

Mouse models of obesity

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Abstract

Insights into the etiology of human obesity have arisen from the study of animal models. Animal models of obesity are also important for the development of future treatments of obesity. An *agouti* mouse mutation resulting in obese, yellow mice was described over a century ago and in 1992 *agouti* was cloned, making it the first obesity gene characterized at the molecular level. The lethal yellow mouse mutation is one of five dominant *agouti* mutations and is an excellent model for human obesity. The molecular categorization of *agouti* was responsible for the elucidation of the melanocortin system's involvement in hypothalamic weight regulation. As genetic knowledge increases many transgenic mice have been created with genes either over-expressed or deleted, models which further enhance the understanding of obesity.

The development of animal models for obesity is important in the development of treatments for obesity. The mouse is an ideal model because it has similar genetics and development as humans and genetic manipulation techniques are now routine. In addition, mice are small and easy to handle and are inexpensive to propagate and maintain. For these reasons, most research into the etiology of human obesity uses mouse models.

Obesity is a phenotype that is readily observable, and mice with naturally arising mutations causing this phenotype have been extensively characterized. The oldest of these mutations is the *agouti* gene, whereas the most well known are mutations in the genes of the satiety hormone leptin and its receptor, *ob* and *db*, respectively.[1, 2 and 3] Mutation of *ob* or *db* results in severe early-onset obesity and insulin resistance, as well as hyperphagia, reduced energy expenditure, infertility, and decreased linear growth. Administration of exogenous leptin to *ob/ob* mice corrects their obesity and restores reproductive function. Mutations in both the leptin gene and its receptor are extremely rare in humans; however, these models are useful in evaluating the downstream effects of leptin. Because *db/db* mice have a receptor mutation, leptin administration has no effect, and thus they can be used as models for leptin resistance.

Other mouse models of obesity include *tubby* and carboxypeptidase E.[4 and 5] The *tubby* mouse develops late-onset obesity with insulin resistance, but the mechanism for this response is unknown. This mutation has also been associated with inherited retinal degeneration and deafness. The protein encoded by the *tubby* gene appears to be a membrane-associated transcription factor activated by G-protein-coupled receptors,[6] although particular genes regulated by *tubby* have not been described.

Homologous mutations in the human *tubby* gene have been found but do not appear to lead to obesity. The carboxypeptidase E gene encodes an enzyme involved in posttranslational processing of many prohormones, including proinsulin and pro-opiomelanocortin. It is thought that disruption of this enzyme reduces the release of ligands for melanocortin receptors in the hypothalamus, which in turn abrogates the melanocortin response to leptin, leading to hyperphagia and late-onset obesity, as well as infertility and hyperproinsulinemia. This indicates not only that leptin's effects are mediated by melanocortins in the hypothalamus, but also that responses to leptin in reproductive tissues may be mediated by the melanocortin system. Rare mutations in the human homologue of this gene have been identified and are associated with development of obesity and type II diabetes, and as such, this model may be useful for investigating therapeutic strategies for these rare cases.

The *agouti* gene

An *agouti* mouse mutation resulting in obese yellow mice was first described more than a century ago. However, it was not until 1992 that *agouti* was cloned, making it the first obesity gene characterized at the molecular level.[7] The *agouti* gene is normally transiently expressed in the follicular melanocyte, where it stimulates production of the red/yellow pigment (pheomelanin) and inhibits production of the black/brown pigment (eumelanin).[8, 9 and 10] Pheomelanin production is regulated by antagonism of the binding of α -melanocyte-stimulating hormone (α -MSH) to melanocortin 1 receptor (Mc1r) from days 4 to 6 of the mouse hair cycle.

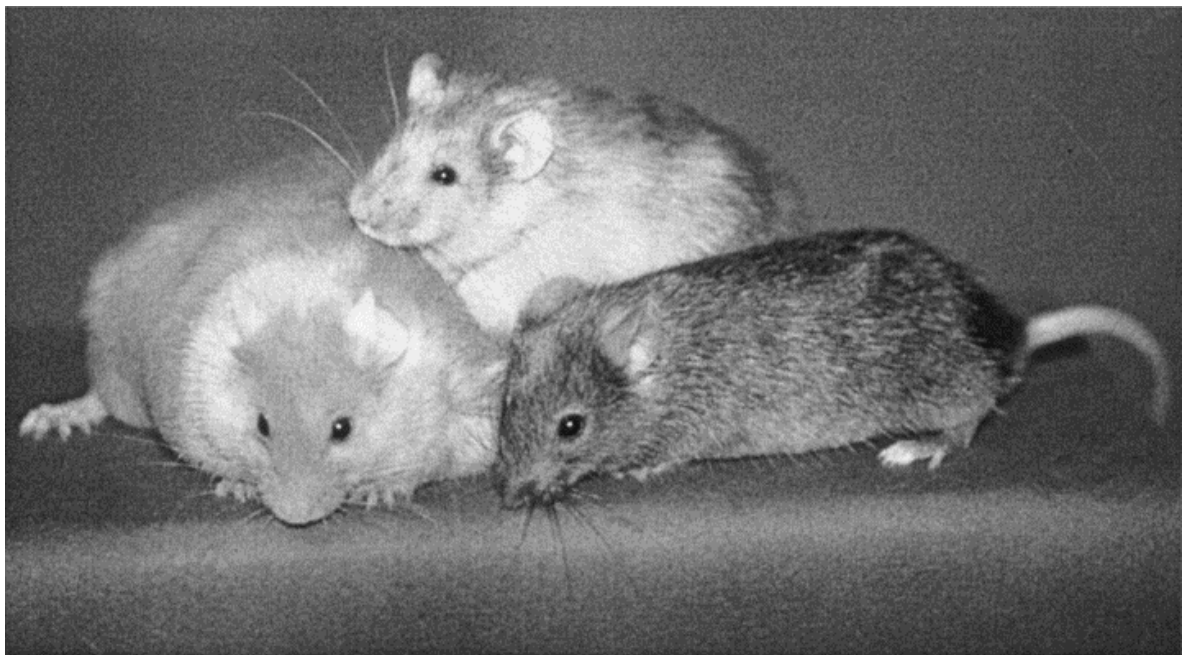


Figure 1. The phenotypes characteristic of the lethal yellow mouse with *agouti* yellow A^y/a (left), *agouti* viable yellow mouse A^{vy}/a (middle), and *agouti* A/a (right).

The lethal yellow (A^y) mutation is one of five dominant *agouti* mutations that result in ectopic *agouti* expression. The A^y genotype results from a 120 to 170 kb deletion that

places it under the control of a ubiquitously expressed promoter.[11, 12 and 13] The *A^y* mouse has a complex phenotype including yellow coat color, mature-onset obesity, type II diabetes, hyperleptinemia, increased linear growth (compared with the *ob/ob* mutation), higher tumor susceptibility, and infertility (Fig 1). Transgenic mice that have been engineered to ubiquitously express *agouti* also have yellow fur, are obese, and develop insulin-resistant diabetes,[14] suggesting that the pleiotropic effects associated with dominant yellow mutations are indeed caused by ectopic *agouti* expression.

In humans, *agouti* is expressed in various tissues, including adipose tissue,[15 and 16] suggesting that it may be involved in the regulation of energy homeostasis. A recent study has found that transgenic mice overexpressing *agouti* in adipose tissue are significantly heavier than nontransgenic mice. Interestingly, food intake is not altered in transgenic mice, suggesting that increased fat mass may be the result of altered energy expenditure.[17] This is a significant finding suggesting that *agouti* expression in human adipose tissue is physiologically relevant. It is likely that ectopic expression of *agouti* in the pancreas stimulates insulin release in the obese yellow mouse. Insulin and *agouti* protein may regulate lipogenesis in an additive manner via calcium signalling. *Agouti* expression in skin has little effect on the obesity phenotype, and *agouti* protein does not circulate as an endocrine factor, because transgenic mice overexpressing *agouti* in skin do not become obese.[18]

Relative to lean controls, *A^y* mice have increased levels of fatty acid synthetase (FAS) and stearoyl-CoA desaturase (SCD), which are involved in fatty acid synthesis and desaturation of saturated fatty acids, respectively.[19] In addition, FAS and SCD mRNA levels increased 1.5- and 4-fold, respectively, in adipocytes treated with recombinant *agouti* protein. [19] Leptin levels are also elevated in *agouti* transgenic mice, suggesting that *agouti* may interact with a number of obesity gene products to play a key role in weight regulation.[20] *Agouti* also up-regulates adipocyte transcription factors, signal transducers and activators of transcription (STAT 1 and 3) and peroxisome proliferator-activated receptor- γ in adipocytes and in transgenic mice expressing *agouti* in adipose tissue.[21]

The molecular categorization of *agouti* was responsible for elucidation of the melanocortin system's involvement in weight regulation, due to its mimicry of *agouti*-related protein activity in the hypothalamus. The melanocortin receptor family comprises five G-protein-coupled proteins, melanocortin 1 receptor (Mc1r) to Mc5r, which demonstrate tissue-specific patterns of expression. Mc4r is expressed in the hypothalamus and plays a key role in the regulation of feeding and metabolism and is normally antagonized by *agouti*-related protein.[22] Mc4r knockout mice are obese but do not have a yellow coat.[23] Novel expression of *agouti* in the brain results in the chronic antagonism of Mc4r, disrupting its function. Administration of a melanocortin agonist to mice overexpressing *agouti* protein does not reverse the obesity phenotype,[24] but administration of an Mc4r agonist inhibits feeding and administration of an Mc4r antagonist increases feeding, suggesting that antagonism of Mc4r plays a role in *agouti*-induced obesity. [21]

The yellow coat color and obesity phenotype of *A^y* mice is suppressed by a mutation in the mahogany gene, recently renamed *Atrnmg*. *Atrn* is therefore likely to be a downstream mediator of *agouti* signalling.[25 and 26] *Atrn* is believed to act

upstream of the melanocortin receptors, because it fails to reverse the respective pigmentation and obesity effects of Mc1r and Mc4rR knockout mice.[25, 26 and 27] Although *Atrnmg* suppresses the obesity phenotype of Mc4r-deficient mice, it does not suppress obesity caused by mutations in leptin receptor (*Lepr^{db}*), carboxypeptidase E (*cpefat*), or tubby mice, indicating that the *Atrn* protein functions with some specificity in the melanocortin-signalling pathway.

Introduction of the *agouti* gene under control of the adipose tissue-specific Ap2 promoter results in high expression of *agouti* in mouse adipose tissue, where it is not normally expressed.[17] Obesity is not seen in this model unless combined with hyperinsulinemia by peripheral administration, suggesting that *agouti* expression primes adipose cells for expansion and lipogenesis via up-regulation of adipogenic transcription factors and lipogenic enzymes. In humans, *agouti* is normally expressed at high levels in adipose tissue, suggesting that hyperinsulinemia or diabetes may stimulate adipogenesis.

Other hormone systems have also been implicated in weight regulation by mouse model studies. Mice that overexpress corticotropin-releasing hormone (CRH) develop hypophagia and increased thermogenesis and energy expenditure.[28] Chronic administration of CRH to obese Zucker rats prevents weight gain but has no effect on lean animals, suggesting that CRH levels may directly regulate weight maintenance. However, CRH levels do not appear to be greatly down-regulated in obese animals, so it is likely that this pathway interacts with the leptin/melanocortin system in regulating weight.

Transgenic mice models

As the pathways involved in weight regulation and energy expenditure are elucidated, a wide range of genes affecting these processes are being identified. Accordingly, many transgenic mice have been created with genes either overexpressed or deleted. In particular, genes involved in the melanocortin pathway of weight regulation have been examined using this approach. Knockout of the *Mc4r* gene in mice is observed to result in early-onset obesity, non-insulin-dependent diabetes, and other obesity-associated syndromes. These symptoms are a parallel to the yellow *agouti* mouse syndrome, indicating that *agouti* expression in the hypothalamus inhibits *Mc4r* function, leading to obesity. Targeted deletion of the *Mc3r* gene also results in a late-onset obesity phenotype, but regulation of appetite and metabolism appear to be intact.[29] *Mc3r* transgenic mouse also seem more susceptible to diet-induced obesity, with high-fat diets leading to significantly increased body weight, especially in female mice. An interesting feature of this diet-induced obesity is that the ratio of adipose mass to lean mass is increased, whereas the overall body weight remains similar to that in *MC3R* knockout mice with normal diets.

Transgenic modification of other genes in the melanocortin pathway has highlighted their importance in weight regulation in mice. Some notable examples include overexpression of *agouti*-related protein, leading to an obesity syndrome similar to that in yellow *agouti* mice but without the pigmentary effects, due to chronic antagonism of hypothalamic *Mc4* receptors.[30] Likewise, up-regulated expression of the syndecan-1 gene produces an obesity syndrome characteristic of melanocortin antagonism in the hypothalamus.[31] This is thought to be due to facilitation of

agouti-related protein binding to Mc3r and Mc4r by syndecan-1, a membrane-bound heparan-sulfate proteoglycan. Similarly, knockouts of the Mc4r, Mc3r, and Pomc genes produce similar obesity phenotypes, again highlighting their importance in weight-regulation pathways.

Transgenic mice have also been used to examine the function of neuropeptide Y (NPY) and its receptors. NPY was the first orexigenic factor from the hypothalamus to be identified, although it appears to be involved in a wide range of central and peripheral processes. Interestingly, intracerebroventricular administration of NPY leads to hyperphagia and increased adiposity,[32] but NPY knockout mice exhibit normal body weight and response to fasting,[33] indicating that NPY is not a necessary part of the hyperphagic fasting response and that NPY may be a redundant signalling molecule in weight regulation. On the other hand, NPY knockout and leptin-deficient mice have a 20%–40% reduction in adiposity and food consumption compared with leptin-deficient mice with an intact NPY gene. NPY-leptin knockouts also display increased oxygen consumption, core body temperature, and locomotive activity relative to leptin-deficient, NPY-positive mice. This suggests that NPY regulates metabolic expenditure of energy but is not the sole factor, because NPY knockout does not totally rescue leptin-deficient mice from the obese phenotype.

Deletion of the NPY receptor genes (of which there are at least six) has produced surprising results.[34, 35, 36 and 37] NPY receptor knockout mice have exhibited a mild late-onset obesity, contrary to what one might expect for receptors of an orexigenic peptide. Knockout of these receptors has also demonstrated their role in a wide range of other processes, including fertility, bone formation, anxiety and stress, and cardiovascular responses. It appears that NPY receptors may maintain a certain level of constitutive activity in the regulation of metabolic pathways, such as thermogenesis and adiposity levels, and that the interaction between NPY and its receptors inhibits this activity. These studies in particular highlight the usefulness of knockout mice in determining the function of particular genes in obesity.

Conclusions

Significant insights into the etiology of human obesity have been gained from the study of animal models. The lethal yellow mouse is an excellent model for human obesity, because *agouti* and MC4R are normally expressed in adipose tissue in humans. In addition, ASIP is a known antagonist of MC4R in humans. Together, this signalling may play a pivotal role in the regulation of lipid metabolism. Mutations at the MC4R gene are the most common genetic cause of morbid obesity in humans.[38 and 39] Identifying the role of *agouti* in human energy homeostasis has important implications for obesity treatment. Attractin is ubiquitously expressed in both mice and humans, and polymorphisms of the attractin gene have been identified that completely reverse the obese phenotype in mice.[25, 26 and 27] Therefore, attractin appears to be a good target for anti-obesity treatment. In addition, melanocortins are obvious targets for obesity treatment, because stimulation of Mc4r results in decreased food intake and antagonism of Mc4r results in increased food intake and body weight.

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