

# The Molecular Pathogenesis of Obesity: An Unfinished Jigsaw Puzzle

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## Abstract

**Introduction:** Obesity is a common but highly complex disease, which evolved from interactions of multiple genes and the environment. In the past decade, there have been major advances in our understanding of the molecular genetics and pathogenesis of obesity, especially with regards to the genetics and functions of chemical mediators and their receptors, such as leptin, the leptin receptor, neuropeptide Y, the melanocortin-4 receptor, agouti-related protein and the peroxisome-proliferator-activated receptor  $\gamma$ 2. **Methodology:** Recent studies and reports on the obesity genes and chemical mediators were reviewed. **Results:** Despite exciting discoveries of single gene mutations with haplo-insufficiency in human subjects, and single-gene disorders resulting in obesity, most cases of obesity are likely the result of subtle interactions of several related genes with environmental factors, which favour the net deposition of calories as fat, culminating in the obese phenotype. Obesity is unlikely to be caused by a single gene defect unless it is extreme (body mass index >60), or present in an isolated population group. However, research has established that genes at multiple loci may interact centrally to determine satiety, and peripherally to influence the metabolic rate of obese individuals. **Conclusion:** The mechanisms of action of these genes in the development of obesity are now being examined, with the aim of eventually discovering a therapeutic intervention for obesity.

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**Key words:** Agouti-related protein, Leptin, Melanocortin-4 receptor, Neuropeptide Y, Uncoupling protein

## Introduction

Obesity is one of the most common health problems in Singapore with escalating prevalence, affecting about 6% of the Singapore population above 18 years of age.<sup>1</sup> The prevalence of obesity is 12.2% in Singapore children aged 6 to 7 years, 12.8% in children aged 11 to 12 years, and 11% in children aged 15 to 16 years.<sup>2</sup> Of particular relevance, obesity in childhood leads to approximately 30% of obesity in adulthood,<sup>3</sup> and the heritability of early-onset obesity may be considerably higher than that of adult-onset obesity. Besides impairment of psychosocial health and self-image, it is a major risk factor for hypertension, diabetes mellitus, coronary heart disease and stroke, and thus is a major source of morbidity and mortality.

Obesity is a common but highly complex disease, which evolved from interactions of the environment, development, behaviour and genotype. The relative long-term constancy of body weight, the difficulty in sustaining intentional weight loss, and the metabolic, endocrine and behavioural alterations that accompany weight change, provide strong evidence that body fat is biologically regulated.<sup>4</sup> The ability to conserve energy in the form of adipose tissue, appears to confer a significant survival advantage,<sup>4-6</sup> and

the human body is enriched with genes which favour the storage of energy, and diminished energy expenditure. However, the availability of cheap, but calorie-dense food in our modern society, as well as an increasingly sedentary lifestyle, have resulted in maladaptation of this physiological mechanism, with serious metabolic consequences.

## Body Fat Mass as a Multifactorial Trait

Body fat mass is known to be a complex, continuous quantitative trait, and regarded as polygenic and multifactorial. The phenotype is the net effect of the contributing genes, acting in concert with environmental and behavioural factors. Common obesity is likely to be due to subtle alterations in the interactions between genetic and environment factors, resulting from an imbalance between energy intake and energy expenditure, which favours the net deposition of calories as fat.

Studies in twins, adoptees and families indicate that as much as 80% of the variance in the body mass index is attributable to genetic factors.<sup>7</sup> These genetic factors which govern body weight include adipose tissue distribution, the resting metabolic rate, changes in energy expenditure in response to overeating, certain aspects of eating behaviour,

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food preferences, lipoprotein lipase activity, maximal insulin-stimulated glyceride synthesis, and basal rates of lipolysis.<sup>7,8</sup> However, the increasing prevalence of obesity also clearly indicates that environmental manipulation, such as alterations in diet and physical activities, may alter or prevent obesity to some extent.

What is the genetic basis of obesity? Obesity has a wide phenotypic variability, ranging from the mildly overweight individual to the individual with morbid obesity. It is now recognised that the obese phenotype is determined by a group of genes which determines metabolic rate and food intake, and is not likely to be attributable to a single gene, unless obesity is extreme (body mass index >60), or present in isolated population groups.<sup>4</sup> A host of environmental and behavioural factors may contribute to the phenotype by an undetermined extent, which may also vary widely between individuals. While the search for candidate obesity genes has posed a major challenge, we have witnessed, in the past decade, several significant milestones in research, which have helped to elucidate the pathogenesis of obesity and the genetic mechanisms of energy management by the human body.

## Advances in the Molecular Genetics of Energy Homeostasis

### *The Elusive Satiety Factor*

The regulation of energy balance has been the focus of discussion dating back to 1783. The body's energy balance was postulated to be controlled by a feedback loop, in which the amount of stored energy is sensed by the hypothalamus, which adjusts the food intake and energy expenditure to maintain a constant body weight. It is now established that the paraventricular nucleus (PVN), ventromedial nucleus (VMN), dorsomedial nucleus (DMN), and arcuate nuclei (ARC) are the satiety control centres of the hypothalamus.<sup>9</sup> Studies on rat models have shown that disruption of ARC, PVN and VMN produced increased food intake and obesity, and disruption of DMN produced decreased food intake.

The classical parabiotic (cross circulation) experiments by Hervey and other investigators demonstrated that there was a circulating satiety factor in the blood stream which regulates food intake:<sup>10-12</sup>

- a) Overfeeding one of a pair of parabiotic mice (which were surgically joined with interchange of blood), reduced food intake and induced weight loss in the partner, apparently because of the transfer of a circulating hormone.<sup>11</sup>
- b) When an ob/ob mouse (obese mouse due to genetic defect and lacking the circulating satiety factor) was surgically joined to a normal animal, it ate less and

gained less weight.<sup>12</sup> This occurred because the ob/ob phenotype which resulted from lack of the proposed satiety hormone, was supplied by the normal animal in the parabiotic pair.

- c) Mice, homozygous for the db mutation, were also obese. This db/db phenotype was due to a deficiency of the hypothalamic receptor for the purported satiety factor. When a normal mouse is paired with a db/db mouse, it rejected food and died of starvation; presumably due to an excess of the satiety factor from the db/db mouse.<sup>12</sup>

### *Leptin*

It is now established that the primary product of the ob gene is the satiety factor termed leptin, and mice with the ob mutation (now designated Lep<sup>ob</sup>) have a deficiency of leptin, while mice with the db mutation (now designated Lep<sup>db</sup>) are deficient in the hypothalamic receptor for leptin.<sup>13</sup> As expected, the ob/ob mouse is characterised by obesity, hyperphagia, hyperglycaemia, hyperinsulinaemia, and insulin resistance, hypothermia, and infertility, and that leptin corrects the endocrine and metabolic defects.

The discovery of leptin<sup>14</sup> and the leptin receptor<sup>15</sup> has opened a new era in obesity research. The protein, "leptin", is derived from the Greek word 'leptos', meaning thin. The etymology of the word "leptin" implies that its physiological role is primarily to suppress body fat, by decreasing food intake and increasing energy expenditure. Leptin is a 167-amino acid peptide made exclusively in adipose tissue in a wide range of animal species, including humans. Northern blot or reverse transcription-polymerase chain reaction (RT-PCR) analysis of the messenger ribonucleic acid (mRNA) for the ob gene showed that it was expressed only in adipose tissue.<sup>14</sup>

Leptin is secreted as an afferent satiety signal, produced in proportion to the mass of adipose tissue, which acts as an endocrine organ. Both human and animal studies have demonstrated the close association between body fat, leptin mRNA and the plasma leptin levels.<sup>16-18</sup> Increase in fat storage will lead to increased leptin, which inhibits the satiety centre in the hypothalamus, and also influences other neuroendocrine systems, including those related to puberty and fertility. There appears to be a lipostatic set point for weight regulation, in which the body will maintain a certain weight and body composition.

In the ob/ob (leptin deficient) mice, systemic or intracerebroventricular administration of leptin reduces food intake and increases energy expenditure, resulting in reduced body fat. It also increases activity of the sympathetic nervous system, decreases insulin, reduces glucose and improves insulin-sensitive glucose disposal.<sup>19-22</sup> Supraphysiological doses of leptin have similar effects in non-obese animals.<sup>23</sup>

In order to promote weight loss in normal lean mice or obese adults, leptin must also be administered in doses that raise serum leptin concentrations by 20 to 30 times the normal level for a given fat mass.<sup>24,25</sup> It is proposed that the principle function of leptin is to maintain the body fat mass, by signalling to the brain that calorie intake and the amount of energy stored as fat are sufficient.<sup>26</sup> In such a model, the depletion of fat mass during starvation leads to reduced leptin synthesis per fat cell. The reduced action of leptin on the hypothalamus initiates compensatory changes in energy intake and output, which favour a return to the usual body weight. The threshold concentration of leptin, below which these changes occur, is highly individualised and influenced by genetic and developmental factors. Any further elevation of serum leptin concentration above the threshold will have little effect on energy homeostasis, except for supra-physiological levels such as leptin administration.

Obesity is a multi-genetic trait with almost unlimited phenotypic variability, which adds complexity to the search for candidate genes. However, Friedman et al<sup>14</sup> took advantage of specific strains of inbred mice in which the complexity of the trait had been reduced to a single gene disorder, where the affected mice (ob/ob) were morbidly obese. To locate the ob gene at its chromosomal location, DNA markers were determined in over 1600 mice cross-bred from obese and non-obese strains. Assuming all genes and their markers segregated independently in such a cross, and that the chance of meiotic recombination was very low, DNA markers consistently found in the obese animals would identify the genome (or a portion) in which the ob gene resides. Of the six genes identified, only one was exclusively expressed in the adipose tissue in normal mice.

This likely candidate gene was sequenced, and found to encode a secreted protein. The investigators thus identified the location of the ob gene within a 650,000 base pair region on the mouse chromosome 6. Recombinant DNA clones from this region were isolated from the DNA library, and the exons (coding sequencing) were identified. Comparison with the ob gene product of normal mice revealed that the mutant ob gene of these obese mice produces a truncated protein due to a premature stop codon. This led to the discovery of the human homologue of the ob gene, which has now been mapped to chromosome 7q31.3.<sup>14,27,28</sup> This elegant work has since been cited as a model for the investigation of genes of complex human diseases using animal models. The validity of such studies using in-bred animal models will allow identification of the genes that contribute to other complex diseases such as hypertension and atherosclerosis.

However, reports of leptin deficiency in obese humans are rare. Montague et al<sup>29</sup> reported the first homozygous mutation in two extremely obese children with undetectable

serum leptin. The deletion of a single guanine nucleotide in codon 133 results in a frameshift mutation leads to 14 aberrant amino acids, followed by a premature stop codon. The mutant leptin was not secreted, but accumulated intracellularly as a consequence of misfolding and aggregation, and was subsequently degraded by the proteasome.<sup>30</sup> The affected girl had marked hyperphagia, was constantly hungry, demanded food continually, and was disruptive when denied food. There was no evidence of substantial impairment in her basal or total energy expenditure, and her body temperature was normal, unlike the ob/ob mice, whose oxygen consumption, energy expenditure, and body temperature were low. Thus, leptin may be less central to the regulation of energy expenditure in humans than in mice. Another difference in all humans with either leptin or leptin receptor mutations is the consistently normal glucocorticoid concentrations, in contrast to the marked excess in ob/ob mice.

Farooqi et al<sup>31</sup> administered daily subcutaneous leptin to a child proven to produce a mutant leptin, and reported an amelioration of hyperphagia, with progressive weight loss, while preserving lean body mass. Strobel et al<sup>32</sup> reported three Turkish patients with marked obesity and hyperphagia, with very low serum leptin levels for their body mass index. Plasma insulin levels were elevated in all, indicative of insulin resistance. One had primary amenorrhea; the adult male homozygote had clinical features of hypogonadism, which were secondary to leptin deficiency, as the gonadotropin levels and testicular responses to testing were normal.

The investigators found a C-to-T transition in the leptin gene, which resulted in an Arg105Trp mutation. All other members of this highly consanguineous kindred were either heterozygous for the mutation, or homozygous for the wild type allele. Individuals heterozygous for the mutation had normal body weight, normal serum leptin levels, fasting blood glucose, and plasma insulin levels, suggesting that a recessive mutation was responsible for this monogenic form of obesity.

Contrary to these isolated reports of leptin deficiency, the vast majority of obese individuals have elevated leptin levels, as reported by Considine et al,<sup>33</sup> with a strong positive correlation between serum leptin concentrations and the percentage body fat, body mass index, and basal insulin concentrations. It has been suggested that there is a resistance to the action of leptin, so that the increase in adipose tissue mass is maintained.

Although leptin exerts anti-obesity effects in leptin-deficient rodents, its role in the pathogenesis or treatment of obesity in humans is still unclear. Linkage studies of some measures of adiposity have mapped to the LEP gene in some groups of extremely obese whites<sup>34,35</sup> and in

Hispanic populations.<sup>36</sup> However, most obese individuals do not have any abnormalities in the coding sequence of leptin.<sup>37,38</sup> Nonetheless, studies of humans with aberrations in leptin action are extremely important in improving our understanding of the physiology of weight regulation, and in the further elucidation of the regulatory pathways.

### *Leptin Receptor (LEPR)*

The leptin receptor is a member of the cytokine family of receptors with several splice variants. The intracellular signalling system for leptin appears to involve activation of the JAK-STAT system, with Stat-3 being the phosphorylated intermediate. Genetic defects in the leptin receptor occur in the diabetic (db/db) mouse. The human LEPR gene has been mapped to chromosome 1p31. Leptin receptors are distributed widely, including brain and many peripheral tissues (lungs, kidneys, muscle, and adipose tissue), suggesting that this peptide may provide a wide range of tissues with information about fat stores. If obesity in humans were due to leptin receptor mutations, then one would expect a much higher leptin concentration than predicted, based on fat mass, but this is not the case.<sup>39,40</sup>

Considine et al<sup>41</sup> examined expression of the LEPR gene in the hypothalamic tissue from 7 lean and 8 obese humans obtained shortly after autopsy. Using RT-PCR, there was no difference in the amount of LEPR mRNA between the lean and obese subjects. A Gln23Arg polymorphism due to A-to-G substitution at nucleotide 668 of the LEPR cDNA was detected, where 11 subjects were heterozygous and 3 were homozygous. The occurrence of the polymorphic allele(s) did not correlate with the body mass index in the patients studied. Neither the mutation in the leptin receptor gene found in db/db mice, nor that found in fa/fa rat, were found in the human obese cases. The results suggested that leptin resistance observed in obese humans is unlikely to be due to a defect in the leptin receptor.

Gotoda et al<sup>42</sup> determined the entire coding sequence of the human leptin receptor cDNA from peripheral blood lymphocytes of 22 morbidly obese patients. Five common DNA sequence variants were found to be distributed throughout the coding sequence at codons 109, 223, 343, 656 and 1019, with one rare silent mutation at codon 986, as well as a novel alternatively spliced form of transcript. None of the five common variants, including three that predict amino acid changes, were null mutations causing morbid obesity, because homozygotes for the variant sequences were also found in lean subjects. Furthermore, the frequency of each variant allele and the distribution of genotypes and haplotypes were similar in 190 obese and 132 lean white British males selected from a population-based epidemiologic survey. The results suggested that these are polymorphisms, and that mutations in the leptin

receptor gene are not a common cause of human obesity.

LEPR mutation was discovered in a consanguineous family of Kabylia origin (in northern Algeria) where three siblings had morbid obesity since early childhood. Clement et al<sup>43</sup> reported that this mutation in the LEPR gene, which involves a G-to-A transition at the +1 position of intron 6, causes obesity and pituitary dysfunction. The affected sisters had normal birth weight but rapidly gained weight in the first few months of life. Manifestations include bizarre eating behaviour, fighting for food, impulsivity and stubbornness, labile emotions and social dysfunction, reminiscent of the Prader-Willi Syndrome. Growth hormone and thyrotropin levels were low, with the presence of hypogonadotropic hypogonadism. These findings suggest that leptin and its receptor are important physiological regulators of several endocrine functions.

### *Neuropeptide Y (NPY)*

Neuropeptide Y (NPY) appears to be the link between the afferent signals including leptin, and the effectors of energy intake and expenditure. The peptide is encoded by the NPY gene located at the chromosome 7p15.1, synthesised in the ARC, and transported axonally to PVN, where the highest concentrations are found. The expression of NPY mRNA is decreased by leptin and estrogen, and increased by insulin and glucocorticoids.<sup>44,45</sup> Food deprivation increased hypothalamic NPY mRNA production in rodents.<sup>46</sup> Furthermore, NPY administered to the PVN of rats stimulates food intake, and increases lipoprotein lipase activity in white adipose tissue, while decreasing sympathetic nervous system activity and thermogenesis in brown adipose tissue.<sup>47</sup>

Based on these findings, leptin exerts its effects on the leptin receptors, and a decrease in leptin levels will result in an increase in NPY. However, NPY-knockout mice were able to maintain normal body fat and food intake, with increased foraging for food when food is withheld.<sup>48</sup> This clearly indicates the presence of alternative pathways regulating calorie storage. Although investigators have found polymorphisms in the NPY gene, these variants did not have any direct association with obesity or energy metabolism, but were consistently associated with cholesterol levels.<sup>49,50</sup>

### *Melanocortin-4 Receptor (MC4-R)*

Pro-opiomelanocortin (POMC) is the precursor of a number of hormones and neuropeptides, and is cleaved tissue-specifically to form different peptides such as adrenocorticotropin (ACTH),  $\alpha$ - and  $\gamma$ -melanocyte stimulating hormone (MSH),  $\beta$ -lipotropin, and endorphin. The MC4-R is a G protein-coupled, seven-transmembrane receptor highly expressed in the hypothalamus.<sup>51,52</sup> The MC4-R gene has been localised to chromosome 18q22.<sup>53,54</sup>

Studies have provided evidence to suggest that MC4-R is involved in weight regulation, and that melanocortin signalling has a key role in energy homeostasis. Inactivation of both copies of the MC4-R genes in mice produced an obesity syndrome with hyperphagia, hyperinsulinaemia, hyperglycaemia, and increased linear growth. Heterozygotes have an intermediate weight between the homozygotes and wild type mice.<sup>55</sup>

The ARC of the hypothalamus consists of POMC-expressing cells, which innervate the neurons of the DMN and the PVN via MC4-R.<sup>56</sup> Melanocortinergic neurons appeared to exert a tonic inhibition on feeding behaviour. A derivative of POMC,  $\alpha$ -MSH is the natural ligand of the MC4-R, and may mediate physiological satiety signals. Rare recessive mutations in humans involving the POMC gene (resulting in associated ACTH deficiency),<sup>57</sup> or the processing of POMC by carboxypeptidase E (CPE)<sup>58</sup> or prohormone convertase 1 (PC1),<sup>59</sup> have also led to early onset morbid obesity. Jackson et al<sup>60</sup> recently reported a novel form of human early-onset obesity in which there is a specific impairment of pro-hormone processing confined to just POMC, and not involving CPE or PC1.

Two recent reports described dominant forms of obesity conferred by frameshift mutations in the human MC4-R gene, leading to haplo-insufficiency.<sup>61,62</sup> One mutation involved a 4-bp deletion at the codon 211 resulting in a truncated protein from the fifth transmembrane domain. The second mutation involved a 4-bp insertion at nucleotide position 732 of the coding sequence, resulting in a truncated receptor lacking the sixth and seventh transmembrane domain, which was non-functional. This mutation co-segregated with severe obesity in the proband's family over three generations.

Hinney et al<sup>63</sup> recently investigated the role of MC4-R as candidate genes in the aetiology of obesity and anorexia nervosa, and concluded that mutations in the MC4-R are not uncommon in obese individuals. The MC4-R coding sequences were screened by single strand conformation polymorphism (SSCP) in 306 extremely obese children and adolescents, 25 healthy but underweight students, 52 normal weight individuals, 51 inpatients with anorexia nervosa, and 27 patients with bulimia nervosa. Sequencing of PCR products for aberrant SSCP patterns was performed. No mutations were found in the group of subjects with anorexia nervosa or bulimia. However, nine different frameshift and missense mutations were discovered in the obese subjects, and all were extremely obese with body mass index above the 99<sup>th</sup> percentile. Some appeared to be dominantly inherited through family studies. The obese individuals with these mutations were not characterised by any other detectable phenotypic abnormalities. Although the mutations are found solely in obese subjects, the small

number of the non-obese controls limits the interpretation and prediction of their role in obesity. Functional studies are warranted to confirm the pathogenicity of these missense mutations.

#### *Human Agouti-Related Protein (AGRP)*

The human agouti-related protein (AGRP) is a neuropeptide implicated in the normal control of body weight downstream of the leptin signalling (post receptor signalling). The agouti protein and the human agouti-related protein (ART/AGRP) are found to be natural antagonists of the MC4-receptors. Ectopic expression of the agouti protein,<sup>64</sup> as well as transgenic overexpression of the ART/AGRP,<sup>65</sup> inhibit hypothalamic melanocortin signalling and result in obesity. The role of the agouti protein as an endogenous antagonist for melanocortin receptors is illustrated by studies on the obese lethal-yellow mice ( $A^y/a$ ).<sup>66</sup>

The gene was cloned by Shutter et al<sup>67</sup> and termed the agouti-related transcript (ART), with 25% identity with human agouti gene. Ollman et al<sup>68</sup> independently isolated the agouti-related protein (AGRP) from an expressed sequence tag database. Shutter et al<sup>67</sup> subsequently mapped the ART gene to human chromosome 16q22.

#### *Peroxisome-Proliferator-Activated Receptor $\gamma$ 2 (PPAR $\gamma$ 2)*

The peroxisome-proliferator-activated receptors (PPARs), especially PPAR $\gamma$ 2, are orphan nuclear receptors, transcription factors and key regulators of adipocyte differentiation and energy storage.<sup>69,70</sup> The PPAR $\gamma$  gene has been mapped to human chromosome 3p25.<sup>71,72</sup> The endogenous ligand for PPAR $\gamma$  is not known.

PPAR $\gamma$ 2 directs the differentiation of preadipocytes to adipocytes, and overexpression can convert fibroblast cell lines to adipocytes.<sup>73</sup> Phosphorylation of the serine residue at position 114 of the human PPAR $\gamma$ 2 reduces its ability to promote differentiation, suggesting that this may be a negative regulatory mechanism.<sup>74</sup> Ristow et al<sup>75</sup> hypothesised that mutations in or around the phosphorylation site would lead to a loss of negative regulation, resulting in obesity. A total of 358 unrelated German subjects were studied, including 121 obese subjects, using PCR-based assay coupled with specific endonuclease digestion. Four obese subjects were each found to be heterozygous for a missense mutation at nucleotide position 344 of the PPAR $\gamma$ 2 gene, causing a Pro115Gln mutation. The four subjects were markedly more obese than the rest. The overall prevalence of the mutation was 1.1% (allelic frequency about 0.5%). Hyperactivity of the mutant PPAR $\gamma$ 2 was confirmed by transfection studies using murine fibroblasts, causing accelerated adipocyte differentiation and greater cellular accumulation of triglyceride.

## Linkage Studies

Despite exciting discoveries of single gene mutations with haplo-insufficiency in human subjects, and aside from several single-gene disorders resulting in obesity (such as Prader-Willi Syndrome), most cases of obesity are likely the result of subtle interactions of several related genes with environmental factors, that favour the net deposition of calories as fat, and culminate in the obese phenotype. Obesity is unlikely to be caused by a single gene defect unless it is extreme (body mass index >60) or present in an isolated population group.<sup>4</sup> Examination of familial transmission of obesity by segregation analysis have provided evidence of major gene segregations that influence body mass index.<sup>7</sup>

Obesity develops when energy intake exceeds energy expenditure, and thus investigators searching for the candidate genes have focused on those with a role in energy metabolism, such as the  $\beta$ -adrenergic receptor, glucocorticoid receptor, and sodium-potassium ATPase. Several investigators have studied inbred strains of rats and mice and mapped some relevant genes to specific locations in the rodent genome, using crosses between high and low susceptibility strains. Some of these trait loci coincide with single genes where mutations have been described and associated with the extremely obese phenotype. Thus, the heritable variation in body fat composition may in part be explained by allelic variation at these loci.

In more recent years, investigators have attempted to identify the obesity genes with linkage studies, looking for linked genetic intervals in the entire genome of subjects from families in which obesity is common. Identifying obesity genes in humans is facilitated by a sample of individuals who demonstrate homogeneity not only for the disease, but also for the environmental factors that modify the disease. However, this approach may not be as promising as previously perceived, because the obesity phenotype is a multiple gene disorder with wide genetic and phenotypic heterogeneity, as well as variable environmental and behavioural influences on its phenotypic expression.

Bray et al<sup>76</sup> recently reported their linkage analysis of candidate genes in a Mexican-American population in Texas. Based on a hypothesised signalling system of afferent circulating signal molecules that respond to changes in adipose stores, and efferent neurochemical and hormonal signals that affect eating behaviour and energy metabolism, the investigators used this model as a framework to select candidate genes for further investigation. Seven genes with regulatory function in control of body fat utilisation have been identified, namely leptin (LEP), leptin receptor (LEPR), neuropeptide Y (NPY), NPY Y1 receptor (NPYY1), glucagon-like peptide-1 (GLP-1), GLP-1 receptor (GLP1R), and uncoupling protein 1 (UCP1).

UCP1 uncouples oxidative phosphorylation from ATP generation, permitting oxidation of fuels without the generation of ATP, and the energy from the proton gradient is dissipated as heat. GLP-1 is a neurochemical shown to influence feeding behaviour, acting directly within the brain to promote satiety without regard to the nutritional state of the animal. Microsatellite markers located within or near these seven candidate genes were typed in 302 non-diabetic individuals from 59 Mexican-American families. Sib-pair linkage analysis was used to examine linkage between these candidate genes and obesity status, as well as obesity-related quantitative variables.

Only NPY was significantly linked to obesity status. NPY was also associated with body weight, abdominal circumference, hip circumference and diastolic blood pressure. LEP was linked to waist/hip ratio and cholesterol, and LEPR was linked to fasting blood glucose and diastolic blood pressure. The study provided evidence for linkage between LEP, LEPR and NPY with several obesity-related measures, and supports further investigation of NPY, LEP and LEPR to identify genetic variations that may influence the obesity phenotype and outcome.

Norman et al<sup>77</sup> searched for linkage to obesity and energy metabolism in an obesity-prone population of Pima Indians, and found other possible candidate genes which included LEPR, agouti-signalling protein, cluster of three matrix metalloproteinase genes (MMP1, 3 and 8), cytosolic glycerol-3-phosphate dehydrogenase, adenosine deaminase, medium chain acyl-CoA dehydrogenase and UCP2. These genes are known to be related to fat and energy metabolism.

## Conclusion

Clinical research, animal studies and linkage analyses, performed in the quest to elucidate the molecular genetics governing obesity, have improved our understanding of weight regulation. Whether this knowledge can be effectively applied to clinical practice remains to be seen. What is clear from these genetic studies is that they have challenged our traditional views of the pathogenesis of obesity and changed our paradigms of how we view obesity as an illness arising from sheer gluttony and poor discipline.

Although much attention has been focused on leptin, there are a large number of other hormones, receptors, enzymes and currently unidentified proteins that are likely to be involved in the complex regulation of body weight. Some individuals may be simply predisposed to obesity at multiple genetic levels and this makes it very difficult for them to lose weight. Current research reinforces the view that obesity is caused by both genetic and environment determinants, and both need to be addressed in the future.

It is hoped that the elucidation of the pathogenesis of obesity will eventually facilitate the design of novel therapeutic agents, which act both centrally and peripherally, with the ultimate aim of using these as adjuncts in the treatment of obesity. Indeed, research in obesity is now being pursued with intense vigour, because at stake is the most highly coveted prize for the discovery of the hormonal and molecular therapy of obesity.

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