Univerzita Karlova v Praze
Přírodovědecká fakulta

Studijní program: Speciální chemicko-biologické obory
Studijní obor: Molekulární biologie a biochemie organismů

Izabela Toman

Monogenní příčiny obezity
Monogenic causes of obesity

Bakalářská práce

Vedoucí závěrečné práce: Mgr. Josef Včelák

Praha, 2019

Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 09.05.2019 Podpis……

Izabela Toman

Abstrakt

V posledních letech dochází k významnému nárustu výskytu obezity a s tím souvisejícímu nárůstu počtu pacientů s diabetem 2 typu, kardiologickými problémy a k předčasnému úmrtí. Mezi nejnebezpečnější formy obezity řadíme její monogenní typ, neboť se jedná o nemoc způsobenou kauzální mutaci jediného genu s typickým fenotypem hyperfagie a morbidní obezity. Cílem této bakalářské práce je shrnutí nejvýznamnějších genových mutací, které mohou způsobovat monogenní formu obezity. První kapitoly nastiňují obecné genetické příčiny obezity a význam Leptin-Melanocortinové dráhy s ohledem na její úlohu v energetické homeostázi. Navazující kapitoly se věnují genům, které s monogenní obezitou souvisejí, a to zejména *MC4R, LEP, LEPR, SIM1* a *BDNF*. Dále je rovněž pojednáváno o nových možnostech léčby včetně nového léku setmelanotidu.

Klíčová slova:

Genetika, Obezita, Monogenní obezita, Leptin-Melanocortinová dráha, MC4R, Setmelanotid

Abstract

In recent years the prevalence of obesity has significantly increased, pursued by multiplication of patients with type 2 diabetes, cardiological problems and premature death. Between the most dangerous forms of obesity belongs its monogenic type as it is a disease caused by single causal mutation with typical phenotype of severe obesity and hyperphagia. The aspiration of this thesis is to summarise the most important genes, mutation of which may cause monogenic type of obesity. The first chapters outline general genetic causes of obesity and importance of Leptin-melanocortin pathway in terms of its role in energy homeostasis. Subsequent sections identify genes involved in monogenic obesity, e.g. *MC4R, LEP, LEPR, SIM1* and *BDNF*. Finally, the thesis summarizes possible new treatments, including new drug "Setmelanotide".

Key words:

Genetics, Obesity, Monogenic obesity, Leptin-Melanocortin pathway, MC4R, Setmelanotide

Abbreviations

AA	Amino acids
ACTH	Adrenocorticotropin
AgRp	Agouti-related protein
ARC	Arcuate nucleus
BBS	Bardet-Biedl syndrome
BDNF	Brain derived neurotrophin factor
BMI	Body mass index
CART	Cocaine and amphetamine-regulated transcript
CCK	Cholecystokinin
CLIP	Corticotropin-like intermediate peptide
CRH	Corticotropin releasing hormone
Db	Diabetes gene
DMN	Dorsomedian nucleus
DMV	Dorsal motor nucleus of the vagus
β-END	Endorphin
ER	Endoplasmic reticulum
GOAT	Ghrelin o-acetyltransferase
JAK	Janus Kinase
LC	Locus coeruleus
LEPR	Leptin receptor
LEP	Leptin
LHA	Lateral hypothalamic area
β-LPH	Beta lipotropin
MC3R	Melanocortin-3 Receptor
MC4R	Melanocortin-4 receptor
MSH	Melanocyte-stimulating hormone
N-POC	N-terminal peptide
NPY	Neuropeptide Y
Ob	Obese gene
PC	Propeptide convertase
PMN	Paramedian nucleus

POMC	Proopiomelanocortin
PVN	Paraventricular nucleus
PWS	Prader-Willi syndrome
REE	Resting energy expediture
SIM1	Single-minded 1
SON	Supraoptic nuclei
STAT	Signal Transducer and Activator of transcription proteins
VMH	Ventromedial hypothalamus
WHO	World Health Organisation

Introduction

Obesity may be one of the most concerning and fastest growing problems of the 21st century. World Health Organisation (WHO) characterises obesity as an abnormal or excessive fat accumulation that presents a risk to health (WHO | Obesity b.r.). But behind these simple words is a multifaceted problem without simple solution. As a disease, obesity touches almost every function of the body, while negatively impacting daily life of those suffering from it. From higher risks of cardiovascular diseases, cancer and diabetes to frail mental health.

The difference between sexes in obesity is a well-known fact. The prevalence of obesity is usually higher in women than in men in all age groups, steadily increasing from 20 years of age and culminating by the age of 50 to 65. The escalated predominance of obesity in developing countries is credited to accelerated changes in socioeconomic status, changes in demographic and transition to mainly sedentary life style and high-energy diet. By 2015 nearly 39 % of world population were estimated to be overweight or obese (Chooi, Ding, and Magkos 2019); predictions conducted by few studies show even more horrifying estimations, that approximately 42 % of the world's population will be affected by obesity and 11 % by severe obesity. Although aim of this particular forecast were adults, childhood obesity have massive influence on in, given the stability of surplus fat mass from childhood into adulthood. (Finkelstein et al. 2012).

Obesity is complex illness, originating from broad spectrum of inheritable and environmental factors. The advances in molecular genetics and bioinformatics contributed to better understanding of molecular and genetical basis of obesity in the last twenty years.

The impact of genetics, life style and environment on different types of obesity varies and estimations differ in studies depending on group of patients and phenotype. The occurrence of monogenic types of obesity is a clear proof for genetic impact on obesity, even if the prevalence of patients with monogenic obesity across the globe is so far low.

Gene identification helps to clarify etiology of obesity and its metabolic consequences, because if it is possible to identify groups or individuals at risk from their genetical profile, it is easier to develop personalized strategies of prevention and treatment.

1. Genetic causes of obesity

Genetic causes of obesity can be generalised into three broad groups:

Syndromic obesity

Polygenic obesity

Monogenic obesity

1.1.Syndromic obesity

Syndromic obesity is an obesity often tied with defined set of phenotypes in addition to the early-onset obesity. The cause may be a single gene change or mutation to more extensive chromosomal region and to this day over 100 gene forms have been identified. There are certain difficulties in determining the etiology of this type of obesity as they are exceedingly rare and have complicated mechanism of inheritance influenced by epigenetics or coordination of genes (Thaker 2017). There is unfortunately no cure or effective drug treatment for syndromic obesity as diet and general management still prove to be most effective (Huvenne et al. 2016). Among the most notable syndromes connected to this type of obesity are Prader-Willi syndrome and Bardet-Biedl Syndrome which will be briefly described as an example.

Prader-Willi syndrome (PWS) is model example of syndromic obesity and with prevalence estimated to 1:22 000 live births is also regarded as the most common cause. Syndrome is caused by the loss of the gene function in an exact region of chromosome 15. Most cases of PWS are not inherited, and genetic changes occur randomly during the formation of reproductive cells (eggs and sperm) or in early embryonic development. Affected people typically have no history of the disorder in their family. Phenotype of this disorder includes neonatal hypotonia, behavioural anomalies and often eating disorders with several stages, ranging from failure to thrive in infancy to hyperphagia often leading to obesity and type 2 diabetes. People with PWS have high ghrelin levels, which seems to directly contribute to the increased appetite hyperphagia, and obesity (see chapter 2 - Leptin-Melanocortin pathway).

Bardet-Biedl syndrome (BBS) is a rare disorder with autosomal recessive inheritance. Estimated incidence is 1:150 000 live births, with higher prevalence in isolated populations in Newfoundland and other small regions. Bardet-Biedl syndrome belongs to a group of ciliopathies that are defined by genetic heterogeneity as multitude of different genes were identified as a cause: *BBS1, BBS2, ARL6 (BBS3), BBS4, BBS5, MKKS (BBS6), BBS7, TTC8*

(BBS8), BBS9, BBS10, TRIM32 (BBS11), BBS12, MKS1 (BBS13), CEP290 (BBS14), WDPCP (BBS15), SDCCAG8 (BBS16), LZTFL1 (BBS17), BBIP1 (BBS18) and *IFT27 (BBS19)*. Phenotype slowly progresses from first decade of life and may include limb abnormalities, gradual night blindness, loss of colour vision and most importantly, even though birth weight may be regular, obesity (Thaker 2017).

1.2. Polygenic obesity

Polygenic obesity, such as we see it in most developed countries is not only affected by environment but also by genetic influences.

Unfortunately, the genetical basis of this type of obesity, even if it is the most common cause of obesity, is not known yet. The explanation may be the exceedingly heterogenic group of patients which it affects. This may extremely complicate the explanation of the genetic nature. As a cause is so far regarded cumulative effect of many genes enforced by obesogenic environment. The relationships between these genes, "polygenes" or "polygenic chromosomal loci", are very complicated. Their effects can be additive, nonadditive or even contradictory. If a genes or polygenes of an individual are studied separately, their contribution to weight gain and obesity development is very small, few hundred grams or less. Recent years have brought many findings, how the obesity development is also impacted by epigenetic mechanisms such as histone modifications, DNA methylation and others. Many chemicals have been identified as obesogens or agents that act as endocrine disruptors. All these reasons explain why molecular genetic diagnosis is not yet possible in the most common polygenic obesity (Hinney, Vogel, and Hebebrand 2010).

1.3. Monogenic obesity

Monogenic obesity is an illness caused mainly by single gene mutation, mostly tied with leptin-melanocortin pathway disrupting signals of hunger and satiety. This bachelor thesis is dedicated to this particular problem that seriously affect life of those suffering from it. Classic mendelian inheritance have been described for this illness as manifestation often requires 2 copies of flawed gene in homozygous form. For successful molecular genetic diagnosis the screening is mainly performed on very young children, where the effects of environment or feeding habits are minor. In current stage of knowledge only 5 % to 7,5 % of the most obese children (BMI higher than 97,5 percentiles in the population of the same age) are successfully diagnosed with causal mutation causing obesity (Huvenne et al. 2016).

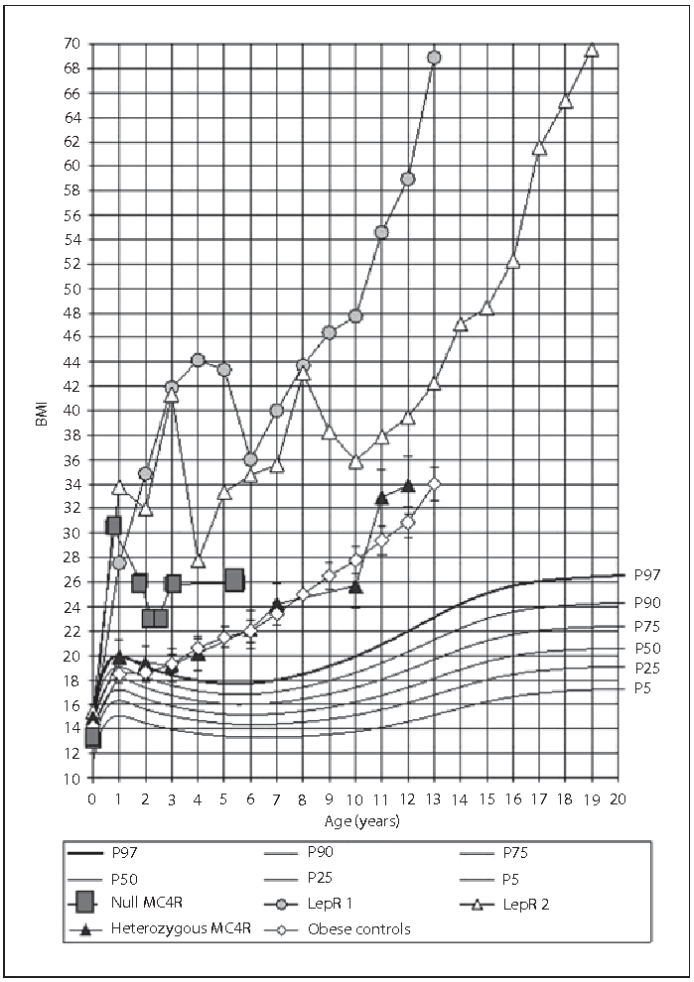

Figure 1 – percentile BMI graph of the juvenile population. Typical cases of carriers with different mutations are shown. Curves of 2 homozygous leptin receptor deficiencies (LepR1 and LepR2) 1 homozygous MC4R deficiency (null MC4R), 6 heterozygous MC4R and for comparison 40 non-mutated obese controls; adopted from (Tounian 2011)

It is also crucial not to forget that, although this sub-chapter is about monogenic obesity (mutation in one distinct gene), environment and other genes also play a large part in monogenic obesity. This is the reason for distinct differences in phenotypes, degree of obesity and its onset with the same mutations.

To this day, more than 30 genes are known, where mutation may clinically be interpreted as monogenic obesity, although many of them were discovered as single cases.

The term "monogenic obesity" has been accepted by the professional community almost two decades ago and is commonly used in medical literature but surprisingly though the term itself is not yet used in disease classification systems.

Orphanet (portal for rare diseases and orphan drugs) labels monogenic obesity under ORPHA code: 98267 Genetic non-syndromic obesity. In WHO International Classification of Diseases, monogenic obesity is not yet classified (ICD-11 - Mortality and Morbidity Statistics b.r.).

Most frequently found mutations in genes are involved in neuronal differentiation of the paraventricular nucleus and leptin-melanocortin pathway. The focus of this bachelor thesis is on the most common causes of monogenic obesity i.e. mutations in MC4R, *MC3R, LEP, LEPR, POMC, BDNF, SIM1, PCI.*

2. Leptin-Melanocortin pathway

Gathered evidence has shown, that central nervous system plays large-scale part in safeguarding against obesity and in its development (Jeong, Kim, and Lee 2014). Leptin-Melanocortin pathway is one of the major aspects in regulation of energy homeostasis and also is a major intersection for signals from peripheral organs and overall dietary condition; every deviation can possibly have severe physiological impact. So, it stands to reason that each and every one of the components must be rigorously controlled and coordinated to achieve equilibrium.

2.1. Hypothalamic nuclei – anatomical description

This controlling effort is centred in diencephalon. Lateral Hypothalamic nucleus with peri-fornical area is suggested to have role in positive energy balance, on the other hand the paraventricular (PVN), dorsomedial (DMN) and paramedian (PMN) nuclei are involved in negative energy balance. Signalling between these two groups of neurons is unified by Arcuate nucleus of the hypothalamus. Arcuate nucleus is handily located on the base of the third ventricle in area without blood brain barrier (Hypothalamic Nuclei b.r.).

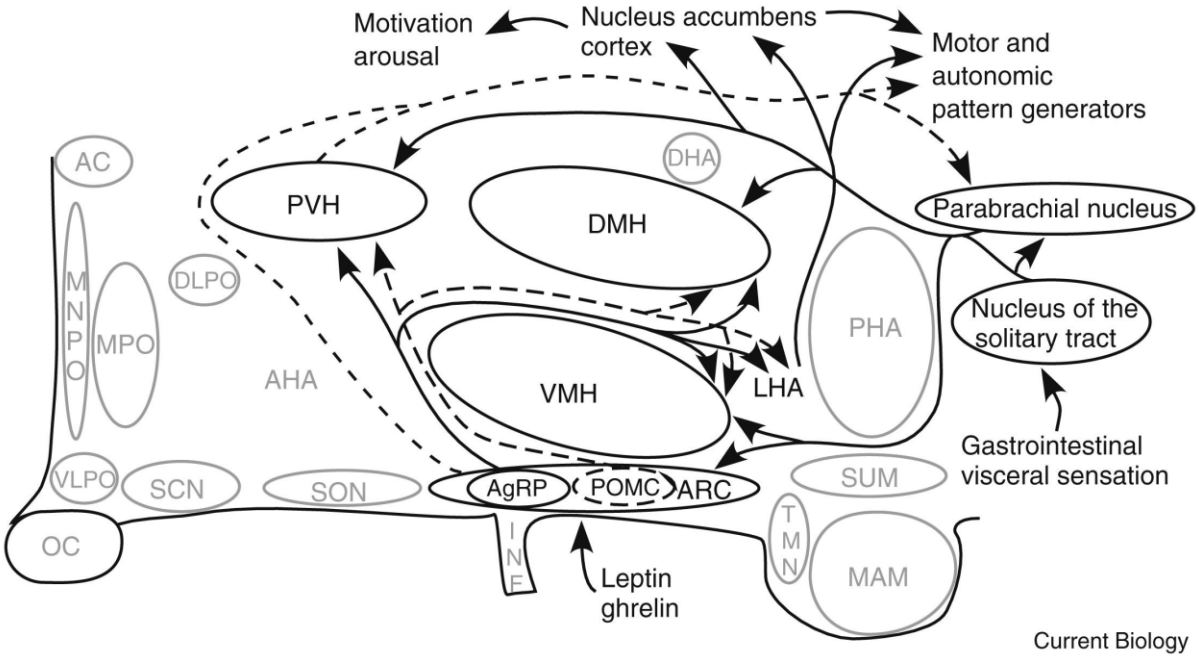

Figure 2- Schematic drawing of the hypothalamic pathways involved in regulation of feeding. Solid lines, pathways promoting feeding; Dashed lines POMC pathways inhibiting feeding. Adopted from (Saper and Lowell 2014)

2.2. Arcuate nucleus

The arcuate nucleus of the hypothalamus is a collection of neurons in the mediobasal hypothalamus, adjacent to the third ventricle and the median eminence. Two groups of neurons are important from an obesiotological point of view. (i) Proopiomelanocortin (*POMC*)/ cocaine and amphetamine-regulated transcript (*CART*) neurons and (ii) co-expressing Agouti-related peptide (AgRP) /neuropeptide Y (NPY) neurons. Both populations will be discussed in depth in next chapters (Minor, Chang, and de Cabo 2009).

2.3. The role of the leptin-melanocortin pathway in the control of food intake

First mentioned neuronal population, POMC is a polypeptide hormone precursor, which provides biologically active peptides such as adrenocorticotropin (ACTH) and then alfa melanocyte-stimulating hormone (α-MSH) by series of enzymatic steps. α-MSH produced by neurons in the ventromedial nucleus have important roles in aforementioned regulation of the appetite (Yang and Tao 2017). POMC functions as a satiety signalling molecule and uses mainly Melanocortin 4-receptor (MC4R) and Melanocortin 3-receptor (MC3R) to inhibit hunger. Both MC3R and MC4R are important part of the path and will be further described.

MC3R is a 7-transmembrane G-protein coupled melanocortin receptor mainly expressed in brain and placenta. For the purpose of this work only brain MC3R will be characterised. Even though MC3R is a one of the more important portions of the pathway, some studies have shown no linkage in mutation in this gene and morbid obesity (Jeong, Kim, and Lee 2014).

Another important 7-transmembrane G-protein coupled melanocortin receptor MC4R is restricted almost only to the brain and is greatly involved in achieving and preserving energy balance by mediating leptin melanocortin pathway. If mutated, this receptor is strongly tied to the increased adiposity and obesity (Qi et al. 2008).

Second neuronal population of arcuate nucleus produces neuropeptide AgRP and NYP. Both work as a neurotransmitter antagonist to POMC and suppress function of MC3R and MC4R and work as an antagonist to α-MSH. They subsequently act as a hunger signal, sensing hunger hormones or lack thereof, like presence of ghrelin, hormonal product of stomach. AgRP/NPY specifically expresses ghrelin receptors to detect aforementioned gut hormone.

Ghrelin is not the only hormone which activates AgRP/NPY since low levels of leptin or insulin may also work as an excitants.

From a time perspective we can divide pathway-important signals into two groups, (i) acute and (ii) long term. The signals of the first group are released based on momentary metabolic needs such as feeling of hunger, appetite, fullness or satiety. To this group may belong cholecystokinin (CCK), ghrelin, and peptide YY for their short-term effects (Garfield et al. 2009). Signals for the second, long term, group are Leptin and Insulin or signals generally tied with adiposity, and as such represents comprehensive metabolic state of the organism (Garfield et al. 2009).

Leptin is protein hormone produced by adipocytes and its amount circulating in the blood is proportional to the amount of white adipose tissue. It has vast share in body weight regulation. This peptide functions in brain as anorexigenic signal and as a sort of a messenger. Leptin brings information about nourishment levels by binding to Leptin receptors (LEPR) expressed on various locations around the brain, most notably in the POMC and AgRP/NPY neurons, various studies has shown that weakened leptin receptor signalling, lower *POMC* gene expression, therefore it is possible to say that leptin is functioning as a regulator for those neurons (Bertile and Raclot 2006).

Over the years considerable progress has been accomplished mainly in understanding of how the adipose tissue engage in energetic homeostasis. White adipose tissue was long mistakenly observed as passive storing organ. Only in last two decades is adipose tissue being taken as metabolically active unit. Secreting many types of adipokines, including leptin, adiponectin and resistin, all of which possess many crucial physiological functions (Sarmento-Cabral et al. 2017).

As to how the pathway is functioning, it is necessary to start with desired hormone such as leptin, insulin or ghrelin, for this example, leptin will be used. Leptin is released from adipocyte and migrates to the brain where it crosses blood-brain barrier by unidirectional system (Banks, DiPalma, and Farrell 1999) and then binds to the leptin receptor located on the POMC neurons. POMC system then subsequently stimulate signal of satiety by producing and releasing α-MSH from its axons terminal parts. α-MSH than activates MC3R and MC4R which leads to decreased eating and larger energy consumption. At the same time leptin also silences AgRP/NPY neurons, which would alternatively antagonise the effect of POMC (Ollmann et al. 1997).

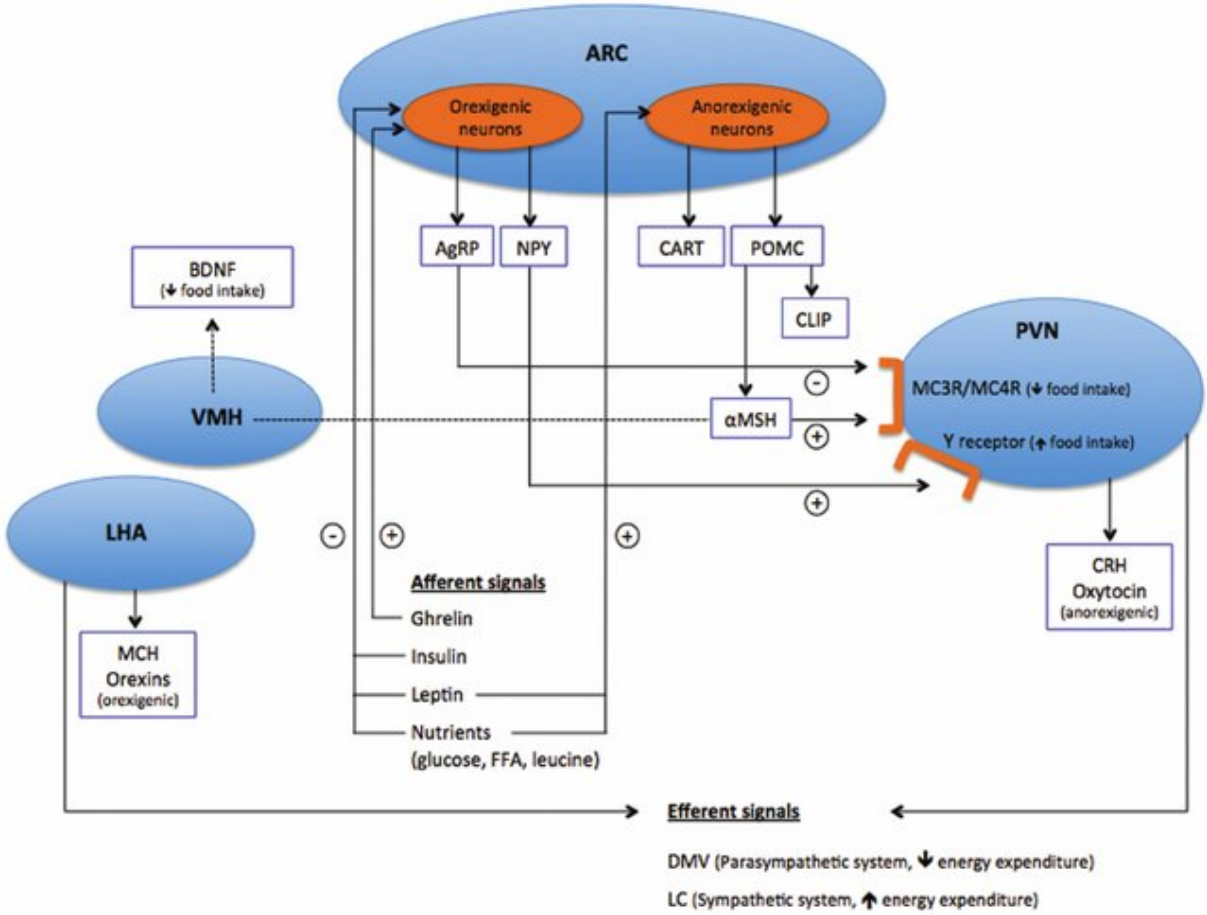

Figure 3 - stylized scheme of regulation of energy homeostasis by the hypothalamus. ARC, arcuate nucleus; PVN, paraventricular nucleus; VMH, ventromedial hypothalamus; LHA, lateral hypothalamic area; AgRP, agouti- related peptide; NPY, neuropeptide Y; CART, cocaine-amphetamine-related transcript; POMC, proopiomelanocortin; CLIP, corticotropin-like-intermediate lobe peptide, α -MSH, alpha-melatonin stimulating hormone; BDNF, brain-derived neurotrophic factor; MCH, melanin concentrating hormone; CRH, corticotropin releasing hormone; DMV, dorsal motor nucleus of the vagus; LC, locus coeruleus. Adopted from (Haliloglu a Bereket 2015)

3. Leptin and Leptin receptor

Out of many genetic disorders connected to the obesity, none have been studied more than *Ob/Ob* and *Db/Db* mice. In 1994 using positive cloning method a molecular defect was determined in *obese* (*Ob)* gene (Zhang et al. 1994). Later on, leptin receptor was discovered in similar manner in *Db/Db* mice.

LEP gene was located on 6th chromosome on mice and in human its location was found on 7q31.3. *Ob* gene was shown to encode 4.5 kilobase long mRNA transcript with a highly conserved 167 amino-acid open reading frame. Upon its isolation the encoding protein was named leptin from Greek *leptos* meaning thin.

As mentioned in previous chapters leptin is found mainly in white adipose tissue, however it can also be found in various tissues such as brown adipose tissue, gastric epithelium, placenta, skeletal muscle and stomach, albeit amount of leptin found is considerably lower (Sobhani 2000).

Numerous drugs and hormones were described to have effect on leptin regulation, be it by protein expression or mRNA. Among them glucocorticoids (Vos et al. 1995), insulin (Tsai et al. 2012) and thyroid hormone triiodothyronine were reported as a mRNA up-regulators and β_3-adrenergic agonist and thiazolidinediones as a down-regulators. Effect of sex hormones was also studied and validated. In this study oestrogens were proved to have positive effect on leptin expression and androgens negative (Machinal et al. 1999). There are also several physiological conditions such as exposure to cold or starvation which can affect level of leptin in plasma.

Obese patients possess raised levels of leptin in serum and abundance of leptin's mRNA in adipocytes. As a result of hyperleptinemia, most people with multifactorial obesity are considered resistant to leptin. Higher level of leptin in serum fails to decrease feeding urges and body weight levels as it would in normal situation (Considine et al. 1996). Many mechanisms were proposed in terms of how does leptin resistance work. Some studies talk about alternations in the crossing the blood brain barrier by leptin or disruptions in developmental programming. The genome-wide association has shown a link with polymorphism near the leptin gene with obesity in general. This confirms just how important leptin really is in the development of obesity (Pan et al. 2012).

Leptin works mainly through activation of cytokine receptor, namely leptin receptor (LEPR) which was identified through cloning technique. It is a member of class I cytokine receptors family, which consist, among others of growth hormone, prolactin or granulocyte-colony stimulating factor. There are 5 known isoforms of LEPR, each product of alternative RNA splicing of *Db (*diabetes) gene and it's possible to sort them into three categories, short, long, and soluble. Long Ob-Rb, short Ob-Ra,Ob-Rc, Ob-Rd and soluble Ob-Rf (Tartaglia et al. 1995).

Structurally all of those contain the same extracellular domain with 820 AA length and transmembrane domain of 34 amino acids (AA); intercellular domain length varies form 303 AA in long form to 32 AA in short variants. Apart from the exact same extracellular and transmembrane domain, OB-R isoforms consist of the same sequence intracellular 29 AA which includes constant box 1 motif and JAK tyrosine kinase. Box 2 motif is only present in long isoform of OB-R, as it ensures maximal activation of OB-Rb. Short forms of Leptin receptor can bind JAK but are not capable of STAT signalling.

LEPR is present in various tissues, with the biggest presence in hypothalamus nuclei such as ARC, DMH and VMH. mRNa of Ob-Rb is highly noticeable especially in ARC as it is found in both NPY/AgRP and POMC neurons.

As is common with cytokine receptors, LEPR does not possess inherent enzymatic activity. Instead it uses signalling by Janus kinase (JAK), tyrosine kinase from JAK family. Binding of leptin to its receptor cause change of conformation and activate receptor-related Janus kinase, ensuring autophosphorylation of JAK1 and JAK2 and tyrosine phosphorylation of cytoplasmic domain and downstream transcription factors named STATs. Those molecules are greatly expressed in hypothalamus and other brain regions controlling food intake (Håkansson and Meister 1998).

Mutations in *leptin* gene as well as in *Leptin receptor* are recognised as a cause of serious early-onset hyperphagic childhood obesity with swift weight increase in first few months of their lives. Mutations in *LEPR* gene are as rare as mutations in *LEP* gene. And since those mutations have autosomal recessive inheritance, most of the described patients are found in countries with high prevalence of consanguineous marriages. They are affected by either the same mutations on both chromosomes or are compound heterozygotes. (Saeed et al. 2015)

3.1. Monogenic forms of obesity in leptin gene and Leptin receptor gene

The first patients with mutation in leptin gene have been described in 1997. For leptin deficiency to manifest, both parental alleles must be affected. So far only few dozen families with homozygous mutations have been described and most often they hail from countries like Pakistan or Turkey (higher chance of consanguinity, as stated before) (Saeed et al. 2012). Classic phenotype includes early onset severe obesity identified with hyperphagia, hypogonadism and increased frequency of infections.

LEP gene mutation can be successfully treated by administrating recombinantly produced injection of leptin once a day. With this medication, hyperphagia recedes and all the other symptoms, obesity included, disappear. Unfortunately, the treatment must continue throughout patients whole life, as without those injections they revert to state before treatment. Regrettably this therapy does not work for *LEPR* gene mutations, but recently new drug called "Setmelanotide" has been approved and there is hope that it will work for patients with *LEPR* mutations, (see chapter 9 - future treatment).

Leptin mutations are reported very sparsely but the most prevalent consist of recessive frame shift mutation in exon 3 in Ob gene, tagged as G133_VfsX14 (398del) which disrupts reading frame and results in 14 abnormal AA and followed by STOP codon. Consequential level of leptin in serum is undetectable or very low.

Another mutation associated with Leptin deficiency is I35del (104_106delTCA). It is a homozygous mutation affecting 3 base pair deletion located on exon 2 in Ob gene and as with the case before leptin level were below detection (Saeed et al. 2012).

Most of the *LEPR* mutations have common symptoms of aberrant eating behaviours, selective deposition of fat mass and in adult patients hypogonadotropic hypogonadism (Farooqi, Wangensteen, et al. 2007).

Among the known mutations of *LEPR* belong frameshift mutation tagged p.C186AfsX27 (c.556delT), this mutation causes premature stop codon and thus aberrant receptor. Another mutation marked as c.2396-1G>T, is an essential splice site mutation, resulting in flawed splicing of Ob transcript by skipping exon 15 and thus encoding shorter and dysfunctional LEPR protein (Saeed et al. 2015).

4. POMC (proopiomenalocortin)

The existence of precursor for molecules such as melanotropins (MSHs), adrenotropin (ACTH) and β-endorphin was confirmed in 1979 by cloning of bovine cDNA. Three POMC exons interlaid with extensive introns were identified in bovine and later also in human genome. The third exon was discovered to contain sequence of previously known active peptides (Slominski 1996). POMC gene is mainly expressed in pituitary, arcuate nucleus of hypothalamus and few peripheral tissues like pancreas, placenta and skin. This gene produces single protein which migrates to Golgi bodies and is then directed by signal peptide sequence into secretory granule where is processed in cell type specific way to generate different neuropeptides (Bateman, Solomon, and Bennett 1990). Between the most important neuropeptides for maintaining healthy body mass belong α-MSH, β-MSH and γ-MSH. Those melanocortin vary in their affinity to melanocortin receptors. For example the β-MSH has highest affinity for MC4R and γ-MSH for MC3R (Grieco et al. 2006).

In POMC neurons the mechanism of intracellular signalling is quite intricate, but it is accepted that steps to achieve individual peptides are under multihormonal control. The processing includes cleaving the peptide by propeptide convertases PC1 and PC2. Both proteinases are able to cleave POMC at distinct pairs of basic residues. It is shown that PC2 has much larger spectrum of distribution in brain than PC1 (Benjannet et al. 1991).

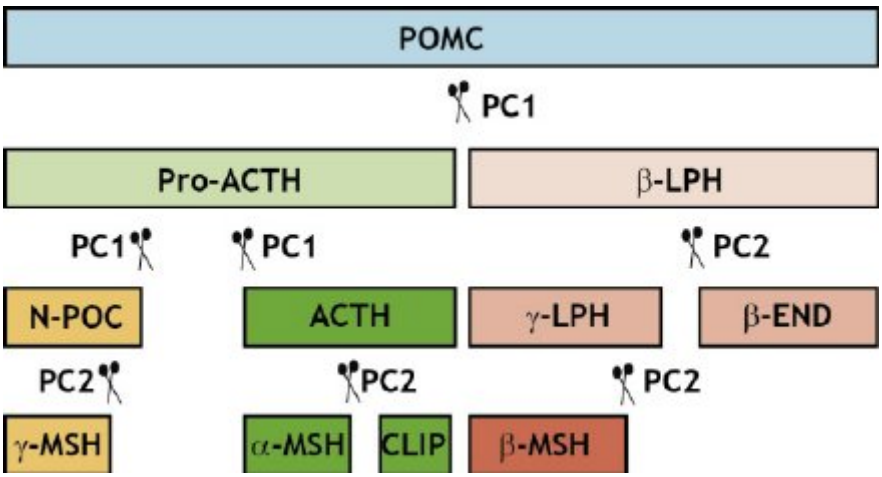

Figure 4 - Hypothalamic post-translational processing of POMC. PC1 cleaves POMC to pro-ACTH and β-lipotropin (β-LPH). Pro-ACTH is than cleaved yet again by PC1 to N-terminal peptide (N-POC) and ACTH. PC2 than cleaves ACTH to α-MSH and corticotropin-like intermediate peptide (CLIP). PC2 also generates Γ-MSH by cleaving N-POC. β-LPH is cleaved by PC2 to γ-LPH and β -endorphin (β-END). B-MSH is cleaved from γ-LPH by PC2. Adopted from (Caruso et al. 2012)

Insulin receptors are expressed in noticeable population of POMC neurons, through those receptors, insulin is able to activate phosphatidylinositol-3 kinase (PI3K) pathway. Both leptin and insulin activate distinctive signalling pathways in POMC neurons in ARC (Benoit et al. 2002). Melanocortin receptors will be covered in depth in the following chapter.

POMC deficiencies phenotype is mostly defined by adrenal failure, hyperphagia, early onset obesity, changed pigmentation and tall statue. Mendelian *POMC* is very rare, but it may take part in predispositions of obesity. Carriers of heterozygous mutation in *POMC* have only slightly higher or even normal weight.

One of the first reported *POMC* deficiency was documented in 1998. Both probands were children with seemingly same phenotype. Both subjects developed severe early-onset obesity with hyperphagia and pale skin with red hair as a result of flawed MC1R signalling in melanocytes in hair follicles. Proband number one had 2 nonsense mutations in exon 3. Proband number two was homozygous for mutation in 5´untranslated region that interfered with initiation of translation (Coll and Loraine Tung 2009).

One of the mutations that may also occur is a missense mutation in the cleavage site between β-MSH and β-endorphin. The resulting gene form an aberrant protein, which then binds to MC4R, and has a reduced ability to activate said receptor and thus cause obesity (Challis et al. 2002).

World-wide, the prevalence of patients with diagnosed mutation in *POMC* gene is very low, for example complete lack of *POMC (*homozygous mutation) was described in only few cases. Fortunately for treatment of obesity due to pro-opiomelanocortin deficiency was approved drug called “Setmelanodide” (chapter 9 - future treatment)

5. Melanocortin receptors

Melanocortin receptors are members of "A" family rhodopsin-like G-protein coupled receptors (GPCR). GPCR are one of vastest class of cell surface proteins and many stimulants like peptides, photons or large glycoprotein hormones depend on those receptors to relay information by activating G-proteins and effectively increase cAMP production. There are 5 melanocortin receptors named from MC1R to MC5R, according to their cloning sequence.
Both MC3R and MC4R are expressed in brain as they are both part of melanocortin pathway. MC3R is expressed in hypothalamus and few regions of brainstem, MC4R on the other hand as stated in previous chapter, is expressed in brain more intensively in regions such as hypothalamus, thalamus, and cortex. MC5R is located in peripheral tissues and has ties to exocrine gland functions (Tao and Segaloff 2003). Neither MC1R nor MC2R do not have significant ties to the monogenic obesity and will therefore not be discussed in this work.

5.1. Melanocortin-4 receptor

Melanocortin-4 receptor (MC4R) is one of the most important parts of the whole leptin-melanocortin pathway. The gene for this receptor is located on chromosome 18q22 has only one exon, and the resulting protein is composed of 332 AA. To be fully functional the receptor must have His-DPhe-Arg-Trp AA sequence (Holder et al. 2002).

Many studies have reported numerous mutations connected to the non-syndromic obesity. In fact *MC4R* mutations are the most common cause of monogenic obesity, with prevalence of 2,4 % in severely obese Czech children who have early-onset obesity (Hainerová et al. 2007). Depending on penetration, the phenotype with exception of obesity, may vary. *MC4R* deficiency includes hyperphagia, food seeking behaviour, linear growth and although, hyperinsulinemia is present, distinct lack of diabetes. Remarkable is that children are more affected by hyperinsulinemia than equally impaired adults. It also seems like MC4R deficiency creates less intensive hyperphagia than deficiency in leptin receptor (Farooqi et al. 2000).In comparison to the other monogenic obesity syndromes, pubertal development and reproduction is not affected in *MC4R* deficient patients. *MC4R* is a prime target for pharmacological research, as a most prevalent mutation tied with obesity.

The first *MC4R* mutation was reported in 1998. And since then 70 mutations were described in patients which varied in ethnicity and age. Mutations included deletions, nonsense, missense and frameshift mutations dispersed though out the whole receptor.

It is possible to divide those mutation into 5 classes.

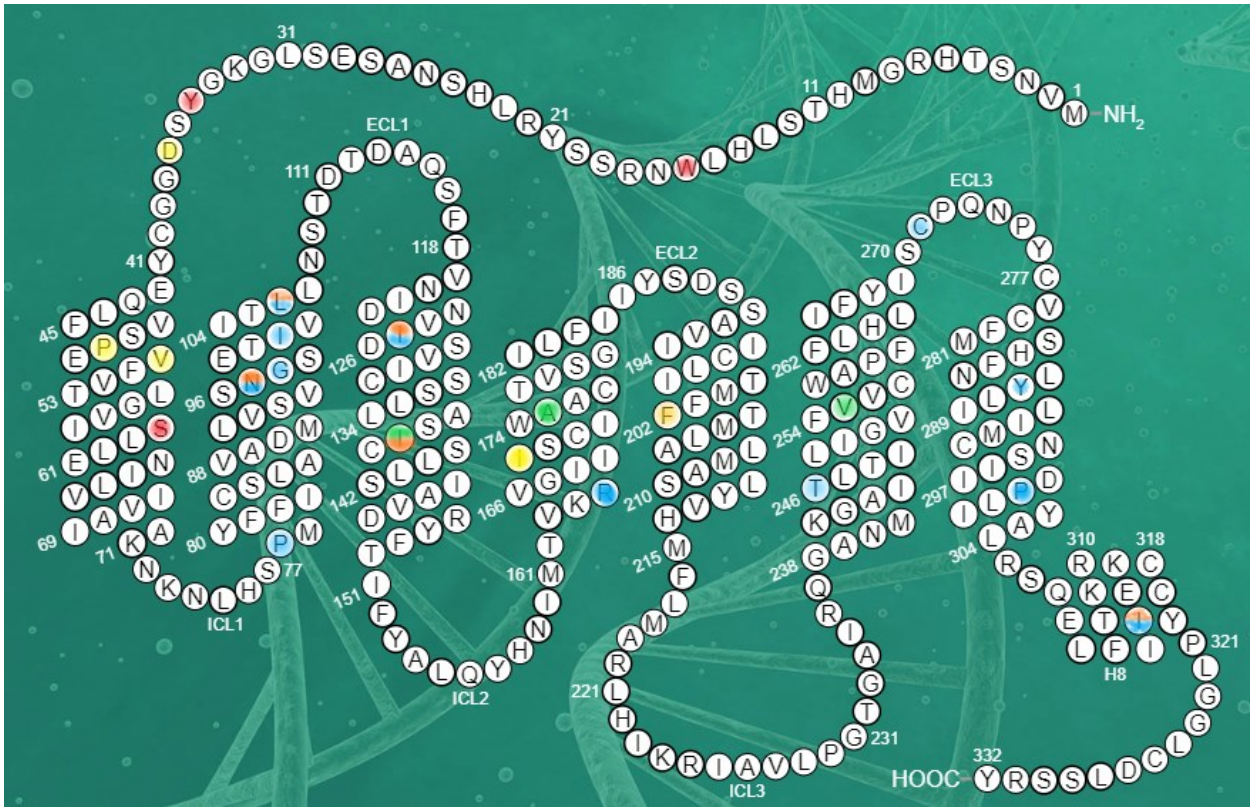

Figure 5 - MC4R schematic, Class I mutations – red color,Class II – blue color, Class III – orange, Class IV - green - Class V – yellow color. adopted from (Melanocortin 4 Receptor | Welcome to the MC4R Gene Website b.r.) edited

5.1.1. Class I. – Null mutations

Mutations W16X, Y35X and L64X are suggested to belong there as these mutants do not yield receptor protein due to defective synthesis or accelerated degradation. There is however little evidence of this.

5.1.2. Class II. – Intercellularly trapped mutants

Mutations L106P, I125K, P299H and many more belong to this class and make it the biggest class of MC4R mutations.

5.1.3. Class III. – Binding defective mutants

Mutations I137T, N97D and I316S show approximately normal expression on the cell surface, but binding of ligands is defective due to decreased amount of maximal binding or impaired binding affinity.

5.1.4. Class IV. – Signalling defective mutants

Mutations I137T, A175T, V253I may belong to this class. These receptor mutations are expressed on cell surface and bind ligands with little difficulty, however agonist-stimulated signalling is defective.

5.1.5. Class V. – Variants with unknown defects

These mutations behave similarly to the wild type in heterogenous system. Some express normal cell surface, ligand binding and even agonist-stimulated cAMP. How and if those mutations cause obesity is still unclear (Tao and Segaloff 2003).

Most mutations were found in heterozygous form with notable exception of mutation N62S. Patients with this mutation in heterozygous form were not obese (Farooqi et al. 2000). During the research of polygenic obesity via Genome wide-association study (GWAS), *MC4R* polymorphisms were discovered, that have statistically slight protective effect against obesity.

5.2. Melanocortin – 3 receptor

Human melanocortin-3 receptor is single exon gene encoding 360 AA protein mapped to the 20q13.2 region. It could be assumed that, from its position in brain as well as its structure, MC3R may be a strong candidate for possible monogenic cause of obesity; however, this receptor has always been controversial in this regard.

Studies have shown that patients with two missense mutations namely, Thr6Lys and Val81Ile in homozygous form, had greater BMI score, leptin and insulin levels and fat tissue mass. Double mutants MC3R had also significantly lower ligand binding capacity. In terms of difference between races, particularly African-American children were partial for being double-homozygous for these variants (Feng et al. 2005).

Another research has uncovered I335S mutation, which has been implied in pathogenic mutation, that mutation leaves receptor without any ligand binding or signalling due to intracellular retention. It is a known fact, that intracellular retentions are one of the most common defects or naturally ensuing mutation (Tao 2007). All this evidence suggests some role of MC3R in obesity, but it is still not clear if the mutations in *MC3R* may be classified as the cause of monogenic obesity.

6. BDNF (Brain derived Neurotrophic factor)

BDNF is a member of neurotrophins, small secretory proteins with significant roles in development and protection of nervous system. These proteins are particularly copious in central nervous system, most notably in VMH; levels of BDNF grow significantly in post-natal development. Studies show that neurotrophins are indispensable for body weight control, for example low circulating levels are correlated with type 2 diabetes and obesity. There is evidence showing that circulating levels of BDNF are regulated by plasma levels of glucose (Krabbe et al. 2007).

Human *BDNF* gene can be targeted to locus 11q14.1 containing 11 exons and 9 functional promoters that are activated tissue and brain-region specifically. *BDNF* gene Encodes 247 AA preprotein which is later cleaved to form fully mature 120 AA protein. This protein is fully conserved across the species. *BDNF* expression is regulated mostly by MC4R signalling.

In *Bdnf* knock-out mice the phenotype consists of obesity, developed hyperphagia and is over all very similar to *MC4R* deficient mice (Friedel et al. 2005).

Missense mutation in *BDNF* receptor TrkB, encoded by *NTRK2* gene on 9 chromosome is associated with weight gain, obesity and hyperphagia. In obese mouse model with type 2 diabetes adding BDNF exogenously and transferring *BDNF* gene leads to weigh loss and decreased insulin resistance. This evidence suggests that BDNF brain deficit is indeed cause of monogenic obesity (Sandrini et al. 2018).

7. SIM1 (Single-minded 1)

Protein single minded 1 (SIM1) is transcription factor with basic helix-loop-helix conformation. It is expressed in developing nervous system and has crucial role in development of several nuclei in hypothalamus, mainly in those which are connected to energy homeostasis like paraventricular nuclei (PVN) and supraoptic nuclei (SON) (Michaud et al. 2001, 1).

Various studies marked *SIM1* as a candidate for a cause of human obesity. *SIM1*-haploinsuficient mice are hyperphagic, obese and exceptionally sensitive to diet-caused obesity, phenotype which closely resembles mice with lack of MC4R. On the other hand, overexpression leads to reduction of food intake, however the correlation between MC4R-null mice and haploinsuficient-*SIM1* is not as straight forward. *SIM1* deficiency is associated with hyperphagic obesity alongside normal energy expenditure.

In humans, the deletions in 6q16 chromosome, which houses *SIM1* gene, have been identified in obese patients with Prader-Willi like (PWL) syndrome characterised by early-onset development delay, hypotonia, and later on by obesity, hyperphagia and facial dysmorphism.

SIM1 non-syndromic mutations were found in many patients with severe obesity, both associated and independent of PWL-related phenotype. Some mutations seriously decrease *SIM1* activity and can be found only in obese people. This suggests that *SIM1* mutations may indeed be part of monogenic causes of obesity. However not every mutation in this gene is responsible for obese phenotype, because it is indicated that environment and genetic background play important part (Bonnefond et al. 2013).

8. PC1/3 – (Prohormone convertase)

Prohormone convertase 1/3 (PC1/3) is a major enzyme tied with processing and maturation of neuropeptide precursors and prohormones. It is encoded by *PCSK1* gene and synthetized in endoplasmic reticulum (ER). Between the most important processing substrates of PC1/3 belong proopiomelanocortin, proinsulin and proglucagon.

PC1/3 is synthetized in inactivated form, and in order to activate this precursor, two significant events have to occur. Firstly, the N-terminal must be cleaved within ER to yield 85 kDa precursor, and secondly another cleaving transpires and generates fully active 66 kDa PC1/3 isoform (Lloyd, Bohan, and Gekakis 2006).

It is intriguing that while human with mutated PC1/3 display obese phenotype, knock-out mice do not display obesity but show multiple growth and endocrinologic problems. This leads to assumption that PC1/3 in human and mice serves slightly different purpose.

Causal unambiguous mutations in PC1/3 are very rare, but for example a missense mutation Ser307Leu was found in Libyan child with severe early-onset obesity, whose parents were consanguineous (Farooqi, Volders, et al. 2007).

9. Future treatment

As the threat of obesity is steadily rising, there is certain pressure on pharmaceutical companies to provide a magical cure in form of a safe and effective drug. This chapter will be dedicated to future possibilities of treatment of monogenic obesity, and perhaps even obesity itself.

For a long time obesitologists did not have any effective and safe drugs to use to treat their patients with obesity; not including those few people in the world who have *LEP* deficiency and therefore may be effectively treated with leptin shots.

MC4R is viewed as one of the promising targets for drug therapy of obesity, currently two approaches of affecting functions or emend the damage of MC4R are tested on experimental models, some are already in clinical stage.

First approach concentrates on a search for appropriate molecular and chemical chaperones to correct the misfolding of MC4R mutants. This approach is however hindered with non-specific effect of chaperones and the necessity of high therapeutic dosage. Out of many tested pharmacological chaperones the most promising seem to be Ipsen 5i and Ipsen 17 (Huang, Wang, and Tao 2017).

Second approach is centred on search for suitable MC4R agonist or a molecule capable of binding receptor and starting signalling cascade resulting in biological response. Aforementioned agonist can be sorted into three groups based on their chemical nature.

1.) Linear peptide agonist

    Various linear peptide analogues of α-MSH with changed AA, are tested for more prominent effect than that of natural α-MSH.

2.) Cyclic peptide agonist

    Majority of cyclic substances tested against MC4R posses short sequence, from four to eight AA of core unit from α-MSH (His-Phe-Arg-Trp), which is vital for MC4R binding and cyclised by disulphide bonds. For example, setmelanotide belongs to this group, containing 8 cyclically bonded AA from natural α-MSH sequence, but with four interchanged AA.

3.) Non-peptide agonist

    Many substances, from the simplest to more complicated organic compounds, were tested for their potential to bind to MC4R (Gonçalves, Palmer, Meldal 2018).

One of the potential medications is a “Setmelanotide”, a cyclic peptide binding with high affinity to human MC4R mostly in lateral hypothalamic area (LHA). It has proved to lower food intake and body weight as well as re-establish insulin sensitivity in mice, dogs and monkeys. Due to test on MC4R knockout mice, where it failed to provide any response in terms of weight loss, it is possible to suggest that the area of effect is indeed MC4R (Collet et al. 2017).

Therapy for Hyperphagia and obesity caused by *POMC* deficiency is based on agonist that substitute lack of MSH binding to MC4R and in result replace the function of *POMC* in leptin-melanocortin pathway. Preliminary studies have shown that such agonist as “Setmalnotide” indeed allowed patients to lose considerable amount of body weight (Kühnen et al. 2016).

Leptin receptor defect may also be cured by “Setmelanotide”, since its small molecules are expected to be able to directly stimulate nerves that control appetite, bypassing the need for leptin., resulting in lower weight and reduced desire for food (Public summary of opinion on orphan designation Setmelanotide for the treatment of leptin receptor deficiency b.r.).

Sermelanotide is also discussed to have effect on PWS, signifficantly increasing resting energy expediture (REE) and increasing fat oxidation in obese individuals. Trials have so far brought satisfying results of weight loss and good tolerability (Crinò et al. 2018).

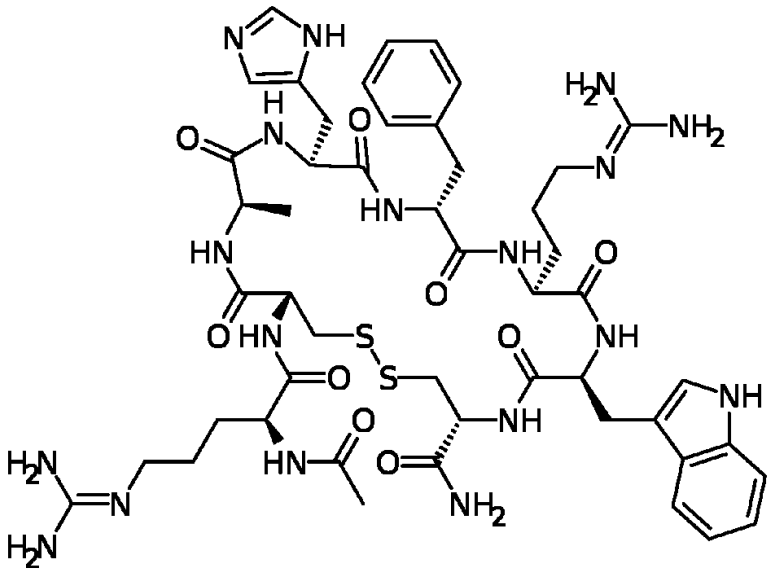

Figure 6 - Chemical structure of Setmelanotide; (4R,7S,10S,13R,16S,19R,22R)-22-[[(2S)-2-acetamido-5-(diaminomethylideneamino)pentanoyl]amino]-13-benzyl-10-[3-(diaminomethylideneamino)propyl]-16-(1H-imidazol-5-ylmethyl)-7-(1H-indol-3-ylmethyl)-19-methyl-6,9,12,15,18,21-hexaoxo-1,2-dithia-5,8,11,14,17,20-hexazacyclotricosane-4-carboxamide (IUPAC nomenclature); adopted from (Setmelanotide|RM-493;BIM-22493;IRC-022493|CAS 920014-72-8 Buy Setmelanotide from supplier medchemexpress.com b.r.)

In the spring of 2019 “Setmelanotide” was approved by European Commission for treatment of rare diseases i.e. obesity due to pro-opiomelanocortin deficiency and obesity due to leptin receptor gene deficiency (Public Health - European Commission b.r.).

In the last stage of clinical testing “setmelanotide” is being used to treat obesity caused by PWS, BBS, Alström Syndrome and POMC Heterozygous Deficiency Obesity.

Another potential drug, that should be mentioned, is RM-853, which inhibits ghrelin o-acyltransferase and is currently in first stages of clinical trials (“OUR PIPELINE.” n.d. Rhythm Pharmaceuticals).

While the hunt for effective drug continues, treatments based on rational diet and, if possible, lifestyle change are still considered the most effective non-invasive way of preventing and reigning in obesity.

10. Conclusion

Obesity as a whole is still growing problem that may well in future endanger millions of people. Diagnosis of obese patients may be divided in to three major categories: Syndromic obesity, Polygenic obesity and Monogenic obesity.

Participation of central nervous system, mainly in monogenic obesity, is non-questionable, as energy homeostasis and regulation of metabolism fall to this organ. Particularly hypothalamus and its neural leptin melanocortin pathway gained much spotlight in this regard.

The discovery of possible obesity-causing genes has brought hope for those suffering from monogenic obesity. Especially afflicted children may now be assessed much easier as the options for the diagnosis are growing.

Most of the genetic mutations share very similar phenotype of increased food intake followed by hyperphagia and obesity. Even though many of the most common gene mutations are known to this day, many more are still waiting to be discovered.

One of the most common causes of ebesity is mutation in receptor MC4R, tied with melanocortin pathway. On the other hand, it's still debated whether or not is MC3R cause of full scale obesity phenotype. LEP and LEPR have been studied thoroughly for their participation in obesity phenotype, mainly through model *Ob/Ob* and *Db/Db* mice. Mutation in another gene, *PC1/3,* is entwined with *POMC* as PC1/3 cleaves POMC into its many neuropeptides. Following this logic mutation in *PC1/3* has to, among others, affect POMC function. *SIM1* is an analogue of Drosophila single-minded gene and is associated with PWL syndrome. *BDNF* plays role in glucose metabolism and has negative impact on obesity and type 2 diabetes.

While main genetic animal model is a mouse, it is very important to remember that not every mutation found in animals is going to cause the same phenotype in human, e.g. mutation in *PC1/3* express obese phenotype in human but not in mice, where it causes growth retardation.

Fortunately for future treatments of obese patients, many new drugs are in process of development or are being already clinically tested. It yet remains to be seen if the developed drugs for monogenic obesity have the same effect on obesity in general. New treatment options emphasise routine gene mutation diagnosis for patients where monogenic type of obesity is suspected, even though it is quite time and financially consuming.

Sources

Banks, William A, Christopher R DiPalma, a Catherine L Farrell. 1999. „Impaired Transport of Leptin across the Blood-Brain Barrier in Obesity☆". *Peptides* 20(11): 1341–45.

Bateman, A., S. Solomon, a H. P. Bennett. 1990. „Post-Translational Modification of Bovine pro-Opiomelanocortin. Tyrosine Sulfation and Pyroglutamate Formation, a Mass Spectrometric Study." *Journal of Biological Chemistry* 265(36): 22130–36.

Benjannet, S et al. „PCI and PC2 Are Proprotein Convertases Capable of Cleaving Proopiomelanocortin at Distinct Pairs of Basic Residues". 1991: 5.

Benoit, Stephen C. et al. 2002. „The Catabolic Action of Insulin in the Brain Is Mediated by Melanocortins". *The Journal of Neuroscience* 22(20): 9048–52.

Bertile, Fabrice, a Thierry Raclot. 2006. „The Melanocortin System during Fasting". *Peptides* 27(2): 291–300.

Bonnefond, Amélie et al. 2013. „Loss-of-Function Mutations in *SIM1* Contribute to Obesity and Prader-Willi–like Features". *The Journal of Clinical Investigation* 123(7): 3037–41.

Caruso, Carla et al. 2012. „Melanocortins: Anti-Inflammatory and Neuroprotective Peptides". *Neurodegeneration*. https://www.intechopen.com/books/neurodegeneration/melanocortins-anti-inflammatory-and-neuroprotective-peptides (7. květen 2019).

Coll, Anthony P., a Y. C. Loraine Tung. 2009. „Pro-opiomelanocortin (POMC)-derived peptides and the regulation of energy homeostasis". *Molecular and Cellular Endocrinology* 300(1): 147–51.

Collet, Tinh-Hai et al. 2017. „Evaluation of a melanocortin-4 receptor (MC4R) agonist (Setmelanotide) in MC4R deficiency". *Molecular Metabolism* 6(10): 1321–29.

Considine, Robert V, Aidas Kriauciunas, Joanna P Ohannesian, a Thomas L Bauer. 1996. „Serum Immunoreactive-Leptin Concentrations in Normal-Weight and Obese Humans". *THE NEW ENGLAND JOURNAL OF MEDICINE* 334(5): 4.

Crinò, Antonino, Danilo Fintini, Sarah Bocchini, a Graziano Grugni. 2018. „Obesity management in Prader–Willi syndrome: current perspectives". *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy* 11: 579–93.

Farooqi, I. Sadaf et al. 2000. „Dominant and Recessive Inheritance of Morbid Obesity Associated with Melanocortin 4 Receptor Deficiency". https://www.jci.org/articles/view/9397/pdf (3. duben 2019).

Farooqi, I. Sadaf, Teresia Wangensteen, et al. 2007. „Clinical and Molecular Genetic Spectrum of Congenital Deficiency of the Leptin Receptor". *New England Journal of Medicine* 356(3): 237–47.

Farooqi, I. Sadaf, Karolien Volders, et al. 2007. „Hyperphagia and Early-Onset Obesity Due to a Novel Homozygous Missense Mutation in Prohormone Convertase 1/3". *The Journal of Clinical Endocrinology & Metabolism* 92(9): 3369–73.

Feng, N. et al. 2005. „Co-Occurrence of Two Partially Inactivating Polymorphisms of MC3R Is Associated With Pediatric-Onset Obesity". *Diabetes* 54(9): 2663–67.

Finkelstein, Eric A. et al. 2012. „Obesity and Severe Obesity Forecasts Through 2030". *American Journal of Preventive Medicine* 42(6): 563–70.

Friedel, S. et al. 2005. „Mutation Screen of the Brain Derived Neurotrophic Factor Gene (BDNF): Identification of Several Genetic Variants and Association Studies in Patients with Obesity, Eating Disorders, and Attention-Deficit/Hyperactivity Disorder". *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics* 132B(1): 96–99.

Garfield, Alastair S. et al. 2009. „Role of Central Melanocortin Pathways in Energy Homeostasis". *Trends in Endocrinology & Metabolism* 20(5): 203–15.

Gonçalves, Juliana Pereira Lopes, Daniel Palmer, a Morten Meldal. 2018. „MC4R Agonists: Structural Overview on Antiobesity Therapeutics". *Trends in Pharmacological Sciences* 39(4): 402–23.

Grieco, Paolo et al. 2006. „Structure–activity studies of new melanocortin peptides containing an aromatic amino acid at the N-terminal position". *Peptides* 27(2): 472–81.

Hainerová, Irena et al. 2007. „Melanocortin 4 Receptor Mutations in Obese Czech Children: Studies of Prevalence, Phenotype Development, Weight Reduction Response, and Functional Analysis". *The Journal of Clinical Endocrinology & Metabolism* 92(9): 3689–96.

Håkansson, Marie-Louise, a Björn Meister. 1998. „Transcription Factor STAT3 in Leptin Target Neurons of the Rat Hypothalamus". *Neuroendocrinology* 68(6): 420–27.

Haliloglu, Belma, a Abdullah Bereket. 2015. „Hypothalamic Obesity in Children: Pathophysiology to Clinical Management". *Journal of Pediatric Endocrinology and Metabolism* 28(5–6). https://www.degruyter.com/view/j/jpem.2015.28.issue-5-6/jpem-2014-0512/jpem-2014-0512.xml (7. květen 2019).

Hinney, Anke, Carla I. G. Vogel, a Johannes Hebebrand. 2010. „From monogenic to polygenic obesity: recent advances". *European Child & Adolescent Psychiatry* 19(3): 297–310.

Holder, Jerry Ryan, Rayna M. Bauzo, Zhimin Xiang, a Carrie Haskell-Luevano. 2002. „Structure-Activity Relationships of the Melanocortin Tetrapeptide Ac-His-DPhe-Arg-Trp-NH(2) at the Mouse Melanocortin Receptors: Part 2 Modifications at the Phe Position". *Journal of Medicinal Chemistry* 45(14): 3073–81.

Huang, Hui, Wei Wang, a Ya-Xiong Tao. 2017. „Pharmacological chaperones for the misfolded melanocortin-4 receptor associated with human obesity". *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* 1863(10, Part A): 2496–2507.

Huvenne, Hélène, Béatrice Dubern, Karine Clément, a Christine Poitou. 2016. „Rare Genetic Forms of Obesity: Clinical Approach and Current Treatments in 2016". *Obesity Facts* 9(3): 158–73.

„Hypothalamic Nuclei". *Obesity and Diabetes*. http://www.diabetesobesity.org.uk/hypothalamic-nuclei.html (30. duben 2019).

Challis, Benjamin G. et al. 2002. „A Missense Mutation Disrupting a Dibasic Prohormone Processing Site in Pro-Opiomelanocortin (POMC) Increases Susceptibility to Early-Onset Obesity through a Novel Molecular Mechanism". *Human Molecular Genetics* 11(17): 1997–2004.

Chooi, Yu Chung, Cherlyn Ding, a Faidon Magkos. 2019. „The Epidemiology of Obesity". *Metabolism* 92: 6–10.

„ICD-11 - Mortality and Morbidity Statistics". https://icd.who.int/browse11/l-m/en#/http://id.who.int/icd/entity/149403041 (30. duben 2019).

Jeong, Jin Kwon, Jae Geun Kim, a Byung Ju Lee. 2014. „Participation of the Central Melanocortin System in Metabolic Regulation and Energy Homeostasis". *Cellular and Molecular Life Sciences* 71(19): 3799–3809.

Krabbe, K. S. et al. 2007. „Brain-Derived Neurotrophic Factor (BDNF) and Type 2 Diabetes". *Diabetologia* 50(2): 431–38.

Kühnen, Peter et al. 2016. „Proopiomelanocortin Deficiency Treated with a Melanocortin-4 Receptor Agonist". *New England Journal of Medicine* 375(3): 240–46.

Lloyd, David J., Sandy Bohan, a Nicholas Gekakis. 2006. „Obesity, Hyperphagia and Increased Metabolic Efficiency in Pc1 Mutant Mice". *Human Molecular Genetics* 15(11): 1884–93.

Machinal, Florence et al. 1999. „In Vivo and in Vitro Ob Gene Expression and Leptin Secretion in Rat Adipocytes: Evidence for a Regional Specific Regulation by Sex Steroid Hormones". 140(4): 8.

„Melanocortin 4 Receptor | Welcome to the MC4R Gene Website". *Melanocortin 4 Receptor*. https://www.mc4r.org.uk/ (7. květen 2019).

Michaud, Jacques L. et al. 2001. „Sim1 Haploinsufficiency Causes Hyperphagia, Obesity and Reduction of the Paraventricular Nucleus of the Hypothalamus". *Human Molecular Genetics* 10(14): 1465–73.

Minor, Robin K., Joy W. Chang, a Rafael de Cabo. 2009. „Hungry for Life: How the arcuate nucleus and neuropeptide Y may play a critical role in mediating the benefits of calorie restriction". *Molecular and cellular endocrinology* 299(1): 79–88.

Ollmann, Michael M. et al. 1997. „Antagonism of Central Melanocortin Receptors in Vitro and in Vivo by Agouti-Related Protein". *Science* 278(5335): 135–38.

"OUR PIPELINE." n.d. Rhythm Pharmaceuticals. Accessed May 5, 2019. https://www.rhythmtx.com/pipeline/product-pipeline/.

Pan, Weihong et al. 2012. „Endothelial leptin receptor mutation provides partial resistance to diet-induced obesity". *Journal of Applied Physiology* 112(8): 1410–18.

„Public Health - European Commission". *Union Register of medicinal products*. http://ec.europa.eu/health/documents/community-register/html/o1688.htm (8. květen 2019).

„Public Summary of Opinion on Orphan Designation Setmelanotide for the Treatment of Leptin Receptor Deficiency". : 4.

Qi, L., P. Kraft, D. J. Hunter, a F. B. Hu. 2008. „The Common Obesity Variant near MC4R Gene Is Associated with Higher Intakes of Total Energy and Dietary Fat, Weight Change and Diabetes Risk in Women". *Human Molecular Genetics* 17(22): 3502–8.

Saeed, Sadia et al. 2012. „High Prevalence of Leptin and Melanocortin-4 Receptor Gene Mutations in Children with Severe Obesity from Pakistani Consanguineous Families". *Molecular Genetics and Metabolism* 106(1): 121–26.

———. 2015. „Genetic Variants in LEP, LEPR, and MC4R Explain 30% of Severe Obesity in Children from a Consanguineous Population". *Obesity* 23(8): 1687–95.

Sandrini, Leonardo et al. 2018. „Association between Obesity and Circulating Brain-Derived Neurotrophic Factor (BDNF) Levels: Systematic Review of Literature and Meta-Analysis". *International Journal of Molecular Sciences* 19(8). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6121551/ (30. duben 2019).

Saper, Clifford B., a Bradford B. Lowell. 2014. „The Hypothalamus". *Current Biology* 24(23): R1111–16.

Sarmento-Cabral, André et al. 2017. „Adipokines (Leptin, Adiponectin, Resistin) Differentially Regulate All Hormonal Cell Types in Primary Anterior Pituitary Cell Cultures from Two Primate Species". *Scientific Reports* 7(1). http://www.nature.com/articles/srep43537 (5. duben 2019).

„Setmelanotide|RM-493;BIM-22493;IRC-022493|CAS 920014-72-8 Buy Setmelanotide from supplier medchemexpress.com". *MedchemExpress.com*. https://www.medchemexpress.com/Setmelanotide.html (7. květen 2019).

Slominski, A. 1996. „The Expression of Proopiomelanocortin (POMC) and of Corticotropin Releasing Hormone Receptor (CRH-R) Genes in Mouse Skin". *Biochimica et Biophysica Acta (BBA) - General Subjects* 1289(2): 247–51.

Sobhani, I. 2000. „Leptin Secretion and Leptin Receptor in the Human Stomach". *Gut* 47(2): 178–83.

Tao, Ya-Xiong. 2007. „Functional Characterization of Novel Melanocortin-3 Receptor Mutations Identified from Obese Subjects". *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* 1772(10): 1167–74.

Tao, Ya-Xiong, a Deborah L. Segaloff. 2003. „Functional Characterization of Melanocortin-4 Receptor Mutations Associated with Childhood Obesity". *Endocrinology* 144(10): 4544–51.

Tartaglia, Louis A. et al. 1995. „Identification and Expression Cloning of a Leptin Receptor, OB-R". *Cell* 83(7): 1263–71.

Thaker, Vidhu V. 2017. „GENETIC AND EPIGENETIC CAUSES OF OBESITY". *Adolescent medicine: state of the art reviews* 28(2): 379–405.

Tounian, Patrick. 2011. „Programming towards Childhood Obesity". *Annals of Nutrition and Metabolism* 58(Suppl. 2): 30–41.

Tsai, Minglun, Akihiro Asakawa, Haruka Amitani, a Akio Inui. 2012. „Stimulation of leptin secretion by insulin". *Indian Journal of Endocrinology and Metabolism* 16(Suppl 3): S543–48.

Vos, Piet De, Régis Saladin, Johan Auwerx, a Bart Staels. 1995. „Induction of Ob Gene Expression by Corticosteroids Is Accompanied by Body Weight Loss and Reduced Food Intake". *Journal of Biological Chemistry* 270(27): 15958–61.

„WHO | Obesity". *WHO*. https://www.who.int/topics/obesity/en/ (7. duben 2019).

Yang, Li-Kun, a Ya-Xiong Tao. 2017. „Biased Signaling at Neural Melanocortin Receptors in Regulation of Energy Homeostasis". *Biochimica et biophysica acta* 1863(10 Pt A): 2486–95.

Zhang, Yiying et al. 1994. „Positional Cloning of the Mouse Obese Gene and Its Human Homologue". *Nature* 372(6505): 425–32.