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Obesity genes: molecular genetic approaches to drug target identification [1]

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Abstract

The environment for developing novel therapeutic agents has undergone dramatic change over the past decade. Innovative strategies for identifying and utilizing molecular targets linked to particular human diseases are replacing the classic approach of screening chemical compounds for potential therapeutic action on unknown targets. Since genetic components are involved in many known diseases, mouse and human genetics, positional cloning and other molecular biology-based approaches are now used to identify genes that are associated with these diseases. It is thought that identification of these disease-linked genes may lead to the discovery and understanding of the physiologically relevant biochemical pathways underlying the disease processes. Clearly, a knowledge of these biochemical pathways will provide future molecular targets, enzymes or receptors, that will offer opportunities to apply modern methods of high throughput screening, medicinal chemistry, parallel synthesis and combinatorial chemistry for drug discovery. In this manuscript, we illustrate how mouse genetics and molecular biology-based approaches have led to the identification of all five known single gene mutations that cause obesity in mice. Additionally, we describe how identification of these genes has helped unravel underlying biochemical pathways that regulate behavioral, metabolic and neuroendocrine responses in rodents.

Keywords: Obesity; Genetics; Molecular targets

Human obesity is considered the most common nutritional disorder in the developed world. It is estimated that over 100 million individuals are considered obese and the overall prevalence for obesity in industrialized and developing countries is increasing. Obese individuals exhibit a characteristic pathophysiology including high rates of lipid deposition in adipose tissue, reduced insulin sensitivity of muscle and fat, hyperinsulinemia and alterations in OB protein/leptin levels. These characteristics are often associated with significant chronic diseases including hypertension, non-insulin-dependent diabetes mellitus, hypercholesterolemia, stroke, sleep apnea, joint diseases and certain cancers [1].

It is generally accepted that human obesity results from an interaction of both environmental (50–65%) and genetic (35–50%) factors [2,3]. However, the precise molecular events that lead to the obese phenotype remain unclear. Considerable evidence points to the fact that obesity is a disease of altered biological regulation (reviewed in [4]). Careful integration of endocrine and metabolic factors is thought to determine an individual's steady-state body weight. This steady-state body weight and body fat content can remain constant in most individuals for decades provided that energy intake and expenditure are properly balanced.

On the whole, energy balance is the result of the control of ingestive behavior, metabolism, energy expenditure and energy storage in adipose tissue [1,5,6]. Food intake or feeding behavior results from the coordination of central and peripheral neural, hormonal and metabolic signals by control centers in the brain. This centrally mediated integration is complex and thought to involve hypothalamic neuropeptides, brain insulin, OB protein, glucose levels, and ascending and descending neural inputs [4,7].

Energy expenditure is a function of resting metabolic rate, dietary and cold-induced thermogenesis and the energy associated with physical activity. As with food intake, regulation of metabolic rate is a complex phenomenon dependent on hormonal and autonomic control [4]. While the control of energy balance is complex and not fully understood, the outcome of imbalance in either direction is predictable. Obesity can occur when energy intake exceeds energy expenditure independent of the genetic or environmental factors leading to this condition (Fig. 1).

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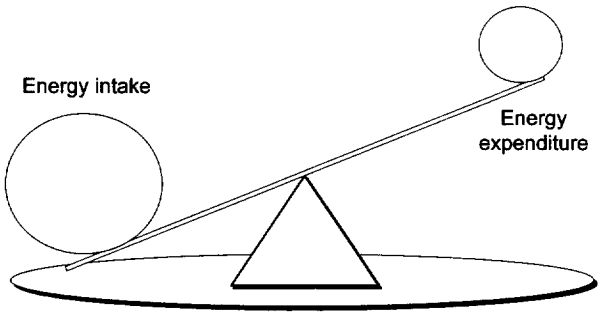

Fig. 1. Energy balance is the result of the control of ingestive behavior, metabolism, energy expenditure and energy storage in adipose tissue. Imbalance in either direction is predictable. Obesity can occur when energy intake exceeds energy expenditure.

From a clinical perspective, obesity is the cause of significant morbidity and exerts a striking impact on the health care industry. It is estimated that some $50–70 billion is spent each year in direct and indirect health care costs associated with obesity. At present, treatment is available in the form of diet and exercise regimens supplemented with centrally acting appetite suppressants. However, long-term maintenance of the modest weight loss achieved (5–10% of the initial weight) is generally hard to accomplish. At present, we do not have a full understanding of the molecular pathways that underlay feeding behavior and energy balance. At least 30 endogenous factors have been found to be involved in appetite or body weight regulation. These include OB protein, insulin, galanin, neuropeptide Y, serotonin, bombesin, melanin concentrating hormone and melanocyte stimulating hormone ([7], reviewed in [8]). Clearly, an understanding of the molecular mechanisms associated with these signaling systems should guide the selection of suitable molecular targets for drug intervention. However, obesity is a multicomponent disease and dissecting these pathways is a formidable task. In recent years researchers have turned toward molecular genetic approaches to discover genes associated with obesity. This approach will guide investigators in the selection of molecular targets for the discovery and development of therapeutic agents for the treatment of obese patients.

Within the past twenty years, recombinant DNA technology has led to the discovery of a large number of disease-related genes. Initial success in this area relied heavily on 'functional cloning' of disease genes that exhibited biochemical defects. Within the last ten years, the technique of 'positional cloning' has emerged that allows the discovery of potential disease genes for which little or no basic knowledge of the underlying molecular defect is required. This method begins by first building a genetic map for the trait of interest. Thus, DNA is collected from families or specific mouse crosses and examined with particular genetic markers for evidence of linkage. Fine mapping the region in which the putative disease gene may reside usually results in the narrowing of the genetic interval to about 1 cM or 1 million base pairs of DNA. Physical maps of this DNA region can be constructed and gross rearrangements of gene structure can be examined. Eventually using information from the physical map and the placement of more molecular markers associated with the disease trait, a smaller genomic region can be isolated from which individual gene clones can be isolated. The putative 'disease genes' are sequenced and compared with normal gene sequences to identify the precise mutation associated with the disease phenotype (reviewed in [9]).

Table 1
Genetic models of obesity in mice

Single gene mutations	Gene product
ob/ob	OB protein (leptin)
db/db	OB receptor
fat/fat	carboxypeptidase E
tub/tub	TUB protein
agouti	AGOUTI protein

In this fashion, researchers have begun to utilize a combination of human and rodent genetic studies to locate and characterize key genes associated with the obese phenotype. Within the last five years, all of the genes associated with single gene defects leading to obesity in mouse models have been cloned. Table 1 shows a summary of the single gene defects in mouse obesity models and the gene products associated with the obese phenotype. The first mouse obesity gene cloned was *agouti* [10]. The AGOUTI protein is an 18.5 kd peptide that binds and antagonizes two of the G-protein coupled melanocortin receptors, MC-1 and MC-4. Researchers found that overexpression of AGOUTI protein produced a syndrome in mice characterized by yellow coat color, obesity, hyperinsulinemia and hyperglycemia ([11], reviewed in [12]). The action of AGOUTI on MC-1 receptors is involved in controling skin color, producing yellow coat color. In addition, it was speculated that the obese phenotype seen following overexpression of AGOUTI is due to its antagonism of MC-4 receptors ([13], reviewed in [4,12]). This speculation is consistent with recent results demonstrating that disruption of the MC-4 receptor gene in mice results in an obese phenotype including hyperphagia, hyperinsulinemia, hyperglycemia and elevated OB protein levels [14]. Taken together, these observations suggest that decreases in signaling through the MC-4 receptor can result in physiological changes leading to obesity.

The *ob/ob* mouse was characterized in 1950 and was the first recessive obesity mutation discovered. These *ob/ob* mice exhibit severe early onset obesity, insulin resistance and strain susceptibility to diabetes, a combination which resembles morbid obesity in humans. In 1994, the *ob* gene was positionally cloned, an event that has had a remarkable impact on the obesity field [15]. The product of the *ob* gene is the OB protein or Leptin, a 16 kd protein, synthesized and secreted by adipocytes. A deficiency in OB protein is thought to be the cause of the obese phenotype in the *ob/ob* mice (reviewed in [4,16,17]). The cloning of the *ob* gene generated a tool, the OB protein, to study the underlying pathways important

in body weight regulation. Shortly after the cloning of the *ob* gene, several teams of investigators demonstrated that injection of OB protein into *ob/ob* mice reduced food consumption and body weight [18–20]. In addition, we showed that intracerebroventricular injection of OB protein reduced food intake and body weight in *ob/ob* animals suggesting that a circulating protein-based signal generated from adipocytes can act on central neuronal networks [18]. Moreover, in diet-induced obese mice, OB protein reduced food intake and body weight, suggesting a possible therapeutic role of OB protein in humans [18].

The phenotype of the *db/db* mice is almost identical to that of the *ob/ob* animals. Parabiosis experiments suggested that *db/db* mice might be defective in a receptor that mediates the action of OB protein ([21], reviewed in [16]). Indeed, it was demonstrated that injection of OB protein failed to result in a reduction of food intake and in the induction of weight loss in the *db/db* mice [18–20], reviewed in [16]). However, we found that OB protein binds to sites within the choroid plexus in wild-type, *ob/ob* and *db/db* mice, suggesting the presence of OB receptor in these mouse strains [22]. Shortly thereafter, the *db* gene was cloned and found to encode a single membrane spanning, class-1 cytokine-related receptor, displaying an intracellular signaling domain [23]. The OB receptor can bind OB protein and trigger intracellular signaling cascades. The *db/db* mice display OB receptor mutations that result in defects in intracellular signaling [24–26]. Therefore, in the *db/db* mice, OB protein is present, binds the receptor, but cannot signal subsequent molecular events (reviewed in [27]).

In 1995, it was discovered that the carboxypeptidase E protein (CPE) was mutated in the *fat/fat* mice [28]. These mice develop obesity at a slower rate than do the *ob/ob* mice. This enzyme is involved in the processing of a number of prohormones. For example, CPE may be involved in the processing of proopiomelanocortin (POMC) resulting in the production of melanocyte stimulating hormone (MSH, [29]). MSH in turn may be an important ligand acting on melanocortin receptors. Thus, it is possible that the obese phenotype found in the *fat/fat* mice may be associated with reduced production of MSH and thus in reduced activation of the MC-4 receptors.

Also in 1995, the *tub* gene product was discovered [30,31]. At present, the activity of the TUB protein remains a mystery and work is underway to link TUB to a known pathway in obesity.

The cloning of these five mouse genes and their human homologues, the initial and ongoing characterization of their corresponding gene products and the linkage to an obese phenotype has renewed interest in tackling problems associated with drug discovery in a complex disease such as obesity. The work performed today in many laboratories around the world has resulted in considerable new insight into the biochemical pathways underlying the regulation of food intake, energy balance and body weight. The most recent reports [32–34] provide evidence suggesting that all five of these known mutations may lie within the same biochemical pathway leading from OB protein in adipocytes to MC-4 receptors in the brain (Fig. 2). Future work will expand our knowledge of these physiologically relevant pathways and will result in the discovery of novel drug targets and therapeutics for the treatment of obesity.

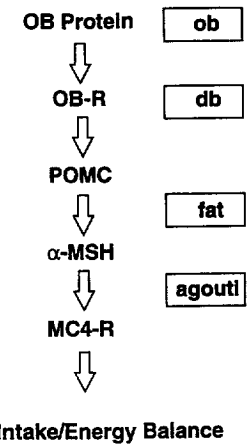

Fig. 2. Four of the known mutations (*ob, db, fat, agouti*) leading to obesity in mice may lie within the same biochemical pathway linking OB protein secreted from adipocytes to activation of MC-4 receptors in the brain.

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