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REVIEW

Mouse models for the central melanocortin system

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Abstract Obesity is characterized by an excess storage of body fat and promotes the risk for complex disease traits such as diabetes mellitus and cardiovascular diseases. The obesity prevalence in Europe is rising and meanwhile ranges from 10 to 20% in men and 15–25% in women. Body fat accumulation occurs in states of positive energy balance and is favored by interactions among environmental, psychosocial and genetic factors. Energy balance is regulated by a complex neuronal network of anorexigenic and orexigenic neurons which integrates peripheral and central hormonal and neuronal signals relaying information on the metabolic status of organs and tissues in the body. A key component of this network is the central melanocortin pathway in the hypothalamus that elicits metabolic and behavioral adaptations for the maintenance of energy homeostasis. Genetic defects in this system cause obesity in mice and humans. In this review we emphasize mouse models with spontaneous natural mutations as well as targeted mutations that contributed to our understanding of the central melanocortin system function in the control of energy balance.

Keywords Energy balance · Melanocortin · Mouse models · Obesity

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Obesity

Obesity is characterized by an excess of body fat and promotes the risk for the development of complex disease traits like diabetes mellitus, cardiovascular dysfunctions, certain forms of cancer and sleep-breathing disorders. World Health Organization defines obesity by a body mass index (BMI) larger than 30 kg/m^2, whereas overweight is defined by a BMI between 25 and 29.9 kg/m^2. The obesity prevalence in Europe ranges from 10 to 20% in men and 15–25% in woman. Body fat mass is influenced by interactions among environmental, psychosocial and genetic factors. Promotion of positive energy balance causes obesity in humans as well as in mice (for review see [4, 20, 33]).

The mouse has proven itself as an excellent model for investigations on human diseases as development and genetics are similar in mouse and man. Furthermore, genetic engineering techniques in the mouse became well-established and reliable tools in the last two decades. In this mini review we highlight different mouse models which have been instrumental to study body weight regulation and the development of obesity.

The melanocortin system and its role in the regulation of energy homeostasis

The lipostatic hypothesis for the control of food intake postulates that adipose tissue produces a hormone in proportion to the amount of fat and acts on the central nervous system to reduce feeding and increase energy expenditure for maintaining energy balance [32]. According to this hypothesis, leptin was identified as the peripheral hormonal signal secreted by adipocytes in

proportion to body fat mass. Positional cloning of leptin substantiated the involvement of the endocrine system in the regulation of body weight [54]. Deficiency of leptin in obese (*ob*/*ob*) mice leads to hyperphagia and reduced energy expenditure [16, 30]. Leptin acts on the leptin receptor, a single-transmembrane-domain receptor of the cytokine receptor family. The *db*/*db* mouse is characterized by severe obesity and carries a naturally occurring mutation in the leptin receptor gene. This mutation causes an aberrant splicing which results in the production of a truncated leptin receptor [11, 28, 37]. Energy-related signals from the periphery (e.g. leptin) are integrated in the arcuate nucleus of the hypothalamus by two subsets of neurons either expressing pro-opiomelanocortin (POMC) or agouti-related protein (AGRP). These neurons project to melanocortin-4-receptor (MC4R) expressing neurons in other hypothalamic regions like the paraventricular nucleus (PVN). In states of high caloric excess, elevated leptin levels stimulate anorexigenic Pomc neurons and inhibit orexigenic Agrp neurons [12, 31]. In second order neurons MC4R-signaling promotes negative energy balance by increasing energy expenditure and reducing food intake [10, 29, 49]. In times of fasting low leptin levels inactivate Pomc neurons and activate Agrp neurons. Agrp release increases food intake and weight gain by inhibiting signaling at the MC4R (for review see [3, 45]) (see Fig. 1).

Mouse models with mutations in the melanocortin system

In this section we point out mouse models that allowed the compilation of the melanocortin system described above. The below-mentioned mouse models are summarized in Table 1.

Intensive studies in several mouse models helped to analyze the function of certain genes that are part of the central melanocortin system. The first hint towards the role of melanocortins in the regulation of body weight arose from the analysis of the agouti yellow (A^y) mouse: this mouse strain encodes a spontaneous and naturally occurring dominant mutation in the agouti gene resulting in a phenotype characterized by yellow fur, increased linear length and severe obesity [2, 7]. Normally, agouti protein is transiently expressed in melanocytes to activate the synthesis of yellow pigment (pheomelanin) and inhibit the production of black pigment (eumelanin) through antagonism of the melanocortin-1-receptor (MC1R) (for review see [17]). The agouti mutation leads to ubiquitous agouti protein expression in A^y mouse which allows it to antagonize MC4R in the brain. Yellow fur color and obesity in the A^y mouse is caused through inhibition of MC1R and MC4R in skin and hypothalamus, respectively [6, 41].

The identification of agouti was the first breakthrough to clarify the role of central melanocortin system in the

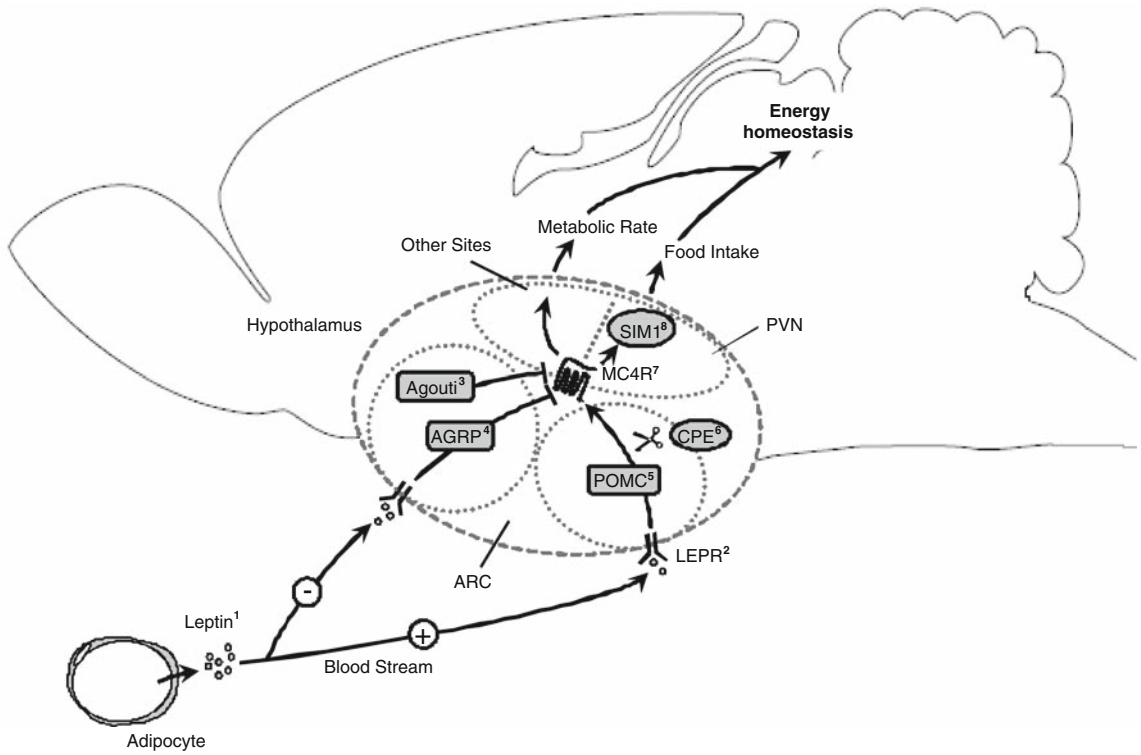

Fig. 1 Schematic representation of the central melanocortin system

Table 1 Mouse models with mutations in the melanocortin system

#	Allele	Gene	Function	Mutation	Effect	Body length	Effect on fur color
1	Obese (ob)	Leptin	Cytokine hormone secreted by adipocytes	Natural	Loss-of-function	↓	–
2	Diabetes (db)	Leptin receptor	Cytokine receptor	Natural	Loss-of-function	↓	–
3	Agouti (A^y)	Agouti	Antagonist of Mc1r	Natural	Gain-of-function	↑	+
4	Agrp	Agouti-related protein	Antagonist of hypothalamic MCRs	Transgenic	Over-expression	↑	–
5	Pomc	Pro-opiomelanocortin	Protein precursor for MCR agonist	Gene targeting	Knock-out	↑	+
6	Fat(fat)	Carbooxypeptidase E	Pomc maturation	Natural	Loss-of-function	–	–
7	Mc4r	Melanocortin-4- receptor	Integration of energy related peripheral signals	Gene targeting	Knock-out	↑	–
8	Sim1	Single-minded 1	Putative target of MC4R signaling	Gene targeting	Loss-of-function,	↑	–

regulation of energy balance. Based on sequence similarity with agouti, a novel gene was identified and named agouti-related peptide (Agrp). Generation of transgenic mice that over-express agouti or Agrp demonstrated that both mouse models develop obesity. But only agouti mutants had a yellow fur color phenotype [46]. This finding indicated that Agrp does not inhibit Mc1r function in the skin but is involved in antagonizing a paralog of Mc1r in the brain.

Subsequently, the identification of Mc4r as the melanocortin receptor in the central nervous system that is antagonized by agouti and Agrp was enabled by a knock-out experiment in mice. Disruption of Mc4r by gene targeting results in a phenotype similar to that observed in the A^y mouse characterized by maturity onset obesity, hyperphagia, hyperinsulinemia and hyperglycemia but without effects on fur color [29]. This knock-out model disclosed a novel signaling pathway for the regulation of body weight and demonstrated that the obesity syndrome of A^y mice is caused by antagonism of Mc4r by agouti protein. Measurements of metabolic rate in young wild-type and Mc4r-null mice with similar body weights showed that mice deficient for Mc4r consumed less oxygen than wild-type animals. Pair-fed Mc4r knock-out mice gained more weight compared to their wild-type littermates when fed the same amount of food, supporting the idea that Mc4r deficient animals have a reduced energy expenditure [49]. Moreover, Mc4r knock-out mice are insensitive to the effect of the synthetic melanocortin receptor agonist melanotan II (MTII). Intraperitoneal administration of MTII increases metabolic rate and reduces food consumption in wild-type animals but fails to promote the same effects in Mc4r-null mice [10]. The inhibitory effect of MTII on feeding is completely blocked by intracerebroventricular co-administration of the synthetic agouti mimetic SHU9119 [13]. These results demonstrate that Mc4r mediates control of food intake and metabolic rate in mice.

Furthermore, Mc4r signaling influences preferences to different macronutrients. Intraperitoneal injection of MTII decreases fat consumption in mice in a dose-dependent manner. This effect requires Mc4r since Mc4r-deficient animals do not respond to MTII treatment. Protein and carbohydrate intake are unaffected by MTII treatment [48].

The endogenous agonists of Mc4r originate from a common precursor polypeptide termed pro-opiomelanocortin (Pomc). Pomc-derived peptides have various physiological functions, including pigmentation, adrenocortical activity and regulation of energy homeostasis. Pomc is a large protein precursor that is cleaved by prohormone convertases to smaller bioactive neuropeptides like the Mc4r-agonists α-/β-melanocyte stimulating hormone (Msh). Deficiency of Pomc in knock-out mice causes multiple dysfunctions in pigmentation and adrenal development. The obesity syndrome of Pomc-null mice resembles characteristics of Mc4r knock-out and A^y mice like increased linear growth and hyperphagia, allegeable by reduced Mc4r signaling in hypothalamus [9]. In humans, Pomc mutations are associated with obesity, adrenal insufficiency and red hair pigmentation [5, 24, 34].

Mutations in enzymes involved in the maturation of melanocortin ligands are responsible for the development of obesity. For example, carboxypeptidase E (Cpe), an exopeptidase involved in processing of preprohormones like Pomc, cleaves C-terminal amino acid residues to generate bioactive molecules. An involvement of Cpe in regulation of energy homeostasis is highlighted by the analysis of Cpe-deficient mice (Cpe^{fat}/Cpe^{fat}). This naturally occurring mutation results in severe obesity accompanied by low levels of the mature α-MSH [8].

Attractin (Atrn) is a widely expressed transmembrane protein whose loss-of-function in mahogany mutant mice ($Atrn^{mg}$) rescues the pleiotropic effects of the A^y mutation, e.g. Atrn suppresses the development of yellow fur color and obesity [2, 19, 43, 44]. Based on this observation Atrn

was described as the first obesity suppressor gene. This finding implicates that Atrn in the A^y mouse is required for agouti to effectively antagonize Mc1r and Mc4r in skin and in the brain, respectively. Furthermore, this finding has raised the possibility that Atrn has a function in Agrp signaling. However, the $Atrn^{mg}$ mutation does not suppress obesity induced by Agrp over-expression not excluding that a molecule similar to Atrn is involved in Agrp action [22]. For example, a yeast two-hybrid screen utilizing the intracellular domain of MC4R as bait led to the discovery of an attractin-like protein (Alp) which is highly co-expressed with MC4R in the PVN of mice [21]. The Alp binding motif in Mc4r contains a putative phosphorylation site indicating that Alp might play a role in Mc4r trafficking. Alp knock-out mice appear normal with no alterations in body weight or pigmentation [51]. Further experiments using double mutants over-expressing Agrp and deficient for ALP could elucidate possible interaction of these two proteins in vivo.

Recently, a putative downstream mediator of Mc4r signaling pathway was investigated in different mouse models. Sim1 (single-minded), a transcription factor involved in neurogenesis, was identified in a girl with early onset obesity most likely caused by increased food intake rather than diminished energy expenditure [26]. Molecular genetics revealed a chromosomal translocation which disrupts the Sim1 gene. Interestingly, Sim1 is highly expressed in the PVN of the hypothalamus [42]. Sim1 heterozygous mice develop hyperphagic obesity, increased linear growth, hyperinsulinemia and elevated feeding efficiency [27]. MTII injection fails to reduce food intake in Sim1 heterozygous mice. But like in wild-type mice, metabolic rate in Sim1 heterozygous mice is increased in response to MTII treatment [35]. BAC transgenic mice over-expressing Sim1 are resistant to diet-induced obesity. Over-expression of Sim1 partially rescues the obese phenotype of A^y mice by normalizing food intake. However, Sim1 over-expression does not affect energy expenditure [36]. These data led to the suggestion that Sim1 acts downstream of Mc4r. Neither Sim1 overexpression nor haploinsufficiency alter metabolic rate thus indicating that the function of Sim1 is limited to the regulation of food intake. Divergence in Mc4r pathways regulating food intake versus energy expenditure was revealed by utilizing a gene targeted mouse carrying a loxP-flanked transcriptional blocker between the transcriptional start site and the start codon of the Mc4r exon. Expression of Cre-recombinase under the control of Sim1 promotor deletes the transcriptional blocker and allows Mc4r expression specifically in neurons of the PVN and the amygdala. Metabolic phenotyping of these mice demonstrated that PVN/amygdala-specific restoration of Mc4r completely rescued hyperphagia but has no effect on energy expenditure [1]. In conclusion Mc4r in PVN and possibly also in the amygdala regulates feeding, whereas Mc4r expressed elsewhere in the central nervous system is responsible for the regulation of energy expenditure.

Mouse models for the analysis of gene variants

Transgenic animals are a powerful tool to investigate the biological function of genes. Furthermore, genetically engineered mouse lines could help to reveal the effect of certain alleles on metabolism. For instance, mutations in the MC4R gene are the most common form of monogenic obesity in humans. Humans encoding MC4R mutations display an obesity syndrome similar to that observed in MC4R-null mice. About 6% of severe obese subjects carry point mutations in or near the MC4R gene [15, 38, 50]. Investigation of signaling activities of these mutant MC4Rs in assays based on cell culture demonstrated that the characteristic of obesity correlates with dysfunctions in receptor properties [14, 25, 39, 52]. The most common MC4R polymorphism V103I is negatively associated with obesity [18], a finding meanwhile replicated in several independent studies [18, 23, 47, 53]. Despite of a negative association of V103I with obesity, a strong pharmacological phenotype of this mutant receptor expressed in cell culture was not identified. Only one report demonstrated a lower potency of orexigenic AGRP on V103I corresponding to the distribution of this allele in population screens [52]. Several point mutations in the Mc4r gene introduced by chemical mutagenesis have been shown to cause different severity of obesity in mice [40]. Mouse lines encoding certain point mutations of interest are required to elucidate the effects of certain alleles on metabolism in vivo.

Mouse models are a valuable tool to gain insights into the function of genes and their implications for the development of metabolic diseases like obesity. Compared to several non-mammalian animal models the mouse permits transferability on human genetic diseases due to the high degree of similarity between the murine and human genome. Furthermore, targeted mutations by homologous recombination are exclusively feasible in murine embryonic stem cells. The introduction of targeted mutations in the mouse may be useful for the functional analysis of certain genes and polymorphisms that are associated with metabolic phenotypes in humans. Nevertheless mouse models show limitations: human diseases like obesity are caused by interplay among several factors like genes, environment, psychological and cultural influences. Though mouse models are not suitable to investigate the impact of complex social factors on the etiology of human diseases they can contribute significantly to elucidate the

role of genetic factors as demonstrated by the function of melanocortins in the regulation of energy balance.

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