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Rare genetic forms of obesity: from gene to therapy.

K Clément^{a,b}, H Mosbah ^a, C Poitou^{a,b}

Affiliations:

^a Assistance Publique-Hôpitaux de Paris, Reference Center for Rare Diseases (PRADORT, Prader-Willi Syndrome and other rare obesity with eating behavior disorders), Nutrition Department, Pitié-Salpêtrière hospital, Paris, France.

^b Sorbonne Université/INSERM, Nutrition and obesities; systemic approaches (NutriOmics) research Unit, Paris, France.

Corresponding author:

Karine Clément

Address: Sorbonne Université/INSERM, Nutrition and obesities; systemic approaches (NutriOmics) research Unit, Paris, France.

Email: karine.clement@aphp.fr

Telephone: 33 (0) 142177031

Fax: 33 (0) 142177961

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Abstract

Monogenic non-syndromic obesity is characterized by severe early-onset obesity with abnormal eating behaviour and endocrine disorders. Genes contributing to these rare forms of obesity are mainly located in the leptin/melanocortin pathway, with typically an autosomal additive inheritance of obesity. The normal function of this hypothalamic pathway is essential for the control of energy balance. Genetic variants are involved in 5 to 30 % of severe early-onset obesity depending on explored populations. Compared to other genes in the pathway especially leptin (*LEP*), leptin receptor (*LEPR*), pro-opiomelanocortin (*POMC*) and prohormone convertase subtilisin/kexin type 1 (*PCSK1*), Melanocortin 4 receptor (*MC4R*)-linked obesity is characterized by obesity of variable severity with no notable endocrine phenotypes. Managing patients with monogenic non-syndromic obesity is clinically challenging since they display complex phenotypes and the obesity is often morbid and refractory to classical treatments. Until recent years, there has been a lack of effective and targeted pharmaceutical molecules except for leptin therapy that was available for leptin deficiency. The picture has changed and new promising molecules acting on the leptin-melanocortin pathway such as setmelanotide -a new *MC4R* agonist- are now emerging as novel targeted therapeutic opportunities.

Key words: obesity; genetics; leptin-melanocortin pathway; monogenic non-syndromic

Introduction

Human obesity is complex and usually results from the interplay between genetic/epigenetic factors (1) and a myriad of environmental triggers, which in some cases are principal causes of obesity development such as in Japanese SUMO Wrestlers in whom the obesity is caused mainly by high fat diet (2). Whereas polygenic forms of common obesity can explain up to 40% of the heritability for obesity clinical phenotypes (3, 4), monogenic obesity causally relates to early-forms of severe obesity. This so-called “genetic obesity” is mostly due to an alteration in the function of critical neuronal populations of the hypothalamus, the central brain area in regulating energy and weight homeostasis.

Historically, major forms of genetic obesity were classified as: 1) monogenic non syndromic obesity, which includes patients with variants in genes involved in the leptin/melanocortin pathway, and 2) syndromic obesity which includes patients with neurodevelopmental disorders or malformative features such as seen in Prader Willi and Bardet-Biedl syndromes, both being the most frequent observed forms. These syndromic obesity forms also involve genes having molecular cross talks with the leptin/melanocortin pathway such as Single-Minded 1 Transcription Factor (*SIMI*), brain-derived neurotrophic factor (*BDNF*), human neurotrophic tyrosine kinase, receptor, type 2 (*NTRK2*), or SH2B Adaptor Protein 1 (*SH2B1*) (5).

This review focuses on main genes contributing to monogenic non-syndromic obesity, directly involved in, or regulating the leptin/melanocortin pathway: leptin (*LEP*), leptin receptor (*LEPR*), pro-opiomelanocortin (*POMC*), prohormone convertase subtilisin/kexin type 1 (*PCSK1*), melanocortin receptor type 3 (*MC3R*) and type 4 (*MC4R*) and its regulator

Melanocortin Receptor Accessory Protein 2 (*MRAP2*), adenylate cyclase 3 (*ADCY3*) and more recently Steroid Coreceptor Activator-1 (*SRC-1*), Semaphorin 3A-G (*SEMA3A-G*), PlexinA1-4 (*PLXNA1-4*), Neuropilin1-2 (*NRP1-2*) and kinase suppressor of ras 2 (*KSR2*).

Our team and others contributed to the discovery of the genes leading to these rare forms of monogenic obesity as a part of intense research led over the last 20 years (5). At the end of the 1990s, we identified the first human variants of the leptin (*LEPR*) and *MC4R* receptor (6-10). We further described new variants of genes in these pathways *POMC* gene (11), *MC3R* (12) and *LEPR* (13). Overall, monogenic obesity represents less than 5% of severe early-onset obesity, but it is probably underdiagnosed in some countries and could be more frequent in specific populations and could reach up to 30% (13-15), for example in populations with a high level of consanguinity (16).

The common phenotype of monogenic non-syndromic obesity includes early-onset (before 5 years of age) severe obesity associated with eating disorders and with a heavy impact on patients' morbidity and mortality. A number of endocrine abnormalities (hypogonadism, growth hormone deficiency, hypothyroidism, corticotropic insufficiency) are often present. Sometimes neurodevelopmental abnormalities (intellectual deficiency, emotional lability, behavioral problems or even autistic traits) are also present such as in syndromic obesities making clinical management even more complex (17). In general, these patients suffer from refractory obesity, meaning that usual standards of care such as life style modification (change in food intake and physical activity) and even bariatric surgery are not efficient in inducing significant weight loss. The discovery of these forms of obesity has not only been critical in the identification of key mechanisms involved in the control of food intake but also led to the discovery of new pharmaceutical drugs. This review provides an updated summary focusing

on non-syndromic monogenic forms of obesity and their available therapeutic options, with a highlight on innovative therapies.

Gene variants in the leptin/melanocortin pathway: mode of transmission and clinical phenotypes

LEP/LEPR

Patients with homozygous or compound heterozygous variants of *LEP* or *LEPR* show a rapid and very early increase in weight gain, as illustrated by the weight curve of *LEPR* deficient subjects (6, 13, 18-22). The examination of children's BMI trajectories identified BMI cut-off points with the recommendation of genetic screening of the leptin/melanocortin pathway when BMI values are >27.0 kg/m² at the age of 2 years or >33.0 kg/m² at the age of 5 years (23). Feeding behaviour in these patients is mainly characterized by severe and uncontrolled hyperphagia (24). Due to the crucial role of leptin in activating the hypothalamic-gonadal axis (25), patients carrying variants in the *LEP* or *LEPR* gene exhibit hypogonadotropic hypogonadism with in addition to their severe obesity. They can display delayed pubertal development, which suggests a recovery of hormonal functions with time in some individuals. For example, long term follow-up of a *LEPR* deficient woman diagnosed at the age of 13 revealed normal pregnancies (26). Given the general importance of leptin in human physiology, somatotrophic insufficiency and thyrotrophic insufficiency were also described in some patients with *LEPR* variants. Impaired T-cell immunity has also been described in leptin-deficient children. Several *LEPR* variants have been observed in severe obesity with an estimated prevalence of 2-4% (13, 20). For example, in the French Reunion Island, a region with high prevalence of obesity, we estimated that the frequency of homozygous *LEPR*

variants reaches 4% of severely obese subjects (13). Genetic screening is thus recommended in patients with severe obesity and hyperphagia presenting endocrine abnormalities (13, 20). Recently, a systematic review found that across 88 patients with *LEPR* mutations, 100% of the patients had early-onset obesity (<5 years), 96% had hyperphagia and 34% had one or more pituitary hormone deficiencies (27).

Measuring serum leptin can sometimes help in leptin deficiency diagnosis (18). However, a congenital leptin deficiency, due to biologically inactive leptin, has been described in a young boy with a severe clinical phenotype including extreme early-onset obesity and hyperphagia. He presented high, but physiological circulating leptin levels in agreement with his obesity level (28). In the same line, we previously reported obese patients carrying homozygous *LEPR* variants with moderately increased serum leptin (13). These reports demonstrate that finding serum leptin at physiological levels in relation to a patient's BMI or fat mass does not rule out the presence of *LEP* or *LEPR* mutations.

Some subjects display *LEPR* heterozygote variants. However, causally associating these latter genetic variants to propensity to obesity development is complex. Looking at families carrying these variants (for example parents of homozygous carriers) revealed heterogeneity in corpulence and obesity onset. Nevertheless, in a French population of severely obese subjects, we observed that BMI of heterozygous carriers was intermediate between *LEPR* wild type and homozygous carriers suggesting that carrying a mutated allele in the *LEPR* favors increased weight (13).

POMC/PCSK1

One major function of leptin is to stimulate hypothalamic POMC neurons via the leptin receptor to stimulate the release of POMC, the precursor of alpha and beta-melanocyte-

stimulating hormone (i.e. α MSH, β MSH) and adrenocorticotrophic hormone (ACTH). As such, obese subjects with complete *POMC* deficiency (homozygous or compound heterozygous variants) have ACTH deficiency and mild central hypothyroidism (19, 29). They also sometimes display alterations in the somatotrophic and gonadotrophic axes or have red hair (11). These modifications in hair color, adrenal function, and body weight are consistent with the lack of *POMC*-derived ligands for the melanocortin receptors MC1R (α MSH), MC2R (ACTH), and MC4R (α MSH) respectively. Beyond complete *POMC* deficiency seen in homozygous or compound heterozygous carriers, some heterozygous variants as well as changes in *POMC* gene methylation could also be involved in obesity development (10, 30). In those cases, *POMC* variants led to defective α or β MSH molecules (10, 31). Carriers of these variants had no endocrine abnormalities or pigmentation peculiarities. However, in our cohort of French subjects with early-onset severe obesity, we found 12 subjects with a heterozygous variant of *POMC*. Among them, two had an hypogonadotropic hypogonadism and a growth hormone (GH) deficiency (personal data). Proconvertase 1 (*PC1*), encoded by the *PCSK1* gene, is an enzyme involved in the processing of *POMC*, proglucagon and insulin. Patients carrying homozygous or compound heterozygote variants in the *PCSK1* gene (leading to PC1 deficiency), exhibit early-onset severe obesity, postprandial hypoglycemia, hypogonadotropic hypogonadism, central hypothyroidism and adrenal insufficiency (32, 33). Severe and persistent diarrhea, secondary to a lack in mature GLP-1 (glucagon-like peptide-1), a derivative of proglucagon, is also described in cases of PC1 deficiency (32, 34). Some patients may also present persistent polydipsia and polyuria due to central diabetes insipidus (35). Some heterozygous variants were also described in *PCSK1* gene. By using next-generation sequencing in 201 participants (71 were obese subjects), Philippe et al described in 2015 the autosomal dominant mode of inheritance of obesity for a null mutation

in the *PCSK1* gene (36). Creemers et al showed positive association between *PCSK1* partial deficiency and obesity (37). In 845 non-consanguineous extremely obese Europeans, the prevalence of *PCSK1* partial deficiency (heterozygous carriers) was found to be 0.83 % of the population (37). More recently, Loffler et al described heterozygous variants of *PCSK1* associated with obesity and impaired glucose metabolism in children (38).

Other genes involved in leptin/melanocortin pathway

After the first discoveries of patients with *LEP*, *LEPR*, *POMC* or *PCSK1* variants, genetic screening in obese population led to the discovery of new monogenic obesities in different countries. By sequencing 2,101 individuals with severe early-onset obesity and 1,536 controls, Pearce et al identified rare variants of the gene coding for *KSR2* in 45 subjects. Unrelated carriers of these variants showed a severe obesity phenotype with hyperphagia in childhood, low heart rate, reduced basal metabolic rate and severe insulin resistance (39). This protein, almost exclusively expressed in the brain, regulates the activity of AMP kinase which controls cell thermogenesis, fat oxidation and glucose metabolism (40). *KSR2* is supposed to be involved in melanocortin signaling while its precise action on this pathway still needs to be deciphered.

Moreover, variants with loss of function of the *MRAP2* gene, a *MC4R* regulatory protein, have been associated with early obesity, hyperphagia, hypertension and hyperglycemia (41). Two recent studies have also found homozygous variants of *ADCY3* in a population with severe obesity and high level of consanguinity (42, 43). The *ADCY3* gene codes for an adenylyl cyclase 3 protein which is involved in the formation of cyclic AMP. This protein could contribute to the function of the neuronal primary cilium, within the hypothalamus. The

protein ADCY3 is co-localized with MC4R, within the hypothalamic neurons. In murine models, the inhibition of ADCY3 in the primary cilia of these neurons induced weight gain. The SRC-1 protein interacts with a target linked to the activation of LEPR, the phosphorylated form of STAT3, in order to potentiate the transcription of POMC. In an exome study performed on more than 2,500 European subjects, 19 new variants of *SRC-1* were identified, including 15 in obese subjects presenting severe and early-onset obesity. Tests are still necessary to understand functional alterations associated with *SRC-1* variants (44). Finally, a recent study has described the role of class 3 semaphorins (SEMA3A-G), via their receptors and co-receptors (plexins: PLXNA1-4 and neuropilins NRP1-2), in the possible regulation of weight and / or fat mass. In mice, SEMA3A-G, via the NRP2 receptor, modulates the development of axonal projections of POMC neurons from the arcuate nucleus to the paraventricular nucleus. Van Der Klaauw et al found 40 rare variants in *SEMA3A-G*, *PLXNA1-4* and *NRPI-2* genes in 573 severely obese individuals (45). All together these new discoveries extend the number of subjects presenting with monogenic obesity possibly related to leptin/melanocortin alterations.

MC4R

MC4R variants-linked obesity follows an autosomal additive mode of inheritance with incomplete age-related penetrance. Homozygous or compound heterozygous carriers of *MC4R* variants are very rare and their phenotype is more severe than in carriers of heterozygous variants (8, 20, 46). Heterozygous variants in *MC4R* are the most common forms resulting in obesity occurring at a later age than in homozygous variants (10, 47., 48-50). This represents approximately 2 to 4% of childhood and adult obesity (51, 52).

However, depending on population background, founder effects were also described in Mexicans where a loss-of-function mutation was found in 1% of them (53).

Patients with obesity related to *MC4R* variants have a more common obesity phenotype with usually no endocrine anomalies compared to patient harboring variants in genes upstream to *MC4R*. In addition to obesity, children carrying *MC4R* mutations often have marked hyperphagia improving with age (54). The association between binge eating disorder (BED) and *MC4R* variants is not found in all studies (47, 55-58). One explanation of this discordance between studies can be the heterogeneous variant functionality as a recent meta-analysis focused on participants with obesity showed that only *MC4R* gain-of-function (GOF) variants are associated with BED (59).

Hundreds of *MC4R* variants have been identified leading to many functional alterations (60) that are not always consistent across variant subtypes. The functional consequences of these variants have been described *in vitro*, finding mostly loss of protein expression, impaired α -melanocyte-stimulating hormone binding, alterations in receptor trafficking or coupling to the stimulatory G-protein, $G\alpha_s$ (47, 60-62). However, approximately a quarter of obesity-associated *MC4R* variants characterized to date do not exhibit such defects. Recent work investigated additional *MC4R*-related pathways such as Gq and signaling bias toward β -arrestin recruitment and increased mitogen-activated protein kinase pathway activation (63, 64) (review in (52)).

Moreover, some studies of patients with heterozygous variants on several genes in the *MC4R* pathway also raise the question of a possible cumulative effect of these variants eventually explaining the phenotype severity. However, detailed phenotypic data on combined heterozygotes are still lacking (65). Overall, based on recent progress and therapeutic potential, it will be critical to obtain a clearer picture of the functional effects of these variants

on *MC4R* function. The involvement of rare variants of *MC3R* in the development of obesity is less clear than for *MC4R*. Modification of body composition has been suggested in *MC3R* variant carriers. Rare variants leading to the receptor functional alterations have been described and the variant T6K+V81I has been, for example, associated with increased adiposity and hyperleptinemia (review in (66)).

Clinical management in patients with leptin/melanocortin pathway variants

In most cases, clinical management of these patients is complex and requires comprehensive, specialized and multidisciplinary management that include dietary advice with supervision of eating behavior, adapted physical activities, psychomotricity, speech therapy, hormone replacement therapy, amongst others. This approach should be implemented in patients with genetic obesity beginning in early childhood and, if possible, in expert centers. In our experience as a reference center for the care of genetic obesities, we observe that these genetic obesity forms are often refractory to usual medical care, meaning that usual standards of care recommending lifestyle modifications are in general inefficient, albeit scarce data are still available on the effect of nutritional/lifestyle interventions on weight loss response in non-syndromic obesity. This might be different between patients with *MC4R* variants and those with gene variant upstream *MC4R* but this needs deep investigation. Implementing a nutritional and physical exercise program for 1 year, investigators found that weight loss in children with *MC4R* variants was equivalent to non-carriers. However, a recovery of full weight 1 year after the end of the program was observed, which was not the case in non-variant carriers (67). The failure of standard of care in patients with variants in the leptin/melanocortin pathway might extend to bariatric surgery, which is a well-known long-term efficient treatment for severe obesity using several operative methods especially laparoscopic

gastric bypass or sleeve gastrectomy. However, variability in the individual weight loss response to bariatric surgery is well described, probably related to lifestyle habits but also individual genetic background. Data in patients with monogenic non-syndromic obesity are limited and controversial. In patients carrying variants in the *LEPR* gene, we reported that, while these patients initially lose weight after bariatric surgery, the magnitude is clearly less when compared to patients with common obesity (68). Moreover, monitoring these patients over the long term in our center, we observed they have all regained the weight initially lost (unpublished data). Recently, a Dutch group published weight loss data after bariatric surgery in a cohort of 1014 patients. In this cohort, 30 patients were diagnosed with heterozygous variants in *POMC* and *PCSK1* genes. Total weight loss after gastric bypass was not significantly different from the non-carriers (69) after 2 years of follow-up. Conversely, in a Chinese cohort of 131 obese subjects, they found 8.4% (11 subjects) with a heterozygous variant located in a gene of the leptin / melanocortin pathway. In patients carrying variants in the *LEP*, *LEPR*, *SIMI* and *PCSK1* genes, the magnitude of weight loss obtained after 6 years of follow-up was significantly lower than that of patients without the variants, also associating with less improvement in metabolic comorbidities (70).

In patients with heterozygous variants of *MC4R*, bariatric surgery induced-weight loss is identical to that of *MC4R* wild type carrier, at least after one year follow-up (71). However, the presence of a GOF variant is associated with a high risk of BED preoperatively and alters weight loss response with more reoperations and postoperative complications (56). In the Dutch cohort, among 11 patients with heterozygous variants, those who had a gastric bypass had a weight loss equivalent to that of the non-mutated patient. However, in those who had a sleeve gastrectomy, the weight loss magnitude was significantly lower, at 2 years after surgery (69). Thus, carriers of leptin/melanocortin pathway variants experience variable responses to

bariatric surgery that is probably multifactorial (review in (52)). Additional studies are necessary to decipher the links between functional characteristics of the variants and weight trajectories (initial weight loss then stabilization / weight gain) especially in the long-term after the surgery. Great caution must be taken before performing bariatric surgery in these patients and probably discussed in detail with a medical reference center for genetic obesity. Regarding obesity drugs, few data exist on drugs tested in non-syndromic obesity. Sibutramine was tested in a patient carrying a functional variant in the homozygous state of *MC4R* without convincing results on weight (72). GLP1 analogs, including liraglutide, have been tested in patients with *MC4R* variants using Liraglutide 3 mg / day for 16 weeks. Interestingly, compared to *MC4R* wild type carriers, the weight loss of *MC4R* variant carriers, was similar (approximately 6%), suggesting that a pro-satiety effect of GLP1 analogs is also present in subjects with *MC4R* genetic alterations (73).

New therapeutic perspectives in the leptin/melanocortin pathway: from genes to targeted therapy

In recent years, research has developed therapeutic innovations in the leptin/melanocortin pathway upstream of the *MC4R* receptor. Before that, pharmaceutical action was limited to the case of leptin deficiency where subcutaneous injection of leptin in children and adults results in weight loss, mainly due to fat mass reductions, with a major and rapid effect on reducing food intake (28, 74). Leptin treatment also induces puberty in these individuals, even in adults (24).

Through *in vitro* models, it was initially reported that *MC4R* receptor agonists IRC-022493 and IRC-022511 could rescue the activity of mutated *hMC4R*. This was especially the case for *MC4R* variants with altered cyclic AMP response to the endogenous agonists (75). Then a series of new pharmacological agonists of *MC4R*, which restore normal mutated receptor activity, were further tested *in vitro* (76). One study demonstrated that the novel human *MC4R* antagonist, Ipsen 17, acted as a pharmacological chaperone of human *MC4R*, increased the cell-surface expression of *MC4R* mutant and their signaling capacity upon α -MSH stimulation (77). Then highly selective *MC4R* agonists (BIM-22493 or setmelanotide) were then tested in obese animal models and showed a decrease in food intake and an increase in total energy expenditure leading to significant weight loss. In contrast to other previously tested *MC4R* agonists, no side effect, especially on blood pressure or heart rate, was observed (78) despite evidence linking central melanocortin signaling to the regulation of blood pressure. Indeed, human *MC4R* deficiency is associated with lower blood pressure and inversely, a previously tested *MC4R* agonist (LY2112688) had an impact on cardiovascular parameters (79), probably through activation of the sympathetic nervous system. While the reason why setmelanotide safety is different from other *MC4R* agonists is not fully understood, several hypotheses have been put forward to explain the differential effects on the cardiovascular system. This includes variability between the different compounds in terms of brain penetration, of specificity (for *MC3R* or *MC4R*), of binding affinity but also probably of *MC4R*-related signaling. These results have stimulated considerable interest in the development of molecules targeting leptin/melanocortin pathway deficiencies. In humans, treatment with RM-493 increases resting energy expenditure without cardiovascular side effects (80). We have also reported that *MC4R* receptor agonists activate the mutated *MC4R* receptor *in vitro* with an altered response to endogenous agonists. Furthermore, previous work also described *MC4R* variants with

functional consequences and the response to agonists specific for this receptor while proposing a predictive score for the response *in vitro* (60). With European colleagues, we have recently shown that setmelanotide, a *MC4R* agonist, restores melanocortin signaling in the presence of homozygous gene variants upstream of *MC4R* (i.e. *POMC* or *LEPR*) while it could also be effective in restoring mutated *MC4R* activity (81, 82). The treatment of two homozygous *POMC* mutation patients with setmelanotide led to weight loss (-51.0 kg and -20.5 kg respectively) over 12 weeks for both patients and improved hyperphagia scores (81), effects we have observed as stable three years later. To date, no increase in blood pressure or severe adverse events related to the study drug has been observed. The most frequently reported adverse effects are increased pigmentation. With these promising results, the medium and long-term side effects should be monitored, especially evaluating the consequences of chronic stimulation of melanocytes with RM-493. Indeed, this pharmaceutical agonist also activates the *MC1R* receptor located on the skin. Trials are now ongoing extending Setmelanotide treatment in patients with homozygous and heterozygous variations of the *LEPR* and *POMC* genes but also in syndromic obesities such as Bardet-Biedl and Alstrom syndrome. Setmelanotide can activate *MC4R*, and rescue its activity when a functional variant is present. Thus, patients with heterozygous variants associated with a loss of function in the leptin/melanocortin pathway might benefit from these innovative treatments in the near future.

Conclusion

Genetic forms of obesity are important to diagnose due to the need for a specific management plan to be set up as soon as possible, and to the multidisciplinary team needed to carry out patient care. In the near future, next generation sequencing (NGS) technologies or whole-

exome sequencing may help physicians to identify new molecular anomalies in patients with early-onset severe obesity in order to improve their care management. Similarly, new treatments have recently emerged that could change the prognosis for these rare severe forms of obesity, giving hope to patients with severe monogenic non-syndromic obesity.

Conflict of interest

The authors have not declared any conflicts of interest.

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Figure Legends

Figure 1: The leptin/melanocortin pathway

POMC-neurons in the arcuate nucleus are activated by leptin and produce α -melanocyte stimulating hormone (α -MSH), which then activates the *MC4R* receptor in the paraventricular nucleus resulting in satiation. A separate group of neurons expressing *NPY* and *AGRP* produce molecules that act as potent inhibitors of *MC4R* signaling.

AGRP: agouti-related protein; *LEPR*: leptin receptor; *NPY*: neuropeptide Y; *POMC*: proopiomelanocortin.

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