to produce a number of hormones in the hypothalamic–pituitary–adrenal axis, such as α -melanocyte-stimulating hormone (MSH), adrenocorticotrophic hormone (ACTH) and β -endorphin. These POMC-derived neuropeptides are physiological agonists of the melanocortin-4 receptor (MC4R). Normally, the hypothalamic synthesis of α -MSH is stimulated by increased leptin levels, and the signal generated by α -MSH at MC4R promotes energy expenditure and decreases food intake (Fig. 4) [73].

Mutations in the *POMC* gene were found in two patients from unrelated families [74]. One patient was a compound homozygote for two mutations in exon 3. A G-to-T transversion in the paternal allele at nucleotide 7013 (G7013T) resulted in a premature truncation at codon 79. This truncation of the protein predicted a complete absence of ACTH, α -MSH and β -endorphin. In the maternal allele, a 1 bp deletion (C7133A) caused a frameshift

mutation which was predicted to disrupt the structure of the receptor-binding core motif of ACTH and α -MSH and introduced a premature termination at codon 131. Compound heterozygosity was also found in the brother of this patient. He died at 7 months of age due to hepatic failure following severe cholestasis, which was caused by adrenal insufficiency due to bilateral adrenal hypoplasia. A second patient was homozygous for a C-to-A transversion at nt 3804 (C3804A) in exon 2, which abolished *POMC* translation. Both patients had developed early-onset obesity with hyperphagia and exhibited a constellation of symptoms reflecting the lack of neuropeptides derived from the *POMC* gene. The lack of α -MSH was responsible for the ensuing obesity (a result of the absence of the melanocortin ligand for MC4R) as well as altered pigmentation and red hair (from the absence of the ligand for the MC1R). Additionally, the lack of ACTH, an MC3R agent, led to adrenal deficiency.

In rodents, no naturally occurring mutations in the *POMC* gene have been reported. However, the role of POMC in the central melanocortin pathways and energy homeostasis has been confirmed by experiments with transgenic animals with a targeted deletion of *POMC* gene [75].

The melanocortin-4 receptor. The importance of the *MC4R* gene in the regulation of human body weight became apparent in 1998 when the first mutations in the human *MC4R* gene were described in some human obese patients [76, 77]. Two groups reported heterozygous frameshift mutations that co-segregated in a dominant fashion with severe early-onset obesity. Subsequently, more than 30 different mutations, including missense, nonsense and frameshift mutations, in the *MC4R* gene have been reported in French, English, German, American, Italian and Spanish individuals [78–90]. The vast majority of subjects thus far described are heterozygotes, with only six homozygotes [81, 89] and one compound heterozygote reported [79].

MC4R is a seven-transmembrane G protein-coupled receptor that activates the cyclic adenosine monophosphate second-messenger system. Functional studies showed that many of the missense mutations, and all three frameshift mutations described so far, lead to a complete or partial loss of function of the *MC4R* gene [78, 80, 87, 89, 91, 92]. In a recent study, Farooqi *et al.* found that the

signalling properties of mutant MC4R receptors, as determined in cell cultures, correlated with the severity of obesity [89]. The study included 500 probands with severe childhood obesity of whom 29 had mutations in the *MC4R* gene. Heterozygotes for mutations that abolished MC4R signalling had a higher average BMI than those for mutations resulting in partial retention of receptor signalling. Similarly, subjects who were homozygous for such *MC4R* mutations had a higher average BMI than heterozygous carriers of the mutations. These findings indicate that the obesity resulting from mutations in *MC4R* is associated with a co-dominant mode of inheritance. In this regard, obesity caused by *MC4R* mutations is similar to the more common forms of obesity in comparison with the previously described single-gene disorder.

The frequency of the *MC4R* mutations causing obesity is controversial, with estimates ranging from under 0.5% [80, 86, 87] to more than 4% [79, 81, 89, 90]. This wide range may be explained by the difference in prevalence of such mutations in certain ethnic groups, but it may also reflect variations in age of onset and severity of obesity.

Carboxypeptidase E and prohormone convertase 1. Impaired processing of POMC appears to be the cause of obesity in the *fat* mouse. The *fat* mutation is an inactivating mutation of the gene encoding for carboxypeptidase E (*Cpe*) [93]. This enzyme is required for the post-translational cleavage of C-terminal amino acid residues from many prohormones and proneuropeptides, such as pro-insulin, proneuropeptide Y, pro-gonadotrophins and POMC. The *fat* mutation encodes a nonfunctional *Cpe*, which results in the secretion of incompletely processed precursors that lack the biological activity of the normal peptides. Consequently, the *fat* mouse exhibits multiple endocrine disorders, including hyperinsulinaemia, infertility and hypoadrenalism, as well as late-onset obesity, the latter ensuing from the lack of α -MSH [93].

Cases of human obesity syndromes caused by mutations of the *Cpe* gene homologue have not yet been identified. However, mutations have been described in the prohormone convertase 1 gene (*PCSK1*). Like *Cpe*, *PCSK1* is involved in the post-translational processing of prohormones and neuropeptides. In a woman who had extreme childhood obesity, abnormal glucose homeostasis, and clinical

manifestations of defective prohormone processing, Jackson *et al.* [94] identified compound heterozygosity for two mutations in the *PCSK1* gene. One mutation was a Gly483Arg substitution, whilst the second was an A-to-C transversion, close to a donor splice site, which resulted in the skipping of exon 5 and the creation of a premature stop codon within the catalytic domain of the enzyme. Three of her four clinically unaffected children had the Gly483Arg missense mutation, whilst the fourth child had the splice site mutation.

In rodents, other naturally occurring single-gene mutations causing obesity have been described, including in the agouti (*Ay*), mahogany (*mg*), tubby (*tub*), growth hormone (*gh*), cholecystokinin A receptor (*Cckar*) and lipin1 (*Lpin1*) genes [34]. So far, mutations in the human homologues of these genes have not been associated with obesity in humans.

It is notable that most of the aforementioned naturally occurring mutations in rodent models of obesity concern genes encoding for molecules that mediate pivotal steps of the same principal regulatory pathway. This complex pathway interacts with a variety of neural circuits of the CNS, implicating a plethora of other neurotransmitters and neuropeptides in the homeostatic weight regulation system, as illustrated in Fig. 4. The intricate functional relationship between these pathways appears to have several critical control points that are presently being examined. So far, no naturally occurring mutations that cause obesity due to reduced metabolic rate have been reported.

Polygenic/common forms of obesity

Individuals affected with Mendelian obesity syndromes or single-gene disorders represent only a small fraction of the obese population and cannot explain the magnitude of the obesity problem that industrialized societies are facing today. Obesity is a complex multifactorial phenotype; interindividual variation in such phenotypes is thought to result from the action of multiple genes and environmental factors.

Human studies to identify the specific genes involved in common obesity are currently dominated by three types of strategies. A first approach is the candidate gene approach that relies on the current understanding of the pathophysiology of

obesity. The candidate genes are selected on the basis of their perceived role or function in biochemical pathways related to the regulation of energy balance or to adipose tissue biology. A second approach is to perform genome-wide linkage scans with a view to identifying chromosomal regions of interest, the so-called QTLs, and eventually genes within these QTLs. A third approach is based on tissue-specific gene expression profile comparing lean versus obese individuals and other informative samples. The latter will not be reviewed here.

Candidate genes

Currently, positive associations with obesity phenotypes have been reported for more than 70 genes [34]. Some of these candidate genes pertain to body mass, body fat, or fat distribution, whereas others are retained for their potential contributions to the regulation of energy intake, energy expenditure, or nutrient partitioning.

Overall, the results of these association studies have not been very consistent and when associations were found, they were relatively weak. Here we focus on a few selected candidate genes for which the positive findings have been replicated in independent studies. Figure 3 also shows the chromosomal location of these obesity genes on the ideogram of the human karyotype. A comprehensive review of all candidate gene studies is beyond the scope of the present paper. However, the reader is referred to the paper by Chagnon *et al.* for a more comprehensive summary of these genes [34].

Candidate genes involved in the regulation of energy expenditure. Although all single-gene disorders causing obesity identified thus far result from defective genes altering primarily food intake, most association studies have focused on genes that are involved in pathways of energy expenditure and lipid and adipose tissue metabolism.

The mitochondrial uncoupling proteins (UCPs) have been examined extensively for association with energy expenditure phenotypes. UCP1 dissipates the proton electrochemical gradient across the mitochondrial membrane, thereby uncoupling substrate oxidation from conversion of adenosine diphosphate to adenosine triphosphate, leading to generation of heat. UCP1 plays an important thermogenic role in brown adipose tissue. The lack of substantial brown

adipose tissue in adult humans suggests that UCP1 has a limited role in thermogenesis in man. A handful of studies have reported a weak, although significant, association between polymorphisms in UCP1 and obesity-related phenotypes such as BMI or changes in body weight and percentage body fat [95–99], but no association has been reported by others (six studies) [34] (to limit the list of references, we refer to the paper by Chagnon *et al.* [34] for the inventory of the negative studies).

Unlike UCP1, UCP2 is widely expressed, whereas UCP3 is predominantly expressed in skeletal muscle, a major tissue contributing to nonshivering thermogenesis in humans. Since the mapping of the UCP2 and UCP3 genes, many researchers have reported association between allelic variation in these genes and resting energy expenditure, respiratory quotient, BMI, waist-to-hip ratio and other obesity-related phenotypes. The coding region of the UCP2 gene exhibits little variability and only one common genetic variant, Ala55Val, has been identified. Sleeping metabolic rate, 24-h energy expenditure and 24-h respiratory quotient were significantly associated with this UCP2 variant [100, 101]. However, no association was found with age of onset of obesity [102]. In the 3' untranslated region (3'UTR), 158 bp downstream of the stop codon, a prevalent 45 bp insertion polymorphism causes a change in the stability of the transcript [103]. Significant associations were found between this UCP2 variant and BMI, body weight, weight gain, percentage body fat, skinfold thickness, 24-h energy expenditure and sleeping metabolic rate in most [100, 101, 103–109] but not all studies (two studies) [34].

In the UCP3 gene and its promoter, at least 14 polymorphisms have been identified [110]. Although functional analyses showed that several of these UCP3 polymorphisms resulted in a truncated protein, an increased expression and a reduced or completely absent activity of the protein, the results of association studies are inconclusive. Positive associations were found with BMI, percentage body fat, waist-to-hip ratio, change in skinfold thickness, respiratory quotient and resting metabolic rate [99, 100, 108, 109, 111–115]. However, many other studies ($n = 7$) reported no effect of these UCP3 variants on obesity-related phenotypes [34].

The adrenergic system plays a key role in the regulation of energy balance through the stimulation

of thermogenesis and lipid mobilization. Lipolysis in fat cells can be stimulated by catecholamines (amongst other hormones) through β -adrenoceptors (β -AR), and inhibited through α_2 -adrenoceptor (α_2 -AR) [116].

The β_2 -adrenoceptor (β_2 -AR) is the most abundant lipolytic adrenoceptor subtype and is downregulated in subcutaneous adipose tissue of obese subjects. Polymorphisms in the β_2 -AR have been associated with obesity-related phenotypes in several ethnic groups [117–125], but these associations could not be confirmed by others (seven studies) [34]. The most commonly studied polymorphisms are Gly16Arg and Gln27Glu. The Gly16 form of the receptor shows enhanced downregulation *in vitro*, whilst the Glu27 allele exhibits attenuated downregulation after exposure to β -agonists [126].

The cloning of the human β_3 -adrenoceptor (β_3 -AR) generated excitement because of its thermogenic, anti-obesity, and antidiabetic properties in animal models [127]. The human β_3 -AR gene is expressed predominantly in infant peripheral brown adipose tissue. In adults, it is expressed at low levels in deep fat, such as perirenal and omental, but at much lower levels in subcutaneous fat [128]. It is also highly expressed in the gallbladder but to a lower extent in the colon, suggesting a potential overall role in the control of lipid metabolism and triglyceride storage and mobilization in adipose tissues [128, 129]. A Trp64Arg mutation located in the first transmembrane domain of the receptor was first weakly correlated with some obesity and insulin resistance phenotypes in Pima Indians [130], and in French [131] and Finnish [132] populations. Significant associations were subsequently reported for the same and other obesity-related phenotypes, such as waist-to-hip ratio, waist circumference, fat mass, abdominal visceral and subcutaneous fat and basal metabolic rate [133–138]. In contrast, a large number of studies failed to replicate these findings in the same and in other populations [34].

Three meta-analyses of the association between the Trp64Arg polymorphism and BMI have been reported [139–141]. The meta-analysis of Allison *et al.* pooled 23 studies including 7399 subjects [139]. Despite the high statistical power, the difference between the Trp64Trp homozygotes and Trp64Arg heterozygotes for mean BMI was not significant (mean difference = 0.19 kg m⁻², $P = 0.07$). Fujisawa *et al.* included eight more

studies (31 studies, 9236 subjects) and found a significantly higher BMI for the Arg-carriers compared with the Trp64Trp homozygotes (mean difference = 0.30 kg m⁻², 95% CI: 0.13–0.47) [140]. In a more recent meta-analysis, data of 27 studies that included only Japanese subjects ($n = 6582$) were pooled [141]. The mean BMI of Arg-carriers was 0.26 kg m⁻² (95% CI: 0.18–0.42, $P < 0.01$) higher than in the Trp64Trp homozygotes.

Despite the fact that the functional correlates of some of these AR polymorphisms (changes in agonist-promoted downregulation, protein expression levels, lipolytic sensitivity, basal metabolic rate, sympathetic nervous system activity) suggest that they may be important in the aetiology of obesity, the data indicate that their role, if any, in human obesity is modest. The end result may depend on population-specific characteristics such as ethnic origin, diet, exercise and environmental factors, i.e. gene–gene or gene–environment interactions.

In this respect, the interactions between polymorphisms in the *UCP1* gene and the β_3 -AR have been studied. The simultaneous presence of the –3826G *UCP1* variant and the Arg64 β_3 -AR variant was associated with a tendency to gain weight in four populations [106, 142–144]. Both the α_2 -AR and β_2 -AR genes showed significant interactions with the glucocorticoid receptor gene in their influence on total abdominal fat area [145]. Gene–gene interactions have also been reported amongst the ARs. Significant interactions have been observed between the β_3 -AR and α_2 -AR genes for total and subcutaneous body fat in the Quebec Family Study [146]. After a 20-week endurance training programme, the decrease in total and subcutaneous body fat was more pronounced when subjects were carriers of both the β_2 -AR Arg16 and β_3 -AR Arg64 polymorphisms [106, 144, 147]. In addition, gene–environment interactions were reported for polymorphism in the β_2 -AR and physical activity. In a large French cohort, highly significant associations between body weight, BMI and waist and hip circumferences, and the Gln27Glu polymorphism were observed in sedentary subjects, but not in the physically active [123].

Another candidate gene is peroxisome proliferator-activated receptor gamma (PPAR γ). PPAR γ is a nuclear receptor, which upon activation with various natural and synthetic ligands, stimulates the transcription of genes responsible for growth and

differentiation of adipocytes. PPAR γ is also the receptor for the thiazolidinedione class of insulin-sensitizing drugs. In humans, three PPAR γ mRNA isoforms have been identified which are formed by alternative promoters and differential splicing [148]. Of the three PPAR γ isoforms, PPAR γ 2 mRNA is the most abundantly and relatively specifically expressed in the adipose tissue. Using real-time RT-PCR, it has been shown that PPAR γ 2 mRNA levels were increased in adipocytes from morbidly obese subjects [149]. This makes PPAR γ 2 a candidate gene for the regulation of body weight [148, 150]. In relation to obesity, the Pro12Ala variant has been most frequently studied. This is a common variant in an alternatively spliced exon B of the PPAR γ 2 isoform and is present in most populations. The Ala-allele results in a reduced *trans*-activation capacity [151]. The role of the Pro12Ala variant in the development of obesity is controversial. The Ala12 allele has been variably associated with lower BMI and greater weight loss [152–154], but more frequently with higher BMI, greater waist circumference, and greater increase in body weight [154–163]. However, many others reported no association between the Pro12Ala variant and obesity-related phenotypes (14 studies) [34].

Although these genes are involved in pathways of energy expenditure and lipid and adipose tissue metabolism, most associations were observed with obesity-related and not energy expenditure phenotypes. Two studies reported significant associations between UCP2 polymorphisms and sleeping metabolic rate [100, 101], 24-h energy expenditure [101], and 24-h respiratory quotient [101]. Other studies found a significant association between UCP3 variants and resting metabolic rate [99, 115] and respiratory quotient [111]. Subjects with the Trp64Arg mutation in the β_3 -AR gene had a lower basal metabolic rate compared with the Trp64Trp homozygotes in two Finnish populations [137, 164].

Candidate genes involved in the regulation of energy intake. Based on the findings in knockout mice and in humans with a single-gene disorder, leptin and its receptor can be considered as legitimate candidate genes for the development of obesity. Several polymorphisms in the coding region and the 5' and 3' flanking region of the *LEP* gene have shown association with body weight, weight loss and BMI

[165–168]. However, not all studies could confirm this association [169]. As described above, leptin circulates at levels proportional to body fat content. Circulating leptin levels are, therefore, accepted as a measure of obesity. Several studies reported associations between *LEP* gene variants and circulating leptin levels [170–179].

Also throughout the length of the *LEPR* gene, a number of sequence variants have been identified. The Gln223Arg polymorphism, which causes an amino acid substitution in the extracellular domain of the leptin receptor, has been associated with adiposity and body composition in a number of populations [178, 180–184], but not in all (three studies) [34]. Recently, a meta-analysis with data pooled from nine studies showed that there was no statistically compelling evidence that any of the three studied *LEPR* alleles (K109R, Q223R and K656N) was associated with BMI or waist circumference in the overall population. However, certain genotypic effects could be population-specific [185]. Although a body of compelling physiological data indicate that leptin might play a significant role in the development of obesity, only a handful of mutations/single-nucleotide polymorphisms appear to be functionally associated.

Whereas rare mutations in the *LEP* gene and *LEPR* cause severe early-onset obesity, more common polymorphisms have only a minor influence on the development of obesity.

Association studies between obesity-related phenotypes and other key proteins of the central signalling system such as POMC, AGRP, MC4R and NPY, are limited, and replication of these results is required. Although these genes are involved in pathways regulating energy intake, no associations between gene polymorphisms in these genes and food intake or eating behaviour have been reported.

Genome-wide scans and positional cloning for obesity-related phenotypes

Genome-wide scans can be used to detect chromosomal regions showing linkage with obesity in collections of siblings, nuclear families and large pedigrees. Linkage analysis identifies QTLs that are co-segregating with a phenotype. It takes approximately 400 markers to cover the whole genome at about 10-cM intervals. When strong evidence for linkage is found, then it becomes useful to screen

that region with a denser map of markers. If the signal remains strong, the relevant chromosomal region can be further scrutinized with a variety of techniques. Thus, a genome scan is used to identify positional candidate genes and offers the potential of identifying new or previously unsuspected genes influencing the phenotype of interest. One can expect that, with appropriate sample sizes and study designs, individual genes accounting for as little as 10% of the variance in a trait can be localized to specific chromosomal regions using the genome-wide scan approach [186].

It has been a little more than 6 years since the publication of the first genome scans focusing on obesity-related phenotypes in humans. Since then, more than 30 genome-wide scans for obesity-related phenotypes have been reported and at least 70 putative loci affecting obesity-related phenotypes have been found, distributed over all chromosomes except the Y chromosome [34]. A handful of studies have reported highly significant linkages. Even more important perhaps is the fact that several of the linkage findings have been replicated as well. Figure 3 shows the QTLs for obesity-related phenotypes that have been identified in at least three genome-wide linkage scans.

The strongest evidence for a QTL influencing obesity-related phenotypes comes from the San Antonio Family Heart Study in which a log odds ratio (LOD) score of 4.9 was observed on chromosome 2p22 for serum leptin levels [187]. The sample included more than 5000 related pairs of Mexican Americans. After typing additional markers in a subsample of this population (337 subjects from 10 families) the LOD score for the QTL increased to 7.5 [188]. This QTL has been weakly replicated in a number of samples, including French (LOD = 2.4–2.7) [189] and African Americans ($0.008 < P < 0.03$) [190]. In white people, the 2p22 region showed also promising linkage (LOD = 2.7) with circulating adiponectin levels [191]. Adiponectin is an adipocyte-derived protein expressed inversely to total fat and is thought to play a role in the obesity-related risks for coronary artery disease and type 2 diabetes mellitus [192, 193]. Although the precise function of the adiponectin remains unclear, the circulating levels are inversely correlated with BMI [194], and the mRNA level is suppressed in the adipose tissue of obese animals and humans [195]. Further, evidence of linkage between

this chromosomal region and diabetes was reported in a French family study (LOD = 2.3) [196]. It is known that obesity is correlated with diabetes, and it is possible that the same genes have pleiotropic effects on both obesity and diabetes. A strong candidate gene for obesity in this region is POMC.

In a large family study that included 2209 individuals from 507 white families, significant linkages were found between markers on chromosome 3 (3q27) and six traits related to obesity (including BMI, waist and hip circumference) with LOD scores ranging from 2.4 to 3.5 [197]. Recently, results from the National Heart, Lung, and Blood Institute Family Blood Pressure Program (6849 individuals from four ethnic groups) supported these findings. They reported a significant linkage (LOD score = 3.4) between the same marker (D3S2427) and BMI [198]. Promising linkage at 3q27 was also reported for BMI in an African American population (LOD = 1.8) [199], and suggestive linkage for plasma leptin levels in Pima Indians (LOD = 1.5) [200]. The 3q27 locus was previously identified as a type 2 diabetes mellitus locus in a French population [196]. An adjacent region on chromosome 3, 3q26, showed promising linkage (LOD = 2.5) with abdominal subcutaneous fat in black people of the HERITAGE Family Study [201]. Several potential candidate genes have been identified in this region. Amongst these genes, the APM1 region encoding adiponectin (at 3q27) seems the most interesting. It has been further suggested that the gene for one of the glucose transporters (*GLUT2*, at 3q26–q27) is also relevant [197]. Another candidate gene is apolipoprotein D (*Apo-D*) (at 3q26). *Apo-D* is a component of high-density lipoprotein and involved in the action of the enzyme lecithin : cholesterol acyltransferase. In an association study, it was found that a marker in *Apo-D* was more common in persons with obesity ($P = 0.006$) [202].

There is a growing body of support for an obesity QTL on chromosome 5p-cen within populations of western European origin and perhaps in African Americans and Pima Indians as well. A genome-wide scan based on 1100 subjects from 170 families of northern European ancestry showed strong evidence (LOD = 4.1) for a QTL at 5p15.3 linked to plasma adiponectin levels [191]. In African Americans, the same locus showed promising linkage (LOD = 1.9) with BMI [199]. A linkage (LOD = 2.9) between markers closer to the centromere (5p13.2) and

plasma leptin levels was observed in French families [189]. In a study with 846 individuals from 235 Pima Indian families, Lindsay *et al.* incorporated parent-of-origin effects in the genome-wide scan to examine the hypothesis that imprinted genes may affect obesity [203]. Weak evidence of linkage of BMI to maternally derived alleles was found on chromosomes 5p13.1–q11.2 (LOD = 1.7).

Three independent studies have provided evidence for a QTL on chromosome 6p. A first genome-wide scan, performed with 770 Pima Indians from 239 families, revealed a linkage for age- and sex-adjusted leptin levels at 6p21.1 (LOD = 2.1) [200]. In 618 subjects from 202 African American families, evidence of linkage (LOD = 2.7) was found for percentage body fat on 6p22.3 [199]. The first genome scan for phenotypes related to eating behaviour was performed in 624 subjects from 28 Amish families. A linkage was found for dietary restraint on chromosome 6p22.1 (LOD = 2.3). These linkages lie in proximity to the genes encoding glucagon-like peptide 1 (GLP-1) receptor, tumour necrosis factor alpha (TNF- α), and lymphotoxin. GLP-1 receptors are found in the hypothalamus and are the target for hypothalamic neurones containing glucose transporter 2 and glucokinase. GLP-1 secretion after a meal stimulates insulin release, lowers blood glucose, and reduces food intake [204]. TNF- α and lymphotoxin are cytokines known to be expressed in adipocytes. TNF- α may have a role in the development of insulin resistance and obesity [205, 206].

Replicated linkages were also reported for a QTL on chromosome 7q. In 672 individuals of 28 Old Order Amish pedigrees of European origin, a QTL spanning 7q31–36 showed linkage (LOD = 1.9) with leptin adjusted for BMI [207]. Genome-wide scans were performed in two samples of families participating in the National Heart, Lung, and Blood Institute Family Heart Study [208]. The first sample included 1184 individuals from 317 sibships, and the second 3027 subjects from 401 three-generation families. Strong evidence of linkage with BMI was found in each sample, as well as in the combined sample on 7q31.3–q32.3 ($3.2 < \text{LOD} < 4.9$). A third genome-wide scan with 1256 black people of the HyperGEN study showed evidence of linkage at 7q22.3 for BMI (LOD = 2.4) [198]. An obvious candidate in this region is the *LEP* gene that maps to 7q31.3. Several studies that targeted the *LEP* gene have reported evidence of linkages for various

anthropometric measures in Mexican Americans and French Canadians [209–211].

Chromosome 10p also shows a pattern of replications for an obesity QTL. Using an affected sib-pair approach, a significant linkage (LOD = 4.9) at 10p12–p11 was reported for BMI in 158 French families [189]. A genome-wide scan in 1100 adults of northern European ancestry showed suggestive linkage (LOD = 1.9) at 10p12–p11 for adiponectin levels [191]. The same locus showed also suggestive linkage (LOD = 2.7) for BMI-adjusted leptin in an Old Order Amish population [207]. Other linkage studies that targeted this chromosomal region have confirmed that 10p12–p11 may harbour genes for obesity-related phenotypes [212, 213].

The region harbouring the largest number of replications is chromosome 11q23–24, with linkages reported in four different studies. In 236 sib pairs from 82 Pima Indian families, a QTL for 24-h energy expenditure was found at 11q23 (LOD = 2) [214]. Two other genome-wide scans in Pima Indians revealed a QTL in this region for BMI [203, 215]. These three genome-wide scans were based on Pima Indians participating in the same survey of diabetes of the National Institutes of Health in the Gila River Indian community in central Arizona [216]. Therefore, these findings are not completely independent. However, a fourth study of 479 male subjects from 14 pedigrees from Utah found linkages ($2.6 < \text{LOD} < 2.8$) for BMI with three markers localized at 11q24.1–24.3 [217]. Three genes/gene clusters in this chromosome 11 interval are candidates for obesity. The first is a cluster of three matrix metalloproteinase genes (*MMP1*, *MMP3* and *MMP8*). Proteinases such as these participate in the processing of TNF- α , which, when overproduced, can lead to insulin resistance and obesity in rodents [218]. The second is the gene for cytosolic glycerol-3-phosphate dehydrogenase (*GPDH-C*). This enzyme and its equivalent in mitochondria play an important role in lipid synthesis. The third is the gene for ataxia-telangiectasia (*ATM*), which is homologous to mammalian phosphatidylinositol-3' kinase, an enzyme that may affect insulin-stimulated glucose transport and perhaps obesity [219].

There is growing evidence of replication for an obesity QTL on chromosome 17p12. The strongest evidence comes from a large study that included 2209 individuals from 507 white families [197]. A QTL with a LOD score of 5 was detected for serum

leptin levels. A genome-wide scan with 924 white subjects from the Genetic Epidemiology Network of Arteriopathy (GENOA) study showed a linkage (LOD = 2) in the same region for BMI [198]. Evidence of linkage (LOD = 1.7) of this locus with adiponectin levels was also found in 1100 subjects from 170 families of northern European ancestries [191]. This region harbours several candidate genes that encode proteins known to influence glucose–insulin homeostasis such as the solute carrier family 4 of the insulin-specific facilitated glucose transporter (GLUT4, at 17p13), and proteins thought to influence nutrient partitioning, lipid metabolism and energy balance, such as the receptor protein known to bind to globular ‘heads’ of the complement C1q (gC1qR, at 17p13.3), and *PPAR α* (at 17p12–p11.2).

Replicated linkages were also found for an obesity QTL on chromosome 20q11–q13 in both native Americans [214] and Caucasians [217, 220]. A genome-wide scan based on 236 sib pairs from 82 Pima Indian families revealed a QTL for 24-h energy expenditure at 20q11 (LOD = 3) [214]. A genome-wide scan in 994 adults from 37 multigenerational families of European descent showed linkage ($2.0 < \text{LOD} < 2.2$) at 20q11.2–q12 for BMI in women [217]. Linkage was also found in 194 Caucasian families between three markers at 20q13 ($3.0 < \text{LOD} < 3.2$) and BMI and percentage body fat [220]. The chromosome region 20q11–q13 contains several genes that are plausible candidates for obesity. For example, the agouti-signalling protein (ASIP), the human orthologue of the *Ay* gene, a potent inhibitor of *MC3R* and *MC4R*. *MC3R* is also located in the 20q11–13 interval. The role of *MC3R* in the development of obesity remains unknown. *MC3R*-KO mice are not significantly overweight but exhibit 50–60% increase in adipose tissue mass compared with wild-type mice [221]. The *GNAS1* (guanine nucleotide-binding protein, α -stimulating activity polypeptide 1) gene is located at 20q13 and, as described above, variants in this gene have been associated with AHO [39]. Because of its role in adipocyte differentiation, the *CEBPB* (CAAT/enhancer-binding-protein beta) is another candidate gene in this region [222].

Recently, new linkage methods have been introduced to better accommodate the polygenic character of obesity. For example, the potential epistatic interactions amongst five regions that have previously

been linked to obesity phenotypes were evaluated by pairwise correlation analyses [223]. The study included 542 siblings from 200 white families and 125 siblings from 44 African American families. Pairwise correlations between these five loci were conducted based on (i) alleles shared by descent (IBD) between affected sib pairs (ASP) and (ii) family-specific nonparametric linkage (NPL) scores. Both the ASP-specific IBD-sharing probability and the family-specific NPL score approach revealed that there were strong positive correlations between 10q (88–97 cM) and 20q (65–83 cM). Subsequently, conditional (BMI \geq 27) linkage analyses were performed for chromosome 20, taking into account the family-specific NPL scores for chromosome 10. The LOD score at 20q rose from 1.53 in the baseline analysis to as high as 3.32 when proportional weights were used.

In another study, the hypothesis that imprinted genes may affect obesity was tested [203]. The evidence of linkage of BMI to loci derived from either father or mother was examined in 846 individuals from 235 Pima Indian families. Evidence of linkage of BMI to maternally and paternally derived alleles was found on chromosomes 5q12–q13 (LOD = 1.7) and 10p15 (LOD = 1.7), respectively.

Is obesity a genetic disorder?

Obesity is a chronic disorder that causes considerable personal suffering in affected individuals and has enormous economic consequences. It increases dramatically the risk of developing type 2 diabetes mellitus, coronary heart disease, hypertension, sleep apnoea, asthma, certain forms of cancer and osteoarthritis of large and small joints [224].

Should obesity be considered as a ‘genetic’ disorder? It clearly is in some relatively rare cases. When obesity is caused by an invalidated gene resulting in the lack of a competent protein affecting a pathway impacting on the regulation of energy balance, then obesity is a disorder with a genetic origin. In such cases, the environment has only a permissive role in the severity of the phenotype. Interestingly, in some cases, the obesity associated with a single-gene mutation could be reversed by administration of the human recombinant protein [63, 68]. It is difficult to conclude firmly on the prevalence of cases of genetic obesity, as there remain undoubtedly a large number of genes to be evaluated in this regard. Based on the body of data accumulated to date, it would seem

that cases of genetic obesity could represent at least 5% of the obesity cases and a large percentage of the severely obese.

For the more common forms of obesity, we propose dividing them into those with a strong genetic predisposition and those with a slight genetic susceptibility. In contrast with the first category (genetic obesity), those with a strong genetic predisposition are not characterized by a clearly defective biology that can be reduced to a gene and a mutation or some other abnormalities. The strong predisposition results from susceptibility alleles at a number of loci. In an environment that does not favour obesity, these individuals would likely be overweight. They become obese and potentially severely obese in an obesogenic environment.

A third group is arbitrarily defined as having inherited a slight predisposition to obesity. In a restrictive environment, they may be normal weight or slightly overweight. An obesogenic environment will result in a large fraction of them becoming obese.

Finally, a fourth group includes those who are genetically resistant to obesity. They remain normal weight or almost normal weight in a wide range of obesogenic conditions. These four types are depicted with respect to differences in obesogenic conditions in Fig. 5.

The obesity epidemic we are facing today occurred only over the past three decades and can clearly not

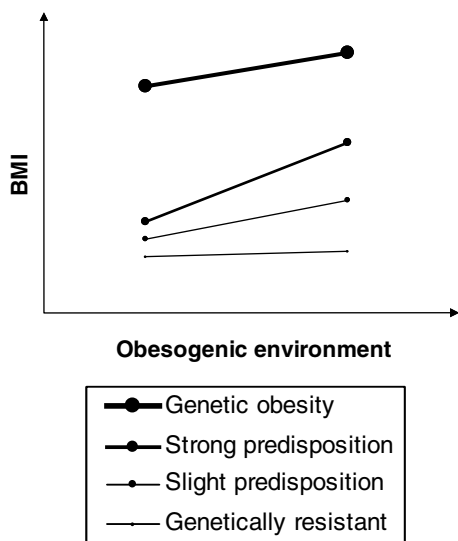


Fig. 5 Four levels of genetic susceptibility to become obese in relation to differences in obesogenic conditions (see text for explanation).

be explained by changes in our genome. The rapid weight gain in the population is more likely due to a changing environment that encourages consumption and discourages expenditure of energy, behaviours that are poorly compatible with our pre-agricultural hunter-gatherer genes. Therefore, most obesity cases come about not as a result of a markedly defective biology but are rather caused by maladaptive behaviours nurtured by an obesogenic environment.

Major behavioural changes would be needed on the part of large segments of the population of industrialized societies to curb the current increase in the prevalence of excess weight. However, we have learned over the past two decades or so that whilst behaviour can be modified in the short term, most people revert back to familiar patterns after a few months. There are considerable environmental and societal forces that make it difficult for most people to adopt a preventive lifestyle that would allow them to achieve and maintain a normal body weight. Without major environmental and societal changes, it is almost certain that the obesity epidemic will continue to spread around the world.

Genes and the risk of becoming obese

Until now, genetic screening for obesity has concentrated on the identification of mutations in specific genes in persons who were severely obese with the greatest success rates being recorded in cases with an early age of onset. An important question is whether genetic tests can be developed for population screening in order to predict the level of risk for the common polygenic forms of obesity.

It has been claimed that genetics will revolutionize clinical practice in that it will lead to the genetic prediction of an individual's risk for common diseases and of responsiveness to drugs [225–228]. According to this view, genomic medicine will revolutionize the diagnosis and treatment of many common illnesses.

This view has however been challenged and the practical usefulness of genetic testing for complex multifactorial diseases has been questioned [229, 230]. This skepticism stems from limitations associated with incomplete penetrance, variable expressivity within and across populations, and the low

magnitude of risks typically associated with a defective genotype in the population [229]. Despite the fact that many 'obesity genes' have been mapped, high-penetrant high-risk genotypes have not been found yet for the most common forms of obesity. Therefore, the contribution of an 'obesity gene' to the development of common obesity will be difficult to quantify and is likely to be low. For example, it has been shown that mutations in the *MC4R* gene are the most common mutations that cause severe early-onset obesity. However, the reported prevalence of these mutations varies by a factor of 10, ranging from 0.5 to 5.8% [85–87, 92]. This observation suggests variable expressivity across populations. Furthermore, the penetrance of the obesity phenotype is incomplete, even in subjects carrying *MC4R* mutations predicted to result in a nonfunctional receptor [86, 92]. The experience with the *MC4R* gene, one of the most promising candidates for a genetic test, strongly suggests that genetic screening may not be as straightforward and productive as originally anticipated. Moreover, gene–gene and gene–environment interactions in the etiology of obesity are still poorly understood and are not taken into account at the moment.

Holtzman and Marteau [229] have argued that the positive predictive value (i.e. the probability that the disease will develop in a person with a positive test result) of a genetic test for a complex disease will likely be low. However, most studies have examined the effect of only one gene at a time to estimate a genetic risk [229–231]. A more productive approach may be to rely on the use of multiple genes [232]. Indeed, the prediction for common diseases, such as obesity, may be improved by considering multiple genes simultaneously [232]. For instance, in one study on the risk of venous thrombosis, the concurrent use of a panel of three genetic tests increased the positive predictive value at least eightfold compared with only a single test. This approach may improve the clinical validity of predictive testing for common multifactorial diseases, such as obesity. However, a major prerequisite for this approach is knowledge about the risk associated with each genotype under various environmental exposures. Multiplex genetic testing needs to be further evaluated for obesity. However, we do not have sufficient data to justify widespread screening for obesity at present.

Conflict of interest statement

Relations with organizations that may constitute potential conflicts of interest for Claude Bouchard: Louisiana State University & Pennington Biomedical Research Center (salary of Executive Director); Endowed George A. Bray Chair in Nutrition at Pennington Biomedical Research Center; Almond Board of California (consultant); Baylor Children's Nutrition Research Center of USDA (external advisory board member); Boston Obesity & Nutrition Research Center of NIH (external advisory board member); Bristol Myers Squibb (consultant); Bristol Myers Squibb (unrestricted grant); Institutes for Pharmaceutical Discovery (scientific advisory board member); Mars, Inc. (Nutritional Research Council Member); Pennington Management of Clinical Trials (member, Board of Directors); Sanofi-synthelabo (member of advisory board for Rimonabant); The Cooper Institute for Aerobic Research (scientific advisory board member); Weight Watchers International (scientific advisory board member).

In addition, as Executive Director of the Pennington Biomedical Research Center, I oversee research undertaken with grants and contracts from pharmaceutical companies and food companies. Finally, I serve also as the institution's official for grants from federal agencies (NIH, USDA, US Army, etc.) and nonprofit foundations (American Heart Association, American Diabetes Association, American Cancer Society, etc.).

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